Article

Chemical Properties of Purified Commercial Humic Acids and Their Comparison with Those of Soil Humic Acids Certified by Japan Humic Substances Society

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Abstract

Commercial humic acids (HAs) have widely been used both as primary and reference material for various research purposes. However, aside from the lack of information on their origin and extraction procedure, their chemical properties are also not fully understood. In this paper, we investigated the chemical characteristics of three commercial HAs, which were purified by alkaline-acid and subsequent HF-HCl treatment. These were compared with two Japan Humic Substances Society (JHSS) standard soil HAs originating from Umbric Andosol and Dystric Cambisol. Ash content of commercial HAs drastically decreased from 18.2-49.8 wt% to 0.4-1.8 wt% by above described purification. Elemental analysis showed that the H/C and N/C atomic ratios of commercial HAs were quite lower than those for soil HAs. Liquid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy revealed that the spectral shapes of commercial and soil HAs also differed markedly. Commercial HAs contained relatively higher aromatics (42.6-65.7%) and lower O-alkyl carbons (9.6-10.7%) than JHSS soil HAs (22.7-41.7% and 23.3-30.6%, respectively). The degree of aromaticity, calculated from the distribution of carbon species based on the ^{13}C-NMR spectra, of commercial samples (60.8-84.2) was quite higher than that of soil HAs (36.2-58.6). The high-performance size-exclusion chromatography (HPSEC) also showed clear differences in the weight and number average molecular weight ($\bar{M}_w$ and $\bar{M}_n$, respectively) and polydispersity of commercial HAs and the standard soil HAs. These results indicate that the origin and chemical properties of commercial HAs are basically different from natural soil HAs. In conclusion, the commercial HAs used in this study are not close representatives of soil HAs. Hence, depending on the purpose of a particular study, basic research application of commercial HAs should be guided by a proper understanding of their chemical property. Also, appropriate pretreatment to remove impurities, including fulvic acid fraction and clay, from the purchased samples is required before use for appropriate comparison between individual and previously published data.

Key words: commercial humic acid, purification, soil humic acid

Introduction

Humic substances (HSs), the major components in soil organic matter, are characterized as dark colored heterogeneous organic macromolecules that are chemically or biologically synthesized secondary from various biomass constituents or their degradation products in terrestrial environments (Stevenson, 1994). HSs can serve as sorbent of essential trace elements and certain pollutants such as heavy metals and organic contaminants, thus playing an important role in controlling their behavior and fate in the soil environment. From the standpoint of global climate, soil carbon pool (2,500 Gt) is 3.3 times and 4.5 times higher than atmospheric (760 Gt) and biotic carbon pool (560 Gt), respectively, and about 60% of soil carbon pool (1,550 Gt) is originated from soil organic carbon (Lal, 2004), so that HSs are also regarded as key substances for global climate change.

Based on their solubility in alkali and acid, HSs are typically divided into three fractions: humic acid

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(HA), fluvic acid (FA) and humin (Aiken *et al.*, 1985). According to Kawahigashi *et al.* (2013), based on the number of publications among these three fractions in 2010, studies on HA (soluble in alkaline but not in acid) are the highest followed by FA (alkaline and acid soluble fraction) and HN (insoluble in both solution). One of the reasons for this is the easier method for obtaining HA relative to other HS fractions. In any cases, many researches have been using a wide variety of HSs derived from soil, sediment, aquatic and commercial products. Among these samples, commercial HAs have widely been used not only as a primary but also as reference material. This is because the procedure for obtaining natural HA is still a time-consuming and laborious processes. However, Malcolm & MacCarthy (1986), in their studies on the chemical properties of five kinds of commercial product including Aldrich HA (AHA), concluded that the use of commercial HAs is not recommended due to (1) the huge difference of their chemical property with those obtained from natural sources and (2) the lack of information on their origin and extraction methods. For the former, they clearly demonstrated that the composition of carbon species and other elements in commercial HAs are markedly different from natural HAs based on the cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance (CPMAS ^{13}C-NMR) spectral and elemental analysis. Many researches have also observed that the physicochemical properties of HSs strongly affect their bioavailability (Perminova *et al.*, 2001) as well as their interaction with other substances such as heavy metals (Xu *et al.*, 2016) and anthropogenic pollutants (Perminova *et al.*, 1999; Fukushima *et al.*, 2005). With regards to the latter, this is a serious problem because the chemical property even of HAs from similar origin may vary depending on the extraction protocol. The International Humic Substance Society (IHSS) and Japan Humic Substances Society (JHSS) have thus specified the recommended extraction procedure for HSs (Swift (1996) for IHSS method and Kuwatsuka *et al.* (1992) for JHSS (NAGOYA) method). Nonetheless, HS suppliers do not clearly indicate the sample origin and the employed extraction process (Malcolm & MacCarthy, 1986). Furthermore, it should be noted that commercial HAs are typically considered to contain impurities, such as clay and acid-soluble FA fraction. For example, Malcolm & MacCarthy (1986) reported that ash content of original commercial HAs ranged from 9.3% to 32.8%. Hence, appropriate purification, as in the case of preparation for soil HS, is very important because these impurities are capable of hindering better understanding of chemical property of a particular HA. Though it has been recommended to remove impurities from commercial HAs prior to use (Fujitake, 2006), the characterization of chemical property of commercial HAs after such treatment has not been well documented except for a few HAs including AHA (Malcolm & MacCarthy, 1986). This is despite the fact that they are still continuously being used as representative HA.

The aim of this research was thus to characterize the chemical properties of three different kinds of commercial HAs purified by the same treatment after procurement. Two standard HAs, which was supplied by JHSS, were utilized as reference samples. From the obtained results, the applicability of commercial HAs for predicting and evaluating various phenomena occurring in natural soil environment as well as the significance of sample pretreatment to remove impurities was also discussed.

Materials and Methods

Humic acids

The commercial HAs were purchased from Sigma-Aldrich (AHA, Lot No. 0001411101, St. Louis, MO, USA), Fluka (FHA, Lot No. 1411101, Buchs, Switzerland) and Wako Chemical (WHA, Lot No. KWM6624, Osaka, Japan). Since these HAs have relatively high ash contents (18.2-49.8%, Table 1), they were first purified to remove acid-soluble FA fraction and other impurities such as clay and then converted into H^+ form according to the recommendation of Fujitake (2006). Briefly, commercial materials were first dissolved in 0.1 M NaOH and then acidified (pH<2) with 6 M HCl. The suspension was stored for 3 days at 4°C and the precipitate was then recovered by centrifugation (8,500×*g*, 25min). This treatment was repeated 3 times to remove fluvic acid fraction. Next, the precipitate was treated (×5) with a mixture of HF and HCl (0.3M HF-0.1M HCl, 1:1, v/v). The resulting precipitate was dialyzed using a 1000 Da dialysis membrane (Spectra/Por 6, MWCO 1000, Spectrum

Table 1. Various properties of HAs.

HAs	Ash (wt%)[c]		log	A_{600}/C	HA type	Elementary composition (atomic%)					Atomic ratio				ω	Molecular size distribution		
	Before	After	(A_{400}/A_{600})			C	H	N	O	S	H/C	N/C	O/C	O/H		$\log \bar{M}_w$	$\log \bar{M}_n$	D
AHA	32.6	1.8	0.72	4.40	B	42.6	34.1	0.7	22.2	0.3	0.80	0.02	0.52	0.65	0.2	4.52[b]	4.24[b]	1.92[b]
FHA	49.8	0.6	0.72	4.80	B	44.3	32.1	0.9	22.5	0.2	0.72	0.02	0.51	0.70	0.3	4.39[b]	4.09[b]	2.00[b]
WHA	18.2	0.4	0.58	8.47	A	51.0	24.7	0.9	23.1	0.2	0.48	0.02	0.45	0.93	0.4	4.16[b]	3.73[b]	2.72[b]
IHA	-	0.5[a]	0.52[a]	6.87[a]	A	40.1[a]	37.2[a]	2.5[a]	20.1[a]	0.1[a]	0.93	0.06	0.50	0.54	0.1	5.00[b]	4.46[b]	3.46[b]
DHA	-	0.7[a]	0.64[a]	2.42[a]	P	36.0[a]	42.5[a]	2.6[a]	18.8[a]	0.1[a]	1.18	0.07	0.52	0.44	-0.1	5.72[b]	4.79[b]	8.36[b]

AHA: Aldrich HA, FHA: Fluka HA, WHA: Wako HA, IHA: Inogashira HA, DHA: Dando HA. [a]Data were quoted from Watanabe (2007). [b]Data were quoted from Kobayashi & Sumida (2015). [c]Ash content before and after alkaline-acid and subsequent HF-HCl treatment. ω: Degree of oxidation defined by Orlov (1985); (2×O (atomic%)-H (atomic%))/C (atomic%). $\bar{M}_w$: weight average molecular weight; $\bar{M}_n$: number average molecular weight; D: polydispersity ($=\bar{M}_w/\bar{M}_n$).

Laboratories Inc., CA) and then freeze-dried. The obtained powder was used for all analyses described below.

Additionally, two standard soil HAs certified by the JHSS were used without further purification. These were prepared from Inogashira soil (Umbric Andosol, IHA; FAO, ISRIC and ISSS, 1998) and Dando soil (Dystric Cambisol, DHA).

Analytical methods

The amounts of carbon, nitrogen, hydrogen and sulfur in commercial HAs were measured with a CE440 elemental analyzer (Exeter Analytical Inc., MA, USA). Ash content was measured by combustion of the dry sample, which was stored in a desiccator over P_4O_{10} *in vacuo*, for 6 hours at 550°C in a muffle furnace. Oxygen composition was calculated by subtracting the C, H, N, S and ash contents from the total weight. This experiment was conducted in duplicate and the average values were reported.

The ultraviolet and visible (UV-Vis) spectra of HAs in 0.1 M NaOH were obtained with a Jasco V-530 type spectrophotometer (Jasco Co., Ltd., Tokyo, Japan). Humification degree was evaluated by the measurement of A_{600}/C, which is the absorbance at 600 nm in 0.1 M NaOH per mg-C mL^{-1}, according to the method of Ikeya & Watanabe (2003). The organic carbon concentration of HA solutions was determined by a Shimadzu TOC-500 analyzer (Shimadzu Co., Kyoto, Japan).

The infrared (IR) spectra were recorded on a Shimadzu FTIR-8600 spectrometer by the KBr pellet method.

Liquid-state NMR spectra of commercial HAs were recorded at 500.16 MHz for ^{1}H and 125.77 MHz for ^{13}C using a JEOL ECA 500 spectrometer (JEOL Ltd., Tokyo, Japan) by the homo-gate decoupling and the inverse gated-decoupling technique, respectively. The spectra were obtained after dissolving 50 mg of HA in a mixture of 0.02mL of 40% NaOD and 0.5mL of D_2O (99.99%, Sigma-Aldrich). Following the method of Fujitake (2007), ^{1}H-NMR spectra were obtained under the following conditions: pulse width 90°; acquisition time 5.4s; pulse delay 4.8s; number of scan 8. Experimental parameters for ^{13}C were as follows: pulse width 45°; acquisition time 0.833s; pulse delay 2.5s; scan number 10,000-30,000. Sodium 3-trimethylsilyl-2, 2, 3, 3-d_4-propionate (TSMP) was used as a chemical shift standard. Chemical shift assignments for ^{1}H-NMR were also referred from Fujitake (2007). The ^{13}C-NMR spectra are divided into six ranges of chemical shifts in alkyl-C (5-48 ppm), O-alkyl-C (48-110 ppm), aromatic-C (110-145 ppm), phenolic-C (145-165 ppm), carboxylic-C (165-190 ppm) and carbonyl-C (190-230 ppm), according to the models proposed by Fujitake & Kawahigashi (1999).

Molecular size distribution of HAs was determined according to the method of Asakawa *et al*. (2008). The chromatographic data were obtained by using the Jasco ChromNav software. A calibration curve was made from standard pullulans (Showa Denko Co,. Tokyo, Japan) of known molecular weights (708k, 334k, 200k, 107k, 47.1k, 21.1k 9.6k and 5.9kDa). The column void volume (V_0) and total permeation volume (V_0+V_i) were determined using blue dextran and acetone, respectively. UV detection was set at 260 nm. For detailed analytical information, please refer to our previous report (Kobayashi & Sumida, 2015). This experiment was conducted in duplicate and average values were reported.

Results

Elemental composition

Ash content in commercial HAs drastically decreased from 18.2-49.8% to below 1.8% after alkaline-acid (×3) and subsequent HF-HCl treatment (×5) as shown in Table 1. The table also shows the elemental composition and atomic ratio of HAs. According to Kumada (1987), the elemental composition of soil HA is 34-52% for C, 22-50% for H, 0.6-3.8% for N and 14-29% for O. All HAs used in this study fell within these ranges. In the two JHSS standard soil HAs, IHA (40.1%) has a higher carbon content than DHA (36.0%), whereas the hydrogen content in the former (37.2%) was lower than that in the latter (42.5%). Comparisons show that the carbon content of the three commercial HAs (42.6-51.0%), particularly WHA, were higher. On the other hand, the nitrogen content of the commercial HAs (0.7-0.9%) was about 3-fold lower than those of the soil HAs (2.5-2.6%). The results of elemental analysis were plotted on the van Krevelen diagram (van Krevelen, 1961) (Fig 1a). The principal reactions such as demethanation, dehydration and decarboxylation are also represented in the figure by straight dotted lines. Areas enclosed by broken lines, referred to as “coal band” (Murata, 1964), were incorporated. This diagram has been widely used by various workers to illustrate compositional and diagenetic changes in coal and related organic materials such as HSs. According to Kuwatsuka *et al*. (1978), the H/C and O/C ratio of soil HAs ranged from 0.5 to 1.5 and from 0.35 to 0.55, respectively. They also described how soil HAs generally undergo dehydrogenation or demethanation in the early to middle stage of humification process, leading to the decline in H/C ratio. Therefore, the lower H/C ratio observed in IHA (0.93) indicates its higher degree of humification over DHA (1.18). On the other hand, all three commercial HAs are placed more towards the dehydrated direction as compared to IHA and DHA (Fig. 2a). It can be observed as well that AHA falls in the area of soil HA, FHA is in the border while WHA, which is the most dehydrated, is placed outside the soil HA region. As described above, the nitrogen contents of commercial and soil HAs were much different so we presented an N/C vs. H/C diagram as well (Fig. 1b). Due to the diversity of soil HAs, the data of 41 soil HAs reported by Yonebayashi & Hattori (1988) were also included in this figure. As is clear from Fig. 1b, all commercial HAs exhibited lower N/C ratio than soil HAs. The degree of oxidation (2×O (atomic%)-H (atomic%))/C (atomic%) defined by Orlov (1985) of commercial HAs follows the order: WHA (0.4) > FHA (0.3) > AHA (0.2) > IHA (0.1) > DHA (-0.1) (Table 1).

UV-Vis spectra and classification of HAs

The UV-Vis spectra of HAs in 0.1M NaOH are shown in Fig. 2. All HAs except for DHA did not exhibit specific absorption bands and their absorbance logarithmically increased from longer to shorter wavelengths. However, in the region below 280 nm, the slope of all spectra from commercial HAs was slightly lower than soil HAs. In DHA, four

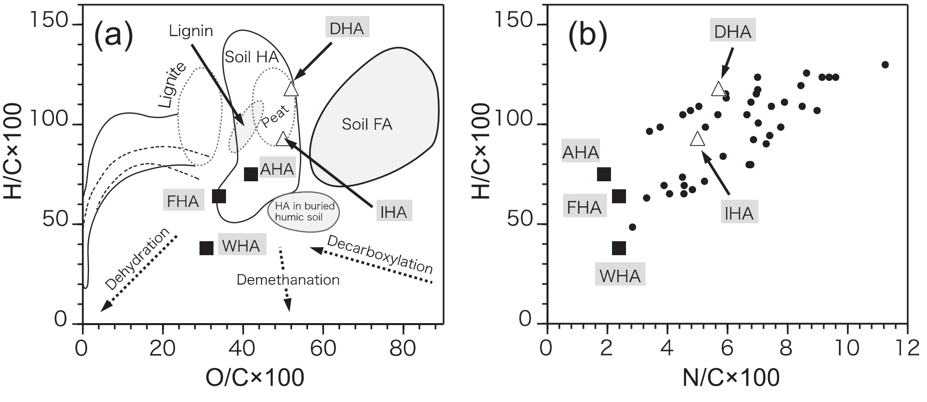

Fig.1. (a) Van Krevelen plot and (b) H/C-N/C diagram for HAs. Enclosed areas in Fig.1a are illustrated with reference to Kuwatsuka *et al*. (1978). Filled circles in Fig. 1b are referred from Yonebayashi & Hattori (1988).

weak absorption bands around 615, 570, 450 and 280 nm, which indicate the presence of the green fraction of HA called Pg (Kumada, 1987), were detected.

Figure 3 shows the A_{600}/C-log (A_{400}/A_{600}) diagram for HAs. The data for 36 soil HAs reported by Ikeya & Watanabe (2003) were also cited in this figure. Kumada *et al*. (1967) classified HA into A, B, Rp and P type based on the log (A_{400}/A_{600}) and RF value (RF=$A_{600}/C\times1000$, where C is the volume of 0.1M $KMnO_4$ consumed by 30 mL of the HA; RF could be replaced with A_{600}/C by multiplying by a factor of 0.0648, Ikeya & Watanabe (2003)). Their humification degree follows the order: Type A > Type B > Type Rp. Since IHA and DHA were placed in Type A and Type P, respectively, IHA was more humified than DHA. WHA had the highest A_{600}/C value (8.9, Table 1) and it belonged to Type A. AHA and FHA was placed in almost the same upper area position in Type B HA. However, the position of AHA and FHA were shifted to the left as compared with the similar A_{600}/C values of soil HAs reported by Ikeya & Watanabe (2003).

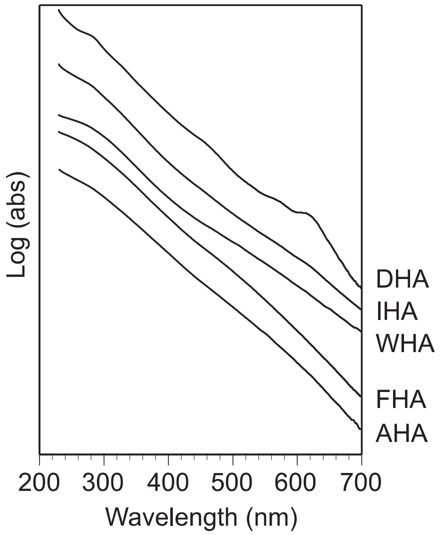

Fig.2. UV-Vis spectra of HAs in 0.1 M NaOH.

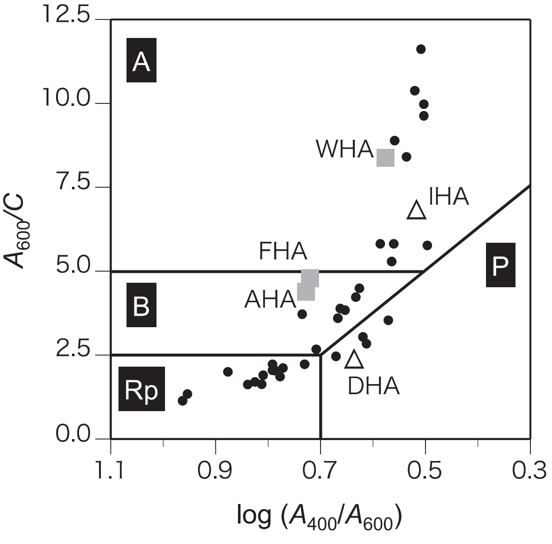

Fig.3. A_{600}/C-log (A_{400}/A_{600}) diagram.
Filled circles are illustrated with reference to Ikeya & Watanabe (2003).

IR spectra

Comparisons among the soil HAs showed that the absorption at around 1720 cm^{-1}, indicating the presence of carboxylic C=O stretching vibrations, was stronger in IHA than DHA, while the absorption near 1540 cm^{-1}, which is derived from N-H bending (amide II bands), was stronger in DHA (Fig.4). There is a clear difference between the IR spectra of soil HAs and commercial HAs at around 1050 cm^{-1}, possibly arising from C-O vibration of alcohols and/or C-O stretching of ethers: the former displayed a clear absorption band at this region whereas the latter have none. Among the commercial HAs, only WHA had no absorption band near 2900 cm^{-1}, which is due

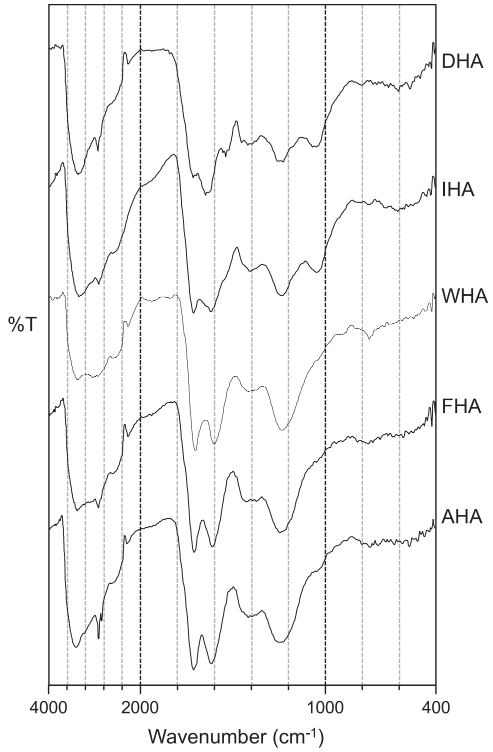

Fig.4. IR spectra of HAs.

to aliphatic C-H stretching. In AHA, there was a relatively strong absorption near 3350 cm^{-1} which arise from alcohols and phenolic O-H stretching as in the case of two soil HAs. Such strong absorption was not found in FHA and WHA.

^{1}H-NMR spectra

It was obvious that the ^{1}H-NMR spectra of all commercial HAs (Fig.5) were totally more broad-shaped when compared with those obtained from soil HAs. Furthermore, the signal due to protons attached to carbons of heteroaromatic and aromatic rings, and carbonyl groups bonded to electronegative groups (H_{Ar}; 6.0-9.0 ppm) was prominent in all commercial HAs. However, the relative intensities of their signals originating from protons on carbon attached to oxygen-sugars, olefins and methoxyl groups ($H_{o\text{-}c}$; 3.0-4.3 ppm) were much weaker than those of soil HAs. Among the commercial HAs, the spectral shape of WHA was very simple; it consists mostly of H_{Ar} and the signals below 6.0 ppm was much weaker than the other commercial HAs. Consequently, the distribution of proton species from commercial HAs (Table 2) was quite different from those from soil HAs. The percentage of H_{Ar} in WHA (75.6 %) was much greater than that of soil HAs (13.1% for IHA and 9.0% for DHA) as well as the other commercial HAs (33.1% for AHA and 37.2% for FHA).

^{13}C-NMR spectra

Liquid state ^{13}C-NMR spectra of HAs and the distribution of carbon species calculated from ^{13}C-NMR spectra are presented in Fig. 6 and Table 2, respectively. As compared with DHA, IHA contains higher aromatic carbon and lower alkyl, O-alkyl and carbonyl carbon (22.7%, 21.3%, 30.6% and 2.3% for DHA and 41.7%, 10.7%, 23.3% and 0.7% for DHA, respectively). As mentioned above, humification process in soil is generally considered as a decline of

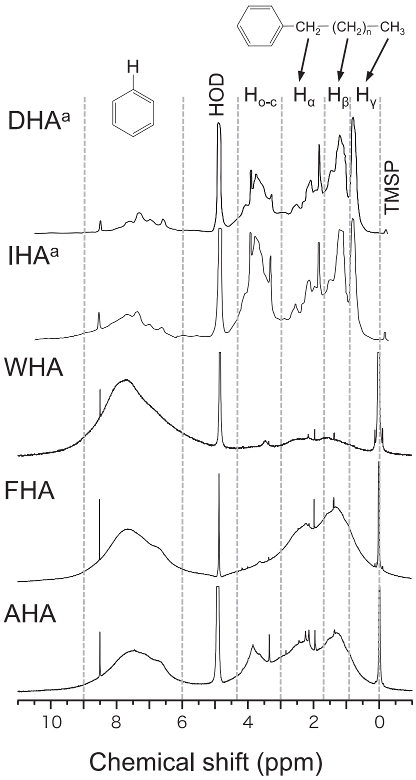

Fig.5. Liquid state ^{1}H-NMR spectra of HAs.
The spectra of IHA and DHA were cited from Fujitake (2007). TMSP: Sodium 3-trimethylsilyl-2, 2, 3, 3-d$_4$-propionate. ^{a}Spectra were cited from Fujitake (2007).

Table 2. Composition of proton and carbon species of HAs.

	Proton species % (δ ppm) from ^{1}H-NMR					Carbon species % (δ ppm) from ^{13}C-NMR						
	H_γ (0-0.9)	H_β (0.9-1.6)	H_α (1.6-3.0)	$H_{o\text{-}c}$ (3.0-4.3)	H_{Ar} (6.0-9.0)	Alkyl (5-48)	Carbohydrate (48-110)	Aromatic (110-145)	Phenolic (145-165)	Carboxylic (165-190)	Carbonyl (190-220)	Aromaticity^{c}
AHA	5.5	15.7	27.2	18.4	33.1	16.4^{b}	10.7^{b}	42.6^{b}	9.2^{b}	18.8^{b}	2.3^{b}	65.7
FHA	8.2	18.8	27.2	8.7	37.2	22.4^{b}	10.0^{b}	44.1^{b}	6.0^{b}	14.0^{b}	3.5^{b}	60.8
WHA	2.4	4.8	11.1	6.1	75.6	3.6^{b}	9.6^{b}	65.7^{b}	4.5^{b}	14.1^{b}	2.5^{b}	84.2
IHA	9.8^{a}	20.2^{a}	21.4^{a}	29.7^{a}	13.1^{a}	10.7^{a}	23.3^{a}	41.7^{a}	6.4^{a}	17.2^{a}	0.7^{a}	58.6
DHA	13.6^{a}	27.8^{a}	25.4^{a}	24.2^{a}	9.0^{a}	21.3^{a}	30.6^{a}	22.7^{a}	6.7^{a}	16.5^{a}	2.3^{a}	36.2

AHA: Aldrich HA, FHA: Fluka HA, WHA: Wako HA, IHA: Inogashira HA, DHA: Dando HA. H_γ: terminal methyl groups attached to saturated aliphatic carbons, H_β: protons on methylene β attached to olefins or aromatic rings, H_α: protons on methyl and methylene α attached to aromatic carbons, carbonyl groups, ester groups, and olefins, $H_{o\text{-}c}$: protons on carbons attached to oxygen-sugars, olefins, and methoxyl groups, H_{Ar}: protons attached to carbons of heteroaromatic and aromatic rings, and to carbonyl groups bonded to electronegative group. ^{a}Data were quoted from Fujitake (2007). ^{b}Data were quoted from Kobayashi & Sumida (2015). ^{c}Percent ratio of aromatic carbon (110-165 ppm) to the sum of the aliphatic (5-48 ppm), carbohydrate (48-110 ppm) and aromatic carbon (110-165 ppm) calculated from ^{13}C-NMR spectra.

alkyl carbon of HA through dehydration and demethanation leading to an increase in aromatic carbon due to the dehydrative condensation reaction of HA. Hence, ^{13}C-NMR data also supported the more humified characteristic of IHA than DHA.

As in the case of ^{1}H-NMR, the ^{13}C-NMR spectral shapes of commercial samples were different from those from soil HAs. Although the shapes for AHA and FHA were relatively similar, the spectra of AHA exhibited sharp and weak signals at around 57 ppm and 160 ppm, respectively. These signals may be due to the presence of methoxyl groups of lignin or C-N bonds in amino-acids (Khan *et al*., 2006; Spaccini & Piccolo, 2009). Also, the relative intensities of carboxylic C (165-190 ppm) in AHA were stronger than that in FHA. In contrast, the spectra of WHA exhibited only two major broad peaks in the regions of aromatic C (110-145 ppm) and carboxylic C (165-190 ppm) except for the sharp peak at 15, 34 and 189 ppm and it was quite different from that of the other HAs. As shown in Table 2, the most predominant carbon species in commercial HAs was aromatic carbon (42.6-65.7%). Particularly, the highest proportion of aromatic carbon and the lowest aliphatic carbon was found in WHA (65.7% and 3.6%, respectively). The low percentage of aliphatic carbon in WHA agreed well with their undetected IR absorption around 2900 cm^{-1} (Fig.4). The proportions of O-alkyl carbon in all commercial HAs were almost the same at about 10%. This value was quite lower than those of IHA (23.3%) and DHA (30.6%). Consequently, the aromaticity of the three commercial HAs (60.8-84.2) was higher than that of the soil HAs (36.2-58.6) as shown in Table 2.

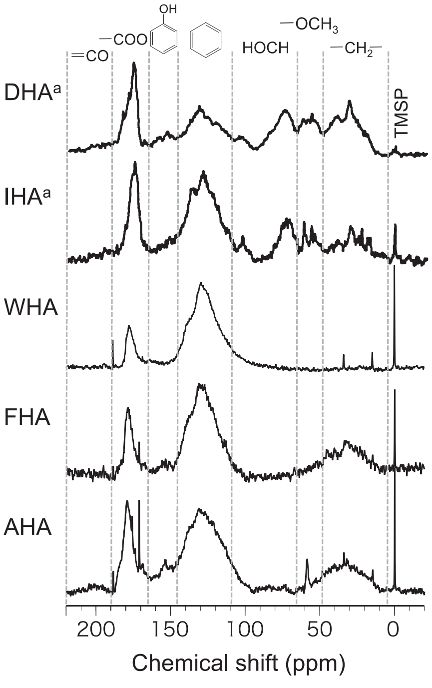

Fig.6. Liquid state ^{13}C-NMR spectra of HAs.
TMSP: Sodium 3-trimethylsilyl-2, 2, 3, 3-d$_4$-propionate. ^{a}Spectra were cited from Fujitake (2007).

HPSEC analysis

Figure 7 shows the HPSEC chromatograms of HAs. The weight and number average molecular weight ($\bar{M}_w$ and $\bar{M}_n$, respectively) of HAs calculated from HPSEC chromatogram are also given in Table 1. All chromatograms monitored by absorbance at 260 nm showed a single broad peak and the samples were almost eluted within the range from void volume (V_0) to total permeation volume (V_0+V_i). The value of $\bar{M}_w$ and $\bar{M}_n$ depend on the experimental condition (e.g. mobile phase, detection wavelength, sample concentration and calibration standard) even when the same HA sample is used. Our data thus cannot be compared with those from previously published literature, but the higher log $\bar{M}_w$ of DHA (5.72) than

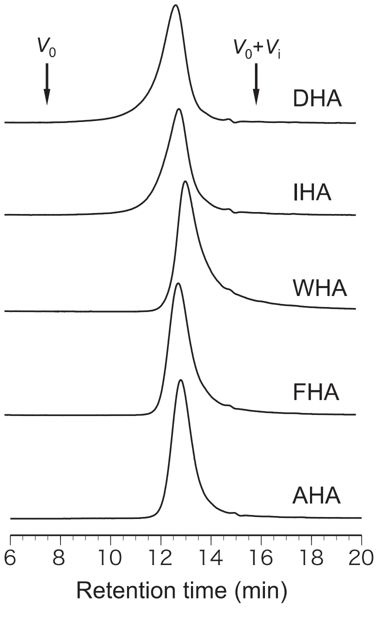

Fig.7. HPSEC chromatograms of HAs.
V_0, void volume; V_0+V_i; total permeation volume.

IHA (5.00) observed in the present study agreed with the report of Fujitake (2007). The values of log $\bar{M}_w$ of commercial HAs were 4.52 for AHA, 4.39 for FHA and 4.16 for WHA. The order of log $\bar{M}_n$ was also consistent with that of log $\bar{M}_w$ (Table 1). These values are lower than that of the soil HAs used in this study. In addition, commercial HAs showed lower polydispersity (1.92-2.72) than soil HAs (3.46-8.36). The value of DHA (8.36) was about 2.5-fold higher than that of IHA (3.46).

Discussion

In this paper, we examined the chemical properties of three purified commercial HAs and compared them with JHSS standard soil HAs. As emphasized earlier, pretreatment to remove FA and ash material, rendering them comparable to JHSS standards, is important to prepare HAs. The alkaline-acid and the HF-HCl treatment have been used for this purpose (in particular, the HF-HCl treatment is part of the IHSS and JHSS protocol). Malcolm & MacCarthy (1986) evaluated the effect of sample pretreatment on the chemical properties of two commercial HAs including AHA. Though their pretreatment method was not well documented in the literature, they used liquid-liquid extraction. Based on the elemental analysis and CPMAS ^{13}C-NMR spectra, they reported a large reduction of ash and oxygen content in both HAs and an increase in relative intensity of aromatic carbon in one commercial product after pretreatment. Such alterations will seriously affect the various HAs properties (e.g. partition coefficient for heavy metals and organic chemicals) thus influencing data interpretation. In the present study, the commercial HAs employed also initially contained high ash material (Table 1), so that such treatment is very important prior to their use. Although we did not conduct any experiments using untreated commercial samples except for ash content determination, the decreasing $\bar{M}_n$, $\bar{M}_w$ and A_{600}/C value due to the presence of FA fraction and the limitation of NMR measurement due to the presence of paramagnetic substance such as iron may be anticipated when using untreated commercial HA.

Our data also clearly indicated that the commercial HAs are not representative of natural soil HA, as previously reported by Malcolm & MacCarthy (1986). All three commercial HAs contained higher aromatic components than JHSS soil HAs. They also exhibited a lower proportion of aliphatic moiety and O-alkyl structure. Furthermore, the commercial HAs are characterized by a high degree of oxidation, low nitrogen content, low molecular weight and low polydispersity. Although HA suppliers do not give important information regarding the source and nature of their products, our results indicated that the origin of commercial HAs was basically different from those of soil HAs. According to Malcolm & MacCarthy (1986), regardless of its suppliers, commercial HAs are obtained from the same source, which is similar to Wyoming dopplerite. Fujitake (2006) considered the origin of commercial HAs to be peat or coal-related substances such as lignite, brown coal and peat moss. Our commercial HA data indicated the difference between WHA and the other HAs (AHA and FHA) in terms of H/C atomic ratio, degree of oxidation, proportion of carbon species, aromaticity and molecular size. The relatively lower log $\bar{M}_w$ value in WHA may be due to their lower amount of aliphatic moiety as indicated in ^{13}C-NMR spectra (Fig.6). In contrast to AHA and FHA, the origin of WHA may thus be a relatively more dehydrated and oxidized coal-related material. However, differences among our commercial HAs data may not be so huge, taking into account the large (about 2.5-fold) difference in polydispersity even in soil-derived HAs (IHA and DHA) (Table 2). The three commercial HAs used in this study are thus supposed to be of similar origin in a broad sense.

Finally, we discuss about the difference in chemical property among various lot numbers using AHA as an example. Malcolm & MacCarthy (1986) indicated that the percentage of total peak area of CPMAS ^{13}C-NMR of AHA samples (Lot Number, LE3601KE) purified by liquid-liquid extraction described above decreases in the order aliphatic > aromatic > carbohydrate and carboxylic carbon. Shin *et al.* (1999) also reported the same distribution of carbon spices of AHA. Watanabe & Fujitake (2008) reported that the aromaticity and the proportions of HA carbon species including alkyl, O-alkyl and aromatic, obtained by ^{13}C-CPMAS NMR can be directly compared to those obtained by the inverse-gated decoupling technique used in this study, meaning that our AHA are clearly different from previously reported AHA data. Specifically, the aromaticity of our AHA sample (66%, Table 2) was much higher than the AHA analyzed by Malcolm & MacCarthy (1986) (29%). Furthermore, the atomic O/C ratio in our AHA (0.52) was about twice higher, whereas the proportion of alkyl carbon in our AHA (16.4%) was 2.7-fold lower. The difference of lot number may thus cause such dissimilarities - this might be one of the possible reasons that HA suppliers do not give detailed information regarding their sources and origin.

Conclusion

The results reported in this paper demonstrated that the chemical properties of purified commercial HAs are quite different from those of JHSS soil HAs. Specifically, commercial HAs are characterized by a high degree of aromaticity and oxidation, low nitrogen content, low molecular weight and low polydispersity. Also, our study showed the significance of sample pretreatment for removing FA and ash materials before use. It has been recognized that the chemical properties of HS influence the fate and behavior of contaminants in the environment (e.g. organic chemical and heavy metal). For example, Chin *et al*. (1997) and Perminova *et al*. (1999) found a positive correlation between carbon normalized partition coefficient (K_{doc}) for PAHs (polycyclic aromatic hydrocarbons) and HA aromacity. Indeed, we previously reported that WHA, which has the highest aromaticity, is the most effective for the sorption of pyrene (4-ring PAH) (Kobayashi & Sumida, 2015). As a result of these differences and with the lack of information regarding the source and detailed preparation method, it is insufficient to evaluate the nature HAs reactivity in natural soil environment through the use solely of commercial samples. Commercial HAs should be purified prior to use and their use must be supported by a clear understanding of their chemical properties in relation to the objectives of a particular study.

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