Soil Science and Plant Nutrition (2007) **53**, 7–11 doi: 10.1111/j.1747-0765.2007.00111.x

SHORT PAPER

Monosaccharide composition of four humus fractions in an Andosol and a Cambisol

Kazuhito ITOH,[1] Akira WATANABE,[2] Kiyoshi TSUTSUKI[3] and Shozo KUWATSUKA[2†]

[1]*Faculty of Life and Environmental Science, Shimane University, Shimane 690-8504,* [2]*Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, and* [3]*Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan*

Abstract

The composition of seven neutral monosaccharides (glucose, galactose, mannose, xylose, arabinose, fucose and rhamnose) released by acid hydrolysis with 0.5 mol L^{-1} H_2SO_4 was compared among four humus fractions, including humic acids (HAs), fulvic acids (FAs), water-soluble non-humic substances (WS-NHS; XAD-8-non-adsorbed fraction of the FA fraction) and humin, for two representative types of Japanese soils, an Umbric Andosol (ando soil) and a Dystric Cambisol (brown forest soil). Although more than 58% of the hexoses and pentoses in the soil were recovered in humin, 29–57% of fucose and rhamnose were found in WS-NHS. In the principal component analysis, humin was separated from the other three fractions because of larger proportions of glucose and xylose and smaller proportions of fucose and rhamnose. The HAs contained a larger proportion of arabinose than the other fractions. The monosaccharide composition of the FAs and WS-NHS was similar in each soil type. As deoxyhexoses and pentoses in soil are known to originate mainly from microorganisms and higher plants, respectively, the contribution of microorganism-derived saccharides to total neutral saccharides was considered to be greater in the order of WS-NHS and FAs > HAs > humin.

Key words: fulvic acid, humic acid, humin, monosaccharide composition, soil saccharide.

INTRODUCTION

Carbohydrates account for 5–25% of soil organic matter (Stevenson 1994). Most of them occur in association with humus fractions in various proportions (Lowe 1978). Cheshire *et al.* (1992) purified the soil polysaccharide fraction by the removal of humic substances (generic fulvic acids [FAs]) from the acid-soluble materials in alkali-extractable humus (FA fraction) using PVP (cross-linked insoluble polyvinylpyrrolidone, "Polyclar") as their adsorbent, followed by precipitation in 75% ethanol. The purified soil polysaccharide fraction accounted for only 15% of the total saccharides in the soil. In contrast, purified humic acid (HA) and FA samples contained saccharides without exception (Clapp and Hayes 1999; Watanabe and Kuwatsuka 1992; Watanabe *et al.* 1994). However, there are few reports on the composition of monosaccharides in humus fractions (Cheshire *et al.* 1992; Clapp and Hayes 1999; Malcolm and McCarthy 1991; Ogner 1980).

Although all the neutral monosaccharides, including hexoses, pentoses and deoxyhexoses, released by acid hydrolysis of soil polysaccharides (Cheshire 1979; Lowe 1978) can be derived both from plants and microorganisms (Cheshire 1979), their composition differs considerably depending on the origin (Oades 1984). It is well known that glucose, galactose and mannose are the major saccharides synthesized by soil microorganisms, as well as rhamnose, fucose and ribose (Murayama 1988). Xylose and arabinose in soil are considered to be derived mainly from higher plants, whereas both higher plants and soil microorganisms can be the major source of glucose (Cheshire 1977; Murayama 1988). The ratio of microorganism-derived saccharides to higher plant-derived

Correspondence: A. WATANABE, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan. E-mail: akiraw@agr.nagoya-u.ac.jp
†Professor Emeritus.

Received 6 December 2004.
Accepted for publication 23 August 2006.

ones has been used to estimate the effect of land-use change on the quality of soil organic matter (Guggenberger *et al.* 1994; Zech *et al.* 1997).

It is considered that the monosaccharide composition may reflect the major source of carbohydrates in humus fractions. In the present study, the composition of monosaccharides released by acid hydrolysis was compared among four major humus fractions using two representative types of Japanese soils, an Umbric Andosol and Dystric Cambisol.

MATERIALS AND METHODS

Soils used and preparation of humus fractions

The soils used were sampled from the A-horizon of Inogashira ando soil (Umbric Andosol; Fujinomiya, Shizuoka, Japan) and Dando brown forest soil (Dystric Cambisol; Shitara, Aichi, Japan) as composites. These two sites have frequently been used in studies of soil organic matter as examples of two soil types widely distributed in Japan (e.g. Kumada 1987; Watanabe *et al.* 1989, 1994, 2004; Yanagi *et al.* 2003). Chemical properties of the soils were reported by Kuwatsuka *et al.* (1992). Major vegetation consisted of *Miscanthus sinensis*, *Sasa* and *Torreya nucifera* at the Inogashira site, and *Quercus crispula*, *Tsuga sicboldii* Carr. and *Fagus crenata* at the Dando site. After plant residues were removed from air-dried soil samples (<2 mm) using tweezers as much as possible, the soil was crushed and sieved (<250 μm) to enhance homogeneity.

Humic and fulvic acid fractions were extracted according to the NAGOYA method (Kuwatsuka *et al.* 1992) with 0.1 mol L^{-1} NaOH at 25°C under N_2. The extracted residue was washed with a 30 g L^{-1} NaCl solution, followed by distilled water, and freeze-dried (humin). The FA fraction was separated from HAs by acidification of the extract and then fractionated using Amberlite XAD-8 (Rohm and Haas, Philadelphia, USA) into adsorbed and non-adsorbed fractions. Because the humic and non-humic substances in the FA fraction are known to be distributed in the XAD-8-adsorbed and non-adsorbed fractions, respectively (Stevenson 1994; Watanabe *et al.* 1994), these two fractions were designated as FAs and water-soluble non-humic substances (WS-NHS) in the present study. The FAs and WS-NHS were desalted using Sephadex G-10, transformed into H^+-form by passing through a column packed with Dowex HCR-W, and freeze-dried (Kuwatsuka *et al.* 1992; Watanabe *et al.* 1994). In contrast, the HA fraction was analyzed for monosaccharide contents without purification. The C content in the samples was determined using a NC analyzer MT-500 (Yanaco, Kyoto, Japan) or a CHN analyzer 2400 (Perkin-Elmer, Wellesley, MA, USA).

Acid hydrolysis and determination of neutral monosaccharides

Two grams of soil or 3–4 g of humin were heated with 80 mL of 0.5 mol L^{-1} H_2SO_4 for 8 h in boiling water (Forsyth 1950). The HAs (300 mg) were heated with 80 mL of 0.5 mol L^{-1} H_2SO_4 for 2 h in boiling water (Tsutsuki and Kuwatsuka 1979). The WS-NHS (16 mg) and FAs (140 mg) were heated with 50 mL of 0.5 mol L^{-1} H_2SO_4 for 2 h in boiling water. Each hydrolysate was filtered and neutralized with a saturated $Ba(OH)_2$ solution. Any barium sulfate formed was removed by centrifugation. The hydrolysate was then mixed with 1 mL of 2.00 μg mL^{-1} *myo*-inositol and reacted with 80 mg $NaBH_4$ for 5 h at room temperature. Excess $NaBH_4$ was degraded by the addition of acetic acid, and the sampled solution was dried up at 50°C using a rotary evaporator. After boric acid and acetic acid were removed by repeated drying up with methanol, monosaccharides in the sample were acetylated by heating at 60°C with 7.5 mL acetic anhydride and 0.5 mL of concentrated H_2SO_4 for 30 min. The acetylated monosaccharides were extracted with 50 mL dichloromethane three times in a separation funnel. The extract was washed with a 50 g L^{-1} $NaHCO_3$ solution, dried up after treatment with Na_2SO_4 for removing water, redissolved in dichloromethane, and subjected to gas chromatography (GC).

A gas chromatograph, Type 063 (Hitachi, Toyko, Japan), equipped with a flame ionization detector and a glass column (length 200 mm; inner diameter 3 mm) packed with 3% ECNNS-M/Gas chrom Q (100–120 mesh) was used. The contents of monosaccharides were determined using *myo*-inositol as an internal standard. Column temperature was raised from 140 to 146°C at a rate of 1°C min^{-1}, then to 182°C at a rate of 3°C min^{-1}. The amount of ribose, a trace component of saccharides in humus fractions (Clapp and Hayes 1999; Ogner 1980), was not determined in the present study.

Statistical analysis

The significance of the difference in the proportion of a monosaccharide among the four humus fractions and of that in the recovery in a humus fraction among seven monosaccharides were subjected to two-factor ANOVA. Principal component analysis was conducted using the software SRISTAT for Excel 97 (Social Survey Research Information, Tokyo, Japan).

RESULTS AND DISCUSSION

The sum of seven neutral monosaccharides yielded from the two soil types was 57.9 and 79.0 mg C g^{-1} soil C (Table 1). Glucose and mannose were the major saccharides in

Table 1 Yield of neutral monosaccharides from soil (mg C g C^{-1}) and their composition (%)

Soil	Glucose	Galactose	Mannose	Xylose	Arabinose	Fucose	Rhamnose	Total
Inogashira	21.9	14.7	18.7	9.2	6.1	4.1	4.3	79.0
	(26.7)†	(18.0)	(22.8)	(13.5)	(8.9)	(4.9)	(5.2)	
Dando	18.7	7.0	14.2	5.3	5.4	2.3	5.0	57.9
	(31.2)	(11.6)	(23.7)	(10.5)	(10.8)	(3.9)	(8.3)	

†Values in parentheses denote molar percentages in total monosaccharides.

Table 2 Recovery of monosaccharides in four humus fractions (%)†

Fraction	Soil	Glucose	Galactose	Mannose	Xylose	Arabinose	Fucose	Rhamnose	Total
Humic acids	Inogashira	9.0	8.0	6.4	10.2	13.0	12.1	15.0	9.1
	Dando	1.3	3.7	2.0	2.6	5.4	8.5	8.8	3.1
Fulvic acids‡	Inogashira	2.2	3.5	3.1	3.3	2.8	5.7	7.6	3.3
	Dando	2.2	4.3	2.2	3.5	3.1	6.2	5.5	3.1
WS-NHS§	Inogashira	12.7	23.7	18.7	18.7	11.8	52.1	48.6	20.5
	Dando	14.2	22.0	12.0	22.8	12.1	37.4	28.5	17.2
Humin	Inogashira	74.1	49.7	49.0	69.4	67.2	25.3	29.5	57.9
	Dando	82.0	62.5	60.1	78.2	80.7	61.7	48.8	70.3

†Yield of each monosaccharide from soil was taken as 100%. ‡XAD-8-adsorbed fraction of the fulvic acid fraction. §Water-soluble non-humic substances (WS-NHS; XAD-8-non-adsorbed fraction of the fulvic acid fraction).

Table 3 Composition of monosaccharides in humus fractions (%)

Fraction	Soil	Glucose	Galactose	Mannose	Xylose	Arabinose	Fucose	Rhamnose
Humic acids	Inogashira	26.2	15.6	15.9	14.9	12.6	6.5	8.4
	Dando	12.4	13.4	14.7	8.4	18.1	10.3	22.8
Fulvic acids†	Inogashira	18.1	18.8	21.4	13.6	7.6	8.6	12.0
	Dando	22.4	16.1	16.6	12.0	10.6	7.6	14.6
WS-NHS‡	Inogashira	16.5	20.7	20.7	12.2	5.1	12.5	12.2
	Dando	25.5	14.7	16.3	13.8	7.6	8.5	13.7
Humin	Inogashira	34.1	15.4	19.3	16.1	10.3	2.2	2.6
	Dando	36.3	10.3	20.2	11.6	12.3	3.4	5.8

†XAD-8-adsorbed fraction of the fulvic acid fraction. ‡Water-soluble non-humic substances (WS-NHS; XAD-8-non-adsorbed fraction of the fulvic acid fraction).

both soil types, as were numerous other soils (Cheshire 1979; Murayama 1980). Table 2 shows the recovery of each monosaccharide in the four humus fractions. After fractionation, 57.9–70.3% of the total monosaccharides in soil were recovered in humin, followed by 17.2–20.5% in WS-NHS, 3.1–9.1% in HAs and 3.1–3.3% in FAs. The recovery of fucose and rhamnose in WS-NHS (28.5–52.1%) was higher than that of the other saccharides, suggesting higher solubility of the microorganism-derived saccharides in alkaline and acid solutions than that of plant-derived ones. Rhamnose was also recovered in the HAs and FAs in a larger proportion than that of hexoses and pentoses ($P < 0.05$).

Table 3 shows the molar composition of neutral monosaccharides in the four humus fractions. Glucose accounted for the largest proportion, 34.1–36.3%, in total monosaccharides in humin. In the other three fractions, the proportion of glucose was largest in one soil, and that of mannose and/or galactose was largest in another soil. The proportions of fucose and rhamnose tended to be smaller for humin than for the other fractions, which was also expressed by their remote positions on the X-axis in the principal component analysis (Fig. 1). These results suggested that the contribution of microorganism-derived saccharides was relatively smaller in humin. In Fig. 1, the characteristic vector of glucose was similar to that of xylose, which may indicate that plant-derived glucose was the major glucose in the humus fractions.

The proportion of arabinose was larger for HAs than for the other three humus fractions ($P < 0.05$).

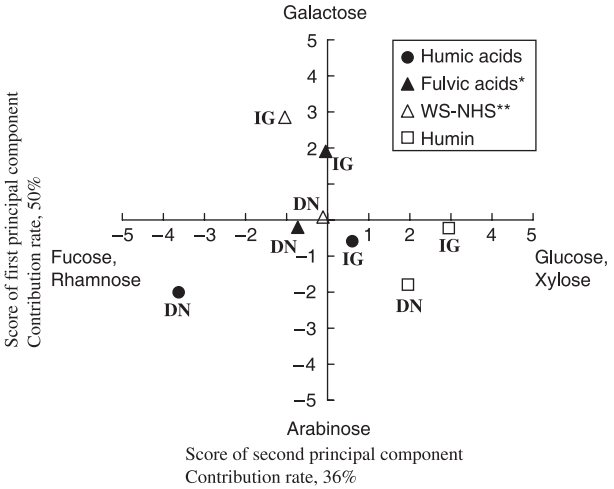

Figure 1 Principal component analysis of the monosaccharide composition in humus fractions. DN, Dando soil; IG, Inogashira soil. *XAD-8-adsorbed fraction of the fulvic acid fraction. **Water-soluble non-humic substances (WS-NHS; XAD-8-non-adsorbed fraction of the fulvic acid fraction).

The larger proportion of arabinose was, however, not observed for the HAs prepared from the same soils using a method recommended by the International Humic Substances Society (IHSS; Watanabe *et al.* 2004). As the degree of humification was higher and the carbohydrate C content was lower in the HAs prepared using the NAGOYA method than in those prepared using the IHSS method (Kuwatsuka *et al.* 1992; Watanabe *et al.* 1994), it is likely that arabinose is more stably associated with HAs.

The monosaccharide composition of FAs was similar to that of WS-NHS in the same soil compared with that of the same fraction in the other soil (Table 3; Fig. 1). The proportion of the sum of two pentoses for the IHSS FAs (Watanabe *et al.* 2004) was in the range of 32.1–42.4%, whereas 21.2–22.6% was recorded in the present samples. The monosaccharide composition of the FAs prepared using the IHSS method in the two soil types was closer to each other. These observations suggest the presence of a stronger interaction of plant-derived saccharides with more hydrophobic FAs because the repetition of the XAD-8 treatment with smaller volumes of resin in the IHSS method may result in the loss of more hydrophilic FA molecules. Although this assumption does not conflict with the above assumption for HAs, confirmation is required because of the differences in the analytical methods used for the monosaccharides by Watanabe *et al.* (2004) and in the present study.

In conclusion, the contribution of microorganism-derived saccharides to total neutral saccharides was considered to be larger in the order of WS-NHS and FAs > HAs > humin, mainly because of two deoxyhexoses in the Dando soil and both deoxyhexoses and hexoses in the Inogashira soil. Arabinose was another key saccharide that characterized the monosaccharide composition of the humus fractions.

REFERENCES

Cheshire MV 1977: Origins and stability of soil polysaccharide. *J. Soil Sci.*, **28**, 1–10.

Cheshire MV 1979: Nature and Origin of Carbohydrates in Soils. Academic Press, London.

Cheshire MV, Russell JD, Fraser AR *et al.* 1992: Nature of soil carbohydrate and its association with soil humic substances. *J. Soil Sci.*, **43**, 359–373.

Clapp CE, Hayes MHB 1999: Characterization of humic substances isolated from clay- and silt-sized fractions of a corn residue-amended agricultural soil. *Soil Sci.*, **164**, 899–913.

Forsyth WCC 1950: Studies on more soluable complexes of soil organic matter. *Biochem. J.*, **46**, 141–146.

Guggenberger G, Christensen BT, Zech W 1994: Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. *Eur. J. Soil Sci.*, **45**, 449–458.

Kumada K 1987: Chemistry of Soil Organic Matter. Elsevier, Amsterdam.

Kuwatsuka S, Watanabe A, Itoh K, Arai S 1992: Comparison of two methods of preparation of humic and fulvic acids, IHSS method and NAGOYA method. *Soil Sci. Plant Nutr.*, **38**, 23–30.

Lowe LE 1978: Carbohydrates in soil. *In*: Soil Organic Matter. Eds M Schnitzer and SU Khan, pp. 65–93, Elsevier, Amsterdam.

Malcolm RL, MacCarthy P 1991: The individuality of humic substances in diverse environments. *In:* Advances in Soil Organic Matter Research: The Impact on Agriculture and the Environment. Ed. WS Wilson, pp. 23–34, The Royal Society of Chemistry, Cambridge.

Murayama S 1980: The monosaccharide composition of polysaccharides in Ando soils. *J. Soil Sci.*, **31**, 481–490.

Murayama S 1988: Microbial synthesis of saccharides in soils incubated with ^{13}C-labelled glucose. *Soil Biol. Biochem.*, **20**, 193–199.

Oades JM 1984: Soil organic matter and structural stability: Mechanisms and implications for management. *Plant Soil*, **76**, 319–337.

Ogner G 1980: Analysis of the carbohydrates of fulvic and humic acids as their partially methylated alditol acetates. *Geoderma*, **23**, 1–10.

Stevenson FJ 1994: Humus Chemistry: Genesis, Composition, Reactions, 2nd edn. John Wiley and Sons, New York.

Tsutsuki K, Kuwatsuka S 1979: Chemical studies on soil humic acids. IV. Amino acid, phenol, and sugar composition in the acid hydrolysable fraction of humic acids. *Soil Sci. Plant Nutr.*, **25**, 29–38.

Watanabe A, Tsutsuki K, Kuwatsuka S 1989: ^{13}C-NMR investigation of humic and fulvic acids obtained from some typical Japanese soils. *Sci. Total Environ.*, **81/82**, 195–200.

Watanabe A, Kuwatsuka S 1992: Ethanol-soluble and insoluble fractions of humic substances in soil fulvic acids. *Soil Sci. Plant Nutr.*, **38**, 391–399.

Watanabe A, Itoh K, Arai S, Kuwatsuka S 1994: Comparison of composition of humic and fulvic acids prepared by the IHSS method and NAGOYA method. *Soil Sci. Plant Nutr.*, **40**, 601–608.

Watanabe A, Maie N, Hepburn A *et al.* 2004: Chemical characterization of Japanese Humic Substances Society standard soil humic and fulvic acids by spectroscopic and degradative analyses. *Humic Sub. Res.*, **1**, 18–28.

Yanagi Y, Hamaguchi S, Tamaki H, Suzuki T, Otsuka H, Fujitake N 2003: Relation of chemical properties of soil humic acids to decolorization by white rot fungus–*Coriolus consors*. *Soil Sci. Plant Nutr.*, **49**, 201–206.

Zech W, Guggenberger G, Zalba P, Peinemann N 1997: Soil organic matter transformation in Argentinian Hapludolls. *J. Plant Nutr. Soil Sci.*, **160**, 563–571.