

CHEMICAL STUDIES ON SOIL HUMIC ACIDS

VIII. Contribution of Carbonyl Groups to the Ultraviolet and Visible Absorption Spectra of Humic Acids

Kiyoshi TSUTSUKI and Shozo KUWATSUKA

Faculty of Agriculture, Nagoya University, Nagoya, Japan

Received January 8, 1979

Forty humic acid (HA) samples and several related substances were reduced with $\text{Na}_2\text{S}_2\text{O}_4$ and NaBH_4 in order to investigate the effect of carbonyl groups including quinone on the ultraviolet and visible absorption spectra of HAs. Difference spectra of Rp and B type HAs as well as lignin due to NaBH_4 reduction had a peak at 320 nm, while those of A type HAs had a peak and a shoulder at different wavelengths. The difference absorbance of A type HAs in the visible range was also higher than that of Rp and B type HAs. There were very significant positive linear correlations between the difference absorbance due to NaBH_4 reduction and carbonyl group contents of HAs. The absorbance of the highly humified HAs at 600 nm increased in 0.1 N NaOH but decreased in the phosphate buffer at pH 7 by the reduction with $\text{Na}_2\text{S}_2\text{O}_4$. Decreasing rates in both cases decreased with increasing degree of humification. It was presumed that the influence of ketonic and aldehydic carbonyl groups on the visible absorption was small and that of quinones was large for the low humified HAs, P₁₋₄ type HAs and the two artificial HAs prepared from hydroquinone and glucose. Though the influence of quinones also increased, that of aliphatic ketone and aldehyde on the visible absorption increased with increasing degree of humification.

Additional Index Words: bathochromic effect, phenolic hydroxyl group, quinone, sodium borohydride, sodium hydrosulfite.

We have studied the elementary (4) and the functional group compositions (8), the products by acid hydrolysis (9, 10) and the degradation with KOH (11) of soil HAs in order to clarify their chemical structures and compositions. From these results, it was presumed that the constituents and structures of natural substances are denatured to produce dark colored amorphous polymers with long conjugate system in the process of humification. Since the ultraviolet and visible absorption of HAs was presumed to be largely contributed by the dark colored polymers, we investigated the ultraviolet and visible absorption, especially their relationship to the dissociation of the phenolic and carboxyl groups of HAs in previous papers (12, 13). Carbonyl groups may largely contribute to the structure of the dark colored polymers, and therefore, it may greatly influence the ultraviolet and visible absorption of HAs. The carbonyl group contents of HAs on carbon basis ranged from 5 to 20% and increased with increasing degree of humification (8). ADLER and MARTIN (2) studied

the change in absorption spectra caused by the reduction with NaBH_4 for lignin and several carbonyl compounds with phenylpropane structures. This type of study has not yet been performed on HAs. In this study, the change in ultraviolet and visible absorption spectra of HAs caused by the reduction with NaBH_4 and $\text{Na}_2\text{S}_2\text{O}_4$ is analysed to clarify the influences of quinones and other carbonyl groups on the ultraviolet and visible absorption spectra of HAs.

MATERIALS AND METHODS

1. *Samples.* Soil HAs: Forty HAs listed in Table 1 were used. The analytical values of these samples such as elementary composition, functional group composition, the products by acid hydrolysis and KOH degradation were reported in previous papers (4, 8-13).

Fulvic acid (FA): Fr. B₄ and Fr. D₁ of a fulvic acid obtained from the Bh layer of a podzolic soil at Hichiso were used. The method of preparation, difference absorption spectra between pH 12.4 and 7 and between pH 7 and 3.5, and KOH degradation products were reported previously (11, 13).

Related substances: As artificial models for humic substances, glucose-HA, hydroquinone-HA and polymaleic acid reported previously (11) were used. Milled wood lignin of rice straw was offered by Prof. N. Terashima of Nagoya University. Elementary composition of this sample was 54.2% C, 4.9% H, and 0.39% N. This sample was also analysed by alkali-hydrolysis and KOH degradation by SHINDO and KUWATSUKA (6, 7). Cercosporin, a derivative of 3,10-dihydroxyperylene-4,9-quinone, isolated from the cultured mycelia of *Cercospora kikuchii* (15) was offered by Prof. S. Yamazaki of the University of Tokyo.

2. *Reduction of HAs with $\text{Na}_2\text{S}_2\text{O}_4$ and the measurement of absorption spectra.*

1) Reduction in 0.1 N NaOH. HA solutions in 0.1 N NaOH having concentration between 2-12 mg/100 ml were prepared. After measuring the absorption spectrum from 400 to 700 nm, about 10 mg of $\text{Na}_2\text{S}_2\text{O}_4$ was added to the solution (about 3 ml) in a measuring cell, and the absorption spectrum of the reduced HA was measured immediately. Difference absorption spectrum was measured by placing the non-reduced HA solution and the reduced solution, with Hitachi 124 spectrophotometer. The absorption spectrum of the reduced HA solution as well as the difference absorption spectrum in the range shorter than 400 nm could not be measured because of the large absorption of $\text{Na}_2\text{S}_2\text{O}_4$ itself in the range.

2) Reduction in phosphate buffer at pH 7. Five to 60 mg of each HA sample were dissolved in 100 ml of 0.1% NaOH. To 2 ml of this solution, 6 ml of 1/12 M phosphate buffer at pH 7 was added. To another 2 ml of the HA solution, 6 ml of the phosphate buffer solution which contained 30 mg of $\text{Na}_2\text{S}_2\text{O}_4$ was added and mixed to prepare the reduced HA solution. Absorption spectra of the reduced and the non-reduced HA solutions and the difference spectrum between them, $\Delta E(\text{Na}_2\text{S}_2\text{O}_4$

at pH 7) spectrum, were measured from 400 to 700 nm. The spectrum of the reduced HA was measured within 3 hr after $\text{Na}_2\text{S}_2\text{O}_4$ addition.

3) Reduction of HAs with NaBH_4 and measurement of the absorption spectra. Five to 60 mg of each HA sample was dissolved in 100 ml of 0.1% NaOH. To 2 ml of this solution, 2 ml of 0.1% NaOH and 4 ml of 0.2 M borate buffer at pH 10.1 were added. To another 2 ml of the HA solution, 2 ml of 0.8% NaBH_4 solution in 0.1% NaOH and 4 ml of the borate buffer were added. The absorption spectra of the reduced and the non-reduced HA solutions and the difference spectrum between them, $\Delta E(\text{NaBH}_4)$ spectrum, were measured from 220 to 700 nm, 15 to 18 hr after NaBH_4 addition.

RESULTS AND DISCUSSION

1. Difference spectra of HAs due to the reduction of carbonyl groups

Difference spectra of HAs due to the reduction with $\text{Na}_2\text{S}_2\text{O}_4$ in the phosphate buffer at pH 7, $\Delta E(\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) spectra, are shown in Fig. 1. There were not so much differences in ΔE spectra except for P_{++++} type HAs (curves 8, 9). The ΔE spectra reached their maxima between 420 and 450 nm and then decreased almost linearly with increasing wavelength. Their maximum peaks shifted to longer wavelength in the order: $\text{Rp} < \text{B} < \text{A} < \text{P}_{++++}$ type. This may suggest that the quinone structure which influence the visible absorbance of HAs becomes unsaturated to form longer conjugate system with increasing degree of humification. The ΔE spectra of P_{++++} type HAs had peaks at 450, 540 and 575 nm due to 3,10-dihydroxyperylene-4,9-quinone(DHPQ). DHPQ also influenced the ΔE spectra of the other HAs, showing that these HAs contain small amounts of Pg pigments. The ΔE spectra of the artificial HAs prepared from hydroquinone and glucose (Fig. 1, curves 10, 11) resembled those of soil HAs except the P_{++++} type HA. However, the difference absorbance per fixed concentration of these artificial HAs were 3.5 to 8 times as large as those of soil HAs at their maximum peaks. This implied that conjugate systems containing quinone play an important role in the visible absorption of these artificial HAs more than in soil HAs.

All the $\Delta E(\text{NaBH}_4)$ spectra of B and Rp type HAs had a peak at 320 nm (Fig. 2, curves 3-6). The $\Delta E(\text{NaBH}_4)$ spectra of the milled wood lignin of rice straw (Fig. 2, curve 13) and the Björkman lignin extracted from *Picea abies* by ADLER and MARTIN (2) also had a maximum at 320 nm and a shoulder at 340 nm. ADLER and MARTIN (2) showed that the $\Delta E(\text{NaBH}_4)$ spectra of lignin are due to coniferylaldehyde with etherified phenolic OH group (ΔE_{max} at 340 nm) and the etherified 4-keto-guayacyl group (ΔE_{max} at 303-314 nm). The similarity between the $\Delta E(\text{NaBH}_4)$ spectra of B and Rp type HAs and lignin suggests that some carbonyl groups in these HAs exist in phenylpropane structures as in lignin. Compared with lignin, HAs are characterized by high absorbance in the visible range. The $\Delta E(\text{NaBH}_4)$ of HAs were also

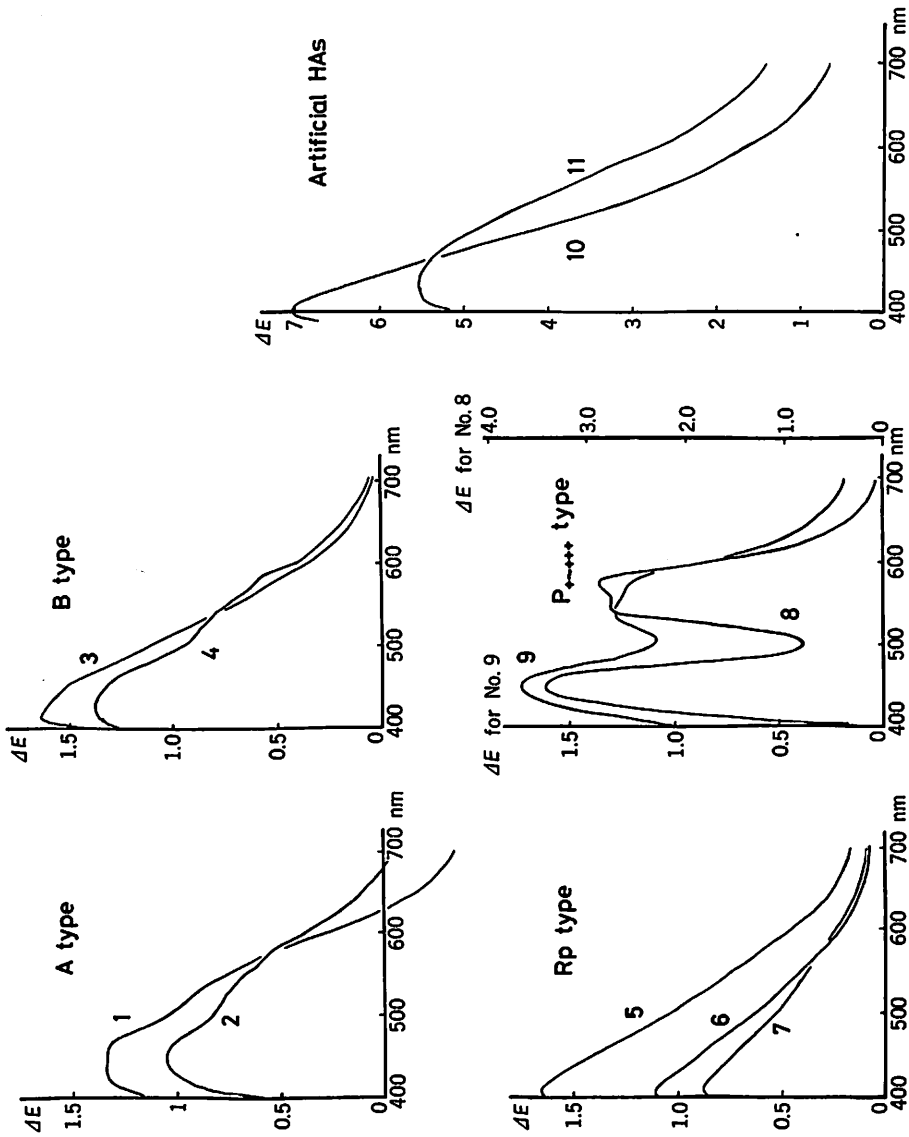


Fig. 1. Difference spectra of humic acids by $\text{Na}_2\text{S}_2\text{O}_4$ reduction in pH 7 phosphate buffer.
 1. Nagara P; 2. Inogashira-1; 3. Kinshozan F; 4. Kuragari 1; 5. Higashiyama (FH); 6. Kinshozan OH; 7. Gifu;
 8. Tsubame Pg; 9. Tsubame-I; 10. H.Q.-HA; 11. Glu.-HA. Concentrations were fixed at 0.1% organic matter.

large while that of lignin was zero in the visible range. Therefore, carbonyl groups are thought to participate in longer conjugate systems in HAs than in lignin and also influence the visible absorption of HAs.

The ΔE spectra of A type HAs (Fig. 2, curves 1, 2) differed from those of B and Rp type HAs. The ΔE spectra had a peak at 280 nm and shoulders near 320 nm and between 450 and 460 nm. Carbonyl groups in A type HAs may influence the visible absorbance in a different way, participating probably in different conjugate systems from those of B and Rp type HAs.

The ΔE spectrum of the hydroquinone-HA (Fig. 2, curve 12) had a broad shoulder around 430 nm and was similar to those of A type HAs especially in the visible range. Therefore, the large $\Delta E(\text{NaBH}_4)$ of A type HAs in the visible range may be related to the polymerized quinone structure. The ΔE spectrum of the glucose-HA had the highest peak at 355 nm and the second peak at 250 nm (Fig. 2, curve 11), and was not similar to those of soil HAs. Therefore, the hydroquinone-HA is supposed to be a suitable model for the dark colored polymers in HAs more than the glucose-HA. The $\Delta E(\text{NaBH}_4)$ per fixed concentration of the hydroquinone-HA and glucose-HA were 1.5 and 2 times larger than those of soil HAs at the wavelengths of maximum absorbance.

The $\Delta E(\text{NaBH}_4)$ spectrum of Tsubame Pg had peaks at 260 and 455 nm and shoulders at 570 and 610 nm (Fig. 2, curve 9). These peaks and shoulders are ascribed to the DHPQ derivatives. The ΔE spectrum of cercosporin, a derivative of DHPQ, had peaks at 305, 485, 600, and 635 nm and minima with minus values at 345, 380, and 395 nm (Fig. 2). Though the maxima of the $\Delta E(\text{NaBH}_4)$ spectrum of cercosporin shifted to longer wavelength than that of Tsubame Pg, the shapes of both spectra were similar to each other. Maximum $\Delta E(\text{NaBH}_4)$ of cercosporin at 485 nm was 2.7 times larger than that of Tsubame Pg at 455 nm. In addition, the $\Delta E(\text{NaBH}_4)$ spectrum of Tsubame Pg did not take minus values like that of cercosporin. These results imply that constituents other than Pg pigment may largely contribute to the ultraviolet and visible absorption spectrum of even Tsubame Pg which was purified for Pg pigment by Sephadex G-50 (5).

The $\Delta E(\text{NaBH}_4)$ spectra of the two fractions of Hichiso FA were very different from those of soil HAs. Furthermore, the spectra of the two fractions were different from each other, though these were fractionated from the same FA. These samples, Fr. B₄ and Fr. D₁, had maximum peaks at 245 and 290 nm, respectively (Fig. 2, curves 7, 8). Both samples have a common peak at 245 nm and a shoulder at 330 nm. ANDERSON and RUSSEL (1) pointed out the similarity between polymaleic acid (PMA) and FAs. We also compared the KOH degradation products of soil HAs and FAs with those of PMA (11). PMA produced only succinic acid which is also the major product from soil HAs and FAs. The wavelengths at the peak (270 nm) and the shoulder (340 nm) of the $\Delta E(\text{NaBH}_4)$ spectrum of PMA (Fig. 2, curve 14) were different from those of the FAs.

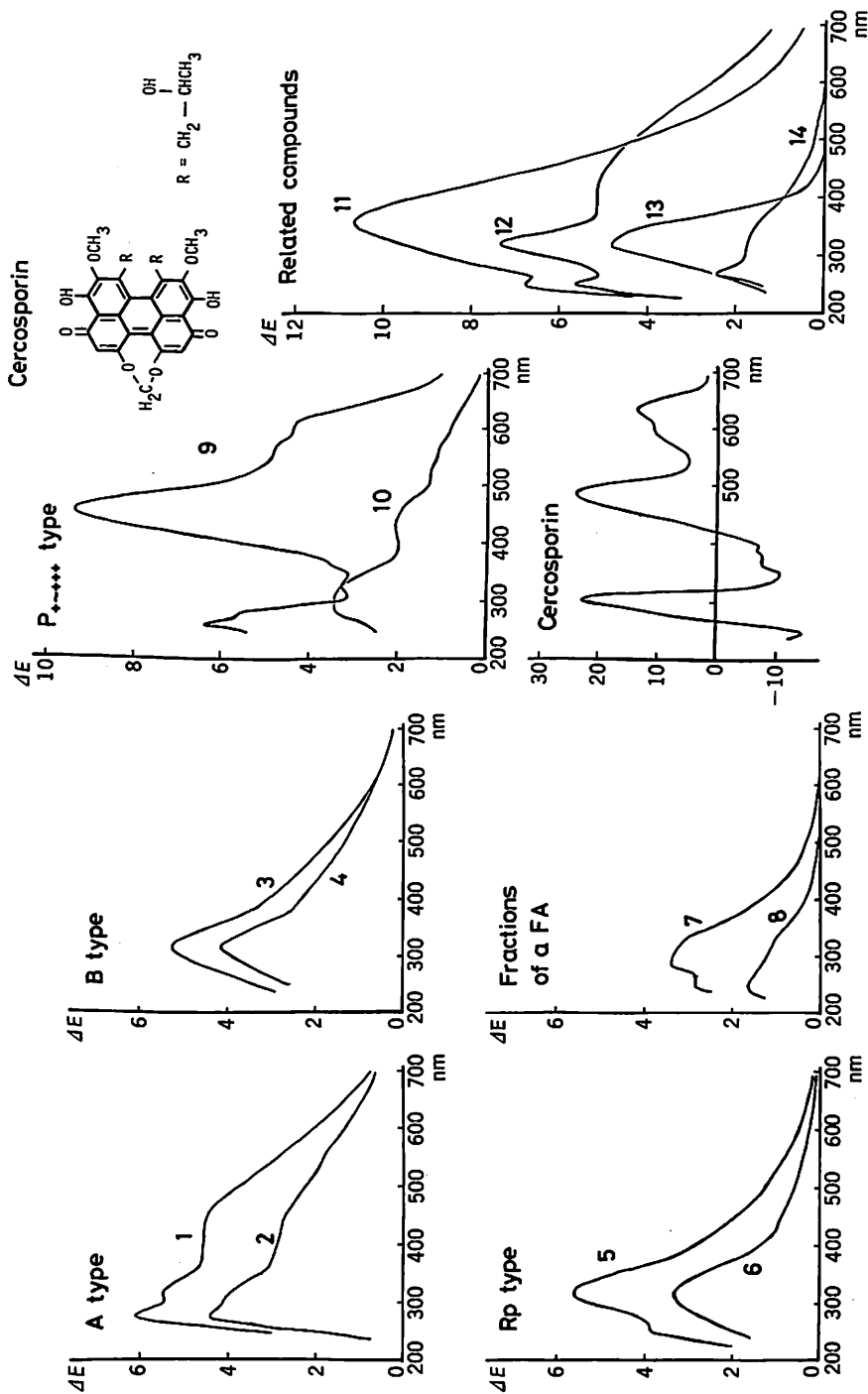


Fig. 2. Difference spectra of humic acids by NaBH_4 reduction.

1. Nagara P; 2. Inogashira-1; 3. Kinshozan F; 4. Kuragari-1; 5. Higashiyama (FH); 6. Gifu; 7. Fr. D₁; 8. Fr. B₁; 9. Tsubame Pg; 10. Tsubame-1; 11. Glu.-HA; 12. H.Q.-HA; 13. milled wood lignin of rice straw; 14. polymaleic acid. Concentrations were fixed at 0.1% organic matter.

2. Relationship between $\Delta E(\text{NaBH}_4)$ and carbonyl group content

The decrease in absorbance of soil HAs due to the reduction with NaBH_4 per fixed concentration, $\Delta E(\text{NaBH}_4)$, was compared at 285, 320, and 600 nm. These wavelengths were selected because $\Delta E/E(\text{NaBH}_4)$ was largest near 600 nm, and the absorbance at 600 nm is used to indicate the degree of humification, and also because $\Delta E(\text{NaBH}_4)$ spectra had peaks at 285 or 320 nm. The $\Delta E(\text{NaBH}_4)$ at 285, 320, and 600 nm increased with increasing E_{600} . In addition, very significant linear correlations were found between carbonyl content and the $\Delta E(\text{NaBH}_4)$ at 285 and 600 nm at 0.1% level (Fig. 3), and at 320 nm at 1% level. Though a very significant linear correlation was also found between carbonyl content and E_{600} ($r=0.708^{***}$), the correlation between carbonyl content and $\Delta E_{600}(\text{NaBH}_4)$ was more significant ($r=0.829^{***}$). These results suggest that $\Delta E(\text{NaBH}_4)$ may be directly attributed to the reduction of carbonyl groups. The $\Delta E_{600}(\text{NaBH}_4)$ of A type HAs were all higher than the estimates by the regression line (Fig. 3), showing that the $\Delta E_{600}(\text{NaBH}_4)$ per fixed concentration of carbonyl group is high for A type HAs. This result suggests that A type HAs have longer conjugate systems than the other types.

3. Relationship between the $\Delta E/E$ by the three reduction procedures and the degree of humification

In this study, HAs were reduced with $\text{Na}_2\text{S}_2\text{O}_4$ and NaBH_4 . The oxidation potential of $\text{Na}_2\text{S}_2\text{O}_4$ (-1.12 V) (14) is low enough to reduce effectively the reversible redox systems such as quinones in HAs producing corresponding diphenols. Because the reduction with $\text{Na}_2\text{S}_2\text{O}_4$ is accompanied with the consumption of OH^- ion, and $\text{Na}_2\text{S}_2\text{O}_4$ is degraded of itself in an acidic medium, it is usually used in an alkaline medium for reduction of quinones. Therefore, HAs were reduced in 0.1 N NaOH. However, bathochromic effect of the phenolate anion formed by the reduction influenced the

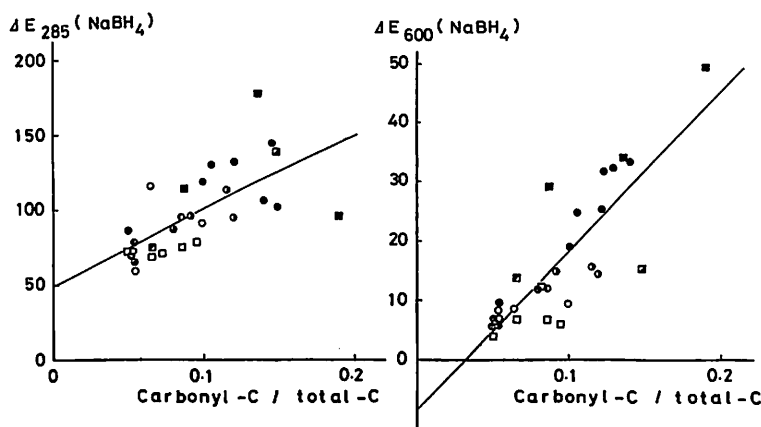


Fig. 3. Relationship between ΔE and carbonyl group content of HAs. Concentrations were fixed at carbon 1 g/100 ml. See Table 1 for the symbols.

Table 1. Decreasing rates of the absorbance of HAs by the reduction of carbonyl groups.

Group ^a	Sample	Type	Symbol ^b	E_{600}^c	$\Delta E/E$ at 600 nm ^d				$\Delta E/E$		Carbonyl/h Atomic ratio
					$\text{Na}_2\text{S}_2\text{O}_8$ NaOH	$\text{Na}_2\text{S}_2\text{O}_8$ pH 7	NaBH_4^e	$\Delta E/E$			
								320 nm	285 nm		
1	Jaana P	A	■	97.7	-0.153	0.043	0.320	0.167	0.153	0.087	
	Nagara P	A	■	89.4	-0.224	-0.005	0.606	0.137	0.108	0.189	
	Kinshozan P-3	A	■	70.7	-0.122	0.084	0.545	0.265	0.219	0.136	
	Kinshozan P-1	A	■	70.5	-0.151	0.098	0.463	0.224	0.153	n.d.	
	Nagara F	A	■	62.1	-0.256	0.034	0.407	0.196	0.173	0.385	
	Nagara OH	A	■	52.4	-0.251	0.027	0.454	0.234	0.198	0.292	
	Jaana F	B	▣	48.0	-0.049	0.153	0.350	0.209	0.176	n.d.	
	Kinshozan F	B	▣	31.7	-0.048	0.302	0.548	0.307	0.238	0.148	
	Ishimakisan P	P ₀	▣	27.2	0.288	0.457	0.591	0.288	0.192	0.066	
	Ishimakisan F	Rp(1)	□	25.1	0.248	0.575	0.590	0.270	0.174	0.083	
	Jaana OH	Rp(1)	□	20.9	-0.061	0.213	0.354	0.266	0.202	0.086	
	Ishimakisan OH	Rp(1)	□	15.2	0.208	0.621	0.534	0.285	0.195	0.066	
	Kinshozan OH-1	Rp(1)	□	13.9	0.090	0.427	0.505	0.273	0.195	0.095	
	Kinshozan OH-3	Rp(1)	□	10.1	-0.111	0.380	0.522	0.325	0.226	0.050	
2	Inogashira-1	A	●	86.2	-0.138	0.051	0.406	0.214	0.199	0.123	
	Temmondai	A	●	82.6	-0.154	0.033	0.430	0.142	0.137	0.129	
	Shitara-black	A	●	80.6	-0.162	0.037	0.451	0.213	0.138	0.140	
	Inogashira-2	A	●	76.0	-0.136	0.024	0.350	0.167	0.153	0.231	
	Fujinomiya-3	A	●	69.7	-0.017	0.148	0.401	0.215	0.190	0.105	
	Komagahara	A	●	63.0	0.021	0.139	0.450	0.235	0.197	0.121	
	Fujinomiya-2	A	●	60.4	-0.028	0.152	0.350	0.433	0.188	0.101	
	Onobaru	P ₀	○	27.6	0.212	0.389	0.496	0.315	0.202	0.085	

3	Shitara-brown	B	⊙	41.4	-0.029	0.164	0.366	0.215	0.179	0.240
	Kuragari-1	B	⊙	37.2	0.086	0.255	0.423	0.267	0.195	0.119
	Higashiyama (A)	B	⊙	32.9	0.342	0.594	0.571	0.336	0.233	0.115
	Hichiso	B	⊙	32.3	0.309	0.532	0.584	0.281	0.204	n.d.
	Kuragari-2	P ₀	⊙	30.6	0.100	0.370	0.528	0.298	0.202	0.092
4	Higashiyama (FH)	Rp(2)	○	20.7	0.260	0.601	0.526	0.351	0.218	0.099
	Higashiyama (L)	Rp(2)	○	15.8	0.281	0.675	0.608	0.342	0.209	0.053
	Kisokoma (F)	Rp(2)	○	14.3	0.266	0.639	0.586	0.329	0.191	0.054
	Yamamoto	Rp(2)	○	13.4	0.259	0.773	0.788	0.426	0.261	0.065
	Anjo black	B	⊕	37.5	-0.032	0.216	0.367	0.217	0.169	0.080
5	Sanage-2	P ₀	⊕	28.4	0.013	0.278	0.379	0.223	0.166	0.054
	Sanage-1	Rp(1)	⊕	20.5	0.092	0.313	0.387	0.257	0.203	0.051
	Gifu	Rp(1)	⊕	13.4	0.158	0.470	0.492	0.290	0.203	0.050
	Anjo	Rp(1)	⊕	13.2	0.218	0.479	0.479	0.271	0.197	0.064
	Tsubame Pg	P ₊₊₊	⊙	114	0.668	0.636	0.886	0.182	0.210	0.134
6	Tsubame 4	P ₊	⊙	59.2	0.421	0.614	0.611	0.268	0.225	0.104
	Tsubame 1	P ₊₊	⊙	38.6	0.467	0.645	0.661	0.281	0.207	0.075
	Shishi	P ₊₊	⊙	25.8	0.394	0.388	0.500	0.222	0.194	0.052
	H.Q.-HA	A		102	0.293	0.654	0.795	0.382	0.239	n.d.
Glu.-HA	A		57.8	0.177	0.696	0.853	0.447	0.319	n.d.	

^a Groups: 1, HAs obtained from calcareous soils; 2, from kuroboku soils; 3, from forest soils; 4, from A₀ layer and rotted wood of forest soils; 5, from paddy soils; 6, P₊₊₊ type HAs; 7, artificial HAs. ^b Symbols are used in Fig. 3. ^c E₆₀₀: the absorption at 600 nm of HAs at the carbon concentration of 1 g/100 ml. ^d ΔE/E: decreasing rate of absorbance. ^e Na₂S₂O₄-NaOH: by Na₂S₂O₄ reduction in 0.1 N NaOH. ^f Na₂S₂O₄-pH 7: by Na₂S₂O₄ reduction in pH 7 buffer. ^g NaBH₄: by NaBH₄ reduction in pH 10.4 buffer. ^h Carbonyl content: ratio of carbonyl carbon to total carbon in HAs.

absorbance of the reduced HAs which makes it difficult to interpret the effect of reduction on the absorption spectra. Therefore, HAs were also reduced with $\text{Na}_2\text{S}_2\text{O}_4$ in the phosphate buffer at pH 7. NaBH_4 is generally used as a selective reducing agent for ketone and aldehyde to obtain alcohols. Unlike $\text{Na}_2\text{S}_2\text{O}_4$, the reduction product of quinone with NaBH_4 is not phenol but dihydroxycyclohexadiene. Carboxyl, ester and amide groups, ethers and C=C double bonds are not reduced with NaBH_4 (3).

In Table 1, the $\Delta E/E$ at 600 nm due to all the three reduction procedures and the $\Delta E/E$ at 285 and 320 nm due to the reduction with NaBH_4 were compared among various soil HAs. The $\Delta E/E$ at 600 nm due to each reduction procedure generally followed in the order: $\Delta E/E$ ($\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH) $<$ $\Delta E/E$ ($\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) $<$ $\Delta E/E$ (NaBH_4). The relationship between the former two may be explained in terms of the bathochromic effect of the phenolate anion on E_{600} values.

$\Delta E/E_{600}$ ($\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH) and $\Delta E/E_{600}$ ($\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) appear to decrease with increasing E_{600} (Table 1). This trend suggests that the rate of contribution of structures other than quinone to the visible absorption increases with increasing E_{600} , that is, the degree of humification. However, the conjugate systems in which quinone structure participates may also develop in the process of humification as seen from the shift of the maximum peaks of ΔE ($\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) spectra to longer wavelength with humification (Fig. 1). Furthermore, quinones and conjugate systems other than quinones may not exist independently but may be conjugated with each other, increasing in degree of conjugation with humification. Because the reduction of quinone with $\text{Na}_2\text{S}_2\text{O}_4$ results in diphenol and does not cut off the conjugate system, it may influence the visible absorption less than the reduction with NaBH_4 which cuts off the conjugate system. Furthermore, the extent of shortening of the conjugate system and the influence of the reduction with $\text{Na}_2\text{S}_2\text{O}_4$ on the visible absorption may become less with increasing degree of conjugation of quinones and structures other than quinones.

The E_{600} values of many HAs, especially of A type HAs, increased after reduction with $\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH and the $\Delta E/E$ ($\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH) had negative values (Table 1). For HAs having long conjugate systems such as A type HAs, the bathochromic effect of the phenolate anion formed by the reduction of quinone may be larger than the decrease in the visible absorbance due to the shortening of the conjugate systems.

Unlike $\Delta E/E_{600}$ ($\text{Na}_2\text{S}_2\text{O}_4$), $\Delta E/E_{600}$ (NaBH_4) had no relation with E_{600} and had considerably larger values. The values of $\Delta E/E$ ($\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) and $\Delta E/E$ (NaBH_4) for the low humified HAs, P₊₊₊₊ type HAs, and artificial HAs were almost similar to each other. This may suggest carbonyl groups which influence the visible absorption of these HAs are mostly composed of quinone groups. On the other hand, the difference between $\Delta E/E$ (NaBH_4) and $\Delta E/E$ ($\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) increased with increasing E_{600} , that is the degree of humification (Table 1). This may suggest that the rate of contribution of carbonyl groups other than quinone to the visible absorption increase with humification.

4. *The effect of the sources and types of HAs on $\Delta E/E_{600}$ due to the reduction of carbonyl groups*

The change in absorbance due to the reduction with NaBH_4 and $\text{Na}_2\text{S}_2\text{O}_4$ was closely related to the sources and types of HAs (Table 1).

The E_{600} of A type HAs increased by the reduction with $\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH except one sample, Komagahara, of which E_{600} decreased only a little. In case of A type HAs, the $\Delta E/E_{600}(\text{NaBH}_4)$ were much higher than the $\Delta E/E(\text{Na}_2\text{S}_2\text{O}_4$ at pH 7). These results suggest the presence of long and complex conjugate systems of quinones and other carbonyl groups in A type HAs.

Not only A type but also B and Rp type HAs extracted from the calcareous soils (Table 1, group 1) increased in E_{600} after the reduction with $\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH, except for the HAs obtained from Ishimakisan soil. In addition, the $\Delta E/E(\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH) and the $\Delta E/E(\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) of the HAs obtained from the calcareous soils were lower than those of HAs from other sources. On the other hand, the $\Delta E/E(\text{NaBH}_4)$ at 600 nm of the HAs obtained from the calcareous soils except Jaana soil were higher than those of the HAs with similar E_{600} values from other sources. High pH condition of the calcareous soils may have promoted the oxidation of HAs and the development of conjugate systems composed not only of quinones but also other carbonyl groups.

The $\Delta E/E_{600}$ due to the reduction of the HAs obtained from forest soils with $\text{Na}_2\text{S}_2\text{O}_4$ and NaBH_4 (Table 1, group 3) ranged between those of the HAs obtained from A_0 layers of forest soils and from kuroboku soils.

The $\Delta E/E_{600}(\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 N NaOH) and the $\Delta E/E_{600}(\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) of the HAs obtained from A_0 layers of forest soils (Table 1, group 4) were the highest next to the $\Delta E/E$ of $P_{+...+++}$ type HAs. In addition, the $\Delta E/E_{600}(\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) was almost equal to or greater than the $\Delta E/E_{600}(\text{NaBH}_4)$. This result suggests that quinones contribute greatly to the E_{600} of these HAs with very little contribution from the other carbonyl groups. This suggestion does not imply that the contents of carbonyl groups other than quinones are small but that carbonyl groups contributing to the visible absorption of these HAs are very few.

In all the three reduction procedures, the $\Delta E/E_{600}$ of the HAs obtained from paddy soils (Table 1, group 5) were smaller than those of other HAs with similar E_{600} . This result suggests that the contribution of both quinones and other carbonyl groups are small because of the small contents of carbonyl groups in these HAs. The values of the $\Delta E/E(\text{Na}_2\text{S}_2\text{O}_4$ at pH 7) and the $\Delta E/E(\text{NaBH}_4)$ of these HAs were similar but the difference between the two values increased with increasing E_{600} . This result suggests that the contribution of carbonyl groups other than quinone to the visible absorption increases with increasing E_{600} though it is much smaller than that of quinones for these HAs.

The $\Delta E/E_{600}$ of $P_{+...+++}$ type HAs (Table 1, group 6) were very high in all the three reduction procedures. This may be due to the high contribution of Pg pigment to the visible absorption of these HAs.

The $\Delta E/E(\text{Na}_2\text{S}_2\text{O}_4 \text{ at pH } 7)$ and the $\Delta E/E_{600}(\text{NaBH}_4)$ of both the artificial HAs made from hydroquinone and glucose (Table 1, group 7) were very high, with the two ratios having nearly similar values, suggesting that quinones contribute highly to the visible absorption of these HAs.

Acknowledgement. We thank Prof. K. Kumada of our laboratory for his valuable advice in this study. Thanks are also due to Prof. N. Terashima of Nagoya University for offering us the sample of milled wood lignin of rice straw, to Prof. S. Yamazaki of the University of Tokyo for offering us the sample of cercosporin, and to Mr. R. Shiroya, Mr. Y. Kawamura, and Dr. O. Sato for offering us their HA samples.

REFERENCES

- 1) ANDERSON, H.A. and RUSSEL, J.D., Possible relationship between soil fulvic acid and polymaleic acid, *Nature*, **260**, 597 (1976)
- 2) ADLER, R. and MARTIN, J., Zur Kenntnis der Carbonylgruppen im Lignin. I, *Acta Chem. Scand.*, **13**, 75-96 (1959)
- 3) CHAIKIN, S.W. and BROWN, W.G., Reduction of aldehydes, ketones and acid chlorides by sodium borohydride, *J. Amer. Chem. Soc.*, **71**, 122-125 (1949)
- 4) KUWATSUKA, S., TSUTSUKI, K., and KUMADA, K., Chemical studies on soil humic acids. I. Elementary composition of humic acids, *Soil Sci. Plant Nutr.*, **24**, 337-347 (1978)
- 5) SATO, O. and KUMADA, K., The chemical nature of the greenfraction of P type humic acid, *Soil Sci. Plant Nutr.*, **13**, 121-122 (1967)
- 6) SHINDO, H. and KUWATSUKA, S., Behavior of phenolic substances in the decaying process of plants. VII. Characteristics of phenolic substances in the humic acids of decayed rice straw and compost-supplied field soil, *Soil Sci. Plant Nutr.*, **23**, 333-340 (1977)
- 7) SHINDO, H. and KUWATSUKA, S., The hydrolysable phenolic components of rice straw lignin, *J. Sci. Soil Manure, Japan*, **49**, 165-166 (1978)
- 8) TSUTSUKI, K. and KUWATSUKA, S., Chemical studies on soil humic acids. II. Composition of oxygen containing functional groups of humic acid, *Soil Sci. Plant Nutr.*, **24**, 547-560 (1978)
- 9) TSUTSUKI, K. and KUWATSUKA, S., Chemical studies on soil humic acids. III. Nitrogen distribution in humic acids, *Soil Sci. Plant Nutr.*, **24**, 561-570 (1978)
- 10) TSUTSUKI, K. and KUWATSUKA, S., Chemical studies on soil humic acids. IV. Amino acid, phenol, and sugar composition in the acid hydrolysable fraction in humic acids, *Soil Sci. Plant Nutr.*, **25**, 29-38 (1979)
- 11) TSUTSUKI, K. and KUWATSUKA, S., Chemical studies on soil humic acids. V. Degradation of humic acids with potassium hydroxide, *Soil Sci. Plant Nutr.*, **25**, 183-195 (1979)
- 12) TSUTSUKI, K. and KUWATSUKA, S., Chemical studies on soil humic acids. VI. Absorbance-pH curves of humic acids, *Soil Sci. Plant Nutr.*, **25**, 365-371 (1979)
- 13) TSUTSUKI, K. and KUWATSUKA, S., Chemical studies on soil humic acids. VII. pH-Dependent nature of the ultraviolet and visible absorption spectra of humic acids, *Soil Sci. Plant Nutr.*, **25**, 373-384 (1979)
- 14) WEAST, R.C. (ed.), Handbook of Chemistry and Physics, 57th Ed. CRC Press, Inc., Cleveland, 1977. pp. D 141-146
- 15) YAMAZAKI, S., OKUBO, A., AKIYAMA, Y., and FUWA, K., Cercosporin, a novel photodynamic pigment isolated from *Cercospora kikuchii*, *Agric. Biol. Chem.*, **39**, 287-288 (1975)