

## CATALYSIS OF NONTRONITE IN PHENOLS AND GLYCINE TRANSFORMATIONS

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**Abstract**—The catalytic power of Ca-nontronite (0.2–2  $\mu\text{m}$ ) for the polycondensation of phenols and glycine and the associated reactions that involve the ring cleavage of phenols and the decarboxylation and deamination of glycine was studied in systems free of microbial activity. At the end of a 90-hr reaction period, the amount of  $\text{CO}_2$  released from the Ca-nontronite-glycine-pyrogallol, Ca-nontronite-glycine-catechol, and Ca-nontronite-glycine-hydroquinone systems were 5.1, 8.7, and 11.6 times higher, respectively, than those from the respective nontronite-free systems, showing the catalytic role of Ca-nontronite in the ring cleavage of phenols and the decarboxylation of glycine. The release of  $\text{CO}_2$  and  $\text{NH}_3$  from the Ca-nontronite-glycine system revealed that Ca-nontronite can catalyze decarboxylation and deamination of glycine. The ability of Ca-nontronite to catalyze the deamination of glycine was substantially enhanced by the presence of a phenol. The visible absorbances at both 472 and 664 nm of the supernatants, the total yields of N-containing humic polymers, and the fractions of glycine converted to nitrogenous polymers indicated that polycondensation of glycine and phenol was greatly catalyzed by Ca-nontronite. The total N-containing humic polymers formed in the systems decreased in the order: Ca-nontronite-glycine-pyrogallol > Ca-nontronite-glycine-catechol > Ca-nontronite-glycine-hydroquinone > glycine-pyrogallol > glycine-hydroquinone > glycine-catechol. The infrared and electron spin resonance spectra of humic acid (HA) and fulvic acid (FA) formed in the supernatants of the reaction systems were quite similar to those of soil HA and FA. The catalytic power of Ca-nontronite in affecting the ring cleavage of phenols, deamination and decarboxylation of amino acids, and formation of humic substances derived from phenols with amino acids in soils and the related environments thus merits attention.

**Key Words**—Catalysis, Deamination, Decarboxylation, Glycine, Nontronite, Phenol, Polycondensation.

### INTRODUCTION

The polycondensation of phenols with nitrogenous compounds, such as amino acids, peptides, and proteins, and the subsequent formation of model humic polymers, mediated by phenolases, was reported by Swaby and Ladd (1962), Haider *et al.* (1965), and Stevenson (1982). Flaig *et al.* (1975) proposed two reaction mechanisms of phenols with amino acids in the presence of phenoloxidases: (1) the addition of amino acid to phenol; and (2) the deamination and decarboxylation of amino acid. Andreux *et al.* (1977) prepared brown, N-containing polymers by auto-oxidation at pH 7.9 from an equimolar mixture of catechol with  $^{14}\text{C}$ -labeled on the aromatic ring and glycine with  $^{14}\text{C}$ -labeled on the carboxyl carbon or on the alkyl carbon of glycine. They found that the  $^{14}\text{CO}_2$  released from the biodegradation of these polymers, when placed in an organic mull of neutral reaction, was mostly derived from carboxyl carbon of glycine incorporated into polymers. Wang *et al.* (1978) used illite, montmorillonite, kaolinite, quartz, and an oxisol as catalysts in the synthesis of humic polymers from a mixture of phenolic compounds, glycine, and ammonia. They reported that pyrophosphate-extractable humic acids (HAs) formed in the reaction systems contained appreciable amounts of nitrogen. Shindo and Huang

(1984b) reported that  $\text{MnO}_2$  promoted the deamination of glycine and the abiotic formation of N-containing HA. Wang *et al.* (1985) reported that Ca-illite and an oxisol catalyzed the formation of N-containing HAs in a solution of 12 phenolic compounds mixed with amino acids or dipeptide. They found that Ca-illite and an oxisol increased both the yields and the nitrogen content of the HAs.

Clay minerals are nearly ubiquitous in soils and sediments. Nontronite is a common smectite in soils and sediments (Borchardt, 1977), and its structural Fe(III) is usually regarded as an electron acceptor in oxidation-reduction reactions (Solomon, 1968; Theng, 1974). Glycine is a common amino acid in soils (Bremner, 1967; McKeague *et al.*, 1986), and pyrogallol, catechol, and hydroquinone are common phenols in soils (Flaig *et al.*, 1975; Martin and Haider, 1980). These three phenols have often been selected as precursors to synthesize humic-like polymers biotically (Schnitzer *et al.*, 1984) and abiotically (Shindo and Huang, 1982, 1984a, 1984b, 1985a, 1985b; Wang *et al.*, 1986); however, the structural differences among pyrogallol, catechol, and hydroquinone in influencing their abiotic polycondensation with glycine and the associated deamination and the decarboxylation of glycine as catalyzed by nontronite still remain to be established. The present study was therefore undertaken to investigate this problem.

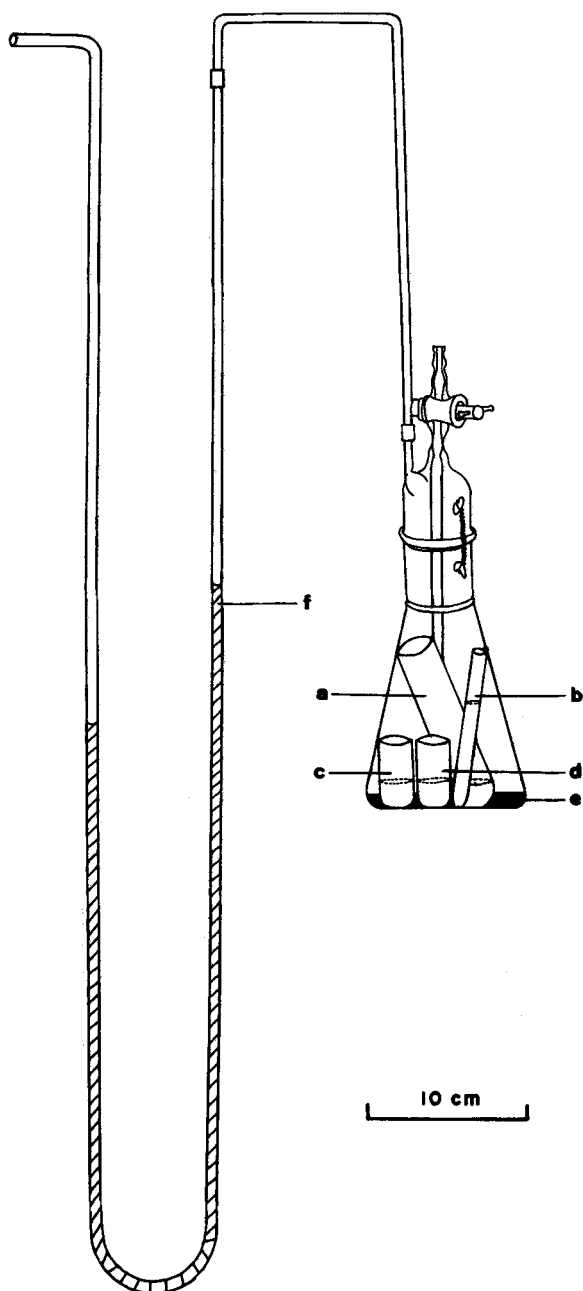


Figure 1. Diagram of apparatus used to determine the amount and distribution of  $\text{CO}_2$  and  $\text{NH}_3$  released upon uptake of  $\text{O}_2$ . (a) 5 ml of 50% KOH, (b) 5 ml of 4%  $\text{H}_3\text{BO}_3$ , (c) 5 ml of 1 M pyrogallol or catechol or 10 ml of 0.5 M hydroquinone, (d) 5 ml of 2 M glycine, (e) 15 or 20 ml of aqueous solution which contained 0.02% (w/v) thimerosal and 1000 mg of autoclaved Ca-nontronite, and (f) Brodie solution.

## MATERIALS AND METHODS

### Materials

The nontronite sample (SWa-1, ferruginous smectites, Grant County, Washington) was obtained from the Source Clay Minerals Repository of The Clay Mineral Society. It was lightly ground and suspended in dilute  $\text{Na}_2\text{CO}_3$  solution (pH 9.5)

at a 1:10 (w/v) ratio (Jackson, 1979). The mineral was dispersed by ultrasonification at 50 watts for 3 min. The 0.2–2- $\mu\text{m}$  particle size fraction was collected by a combination of sedimentation and centrifugation methods (Jackson, 1979).

Thirty grams of the mineral sample was washed five times with 180 ml of 0.5 M  $\text{CaCl}_2$  solution, adjusted to pH 6.5 with 0.01 M HCl, washed with water, dialyzed against deionized-distilled water until  $\text{Cl}^-$  free, and then freeze-dried. The freeze-dried mineral was examined by X-ray powder diffraction (XRD) and infrared (IR) absorption analyses. The specific surface area of the freeze-dried mineral sample (0.2–2  $\mu\text{m}$ ) was estimated by the ethylene glycol monoethyl ether (EGME) retention method (Eltantawy and Arnold, 1973).

Pyrogallol, catechol, hydroquinone, and glycine of analytical grade were obtained from Sigma Chemical Company.

### Determination of released $\text{CO}_2$

To establish that all the reaction systems in this study were abiotic, one gram of each freeze-dried Ca-nontronite was sterilized at 121°C under 15 lb steam pressure for 15 min in an autoclave. One gram of the autoclaved Ca-nontronite (0.2–2  $\mu\text{m}$ ) was suspended in a plastic vial in about 10 or 15 ml of a sterilized aqueous solution containing 0.02% (w/v) thimerosal (an antiseptic). The pH of the suspension was adjusted to 6.00 with 0.01 M HCl over a period of several days until the pH stabilized. An apparatus (Figure 1) was designed to determine the amounts and distribution of  $\text{CO}_2$  and  $\text{NH}_3$  released upon the uptake of  $\text{O}_2$  in the systems. All the suspension was transferred to the autoclaved 500-ml, wide-mouth Erlenmeyer flask (Figure 1). A small amount of sterilized distilled water (pH 6.00) was used to rinse the plastic vial, and the suspension was then transferred to the Erlenmeyer flask. The flask containing the suspension was weighed, and sterilized deionized-distilled water (pH 6.00) was added to adjust the volume of the suspension to 15 or 20 ml. An autoclaved 45-ml polyethylene centrifuge tube containing 5 ml of 50% KOH and an autoclaved 8-ml polyethylene tube containing 5 ml of 4%  $\text{H}_3\text{BO}_3$  were placed in the flask in such a way that they leaned against the wall of the flask. Two 15-ml autoclaved polyethylene tubes (flat bottom), one containing 5 ml of 1 M pyrogallol or catechol or 10 ml of 0.5 M hydroquinone and the other containing 5 ml of 2 M glycine, were similarly placed in the flask. Finally, each flask was capped tightly with a ground cap which was then fastened by a pair of steel springs to the flask. The cap of the flask was connected by Tygon tubing to a manometer containing the same manometer fluid, Brodie solution (23 g of NaCl and 5 g of sodium choleate dissolved in 500 ml of water) as that used in a Gilson Respirometer (Umbreit *et al.*, 1964). Each flask was shaken slowly to mix the pyrogallol, catechol, or hydroquinone and glycine solutions with the Ca-nontronite suspension, and then gently agitated in a water bath at 25°C for 90 hr. All the experiments including the controls were duplicated. At the end of the reaction period, the end of the Tygon tubing that was connected to the manometer was closed with a clamp. Each flask was disconnected from the manometer and flushed with  $\text{N}_2$  to sweep the gases present into a tube containing 20 ml of 0.15 M KOH to absorb the  $\text{CO}_2$  present. By the same procedure used for flushing the  $\text{CO}_2$  present in the flasks, another duplicated set of reaction flasks were flushed with  $\text{N}_2$  to sweep the gases that were present into tubes containing 10 ml of 4%  $\text{H}_3\text{BO}_3$  to absorb the  $\text{NH}_3$  present. The amount of the  $\text{CO}_2$  absorbed by the KOH solution after flushing  $\text{N}_2$  through the system and by the KOH solution that was placed in a flask to absorb the released  $\text{CO}_2$  during the reaction period were determined by titration (Tiessen *et al.*, 1981). The released  $\text{NH}_3$  absorbed by the  $\text{H}_3\text{BO}_3$  solution after flushing  $\text{N}_2$  through the system and by the  $\text{H}_3\text{BO}_3$  solution that was placed in the flask to absorb the released  $\text{NH}_3$  during the reaction period

Table 1. Examination of growth of microorganisms in the reaction systems during 40-day incubation period.

Nontronite	Glycine	Phenol	Thimerosal <sup>1</sup>	Inoculum <sup>2</sup>	Growth of microorganisms in various media <sup>3</sup>		
					GYEN	AIA	Czapek-Dox
P <sup>4</sup>	P	Pyrogallol	P	A <sup>5</sup>	— <sup>6</sup>	—	—
A	P	Pyrogallol	P	A	—	—	—
P	P	Catechol	P	A	—	—	—
A	P	Catechol	P	A	—	—	—
P	P	Hydroquinone	P	A	—	—	—
A	P	Hydroquinone	P	A	—	—	—
P	P	A	P	A	—	—	—
A	P	A	P	A	—	—	—
A	A	A	A	P	+ <sup>7</sup>	+	+

<sup>1</sup> This antiseptic does not affect the oxidation process of phenolic compounds (Wang *et al.*, 1983).

<sup>2</sup> Centrifugate obtained at 1000 *g* from a soil-water suspension (soil: water = 1:10) was used as the inoculum. Fresh soil was taken from the Ap horizon of a sandstone-shale noncalcareous older alluvial soil (Aeric Haplaquepts), Tali Series (120°40'E, 24°7'N) (Hsieh and Wang, 1989).

<sup>3</sup> GYEN = agar plate made of glucose, yeast extract, nutrient broth and agar for incubation of bacteria (Germida and Casida, 1980); AIA = Actinomycete isolation agar for incubation of actinomycetes (Olson *et al.*, 1984); Czapek-Dox = agar plate for incubation of fungi (Olson *et al.*, 1984).

<sup>4</sup> In the presence.

<sup>5</sup> In the absence.

<sup>6</sup> No growth.

<sup>7</sup> Growth detected.

were determined by Kjeldahl method (Bremner and Mulvaney, 1982).

#### Examination of microbial activity

To establish that all the reactions in this experiment were abiotic, a small amount of individual suspensions containing autoclaved Ca-nontronite, sterilized water, thimerosal, glycine, and pyrogallol, catechol, or hydroquinone was inoculated to the agar plates of GYEN (plate made of glucose, yeast extract, nutrient broth, and agar for incubation of bacteria) (Germida and Casida, 1980), AIA (actinomycete isolation agar for incubation of actinomycetes), and Czapek-Dox (plate for incubation of fungi) (Olson *et al.*, 1984), both at the beginning and at the end of the 90-hr reaction period to check for microbial growth in the systems. In addition, the centrifugate obtained at 1000 *g* from a soil-water suspension (soil: water = 1:10) was used as the inoculum in one of the treatments (Table 1). The fresh soil was taken from the Ap horizon of a sandstone-shale noncalcareous older alluvial soil (Aeric Haplaquepts), Tali Series (120°40'E, 24°7'N) (Hsieh and Wang, 1989). All the inoculated plates were incubated at 25°C for 40 days.

#### Formation of N-containing humic polymers

At the end of the 90-hr reaction period, the pH of the suspensions in the 500-ml Erlenmeyer flasks (Figure 1) was measured. The suspensions were then centrifuged at 7800 *g* for 10 min to separate the supernatants from the sediments. The absorbances of the supernatants at 472 and 664 nm were determined, and the sediments were washed with deionized-distilled water twice at the 1:10 (w/v) ratio to remove water-soluble NH<sub>4</sub><sup>+</sup>. The washings were combined with the supernatants. The washed sediments were treated with 2 M KCl at the 1:10 (w/v) ratio to extract the exchangeable NH<sub>4</sub><sup>+</sup> adsorbed on humic polymer-mineral complexes (Keeney and Nelson, 1982). The KCl extracted sediments were then treated with deionized-distilled water several times at the 1:10 (w/v) ratio to extract the water-soluble humic polymers until the extracts were clear. All the extracts were combined with their respective supernatants. The combination solutions were acidified to pH 1.0 with 6 M HCl, according to the method

recommended by the International Humic Substances Society (Aiken, 1985; Hayes, 1985; Leenheer, 1985; Swift, 1985). The acidified solution was equilibrated at room temperature for 24 hr and then centrifuged at 2000 *g* to separate the HA fraction from the fulvic acid (FA) fraction (supernatant). The precipitated HA fractions were suspended in a 0.1 M HCl: 0.3 M HF solution in plastic containers and shaken overnight at room temperature. The suspensions were centrifuged at 2000 *g* to precipitate HA fractions, which were then suspended in deionized-distilled water and transferred to dialysis tubes with a molecular-weight cutoff of 1000 to separate the fractions of HA (MW > 1000). The supernatants containing the FA fractions were passed through XAD-8 resin to adsorb FA, and the effluents were analyzed for NH<sub>4</sub><sup>+</sup> by the Kjeldahl method (Bremner and Mulvaney, 1982). The FA fractions adsorbed on XAD-8 were eluted with 0.1 M NaOH, the NaOH eluates were immediately acidified with 6 M HCl to pH 1.0, and HF was added to each eluate to make 0.3 M HF. The solutions were stirred for at least 48 hr in a fumehood and passed through XAD-8 resin to adsorb FA; the effluents were discarded. The FA adsorbed by XAD-8 was eluted with 0.1 M NaOH; the NaOH eluates were then immediately passed through H<sup>+</sup>-saturated cation-exchange resin (Bio-Rad AG MP-50) to convert all FA to H<sup>+</sup>-saturated FA. The H<sup>+</sup>-saturated FA solutions were concentrated by using a rotary evaporator to an adequate volume. The concentrated FA solutions were transferred to dialysis tubes with molecular-weight cutoff of 1000 to separate the fractions of FA (MW > 1000). The equilibrated solutions outside the dialysis tubes were then concentrated by using a rotary evaporator to collect the fraction of FA (MW < 1000). All HA and FA samples were freeze-dried, their yields determined, analyzed for N contents (Bremner and Mulvaney, 1982).

#### Characterization of humic polymers

The IR spectra of the freeze-dried HA and FA samples were obtained from KBr discs (each disc contained 2 mg sample and 300 mg KBr). The spectra were recorded in the 300–4000-cm<sup>-1</sup> range on a Perkin-Elmer 457A IR spectrophotometer. The electron spin resonance (ESR) spectra of freeze-dried powders of HA and FA were obtained with a Bruker ER 200D

Table 2. Release of carbon dioxide in nontronite-glycine-phenol systems at end of 90-hr reaction period.

Phenol	CO <sub>2</sub> release (μmole) <sup>1</sup>
Pyrogallol	345 ± 7 (67 ± 2) <sup>2</sup>
Catechol	365 ± 1 (42 ± 2)
Hydroquinone	348 ± 2 (30 ± 2)
None	4 ± 2 (N.D.) <sup>3</sup>

<sup>1</sup> The amounts of CO<sub>2</sub> released in the systems which contained one gram of Ca-nontronite (0.2–2 μm), 10 mmole of glycine, and 5 mmole of pyrogallol, catechol or hydroquinone in 30 ml of aqueous solution adjusted to pH 6.00.

<sup>2</sup> Values in parentheses are the CO<sub>2</sub> values obtained in the absence of nontronite.

<sup>3</sup> Not detectable.

10/12 spectrometer operating at 100 kHz and a nominal frequency of 9620 MHz at room temperature.

#### Determination of organic-C and N contents of nontronite-humic polymer complexes

After extraction of HA and FA with deionized-distilled water, the sediments were freeze-dried and their organic-C contents (Tiessen *et al.*, 1981) and the total N (Bremner and Mulvaney, 1982) determined.

## RESULTS AND DISCUSSION

Growth of microorganisms in the systems studied was not evident (Table 1); all the reactions that occurred in this study were thus abiotic in nature.

At the end of a 90-hr reaction period, the amounts of the CO<sub>2</sub> released from the following eight systems were determined: (1) Ca-nontronite-glycine-pyrogallol, (2) glycine-pyrogallol, (3) Ca-nontronite-glycine-catechol, (4) glycine-catechol, (5) Ca-nontronite-glycine-hydroquinone, (6) glycine-hydroquinone, (7) Ca-nontronite-glycine, and (8) glycine. The amounts of the CO<sub>2</sub> released from the Ca-nontronite-glycine-pyrogallol, Ca-nontronite-glycine-catechol, and Ca-nontronite-glycine-hydroquinone systems were 5.1, 8.7, and 11.6 times greater, respectively, than those from the glycine-pyrogallol, glycine-catechol, and glycine-hydroquinone systems (Table 2). Another investigation in this laboratory has found that Ca-nontronite greatly promotes the ring cleavage of pyrogallol, catechol, and hydroquinone and the subsequent release of CO<sub>2</sub> in the reaction systems. The data in Table 2 show that the amounts of CO<sub>2</sub> released from the Ca-nontronite-glycine-phenol and glycine-phenol systems were much higher, respectively, than those from the Ca-nontronite-phenol and phenol systems. Thus, Ca-nontronite greatly promoted the ring cleavage of phenols and the decarboxylation of glycine. The amount of CO<sub>2</sub> released from the Ca-nontronite-glycine system was 4

μmole, whereas that from the pure glycine system was not detectable (Table 2). Thus, Ca-nontronite promoted the CO<sub>2</sub> release from glycine in the system.

At the end of the reaction period, except for the glycine-catechol and the Ca-nontronite-glycine systems, the pH of the suspensions, which were initially adjusted to pH 6.00, decreased substantially (Table 3). This decrease was likely due to the generation of protons during the polycondensation of glycine and phenols either in the presence or in the absence of Ca-nontronite. The final pH of the suspensions in the Ca-nontronite-glycine-phenol systems were much lower than those in their corresponding glycine-phenol systems (Table 3), apparently because of a higher degree of humification of glycine and phenol in the presence of Ca-nontronite. A decrease in the final pH in the glycine-pyrogallol and glycine-hydroquinone systems was observed, but not in the glycine-catechol system, indicating that the structures and functionality of phenols played an important role in determining their polycondensation with glycine. Moreover, they were apparently related to the final pH of the reaction systems. An increase in the final pH in the Ca-nontronite-glycine system (Table 3) may have been due to the deamination of glycine as catalyzed by Ca-nontronite. The pH of the glycine system was stable during the reaction period (Table 3).

The presence of Ca-nontronite in the solutions of the glycine-phenol systems greatly enhanced the absorbance of the supernatants at 472 and 664 nm (Table 3). The absorbances of the supernatants at 472 nm in the Ca-nontronite-glycine-pyrogallol, Ca-nontronite-glycine-catechol, and Ca-nontronite-glycine-hydroquinone systems were 10.6, 190, and 86.5 times greater, respectively, than those in the glycine-pyrogallol, glycine-catechol, and glycine-hydroquinone systems, whereas the absorbances at 664 nm in the systems were 30.8, 409, and 184 times greater, respectively, (Table 3). The total yields of synthesized humic polymers formed in the reaction systems decreased in the order: Ca-nontronite-glycine-pyrogallol >>> Ca-nontronite-glycine-catechol > Ca-nontronite-glycine-hydroquinone > glycine-pyrogallol > glycine-hydroquinone > glycine-catechol (Table 3). The total yields of synthesized humic polymers formed in the Ca-nontronite-glycine-pyrogallol, Ca-nontronite-glycine-catechol, and Ca-nontronite-glycine-hydroquinone systems were 2.9, 2.5, and 1.9 times greater, respectively, than of those formed in the glycine-pyrogallol, glycine-catechol, and glycine-hydroquinone systems (Table 3). In the Ca-nontronite-glycine-phenol systems, the sequences for the absorbances of the supernatants at both 472 and 664 nm were not in good agreement with that for the total yields of synthesized humic polymers formed. This may be attributable to the structures and functionality of phenols and the properties of the humic polymers formed; however, the data still indicate that



Ca-nontronite was a strong catalyst for the transformations of glycine and phenols to form humic polymers. The humic polymers formed in the Ca-nontronite-glycine system were not detectable; the glycine was very stable during the reaction period (Table 3).

Most of the glycine converted in the reaction systems were in the forms: (1)  $\text{NH}_4^+$  in supernatants of the suspensions, (2)  $\text{NH}_4^+$  adsorbed on organo-mineral complexes, (3) N in humic polymers (HA and FA), and (4) N in nonextractable humic substances (Table 4). The  $\text{NH}_4^+$  present is due mainly to the deamination of glycine in the reaction systems. The deamination from the Ca-nontronite-glycine-pyrogallol, Ca-nontronite-glycine-catechol, and Ca-nontronite-glycine-hydroquinone systems were 2.3, 14.5, and 12.0 times greater, respectively, than that from the glycine-pyrogallol, glycine-catechol, and glycine-hydroquinone systems (Table 4). The conversion of glycine-N to polymer-N in the Ca-nontronite-glycine-pyrogallol, Ca-nontronite-glycine-catechol, and Ca-nontronite-glycine-hydroquinone systems were 2.8, 4.3 and 6.1 times greater, respectively, than that in the glycine-pyrogallol, glycine-catechol, and glycine-hydroquinone systems (Table 4). The deamination from the Ca-nontronite-glycine system was only 3.0  $\mu\text{mole}$ , whereas that from the glycine system was not detectable during the reaction period (Table 4). Table 4 also shows that the amounts of total glycine-N converted in the Ca-nontronite-glycine-pyrogallol, Ca-nontronite-glycine-catechol, and Ca-nontronite-glycine-hydroquinone systems were 2.7, 9.6, and 10.0 times greater, respectively, than those in the glycine-pyrogallol, glycine-catechol, and glycine-hydroquinone systems. The amount of total glycine-N converted in the Ca-nontronite-glycine system was only 3  $\mu\text{mole}$ , whereas that in the glycine system was not detectable (Table 4). Thus, Ca-nontronite apparently promoted the conversion of glycine in the glycine-phenol solutions and that phenols greatly accelerated the deamination of glycine and its conversion to N-containing humic polymers. The small amount of glycine converted in the nontronite-glycine system can be regarded as a catalytic effect of Ca-nontronite. The data in Table 2 and 4 also show that glycine was oxidatively decomposed by the catalysis of Ca-nontronite and that the release of  $\text{CO}_2$  through decarboxylation was accompanied by the evolution of  $\text{NH}_3$  through deamination. In addition to the differences in the absorbances of the supernatants between the Ca-nontronite-glycine-phenol systems and their corresponding glycine-phenol systems shown in Table 3, the great enhancement in the conversion of glycine to N-containing humic polymers in the Ca-nontronite-glycine-phenol systems also indicates that polycondensation of glycine and phenols was catalyzed by Ca-nontronite. The absorbances of supernatants of the suspensions (Table 3), the total yields of polymers formed in the reaction systems (Table 3), and the con-

Table 3. Final pH of suspensions, absorbance of supernatants, and yield of polymers for nontronite-glycine-phenol systems.

Nontronite	Reaction condition <sup>1</sup>		Final pH		Absorbance		Supernatant		Humic polymers adsorbed in sediment <sup>2</sup> (mg)	Total humic polymers (mg)
	Glycine	Phenol	472 nm	664 nm	472 nm	664 nm	Humic acid (MW > 1000) (mg)	Fulvic acid (MW > 1000) (mg)		
P <sup>3</sup>	P	Pyrogallol	4.04 ± 0.00	4.01 ± 0.06	7.83 ± 0.08	4.01 ± 0.06	16.3 ± 0.2	80.6 ± 0.3	9.7 ± 0.8	107 ± 1
A <sup>4</sup>	P	Pyrogallol	5.39 ± 0.01	0.13 ± 0.01	0.74 ± 0.03	0.13 ± 0.01	5.9 ± 0.1	30.8 ± 0.2	N.A. <sup>5</sup>	36.7 ± 0.3
P	P	Catechol	4.46 ± 0.01	4.09 ± 0.09	11.4 ± 0.1	4.09 ± 0.09	7.5 ± 0.1	32.3 ± 0.2	6.0 ± 0.8	46 ± 1
A	P	Catechol	6.32 ± 0.02	0.06 ± 0.00	0.06 ± 0.00	0.01 ± 0.00	0.8 ± 0.0	17.6 ± 0.1	N.A.	18.4 ± 0.1
P	P	Hydroquinone	4.47 ± 0.00	3.67 ± 0.09	14.7 ± 0.1	3.67 ± 0.09	7.3 ± 0.1	29.6 ± 0.2	5.0 ± 0.2	41.9 ± 0.5
A	P	Hydroquinone	5.26 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.02 ± 0.00	2.4 ± 0.0	19.2 ± 0.1	N.A.	21.6 ± 0.1
P	P	A	6.20 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N.D. <sup>6</sup>	N.D.	N.D.	N.D.
A	P	A	6.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N.D.	N.D.	N.A.	N.D.

<sup>1</sup> 1 g of Ca-nontronite was suspended in 30 ml aqueous solution which contained 10 mmole glycine, and 5 mmole of pyrogallol, catechol or hydroquinone; pH adjusted to 6.00.

<sup>2</sup> Weights of humic polymer-mineral complexes formed in the nontronite-glycine-pyrogallol, nontronite-glycine-catechol, and nontronite-glycine-hydroquinone systems were 1004 ± 2, 961 ± 1, and 998 ± 2 mg, respectively; the organic C contents of the complexes were 0.79 ± 0.03, 0.62 ± 0.03, and 0.56 ± 0.00%, respectively. Organic-C contents of nontronite was 0.31 ± 0.01%. Organic-C content of the nontronite was subtracted, then amounts of humic substances in the sediments were estimated on the basis of the previous finding that synthesized humic substances contain about 50% organic C (Wang *et al.*, 1983).

<sup>3</sup> In the presence.

<sup>4</sup> In the absence.

<sup>5</sup> Not applicable.

<sup>6</sup> Not detectable.

Table 4. Distribution of the converted glycine-N to  $\text{NH}_4^+$ ,  $\text{NH}_3$ , and N-polymers as influenced by nontronite and phenols at end of 90-hr reaction period.

Nontronite	Reaction condition <sup>1</sup>		$\text{NH}_4^+$			Polymer N			Total converted glycine-N ( $\mu\text{mole}$ )		
	Glycine	Phenol	Supernatant ( $\mu\text{mole}$ )	Exchangeable form ( $\mu\text{mole}$ )	$\text{NH}_3$ ( $\mu\text{mole}$ )	Sum ( $\mu\text{mole}$ )	Humic acid (MW > 1000) ( $\mu\text{mole}$ )	Fulvic acid <sup>2</sup> ( $\mu\text{mole}$ )		Non-extractable humic substances ( $\mu\text{mole}$ )	Sum ( $\mu\text{mole}$ )
P <sup>3</sup>	P	Pyrogallol	78 ± 3	8.6 ± 0.8	4.9 ± 0.0	92 ± 4	66.0 ± 0.8	143.2 ± 0.6	83.3 ± 0.2	293 ± 2	385 ± 6
A <sup>4</sup>	P	Pyrogallol	13 ± 1	2.4 ± 0.2	6.1 ± 0.0	40 ± 1	24.4 ± 0.4	78.5 ± 0.4	N.A. <sup>5</sup>	102.9 ± 0.8	143 ± 2
P	P	Catechol	956 ± 8	112 ± 4	4.4 ± 0.0	1072 ± 12	40.5 ± 0.5	90.0 ± 0.7	175.1 ± 0.2	306 ± 1	1378 ± 13
A	P	Catechol	61 ± 2	5.1 ± 0.4	8.1 ± 0.0	74 ± 2	4.5 ± 0.0	65.9 ± 0.3	N.A.	70.4 ± 0.3	144 ± 2
P	P	Hydroquinone	854 ± 7	102 ± 3	6.0 ± 0.0	962 ± 10	28.8 ± 0.4	75.4 ± 0.6	142.8 ± 0.3	247 ± 1	1209 ± 11
A	P	Hydroquinone	70 ± 2	5.8 ± 0.4	4.3 ± 0.0	80 ± 2	10.0 ± 0.0	30.5 ± 0.2	N.A.	40.5 ± 0.2	121 ± 2
P	P	A	3.0 ± 0.1	N.A.	0.0 ± 0.0	3.0 ± 0.1	N.D. <sup>6</sup>	N.D.	N.D.	N.D.	3.0 ± 0.1
A	P	A	N.D.	N.A.	N.D.	N.D.	N.D.	N.D.	N.A.	N.D.	N.D.

<sup>1</sup> Explained in Table 3.

<sup>2</sup> Including fulvic acid (MW > 1000) and fulvic acid (MW < 1000) formed in the supernatants.

<sup>3</sup> In the presence.

<sup>4</sup> In the absence.

<sup>5</sup> Not applicable.

<sup>6</sup> Not detectable.

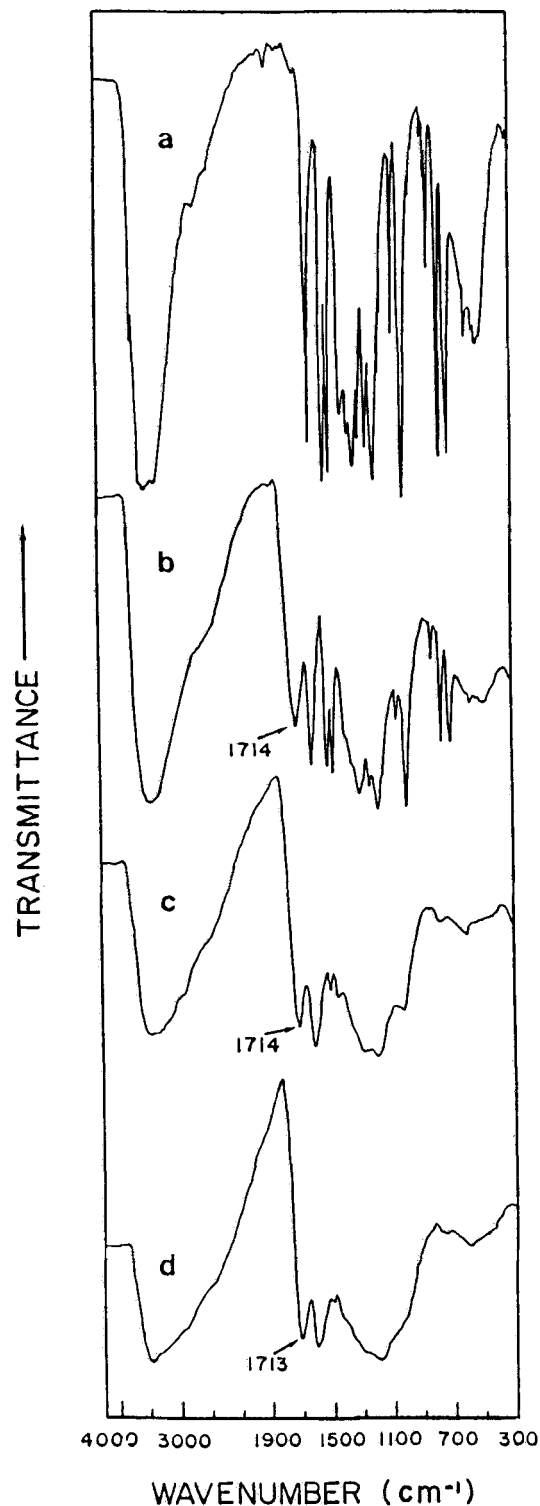


Figure 2. Infrared spectra of pyrogallol and synthesized N-containing humic polymers formed in the Ca-nontronite-glycine-pyrogallol system. (a) unpolymerized pyrogallol, (b) fulvic acid (MW < 1000), (c) fulvic acid (MW > 1000), and (d) humic acid (MW > 1000).

Table 5. Electron spin resonance spectroscopic properties of humic substances formed in nontronite-glycine-phenol systems.<sup>1</sup>

Nontronite	Reaction condition		Humic acid (MW > 1000)		Fulvic acid (MW > 1000)	
	Glycine	Phenol	g-value	Breadth (G)	g-value	Breadth (G)
P <sup>2</sup>	P	Pyrogallol	2.0031	3.7	2.0043	3.6
A <sup>3</sup>	P	Pyrogallol	2.0040	3.5	2.0036	3.4
P	P	Catechol	2.0030	3.8	2.0031	4.2
A	P	Catechol	2.0034	3.6	2.0037	4.1
P	P	Hydroquinone	2.0033	3.9	2.0033	3.5
A	P	Hydroquinone	2.0036	4.1	2.0032	3.9

<sup>1</sup> At the same signal and modulation settings, the electron spin resonance spectra of fulvic acid (MW < 1000) were not obtainable, indicating negligible free radical content.

<sup>2</sup> In the presence.

<sup>3</sup> In the absence.

version of glycine to N-containing humic polymers (Table 4) clearly indicate that Ca-nontronite strongly catalyzed the polycondensation of glycine and phenols.

Except for the relative intensity of the IR absorption band at about 1715 cm<sup>-1</sup>, the IR spectra of humic polymers formed in the reaction systems were similar. Therefore, only the IR spectra of humic polymers formed in the Ca-nontronite-glycine-pyrogallol system are shown in Figure 2. The IR spectrum of FA (MW < 1000) (Figure 2b) is similar to that of unpolymerized pyrogallol (Figure 2a), but this FA (MW < 1000) fraction displays an IR absorption band at about 1715 cm<sup>-1</sup>, which may be attributable to carboxyl groups. The IR spectra of FA (MW > 1000) (Figure 2c) and HA (MW > 1000) (Figure 2d) are completely different from that of unpolymerized pyrogallol (Figure 2a), but very similar to those of natural humic substances (Schnitzer, 1977; Hatcher *et al.*, 1980; Schnitzer and Ghosh, 1982). Absorption bands were present at 3000–3400 cm<sup>-1</sup> (hydroxyl groups with varying degrees of H bonding), 2800–2900 cm<sup>-1</sup> (stretching vibrations of aliphatic CH, CH<sub>2</sub>, and CH<sub>3</sub>), 1715 cm<sup>-1</sup> (carboxyl and aldehydic and ketonic carbonyl), 1610 cm<sup>-1</sup> (C=C-stretching vibrations in aliphatic and aromatic compounds), 1380 cm<sup>-1</sup> ( $\delta$ , CH<sub>3</sub>, salts of carboxylic acid and/or aliphatic CH vibration), 1230 cm<sup>-1</sup> (C–O-stretching vibrations of esters, ethers, and phenols), and 1030 cm<sup>-1</sup> (ethers).

The IR spectra of the synthesized FA (MW > 1000) and HA (MW > 1000) (Figures 2c and 2d) formed in the reaction systems show that the sequence for the intensity of IR absorption at 1714 cm<sup>-1</sup> is: Ca-nontronite-glycine-pyrogallol > Ca-nontronite-glycine-catechol > Ca-nontronite-glycine-hydroquinone (not shown). The titration of humic polymers (MW > 1000) formed in the Ca-nontronite-glycine-phenol systems to pH 7.0 resulted in virtual disappearance of the absorption band at about 1714 cm<sup>-1</sup>, concomitant appearance of a band at about 1610 cm<sup>-1</sup>, and intensification of the band at about 1380 cm<sup>-1</sup> (not shown). On the basis of the existing information on the nature

of soil humic substances (Schnitzer and Skinner, 1963; Wagner and Stevenson, 1965; Theng *et al.*, 1966), such shifts indicate that the absorption band at about 1714 cm<sup>-1</sup> in Figures 2b, 2c, and 2d is due mainly to carboxyl groups in the synthesized humic polymers.

No significant differences were noted in the ESR spectra of the FA (MW > 1000) and HA (MW > 1000) synthesized in the reaction systems. All the spectra were single lines, devoid of hyperfine splitting (not shown). The g-values and breadths between the two extreme peaks on the derivative curves are summarized in Table 5. The ESR spectra are similar to those of natural FAs and HAs (Schnitzer, 1977; Senesi and Schnitzer, 1977; Schnitzer and Lévesque, 1979).

Semi-quinone radicals (Table 5) appear to form through electron transfer from pyrogallol, catechol, or hydroquinone to Fe<sup>3+</sup> or other variable-valence transition metal ions on the edge or in the structure (Solomon, 1968) of Ca-nontronite. Electrons seem to be able to diffuse or tunnel to octahedral sites from layer edges or basal surfaces (Tennakoon *et al.*, 1974). Strong oxidation power of chemisorbed O<sub>2</sub> on silicate minerals (Solomon and Hawthorne, 1983), such as Ca-nontronite, appear to have caused the ring cleavage of pyrogallol, catechol, or hydroquinone (Table 2; Figure 2) and to have released of CO<sub>2</sub> and NH<sub>3</sub> from glycine (Tables 2 and 4). Semi-quinone radicals, aliphatic fragments, and glycine apparently underwent polycondensation to form N-containing humic polymers (Tables 2, 3, and 4; Figure 2). Under an N<sub>2</sub> atmosphere, the amounts of CO<sub>2</sub> and humic polymers formed drastically decreased (author's unpublished work).

In summary, Fe(III) on the edges and other electron accepting sites of Ca-nontronite contributed to the polycondensation of pyrogallol, catechol, or hydroquinone with glycine and the subsequent formation of N-containing humic polymers, the IR and ESR spectra of which resembled those of natural humic substances. Ring cleavage of phenols and the release of CO<sub>2</sub> from glycine as catalyzed by Ca-nontronite can be a pathway for carbon turnover. Furthermore, the enhancement of

phenols on the deamination of glycine as catalyzed by Ca-nontronite may be a pathway of N transformations that are significant in nature. Mineralization of N via abiotic processes thus deserves close attention.

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