

## CHEMICAL STUDIES ON SOIL HUMIC ACIDS

### V. Degradation of Humic Acids with Potassium Hydroxide

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Seventeen samples of soil humic acids, two fractions of soil fulvic acid sample, and several related compounds such as lignin, tannin, flavonoid and artificial humic substances were decomposed in conc. KOH solution at 180°C. Succinic acid, glutaric acid, phloroglucin, *p*-hydroxybenzoic acid, vanillic acid, protocatechuic acid, 3,4-dihydroxy-5-methoxybenzoic acid, and gallic acid were detected in the degradation products of humic acids. The amounts of these degradation products were discussed in relation to the degree of humification or the sources of the humic acid samples. Succinic acid also resulted from glucose, polymaleic acid, and the humic acid and humin prepared from glucose, but glutaric acid resulted only from glucose humic acid and glucose humin but not from glucose and polymaleic acid. Succinic acid and glutaric acid were supposed to result from the same structural portions in humic acids because of the very significant positive linear correlation between their amounts. *p*-Hydroxybenzoic, vanillic, protocatechuic, and 3,4-dihydroxy-5-methoxybenzoic acids were presumed to result mainly from lignin structure in humic acids. Soil humic acids yielded small amounts of gallic acid although the yields by hydrolysable tannins were in large amounts. The yields of above-mentioned degradation products from humic acids decreased with increasing degree of humification. Phloroglucin resulting from flavonoids including condensed tannins were also found in the degradation products of humic substances. Its yield showed no linear correlation with *RF* value of humic acid, and is presumed to be rather related to the vegetation at the sites of soil sampling.

*Additional Index Words:* alkaline degradation, artificial humic substances, humic acids, lignin structure.

Although humic acids have been investigated by many researchers, their chemical structure has been elucidated little. Through the studies on the compositions of C, H, N and O (7), oxygen-containing functional groups (11), nitrogenous constituents (12), phenolic substances and sugars in acid-hydrolysable fraction (13) of humic acids, we have found that these compositions were closely related to the degree of humification and soil types.

In this study, KOH degradation products of 17 humic acid samples, two fractionated preparations of fulvic acid, and several related compounds were compared in order to understand the chemical structure and composition of humic acids and its relationship to the degree of humification and to the sources of soil humic acids.

## MATERIALS AND METHODS

*1. Humic acid and fulvic acid samples*

Seventeen humic acid samples (Table 3) comprising 4 A type, 4 B type, 4 Rp(1) type, 2 Rp(2) type, 1 P<sub>0</sub> type, 2 P<sub>+</sub>~# type humic acids were used. The sources of these humic acids are grouped as follows, Kuroboku soils (Nos. 2, 4, 13 and 36), A-layers of brown forest soils (Nos. 18 and 21), A<sub>0</sub>-layers of forest soils (Nos. 33 and 38), Bh-layer of a podzolic soil (No. 40), plowed layers of paddy soils (Nos. 29, 35 and 36), rendzina-like calcareous soils (Nos. 12, 19 and 32) and a humic buried soil (Nos. 1 and 11). The method of isolation and purification, and other analytical data of these samples are available in our previous papers (7, 11-13).

Two samples of fulvic acid fraction, Fr. B<sub>4</sub> and Fr. D<sub>1</sub>, were prepared from the Bh-layer of a podzolic soil, Hichiso soil. The humic and fulvic acid samples of this soil were prepared as follows. Air-dried soil (4 kg) was passed through a 2 mm sieve and extracted with 0.5 N NaOH (14 liters) by intermittent shaking under nitrogen at room temperature for 24 hr. From this extract, the humic acid sample was prepared according to KUMADA and KAWAMURA (6), except that the final humic acid solution in 0.1 N NaOH was treated with cation exchange resin, Diaion SK1B, before acidifying the solution with HCl to collect the humic acid. The yield of the humic acid sample (No. 40) was 11.0% of total carbon in the soil and the elementary composition was C: 51.6%, H: 4.89%, N: 3.35%, O: 40.2% and C/N: 15.4. The fulvic acid fraction which remained soluble after acidification was adsorbed on charcoal, and eluted with acetone-water (9 : 1), water, and 0.1 N NaOH successively to obtain the fractions B, C, and D respectively according to FORSYTH (3). Fraction B was redissolved in ethanol and fractionated further by adding successively 1, 3 and 5 time volumes of ether to the ethanol solution to obtain precipitations, Fr. B<sub>1</sub>, Fr. B<sub>2</sub> and Fr. B<sub>3</sub>, respectively. Ethanol was evaporated off from the remaining solution to become sticky syrup, and an excess volume of ether was added to it to precipitate Fraction B<sub>4</sub> which was used in this study. The yield of Fr. B<sub>4</sub> was 2.45% of total carbon in the soil, and the elementary composition was C: 48.8%, H: 4.93%, N: 0.8%, O: 45.5% and C/N: 61. Fraction D was treated with the cation exchange resin, Diaion SK1B, and evaporated to dryness by a rotary evaporator below 60°C. The remainder was extracted with ethanol, and the extract was dried by a rotary evaporator to obtain Fraction D<sub>1</sub> which was used in this study. The yield was 6.8% of total carbon in the soil and the elementary composition was C: 46.0%, H: 3.92%, N: 0.51%, O: 49.5% and C/N: 90.3.

*2. Related compounds*

Commercial lignin (lignosulfonic acid extracted from coniferae, Nakarai Chemicals, Extra Pure), tannic acid (gallotannin, Dainihonsei-yaku, Guaranteed Reagent) and rutin (3,3',4',5,7-pentahydroxyflavon-3-rutinoside, Tokyo Kasei, Guaranteed Reagent)

were purchased. Wattle tannin and chestnut tannin were crude extract samples. Artificial models for humic substances were prepared as follows.

*Glucose humic acid and glucose humin* Glucose (50 g) was refluxed with 6 N HCl (500 ml) for 4 hr. The resulting precipitate was filtered through a filter paper and washed thoroughly with water. The residue on the filter paper was dissolved again in 0.1 N NaOH (humic acid fraction). The humin fraction remaining on the filter paper was washed thoroughly with water and dried at 80°C. The humic acid fraction in 0.1 N NaOH was precipitated at pH 1 by adding HCl, collected by filtration, washed thoroughly and dried at 80°C. The yields of the humin and the humic acid were 4.4 g and 3.8 g respectively. The elementary composition of the humic acid was C: 63.5%, H: 4.36%, N: 0%, and O: 32.2%.

*Hydroquinone humic acid* Hydroquinone (50 g) and NaOH (25 g) were dissolved in water (50 ml) and allowed to stand at 55°C for 54 hr in an evaporating dish compensating for the loss of water by evaporation at times. The solution was then acidified to pH 1 with HCl and filtered. The residue on the filter paper was washed thoroughly with water and dried at 80°C. The yield was 4.3 g and the elementary composition was C: 58.0%, H: 2.92%, N: 0% and O: 3.91%.

*Polymaleic acid* Polymaleic acid was prepared according to the method of "Experiment No. 1" by BRAUN and POMAKIS (2), except that the reaction temperature was raised to 113°C (b.p. of toluene) instead of 100°C. Maleic acid anhydride (20 g) and pyridine (0.92 ml) in toluene (100 ml) were refluxed for 29 hr. The resulting brown precipitate was collected by decantation, dissolved again in acetone, precipitated by adding ether, and filtered. The residue on the filter paper was washed thoroughly with ether, and dried over silica-gel overnight. The yield was 13.1 g, and the elementary composition was C: 52.1%, H: 3.90%, N: 0.78%, O: 44.1% and C/N: 65.7.

### 3. Successive ether extraction, acid hydrolysis and KOH degradation of humic acids

Three soil humic acids comprising No. 2 (A type), No. 18 (B type), and No. 38 (Rp(2) type), and the glucose humic acid (97, 73, 77 and 65 mg respectively) were dispersed in 6 N HCl (10 ml) by ultrasonics, and extracted three times with ether. The acid solutions were heated at 100°C in flasks with reflux condensers. The hydrolysates were extracted three times with ether. The residual mixtures were dried by a rotary evaporator, weighed, degraded with KOH at 180°C as described later, and extracted with ether. The carbon content of each extract was determined colorimetrically by  $K_2Cr_2O_7$ -oxidation (10) after drying, and the composition of degradation products was analyzed by gas-chromatography.

### 4. KOH degradation and analysis of the products

Sample (10–100 mg), water (0.4 ml) and KOH pellets (2.0 g) were placed in a hard glass test tube. The air in the tube was completely replaced by nitrogen. The test tube was sealed and heated at 180°C for 2 hr. The content was acidified with 35% HCl (3.5 ml) in the opened tube while being cooled with ice water. It was trans-

ferred to a glass centrifuge tube with about 10 ml water and shaken with ether (30 ml). The mixture was centrifuged to separate the ether extract from the emulsion. The extractions were repeated two times. The combined ether extracts were dehydrated with  $\text{Na}_2\text{SO}_4$ , transferred to a flask by washing with ether, and evaporated to dryness. The dried extract was dissolved in acetone, transferred into a 10 ml volumetric flask, and made to volume with acetone. To a 0.5 ml portion of this solution in a small vial, 0.5 ml of 0.01% adipic acid solution in acetone was added as an internal standard. Acetone was evaporated and N,O-bis(trimethylsilyl)acetamide (0.1 ml) was added to this and stoppered tightly. After standing for 20 min at room temperature, 5  $\mu\text{l}$  portion was injected into a gas-chromatograph: Hitachi model 063, flame ionization detector, 2,000  $\times$  3 mm coiled stainless column packed with 1.5% SE 30 on Chromosorb W 60–80 mesh, programmed from 100–250°C at a rate of 5°C/min,  $\text{N}_2$  flow rate 50 ml/min. Some of the trimethylsilylated samples were also analyzed by a gas-chromatographic–mass spectrometric (GC–MS) system (JGC-20K-JMS-D100). Conditions for gas chromatography in the GC–MS system were: JEOL JGC-20K, 1,000  $\times$  3 mm glass column packed with 1.5% OV 1 on Shimalite W 80–100 mesh, programmed from 100–250°C at a rate of 5°C/min, He flow rate 30 ml/min. Though many small peaks appeared in the gas chromatogram of GC–MS system than in FID gas chromatogram, areas of the peaks of major degradation products did not differ. Degradation products were identified by matching mass spectra and retention times with those of known standard chemicals.

Carbon content of the ether-extract was determined colorimetrically by  $\text{K}_2\text{Cr}_2\text{O}_7$  oxidation (10). A fixed portion (0.3–0.5 ml) of the acetone solution of the extract was placed in a test tube. Acetone was evaporated off completely by heating. Two ml of 0.1 N NaOH and 4 ml of 0.5 N  $\text{K}_2\text{Cr}_2\text{O}_7$  in sulfuric acid were added in this order. After 30 min, the color of the solution was measured at 645 nm. The standard curve was made with glucose in 0.1 N NaOH.

## RESULTS AND DISCUSSION

### 1. Yields of ether extracts by the successive treatments with 6 N HCl and hot KOH (Table I)

The degradation of humic acids with KOH at 180°C made 12–38% of the humic acids extractable with ether from acidic solution. The yields decreased in the order Rp, B, and A type with increasing degree of humification. The yield of ether extractable product from glucose humic acid was 26%. By the successive treatments of humic acids, suspending in 6 N HCl at room temperature made 2–12%, hydrolysis with 6 N HCl at 100°C made 3–6%, and following degradation with KOH at 180°C made 8–17% extractable with ether from acidic solutions. The sum of the yields by these successive treatments was almost the same as the yield obtained by direct degradation with KOH for each sample. Total amounts of the identified compounds were about 10% of the ether-extractable fractions, and about 1–3% of the total weight

of humic acids. No peak was observed on the gas chromatograms of the ether extracts of humic acid suspensions in 6 N HCl and the humic acid hydrolysates with 6 N HCl under the GC condition used here. The fat soluble fraction in humic acids is thought to be extracted with ether from the humic acids suspended in 6 N HCl. The absence of the low molecular compounds in the fat soluble fraction may suggest that compounds detected in the KOH degradation under the GC condition used here did not exist in free or easily hydrolyzable form in these humic acids. Other researchers (1, 4) detected degradation products such as levulinic, succinic, and fumaric acids (1),

Table 1. Yields of ether-extracts before and after acid hydrolysis, after successive KOH degradation, and after the direct KOH degradation of humic acids.

No.	Sample	Type	Yields (mg/g) of ether extracts			
			(1)	(2)	(3)	(4)
2	Temmondai	A	23.0	33.7	77.0	120
18	Kuragari	B	62.3	49.9	113	224
38	Kisokoma (F)	Rp (2)	117	56.1	171	375
	Glucose HA		28.8	34.6	167	255

Notes: (1) Yields of total ether-extracts from the humic acid suspensions in 6 N HCl. (2) Yields from the successive 6 N HCl hydrolysates of humic acids. (3) Yields from the successive KOH degradation products of humic acids. (4) Yields from the direct KOH degradation products of humic acids.

Table 2. Yields of KOH degradation products from the pretreated humic acids<sup>a</sup> and from the original humic acids.

	Yields (mg/g) of products <sup>b</sup>									Ether extracts
	Succi.	Glutar.	<i>p</i> -H.b.	Phlo.	Vanil.	Proto.	Dih.m.	Gallic	Total	
Pretreated preparations <sup>a</sup>										
Temmondai	3.10	0.93	0.41	0.095	0.42	0.96	tr.	tr.	5.92	77.0
Kuragari	4.74	0.93	1.30	1.21	0.43	1.17	tr.	tr.	9.78	113
Kisokoma (F)	5.25	0.86	1.26	3.42	0.49	5.03	tr.	tr.	16.3	171
Glucose HA	5.12	1.23	0	0	0	0	0	0	6.35	167
Original preparations										
Temmondai	4.72	1.02	0.74	0.22	0.56	1.44	tr.	tr.	8.70	120
Kuragari	11.42	1.74	2.19	2.36	0.79	5.60	0.38	0.15	24.6	224
Kisokoma (F)	8.57	1.78	1.99	3.92	1.23	7.28	1.03	0.27	26.1	375
Glucose HA	9.25	1.04	0	0	0	0	0	0	10.3	255

<sup>a</sup> Humic acid preparations which were extracted with ether, hydrolyzed with 6 N HCl, and extracted again with ether, successively, before KOH degradation. <sup>b</sup> Succi.: Succinic acid, Glutar.: Glutaric acid, *p*-H.b.: *p*-Hydroxybenzoic acid, Phlo.: Phloroglucin, Vanil.: Vanillic acid, Proto.: Protocatechuic acid, Dih.m.: 3,4-Dihydroxy-5-methoxybenzoic acid, Gallic: Gallic acid.

*p*-hydroxybenzoic, vanillic, and protocatechuic acids (1, 4) and vanillin (4) from the 6 N HCl hydrolysates of humic acids. The absence of the low molecular compounds in the ether extracts after the HCl hydrolysis under the GC condition used here may be because considerable amounts of fat soluble fractions were removed before the hydrolysis and furthermore acid hydrolysis is not so strong as KOH degradation. By the KOH degradation one-half the products from the original humic acids were obtained from the pretreated humic acids which were hydrolyzed with 6 N HCl and extracted with ether (Table 2). This result suggests that large portions of the original structure of the KOH degradation products were combined very strongly in humic acid structure.

## 2. Identification and determination of the KOH degradation products

Gas-chromatogram of the KOH degradation products of a humic acid sample (No. 18, Kuragari brown forest soil, B type) is shown in Fig. 1. Main peaks were all identified. The major peaks identified were succinic acid [1] in Fig. 1 and protocatechuic acid [6]. Glutaric acid [2], *p*-hydroxybenzoic acid [3], phloroglucin [4] and vanillic acid [5] were medium products, while the minor products were gallic acid [8] and 3,4-dihydroxy-5-methoxybenzoic acid [7]. The low yields of vanillic acid can be attributed to the transformation of vanillic acid to protocatechuic acid under the given degradation condition.

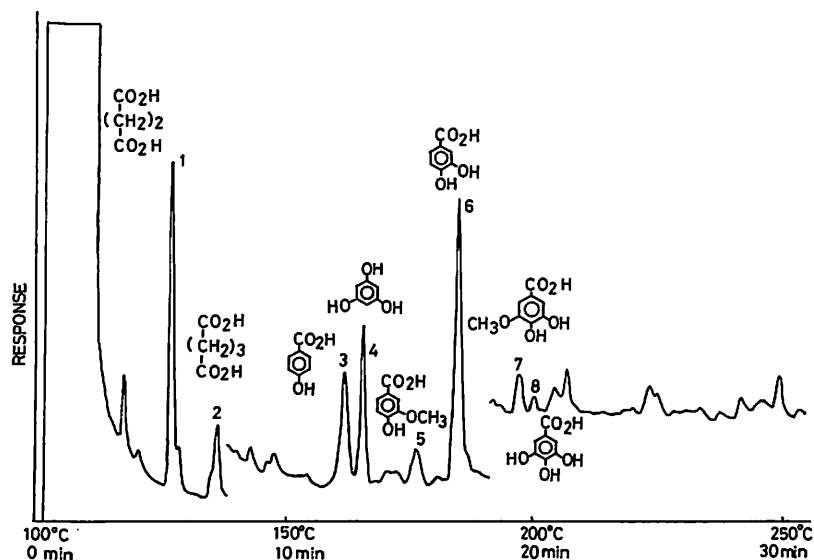


Fig. 1. GC analysis of the KOH degradation products of Kuragari (No. 18) humic acid.

GC conditions as defined on 1.9-13, p. 186.

NEYROUD and SCHNITZER (8) also hydrolyzed humic and fulvic acids in 3 N NaOH at 170°C for 3 hr. Though the hydrolytic condition used by them was milder, more amounts of degradation products were obtained than in this study. The kinds and yields of the degradation products were quite different in both results. *p*-Hydroxybenzoic, vanillic, protocatechuic and gallic acids detected here were also detected in their methylated forms by NEYROUD and SCHNITZER (8), but differed in yields. Though protocatechuic acid was the major and gallic acid was the minor degradation product in our study, the yield of the former was less than the latter in their methylated forms in their study. In addition, *n*-fatty acids (C<sub>14</sub>-C<sub>20</sub>) were not detected in this study. As standards of *n*-fatty acids were detectable under the gas chromatographic conditions used here, the absence of *n*-fatty acids in the degradation products may be due to the degradation conditions or the method of sample preparation. On the other hand, succinic acid, glutaric acid and phloroglucin were detected in this study but not in theirs. Our results also differed from the results of JAKAB *et al.* (5). The reason for the difference in degradation products should be examined by changing the conditions for degradation, extraction and analysis using the same sample.

### 3. KOH degradation products of humic acids and their original structures (Table 3)

Commercial lignin, which is a lignosulfonic acid, yielded 2.26% protocatechuic acid and much smaller amounts of succinic, glutaric, *p*-hydroxybenzoic, vanillic and 3,4-dihydroxy-5-methoxybenzoic acids by the KOH degradation (Table 5). The small quantities of succinic and glutaric acids are presumed to have resulted from sugar contained in the lignin reagent, as mentioned later. Besides, SHINDO and KUWATSUKA (9) decomposed a milled wood lignin of rice straw with KOH at 180°C in an open tube with a reflux condenser, and detected 3.5% *p*-hydroxybenzoic acid, 2.0% vanillic acid, 3.6% syringic acid, and 1.1% protocatechuic acid. Though the compositions of degradation products of different lignins may differ according to their origins, these results suggested that *p*-hydroxybenzoic, vanillic, protocatechuic, and 3,4-dihydroxy-5-methoxybenzoic acids resulted from lignin structure in humic acids. 3,4-Dihydroxy-5-methoxybenzoic acid is presumed to result from syringyl moiety in lignin by partial demethylation. Among these degradation products, the yields of *p*-hydroxybenzoic acid were low from the humic acids obtained from the forest soils (Nos. 18, 21, 33, 38 and 40), and high from those obtained from the soils of gramineous vegetation (Nos. 19, 26, 29, 32, 35 and 36, Table 3). This result corresponds well to the above-mentioned result that the yield of *p*-hydroxybenzoic acid was low from the commercial lignin obtained from conifer woods while it was high from the milled wood lignin of rice straw (9). It is suggested that the composition of the degradation products of humic acids reflects well their original lignin-structure.

The yields of these phenolic acids decreased with increasing degree of humification. Correlation coefficients were calculated between the yields of degradation products and *RF* value where P<sub>+</sub>~# type humic acids were excluded from the samples (Table 4). The yields of protocatechuic acid (1.17-13.3 mg/g) and vanillic acid (0.35-1.51

Table 3. Yields of KOH degradation products from various soil humic acids.

Humic acids		Yields (mg/g) of products <sup>a</sup>											Succi. Proto.	
Type	No.	RF	Succi.	Glutar.	p-H.b.	Phlo.	Vanil.	Proto.	Dih.m.	Gallic	Total	Ether extracts	Succi.	Proto.
A	2	Temmondai	144	4.72	1.02	0.74	0.22	0.56	1.44	tr.	tr.	8.70	120	3.28
	4	Inogashira	138	5.86	1.39	0.87	0.19	0.35	1.17	tr.	tr.	9.83	134	5.01
	12	Kinshozan P	92	6.80	1.19	1.80	0.62	0.60	4.34	0.36	0.31	16.0	203	1.57
	13	Komagahara	89	8.28	1.01	2.09	0.23	0.74	6.66	tr.	tr.	19.0	261	1.24
B	18	Kuragari	61	11.42	1.74	2.19	2.36	0.79	5.60	0.38	0.15	24.6	224	2.03
	19	Kinshozan F	60	12.10	2.25	2.98	1.89	0.92	6.44	0.82	0.81	28.2	245	1.87
	40	Hichiso	51	14.2	3.56	2.18	4.23	0.50	4.23	tr.	tr.	28.9	349	3.36
	21	Higashiyama (A)	47	6.90	1.38	1.86	4.18	1.01	7.07	0.69	0.25	23.3	304	0.976
Rp(1)	29	Sanage	32	6.74	2.63	4.64	0.69	1.27	7.72	2.90	1.45	28.0	253	0.873
	32	Kinshozan OH	29	10.52	2.90	4.96	0.38	1.51	9.14	1.74	0.97	32.1	222	1.15
	35	Gifu	22	10.22	2.96	5.84	0.81	1.36	7.67	3.00	1.35	33.2	258	1.33
	36	Anjo	21	11.24	2.72	7.13	0.39	1.31	13.3	1.18	0.29	37.6	260	0.845
Rp(2)	33	Higashiyama (L)	23	8.19	1.56	2.38	5.74	1.22	10.27	1.15	0.24	30.8	382	0.798
	38	Kisokoma (F)	20	8.57	1.78	1.99	3.92	1.23	7.28	1.03	0.27	26.5	375	1.18
Po	26	Onobaru	39	9.94	1.69	3.49	2.73	0.57	4.81	0.55	0.47	24.3	229	2.07
P+++	1	Tsubame Pg	202	7.28	0.73	1.52	0.04	0.16	4.09	tr.	tr.	13.8	184	1.78
P++	11	Tsubame	94	7.28	1.68	1.40	0.11	0.48	2.32	tr.	tr.	13.3	203	3.28
		Max. yield		14.2	3.56	7.13	5.74	1.51	13.3	3.00	1.45	37.6	382	
		Min. yield		4.72	0.73	0.74	0.04	0.16	1.17	trace	trace	8.70	120	
		Mean yield		8.83	1.89	2.83	1.69	0.86	6.09	0.81	0.39	23.4	247	

<sup>a</sup> See the footnote of Table 2, b.



Table 4. Correlation coefficients<sup>a</sup> between the yields of each degradation product and *RF* values of soil humic acids (A) and between the yields of each degradation product and that of protocatechuic acid from soil humic acids (B).

	Degradation products <sup>b</sup>										Total	Ether extracts	Succi. Proto.
	Succi.	Glutar.	<i>p</i> -H.b.	Phl.	Vanil.	Proto.	Dih.m.	Gallic					
A <sup>c</sup>	-0.536*	-0.592*	-0.683**	-0.448 <sup>N.S.</sup>	-0.774***	-0.809***	-0.645**	-0.514*	-0.927***	-0.764***	0.744***		
B <sup>d</sup>	0.380 <sup>N.S.</sup>	0.433 <sup>N.S.</sup>	0.778***	0.258 <sup>N.S.</sup>	0.832***	1	0.593**	0.425 <sup>N.S.</sup>	N.D.	N.D.	N.D.		

<sup>a</sup> Significant at \*\*\* 0.1% level, \*\* 1% level, \* 5% level; N.S., regarded not significant because probability >5%; N.D., not calculated because unnecessary. <sup>b</sup> See the footnote of Table 2, b. <sup>c</sup> *n*=15, Tsubame Pg and Tsubame humic acids were excluded because of the absorption by Pg at 600nm. <sup>d</sup> *n*=17.

Table 5. Yields of KOH degradation products from humic acid and two fractions of a fulvic acid of Hichiso soil and those from related compounds.

	Yields (mg/g) of products <sup>a</sup>											Succi.	Ether extracts	Proto.
	Succi.	Glutar.	<i>p</i> -H.b.	Phlo.	Vanil.	Proto.	Dih.m.	Gallic	Total					
<b>FAs and HA of Hichiso soil</b>														
Hichiso FA-Fr. B <sub>4</sub>	17.8	2.43	6.47	1.77	3.17	8.57	tr.	tr.	40.2	264	2.08			
Hichiso FA-Fr. D <sub>1</sub>	14.9	1.53	5.03	1.33	2.39	6.85	tr.	tr.	32.0	296	2.18			
Hichiso HA	14.2	3.56	2.18	4.23	0.50	4.23	tr.	tr.	28.9	349	3.36			
<b>Related compounds</b>														
Polymaleic acid	43.4	0	0	0	0	0	0	0	0	0	0			
Glucose humic acid	9.25	1.04	0	0	0	0	0	0	0	0	0			
Glucose humin	9.20	0.92	0	0	0	0	0	0	0	0	0			
Glucose	10.5	0	0	0	0	0	0	0	0	0	0			
Hydroquinone H.A.	0	0	0	0	0	0	0	0	0	0	0			
Commercial lignin	2.19	0.67	0.57	0	1.63	22.6	1.73	0						
Rutin	7.03	0	6.39	76.8	0	45.1	0	0						
Wattle tannin	5.88	0	0	24.1	0	46.2	0	0						
Chestnut tannin	3.28	0	0	0	0	3.82	0	87.2						
Tannic acid	2.30	0	0	0	0	1.90	0	438						

<sup>a</sup> See the footnote of Table 2, b.

mg/g) correlated negatively with *RF* value at 0.1% level, and the yields of *p*-hydroxybenzoic acid (0.74–7.1 mg/g) and 3,4-dihydroxy-5-methoxybenzoic acid (trace–3.00 mg/g) at 1% level. Total yields of the fraction extracted with ether and of the identified compounds also correlated negatively with *RF* value at 0.1% level. In our previous study (11), rapid demethylation of humic acids was observed with increasing degree of humification. Decomposition of the frame structure of lignin, as deduced from the decrease in the yields of degradation products, does not proceed so fast as demethylation. Correlation coefficients were also calculated between the yield of each degradation product and that of protocatechuic acid (Table 4). Very significant positive linear correlations were found between protocatechuic acid and *p*-hydroxybenzoic acid, vanillic acid and 3,4-dihydroxy-5-methoxybenzoic acid. However, no significant correlation was found between protocatechuic acid and succinic acid, glutaric acid, phloroglucin and gallic acid. These results also suggest that *p*-hydroxybenzoic, vanillic, protocatechuic and 3,4-dihydroxy-5-methoxybenzoic acids resulted from the same structural parts which may be of lignin-origin, but that succinic acid, glutaric acid, phloroglucin and gallic acid resulted from the different structural parts.

The yield of succinic acid by the KOH degradation of soil humic acids increased in the order, Rp(2), Rp(1) and B type, but decreased with increasing *RF* value among A type humic acids (Table 3). Negative linear correlation between the yields of succinic acid and *RF* value was significant at 5% level, which was lower than the levels of the negative correlation between the yields of the phenolic acids and *RF* value (Table 4). The ratio of the yields of succinic acid to protocatechuic acid increased with increasing *RF* value (Table 3), and the positive linear correlation between them was significant at 0.1% level (Table 4). Therefore, the original structure of succinic acid may be degraded more slowly than that of protocatechuic acid during humification. On the other hand, a positive linear correlation was found between the yields of succinic acid and glutaric acid at 0.1% level ( $r=0.772$ ,  $n=17$ ). Therefore it is suggested that the original structures for these two compounds are closely related with each other but different from lignin-like structure.

Succinic acid was the major KOH degradation product of glucose humic acid and glucose humin. Succinic acid also resulted from glucose and compounds which have sugar moieties such as tannic acid, chestnut tannin, wattle tannin, and rutin. Polymaleic acid also produced 4.34% succinic acid, which was the highest yield obtained from the various related compounds examined here (Table 5). ANDERSON and RUSSEL (1) proposed polymaleic acid as a model for soil fulvic acid because of the similarities in their infrared absorption spectra, elementary composition, and acid-hydrolysis products.

Glutaric acid also resulted from glucose humic acid and glucose humin (Table 5). The yield of glutaric acid from glucose humic acid increased from 1.04 to 1.23 mg/g while that of succinic acid decreased from 9.25 to 5.12 mg/g when glucose humic acid was heated in 6 N HCl at 100°C for 24 hr (Table 2). Compared to succinic acid, glutaric acid did not result from glucose, the compounds with sugar moieties, and

polymaleic acid (Table 5). Therefore glutaric acid was presumed to result from the browned polymer of sugars in soil humic acids.

Succinic acid resulted from browned polymer of sugars, the compounds with sugar moieties, polymaleic acid structure or some others in humic acids. But the sugar contents in humic acids (0.5–5%) (13) may be too low to account for all the yields of succinic acid. Browned polymer resulting from sugars is an attractive model structure for the origin of succinic acid, because a very high positive correlation was found between the yields of succinic and glutaric acids. The yields of succinic acid from soil humic acids (4.7–14.2 mg/g) were comparable to the yields from glucose, glucose humic acid and glucose humin (10.5, 9.25 and 9.20 mg/g, respectively). However, this result does not necessarily mean that most part of the humic acid structure is accounted for by the compounds related to sugars, because the yields of succinic acid from soil humic acids and related compounds are too low to be compared exactly and other possibilities of the partial structure of humic acids (e.g. lignin structure and unknown unsaturated structure) can not be excluded. Polymaleic acid is supposed to be an alternative model with respect to the high yield of succinic acid (43.4 mg/g). However, sufficient proof is needed to certify its structural similarity to soil humic substances.

No significant linear correlation was found between the yield of phloroglucin (0.19–5.74 mg/g) in the KOH degradation products and *RF* value of humic acids (Table 4). The yield of phloroglucin was high for the humic acids from forest soils (Nos. 18, 21, 33, 38 and 40), and low (<0.81 mg/g) for the other humic acids (Table 3). The yield of phloroglucin may depend on the amount of flavonoids and condensed tannins supplied to the soils, because they are richer in wood plants and supplied in large amount from fallen leaves and twigs, and rotted woods in forest. Among the related compounds, wattle tannin (a condensed tannin) and rutin (rutinoside of quercetin) produced phloroglucin by KOH degradation (Table 5). In the Bh layer of podzolic soil, larger amount of phloroglucin resulted from the humic acid than from the fulvic acid fractions (Table 5). In addition, comparing the humic acids (Nos. 19 and 32, Table 3) extracted successively with 0.1 N NaOH and 0.1 N NaF from a calcareous soil, greater amount of phloroglucin resulted from the humic acid extracted with 0.1 N NaF which is presumed to dissolve even the humic acids combined with Ca ion in soil. It is suggested that humic substances resulting from flavonoids and condensed tannins are stabilized by complex formation with metal ions. The higher yield of phloroglucin from the humic acid fraction than that from the fulvic acid fractions may be partially because flavonoids and condensed tannins are soluble in alkali but hardly soluble in acid solution, like humic acids.

The yields of gallic acid (trace–1.5 mg/g, Table 3) were low from soil humic acids, but their negative correlation with *RF* value was significant at 5% level (Table 4). Hydrolysable tannins such as tannic acid and chestnut tannin, which mainly consist of galloyl moieties, yielded high amounts of gallic acid by the KOH degradation (Table 5). Syringyl moiety in lignin structure may also contribute to the formation

of gallic acid by demethylation during the KOH degradation. Therefore, the contribution of hydrolysable tannins to the structure of soil humic acids is presumed to be very small. It may be because hydrolysable tannins are easily polymerized in soil.

The artificial humic acid prepared from hydroquinone yielded hydroquinone (44.9 mg/g) and gentisic acid (2,5-dihydroxybenzoic acid, 11.2 mg/g) by the KOH degradation. No other peak for the degradation product was observed on the gas chromatogram (Table 5). About 59% of the original hydroquinone was recovered when hydroquinone was subjected to KOH degradation.

#### 4. *Difference in the KOH degradation products between humic acid and fulvic acid fractions from a Bh layer of podzolic soil*

Compositions of the KOH degradation products were compared among the humic acid and the two fractions of a fulvic acid obtained from a Bh layer of podzolic soil (Table 5). The same kind of degradation products were obtained from the humic acid and the fulvic acid fractions, but the amounts of individual products differed between them. Between the two fulvic acid preparations, the quantitative compositions were similar, but the total yield of degradation products was higher from the less polar Fr. B<sub>4</sub> fulvic acid than from the more polar Fr. D<sub>1</sub> fulvic acid. The yields of *p*-hydroxybenzoic acid, vanillic acid, and protocatechuic acid were remarkably higher, and that of succinic acid was also higher from the fulvic acid preparations than from the humic acid. Therefore, lignin structure and browned polymers resulting from sugars are thought to contribute more largely to the fulvic acid than to the humic acid. On the other hand, the yields of glutaric acid and phloroglucin were higher from the humic acid than from the fulvic acid preparations. Difference in the yields of phloroglucin was discussed already. The higher yield of glutaric acid obtained from the humic acid than from the fulvic acid preparations is presumed to correspond to the result that the yield of glutaric acid from glucose humic acid by the KOH degradation increased when glucose humic acid was heated in 6 N HCl (Table 2). Thus, larger amount of glutaric acid is thought to result from more browned material which may have higher molecular weight. Therefore, the yields of glutaric acid were higher from the humic acid than from the fulvic acid preparations.

#### 5. *Conclusion*

Phenolic acids such as protocatechuic, *p*-hydroxybenzoic, vanillic, and 3,4-dihydroxy-5-methoxybenzoic acids were the main KOH degradation products from soil humic acids. These phenolic acids were presumed to result from lignin structure in humic acids. Succinic acid, which is one of the major degradation products, and glutaric acid were presumed to result from browned polymer of sugars. The yields of the above-mentioned degradation products decreased with increasing degree of humification. Residual substances from decayed plants and microorganisms including lignin, browned polymer of sugars and tannins are presumed to contribute largely to the chemical structure of the low humified humic acids. The decrease in the yields

of the degradation products suggests that with increasing degree of humification the original structure of such humic acids may undergo oxidation, condensation and polymerization as well as the consumption of the easily decomposable fractions and may be transformed to the dark colored amorphous polymer which has very stable structure and produces no special compounds even by the KOH degradation. Phloroglucin in the degradation products implied that flavonoids and condensed tannins contribute to the structure of soil humic acids. Its yields were presumed to depend on the vegetations at the sampling sites of the soils for humic acid preparation. Gallic acid was presumed to result from hydrolysable tannins and lignin structure in humic acids, but with lower yields.

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