Determination of Hydroxylated Fatty Acids from the Biopolymer of Tomato Cutin and their Fate during Incubation in Soil

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ABSTRACT:
Introduction – The plant cuticle is a thin, predominantly lipid layer that covers all primary aerial surfaces of vascular plants. The monomeric building blocks of the cutin biopolymer are mainly \(\omega\)-hydroxy fatty acids.

Objective – Analysis of \(\omega\)-hydroxy fatty acids from cutin isolated from tomato fruits at different stages of decomposition in soil.

Methodology – Preparation of purified cutin involving dewaxing and HCl treatment. Incubation of purified cutin for 20 months in soil. Pentfluorobenzoyl derivatives were used for GC/MS operated in the electron capture negative ion (ECNI) mode and trimethylsilyl ethers for GC/MS operated in the electron ionisation (EI) mode for analysis of \(\omega\)-hydroxy fatty acids.

Results – Six \(\omega\)-hydroxy fatty acids were detected in the purified cutin, three of which were identified as degradation products of 9,16-dihydroxyhexadecanoic acid as a consequence of the HCl treatment involved in the purification step. Incubation of the isolated cutin in soil was accompanied with decrease in concentration of all hydroxyl fatty acids.

Conclusion – We produced evidence that the HCl treatment only affected free hydroxyl groups and thus could be used for proportioning free and bound OH-groups on cutin fatty acids. The method enabled a direct quantification of the \(\omega\)-hydroxy fatty acids throughout the incubation phase. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: tomato; cuticle; hydroxylated fatty acids; GC/MS; cutin transformation
Experimental

Chemicals and standards

n-Hexane (HPLC gradient grade), silica gel 60 (particle size 0.063–0.2 mm, 70–230 mesh), ethanolic BF₃ (~10%) and tetrahydrofuran (THF) were ordered from Fluka; methanolic BF₃ (~13%) was from Riedel-de-Haën, and PdO₂ (Adam’s catalyst) was from Sigma Aldrich (all located in Taufkirchen, Germany). Acetonitrile (HPLC gradient grade) was from Roth (Karlsruhe, Germany), triethylamine (TEA) was from Merck (Darmstadt, Germany), pentafluorobenzoyl chloride (99%, PFBO-CI) was from Sigma-Aldrich (Steinheim, Germany) and bis-N-O-trimethylsilyl trifluoroacetamide + trimethylchlorosilane (BSTFA + TMCS; 99%) and celite 545 were from Supelco (Deisenheim, Germany). Ricinoleic acid was from Larodan (Malmö, Sweden). Oxalic acid, ammonium oxalate (source), chloroform and methanol (both HPLC grade), and 6 M HCl were from Sigma (Rehovot, Israel).

Isolation of the cutin biopolymer from tomato

The cutin biopolymer was isolated from fruits of tomato (*Lycopersicon esculentum* Mill) using the method reported previously by Chefetz (2003) and Schechter *et al.* (2006). Briefly, cuticle sheets were peeled manually from the fresh fruits after boiling in water. Then the bulk cuticle sheets were treated with an oxalic acid (4 g/L) and ammonium oxalate (16 g/L) solution at 90°C for 24 h and washed to remove any residual materials. Waxes were removed by Soxhlet extraction with chloroform:methanol (1:1, v/v) for 6 h. The dewaxed tomato cuticles were hydrolysed with 6 M HCl (6 h under reflux) to remove carbohydrates and proteins. All treatments were performed twice to ensure complete removal of the desired fraction. All purified fractions were washed with deionised water, freeze-dried, ground and sieved.

Decomposition of isolated cutin biopolymer from tomato in soil

Cutin biopolymer samples (4–6 g) were placed in nylon bags (20 × 10 cm) with a mesh size of 45 μm to prevent loss of cuticle fragments to the soil and to allow microbial activity (Schädler & Brandl, 2005). The bags were placed in plastic containers containing 2500 g of previously air-dried and sieved (<2 mm) sandy loam soil with an organic matter content of 0.22 ± 0.01% (w/w). To ensure sufficient nutrient supply, N, P and K were added (0.01% (w/w)). To ensure sufficient nutrient supply, N, P and K were added (0.01% (w/w)).

Fatty acid clean-up

The sample clean-up was performed according to Franke *et al.* (2005), except for the internal standard used in the present study: ricinoleic acid (12-0H:18:1(9:1)). About 3 mg cutin, 0.3 mg ricinoleic acid and 1 mL BF₃ solution were heated for 16 h at 70°C to transfer cutin-derived fatty acids into the corresponding methyl esters (MES). The cooled solution was extracted with 3 mL n-hexane. Aliquots containing the cutin-derived fatty acid methyl esters (FAMES) were directly analysed or subjected to derivatisation procedures.

Trimethylsilyl (TMS) ether derivatisation

This procedure was carried out with modifications according to Kawamura and Gagosian (1988): 0.5 mL of the cutin-derived FAMES in n-hexane was mixed with 0.5 mL solution of BSTFA + 1% TMCS and heated for 15 min at 90°C. The resulting solution containing the TMS derivatives of hydroxy FAMES was analysed by GC/EI-MS.

Pentafluorobenzoxlyoxy (PFBO) derivatisation

This procedure was carried out according to Jenske and Vetter (2008): 150 μL of 35% PFBO-CI solution and 100 μL of 2% TEA solution (both in acetonitrile) were added to the dried samples (obtained from a 1 mL aliquot of cutin-derived fatty acids in n-hexane) and then heated for 1 h at 100°C (Mielniczuk *et al.*, 1993). After the sample was cooled to room temperature, distilled water (2 mL) and n-hexane (3 mL) were added, and the products were separated with the organic phase. For GC/ECNI-MS analysis, the PFBO derivatives of the cutin-derived fatty acids were diluted 1:10.

Ethyl esters of cutin-derived fatty acids

Cutin-derived fatty acid ethyl esters (EEs) and the internal standard 14:0-EE were produced similarly to the synthesis of MES except for the use of ethanolic BF₃ (Thurnhofer & Vetter, 2006). The EEs of the cutin-derived fatty acids were used for interpretation of the mass spectra. Fragment ions with identical m/z values in the mass spectra of MES and EEs are formed by elimination of the ester moiety whereas those containing the ester group are shifted by 14 to high mass in the case of EEs (Jenske & Vetter, 2008). The 14:0-EE was added as an internal standard for compensation of (minor) instrumental instabilities from run to run throughout the evaluation (Thurnhofer & Vetter, 2006).

Separation of hydroxlylated and non-hydroxyalted fatty acids

Cutin-derived FAMES were dissolved in 1 mL of a 98:2 (v/v) mixture of n-hexane and ethyl acetate (Jenske & Vetter, 2008). After equilibration with 3 mL of n-hexane:ethyl acetate (98:2, v/v), the sample was loaded onto a solid-phase extraction (SPE) column containing 0.8 g of dried silica gel in a Pasteur pipette. The column was washed with 10 mL of the n-hexane:ethyl acetate mixture (98:2, v/v) to elute the non-hydroxy FAMES. Hydroxy FAMES were eluted into another fraction with 6 mL of pure ethyl acetate (Jenske & Vetter, 2008). After evaporating to dryness, the fatty acids in the two fractions were subjected to the TMS derivatisation as described above.

Hydrogenation of unsaturated fatty acids

Dry cutin-derived FAMES were diluted in ~2 mL dry THF and 2 mg of the catalyst PdO₂ in a two-necked flask and the solution was stirred for 5 h in a H₂ atmosphere, maintained with a H₂-filled balloon on one side of the flask and a tight glass stopper on the other side (Glass *et al.*, 1975). The hydrogenated derivatives were filtered through celite 545 to remove the catalyst before evaporating the THF. The FAMES were converted into TMS derivatives (see above) and made up with n-hexane for further GC/EI-MS measurements.

Gas chromatography/mass spectrometry

GC in combination with electron ionisation mass spectrometry (GC/EI-MS). GC/EI-MS analysis of cutin-derived TMS-O-FAMES was performed with an HP 6890 GC coupled to an HP 5972 mass selective detector (Hewlett-Packard/Agilent, Waldbronn, Germany). Samples were splitless injected at 250°C with an HP 7673 autosampler. An HP-5ms column (30 m, 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Agilent) was installed in the oven. The following GC oven program was applied: after injection, the temperature was held for 1 min at 60°C, raised at 10°C/min to 300°C and then held for 15 min. The total run time was 40 min. The transfer-line and ion source temperatures were set at 280 and 170°C, respectively. Helium 5.0 was used as the carrier gas at a constant flow of 1 mL/min. After a solvent delay of 8 min, measurements were carried out in the full-scan mode, recording m/z 50–650. Additional full scan measurements included ranges m/z 200–500 and m/z 200–650. The samples were analysed in the selected ion monitoring (SIM) mode using the following values: m/z 73 and m/z 103 were recorded throughout the run, whereas m/z 187, m/z 311, m/z 309, m/z 343, m/z 341 were recorded.
from 8 to 20 min and m/z 273, m/z 259, m/z 245, m/z 231 from 20 to 40 min.

Quantification of the TMS-O-FAMEs by GC/EI-MS-SIM was carried out as follows. The GC/EI-MS response in the full scan mode is directly related to the carbon content of a compound. The response factors of the hydroxy fatty acid derivatives in the sample at the start were determined in full-scan mode. These responses were then related to, and corrected by, the responses of m/z 103 (characteristic for ω-hydroxy fatty acids) as determined in the SIM mode used for quantification owing to its higher sensitivity and better reproducibility, especially for low abundant peaks. Based on these individual correction factors, the quantification was carried out using the m/z 187 response of ricinoleic acid taking into account the individual carbon contents of 61–69%. Final corrections to even out instrumental variations were made according to the area of the standard 14:0-EE.

GC in combination with electron capture negative ion mass spectrometry (GC/ECNI-MS). Analyses were carried out with a 3800/1200 GC/MS system in combination with an 8400 auto injector (Varian, Darmstadt, Germany). An HP-5ms column (30 m, 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Agilent) was installed and heated with the following GC oven program: after 2 min isothermal at 60°C, the temperature was increased by 10°C/min to 300°C (hold time 14 min). The total run time was 40 min. He (5.0, Sauerstoffwerke, Friedrichshafen, Germany) was used as the carrier gas at a constant flow rate of 1.2 mL/min. The electron energy was set to 70 eV. The injector, transfer-line, and ion source temperatures were set at 280, 250 and 200°C, respectively. A scan rate of 2 cycles/s was applied, and the filament emission current was set at 50 μA. Nitrogen (5.0, Air Liquide, Bopfingen, Germany) was used as the reagent gas with a constant flow rate of 1.2 mL/min. The electron energy was set to 70 eV. The injector, transfer-line, and ion source temperatures were set at 280, 250 and 200°C, respectively. A scan rate of 2 cycles/s was applied, and the filament emission current was set at 50 μA. Nitrogen (5.0, Air Liquide, Bopfingen, Germany) was used as the reagent gas with a pressure of 7 Torr (Rosenfelder & Vetter, 2009).

In the full-scan mode, m/z 50–650 was recorded throughout the run. In the SIM mode, PFBO-O-FAMES were determined as follows: for the saturated PFBO-O-FAMES, we monitored m/z 452/453 (PFBO-O-14:0-ME), m/z 466/467 (PFBO-O-15:0-ME), m/z 480/481 (PFBO-O-16:0-ME), m/z 494/495 (PFBO-O-17:0-ME), m/z 508/509 (PFBO-O-18:0-ME), m/z 522/523 (PFBO-O-19:0-ME), and m/z 536/537 (PFBO-O-20:0-ME) throughout the run. Likewise, monounsaturated PFBO-O-FAMES were determined with m/z 450/451 (PFBO-O-14:1-ME), m/z 464/465 (PFBO-O-15:1-ME), m/z 478/479 (PFBO-O-16:1-ME), m/z 492/493 (PFBO-O-17:1-ME), m/z 506/507 (PFBO-O-18:1-ME), m/z 520/521 (PFBO-O-19:1-ME) and m/z 534/535 (PFBO-O-20:1-ME).

Results and Discussion

Analysis of the PFBO and TMS derivatives of hydroxy fatty acids from the cutin biopolymer by GC/MS

The GC/EI-MS chromatogram of the TMS-O-FAMEs obtained from the intact (non-incubated) cutin sample showed the TMS derivative of the internal standard ricinoleic acid ME (Fig. 1a) along with the six additional characteristic peaks (Fig. 2a). To verify that all peaks originated from hydroxy fatty acids, one sample aliquot was fractionated on silica before derivatisation. The six cutin-characteristic peaks (Fig. 2a) were identified as hydroxy fatty acids, confirming that all compounds were hydroxy fatty acids. However, 16:0 was not detected.

The six hydroxy fatty acids and the internal standard were also detected in the form of PFBO derivatives by GC/EI-MS (Fig. 2b). However, the fragmentation in the mass spectra was different. Whereas the GC/EI-MS spectra of the TMS-O-FAMES showed the known fragmentation of silyl ethers, the GC/EI-MS spectra of the PFBO derivatives were characterised by the dominant molecular ion typical of PFBO derivatives of PFBO-ME (Jenske & Vetter, 2008). We used the highly sensitive GC/EI-MS SIM technique (Jenske & Vetter, 2008) to screen for traces of further mono-hydroxylated, saturated and monoenoic fatty acids. Since the GC/EI-MS spectra of PFBO-O-FAMES feature the molecular ion (Jenske & Vetter, 2008), the lack of response to m/z 508 and m/z 506 demonstrated that neither OH-18:0 nor OH-18:1 isomers were present in the samples.
both isomers are also α-hydroxy fatty acids with double bonds in different positions in the hydrocarbon chain.

The most abundant peak (no. 6, Fig. 2) in the GC/EI-MS of the TMS-derivatised sample was identified as 10,16-di-OH-16:0 (Fig. 1c) by an excellent match with the mass spectrum of the authentic compound. Both the reference and our peak featured m/z 309, m/z 289 and the characteristic m/z 273 and m/z 275 (Fig. 3b). While m/z 273 is formed by cleavage between C-10 and C-11 ([M – 173]+; charge on the tail), m/z 275 is formed by cleavage between C-9 and C-10 ([M – 171]+; charge on the head group). Furthermore, peak no. 5 (Fig. 2c) was tentatively identified as 8,16-diOH-16:0 (m/z 245, Fig. 1d) and/or 9,16-di-OH-16:0 (m/z 259, Fig. 1e) because of the presence of the diagnostic fragment ions listed in parentheses. The m/z 259 corresponds with m/z 273 of the 16,10-diOH-16:0, except that it has its chain-hydroxyl group on C-9, and with m/z 245, which has this group on C-8. Gérard et al. (1992) reported the presence 9,16-diOH-16:0 in tomato cutin. Interestingly, Riederer et al. (1993) noted that 8,16-diOH and 9,16-diOH-16:0-ME do not separate on a CP-Sil 5 (equivalent to DB-1) GC stationary phase. It is thus possible that both positional isomers were present in the sample. Application of GC oven programs with slower heating rates did not provide the desired resolution of the potentially co-eluting isomers.

The GC/EI-MS spectrum of peak no. 7 (Fig. 4a) did not match any literature spectrum available to us. As shown above, the non-derivatised compound eluted into the OH-FAME fraction and thus peak no. 7 originated from a hydroxy fatty acid. More information was directly obtainable from the GC/ECNI-MS spectrum of the PFBO derivative. The M⁺ at m/z 514 showed a remarkable isotope pattern: in addition to the ¹³C satellite peak [M + 1]⁺, it also featured an abundant isotopic peak at [M + 2]⁺ along with a smaller isotopic peak [M + 3]⁺ (Fig. 4b). The ratio of the isotopic peaks fully matched the presence of a chlorine atom and the elemental composition of C₂₀H₃₄ClF₅O₄ for the PFBO-O-FAME (Fig. 4b and c). Subtraction of the mass derivatisation groups led to a chloro,hydroxy-16:0 isomer for peak no. 7. This prediction was supported by the GC/EI-MS measurements. The molecular mass of the proposed chloro,hydroxy-16:0 isomer (analysed as silyl ether and ME) is m/z 392. In agreement with all other hydroxy fatty acids in the sample, M⁺ was not detected and the highest mass at m/z 377 corresponded with the [M – CH₃]⁺ fragment ion (Fig. 4a). This fragment ion also featured the chlorine isotope peaks (Fig. 4a). Likewise, m/z 345 corresponded with [M – CH₃·HOEt]⁺, which is also typical of the other TMS-O-FAMES found in this sample. Moreover, the corresponding EE (see Experimental section) also showed m/z 361 [M – HOEt]⁺ and m/z 345 [M – CH₃·HOEt]⁺, confirming formation of both ions via elimination of the alkoxy group on the ester side. Loss of HCl from m/z 345 of the TMS-O-FAME led to m/z 309 (IM – CH₃·MeOH – HCl)⁺. The m/z 309 was also detected in the mass spectra of the OH-16:1 isomers (see above). The loss of chlorine is clearly evidenced by the absence of the chlorine isotope peak because it displayed no peak at 2 u higher than the monoisotopic peak. HCl elimination was also involved in the formation of m/z 235 and m/z 217, both of which were present in the GC/EI-MS spectra of the ME and EE. The m/z 235 likely represents [M – HOMe – OSMes – HCl]⁺ (C₁₅H₂₁O⁺), whereas m/z 217 is formed by loss of CO from the former (C₁₃H₂₇O⁺). The high intensity of m/z 103 supported the
Figure 3. GC/EI-MS spectra of the TMS derivatives of (a) 16-hydroxy-hexadecanoic acid (16-OH-16:0) methyl ester, (b) 10,16-dihydroxy-hexadecanoic acid (10,16-diOH-16:0) methyl ester, and (c) the potential 9,16-dihydroxy-hexadecanoic acid (9,16-diOH-16:0) methyl ester and/or 8,16-dihydroxy-hexadecanoic acid (8,16-diOH-16:0) methyl ester.

Figure 4. Mass spectra of peak no. 7 eventually identified as chloro,16-hydroxy-hexadecanoic acid (Cl,16-OH-16:0) methyl ester. (a) GC/EI-MS full-scan spectrum (m/z 50–650) of the TMS derivative with the inclusion of a full-scan spectrum (m/z 200–500) recorded for higher abundance in the high mass range. (b) GC/ECNI-MS spectrum (excerpt) of the molecular ion of the PFBO derivative as measured and (c) a drawing of the theoretical isotope distribution of C$_{24}$H$_{32}$ClF$_{5}$O$_{4}$. 
presence of the hydroxyl group on the terminal primary position, whereas the carbon position of the chlorine atom on the carbon chain could not be resolved. Note that chloro,hydroxy fatty acids have not been described as constituents of cutin. It is thus rather likely that the compound was formed during the sample preparation, in particular during the initial preparation of the sample, which included treatment with HCl (see below), used to remove carbohydrates and proteins (Chefetz, 2003; Shechter et al., 2006). Riederer and Schönherr (1986) have shown that 0.2 M HCl is suitable for transforming epoxylated fatty acids into chlorohydrin derivatives. The harsher conditions of the HCl treatment as used in this and other studies probably initiated both the direct substitution of OH by Cl and the loss of water (formation of OH-16:1 isomers). It is thus very likely that the chlorine atom is located on C-10, but this could not be proven.

There is strong evidence that the chloro,16-OH-16:0 and the two 16-OH-16:1 isomers originated from the decomposition of 10,16-diOH-16:0 (Fig. 1c). Since they were already present in the bulk cutin used for the soil experiment, they were considered in assessments of the relative amounts of cutin-derived fatty acids. It should be noted that the sample pretreatment with 6 M HCl is widely used in the literature (Chaumat & Chamel, 1991; Chefetz, 2003; Benitez et al., 2004; Chen et al., 2008). Therefore, it can be assumed that the degradation described above is a common feature in cutin research.

Changes in peak patterns during incubation of the cutin biopolymer sample

The amounts of the six hydroxy fatty acids were quantified throughout the incubation time (Table 1). The highest relative amounts of the six fatty acids originated from 10,16-diOH-16:0 and chloro,16-OH-16:0, followed by the two OH-16:1 isomers, 16-OH-16:0 and the proposed 9,16-diOH-16:0 isomer (Fig. 5). In the literature, the bulk tomato cutin is represented by 10,16-diOH-16:0 (~93%; Gérard et al., 1992). Under the realistic premise that the unknown OH-16:1 isomers and the chloro,16-OH-16:0 are artefacts of the sample pretreatment (see above), 10,16-diOH-16:0 and its three degradation products represented 90% of the hydroxy fatty acids (Table 1). From a chemical point of view, it is evident that acid-catalysed elimination of H\textsubscript{2}O and substitu-

![Figure 5](image-url)  
**Figure 5.** Course of abundance of the cutin-derived fatty acids during incubation in soil.
Table 1. Structure and mass spectrometric details of the hydroxy fatty acids studied along with their fate in soil

<table>
<thead>
<tr>
<th>Peak</th>
<th>Structure</th>
<th>Contribution of m/z 103 to total ion current (TIC)</th>
<th>M⁻ (m/z) of the PFBO-methyl ester derivative</th>
<th>M⁺/[M – CH₃]⁺ (m/z) of the TMS-methyl ester derivative (no. of carbons)</th>
<th>Carbon content of the derivative (correction relative to internal standard)</th>
<th>Start (t0) amounts in g/100 g</th>
<th>Sample at start</th>
<th>Amounts left relative to the starting sample after exposure to soil (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16-OH-16:1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.73%</td>
<td>478</td>
<td>356/341 (20)</td>
<td>67.4 (1.021)</td>
<td>2.92</td>
<td>100%</td>
<td>68.4% 61.9% 67.8% 63.4% 36.9%</td>
</tr>
<tr>
<td>2</td>
<td>16-OH-16:1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.06%</td>
<td>478</td>
<td>356/341 (20)</td>
<td>67.4 (1.021)</td>
<td>6.22</td>
<td>100%</td>
<td>71.2% 62.3% 55.7% 53.8% 33.9%</td>
</tr>
<tr>
<td>3</td>
<td>16-OH-16:0</td>
<td>3.95%</td>
<td>480</td>
<td>358/343 (20)</td>
<td>67.0 (1.027)</td>
<td>2.87</td>
<td>100%</td>
<td>86.7% 72.4% 73.9% 63.2% 29.8%</td>
</tr>
<tr>
<td>4</td>
<td>12-OH-18:1(9) (IS)</td>
<td>4.47%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>508</td>
<td>386/371 (22)</td>
<td>68.8 (1.000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9,16-diOH-16:0&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.62%</td>
<td>524&lt;sup&gt;a&lt;/sup&gt;</td>
<td>446/431 (23)</td>
<td>61.9 (1.111)</td>
<td>3.23</td>
<td>100%</td>
<td>111.8% 73.2% 57.1% 81.7% 29.9%</td>
</tr>
<tr>
<td>6</td>
<td>10,16-diOH-16:0</td>
<td>2.57%</td>
<td>524&lt;sup&gt;a&lt;/sup&gt;</td>
<td>446/431 (23)</td>
<td>61.9 (1.111)</td>
<td>21.7</td>
<td>100%</td>
<td>86.3% 66.0% 48.8% 73.2% 18.4%</td>
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<tr>
<td>7</td>
<td>Cl,16-OH-16:0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.71%</td>
<td>514</td>
<td>392/377 (20)</td>
<td>61.2 (1.124)</td>
<td>24.6</td>
<td>100%</td>
<td>100.4% 82.7% 75.7% 75.1% 20.8%</td>
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<tr>
<td>SUM</td>
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<sup>a</sup> Tentatively identified.
<sup>b</sup> Position of double bond or chlorine not resolved.
<sup>c</sup> m/z 187 in the case of the internal standard ricinoleic acid.
<sup>d</sup> May also contain 8,16-diOH-16:0.
<sup>e</sup> M⁻ (m/z 690) not detected; ion represents the [M – C₆F₅ + H]⁺ fragment ion.
<sup>f</sup> Contribution of the hydroxy fatty acids to the cutin biopolymer based on the quantitative results.
the present degradation study we used the purified biopolymers, while Riederer et al. (1993) and Stimler et al. (2006) estimated decomposition and degradation rates of cutin from the decomposition of leaf and whole-cuticle residues.

At the end of the experiment, the lowest amounts were detected for the di-substituted fatty acids, whereas the monoenoic fatty acids appeared to be most recalcitrant (Fig. 5). Since both di-substituted fatty acids were present at about an order of magnitude higher concentrations, they may have been more effectively assessed by bacterial decay. The significant change in the composition of the cutin biopolymer upon ageing in soil is likely to have an impact on the sorption properties of this relevant soil organic matter (Chefetz, 2003; Shechter et al., 2006). The methods described here are suitable for studying this phenomenon in future work, and they are expected to be helpful in identifying potential artefacts in the sample preparation method as shown in the present study.

Acknowledgements

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References


Schädler M, Brandl R. 2005. Do invertebrate decomposers affect the decomposition and degradation rates of cutin from the decomposing and degradation rates of cutin from the decomposing植物体と環境。物質の化学的な変化を理解するためには、これらの方法が有用であることが示唆されている。