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Identification of intact long-chain *p*-hydroxycinnamate esters in leaf fibers of abaca (*Musa textilis*) using gas chromatography/mass spectrometry

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The study of acetone-extractable components from the leaf fibers of the non-wood plant abaca (*Musa textilis*) resulted in the isolation and identification of series of intact hydroxycinnamate esters consisting of ferulic and *p*-coumaric acids esterified to long-chain fatty alcohols (C_{20} to C_{28}) and *ω*-hydroxyfatty acids (C_{22} to C_{28}). These series of compounds were characterized by high-temperature gas chromatography/mass spectrometry (GC/MS) using capillary columns (12 m length) with thin films that allowed the analysis of intact (i.e., without prior saponification) hydroxycinnamate esters. Characterization of intact individual compounds was achieved based on the mass spectra obtained by GC/MS of the underivatized compounds and their methyl and/or trimethylsilyl ether derivatives. Copyright © 2004 John Wiley & Sons, Ltd.

p-Hydroxycinnamic acids, namely ferulic (4-hydroxy-3-methoxycinnamic) and *p*-coumaric (4-hydroxycinnamic) acids, have been observed in plants in free form or covalently bound to different moieties such as lignin, polysaccharides, amino acids and lipids.[1–4] Amongst lipid compounds, ferulic acid esters have been widely reported in different plant families in a variety of structural tissues (wood, bark, pine needles, leaves, roots, etc.). Esters of ferulic acid with long-chain *n*-fatty alcohols are the most abundant,[1,5–9] although ferulic acids esterified with *ω*-hydroxyfatty acids[8,10] and sterols[4,11] have also been reported. Esters of *p*-coumaric acid, particularly alkyl *p*-coumarates, have also been found in plants although less frequently and in lower amounts.[1,9,12,13]

The aim of this study was to characterize the esters of hydroxycinnamic acids (ferulic and *p*-coumaric acids) with long-chain fatty alcohols and *ω*-hydroxyfatty acids (Fig. 1) in the leaf fibers of abaca (*Musa textilis*). Abaca fiber is an agronomically important source of natural fibers. Its long fiber length, high strength and fineness make it a superior material for the production of high-quality papers of high porosity.[14] The intact (i.e., without prior saponification) hydroxycinnamate esters from abaca fibers were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), using short-and medium-length high-temperature capillary columns, respectively, with thin films, according to the method previously described,[15] which enables the elution and analysis of intact high molecular weight lipids. The total extracts were also fractionated using a solid-phase extraction (SPE) protocol to concentrate and separate the *p*-hydroxycinnamate esters of long-chain fatty alcohols from those of *ω*-hydroxyfatty acids. The different series were analyzed as either underivatized, methylated and/or silylated compounds, and the corresponding mass spectra are discussed.

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EXPERIMENTAL

Samples

The plant material used for this study consisted of leaf fibers from abaca (*M. textilis*). The air-dried fibers were milled using a knife mill (Janke and Kunkel, Analysenmühle) and extracted with acetone in a Soxhlet apparatus for 8 h. The extracts were evaporated to dryness, and redissolved in chloroform for chromatographic analysis of the lipophilic fraction. Two replicates were used for each sample, and all of them were subjected to GC and GC/MS analyses.

SPE fractionation

The lipid extracts were fractionated by a SPE procedure using aminopropyl-phase cartridges (500 mg) from Waters. The dried chloroform extracts were taken up in a minimal volume (<0.5 mL) of hexane/chloroform (4:1) and loaded into the cartridge column previously conditioned with hexane (4 mL). The cartridge was loaded and eluted by gravity. The column was first eluted with 8 mL of hexane and subsequently with 6 mL of hexane/chloroform (5:1), then with 10 mL of chloroform and finally with 10 mL of diethyl ether/acetic acid (98:2). Each isolated fraction was dried under nitrogen and analyzed by GC and GC/MS. The long-chain

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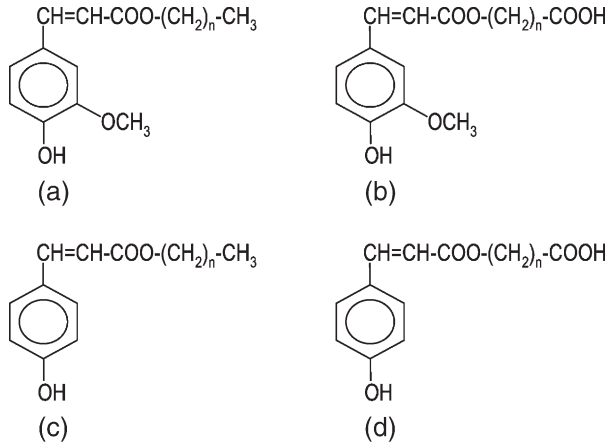

Figure 1. Chemical structures of the different series of *p*-hydroxycinnamate esters: (a) ferulates of fatty alcohols, (b) ferulates of ω-hydroxyfatty acids, (c) *p*-coumarates of fatty alcohols, and (d) *p*-coumarates of ω-hydroxyfatty acids.

hydroxycinnamoyl esters of long-chain alcohols eluted in the chloroform fraction while the hydroxycinnamoyl esters of the ω-hydroxyfatty acids eluted in the acidic fraction.

GC and GC/MS analyses

The GC analyses of the extracts were performed using a Hewlett-Packard HP-5890 (Hewlett-Packard, Hoofddorp, Netherlands) with a short fused-silica capillary column (DB-5HT; 5 m × 0.25 mm i.d., 0.1 μm film thickness) from J&W Scientific (Folsom, CA, USA). The temperature program started at 100°C with a 1 min hold, and then was raised to the final temperature of 350°C at 15°C/min, and held for 3 min. The injector (split-splitless) and detector (flame ionization detector, FID) temperatures were set at 300 and 350°C, respectively. The carrier gas was helium at a rate of 2 mL/min and the injection was performed in splitless mode. Peaks were quantified by GC/FID peak area. Due to the lack of appropriate hydroxycinnamate ester standards, quantitation was performed against sitosterol.

The GC/MS analyses were performed using a Varian Saturn 2000 gas chromatograph (Varian, Walnut Creek, CA, USA), equipped with a fused-silica capillary column (DB-5HT, J&W; 12 m × 0.25 mm i.d., 0.1 μm film thickness), and with an ion trap detector. The oven was heated from 120°C (1 min) to 380°C at 10°C/min and held for 5 min. The transfer line was kept at 300°C. The injector was temperature programmed from 120°C (0.1 min) to 380°C at a rate of 200°C/min and held until the end of the analysis. Helium was used as carrier gas at a rate of 2 mL/min. Trimethylsilyldiazomethane methylation and bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation, in the presence of pyridine, were used to produce the appropriate derivatives, when required.

RESULTS AND DISCUSSION

The total lipophilic extracts of abaca accounted for 0.4% of the leaf fiber. Figure 2 shows the total ion current chromatogram

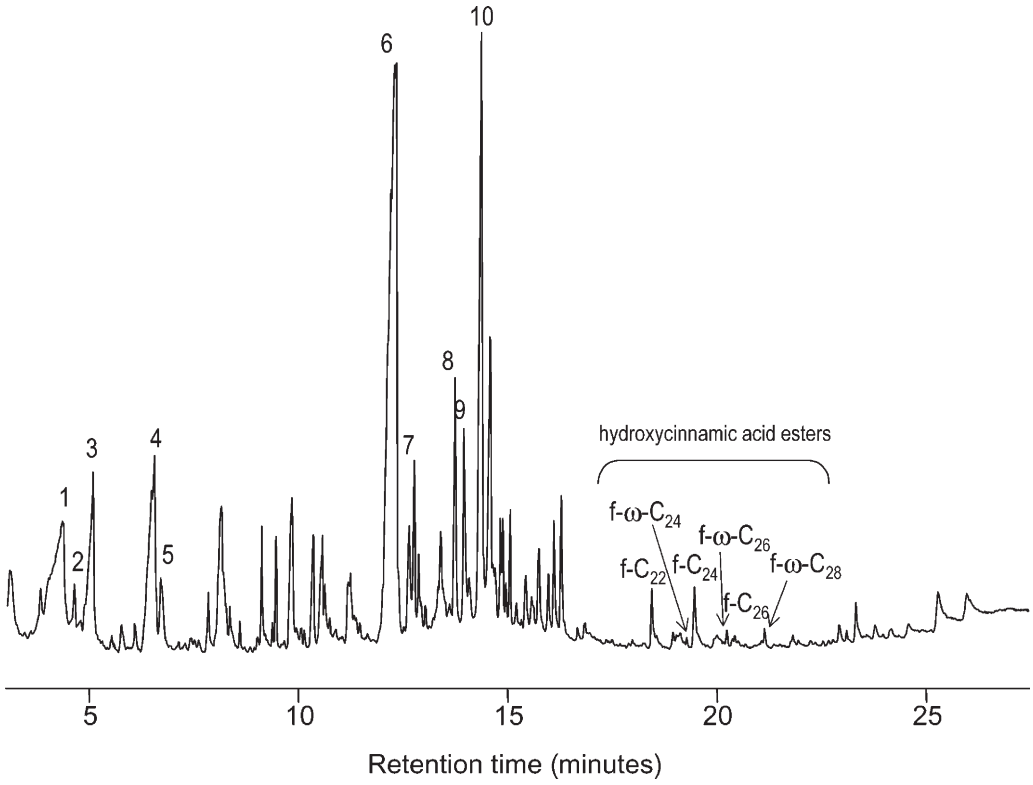

Figure 2. Total ion current chromatogram of the total underivatized lipophilic extracts from abaca leaf fibers. Labels: f-C_n, indicates *trans-n*-alkyl ferulates; f-ω-C_n, indicates *trans*-feruloyl esters of ω-hydroxyfatty acids. *p*-Coumarates are present in minor amounts and are not indicated in the chromatogram. Other compounds identified: 1, *p*-coumaric acid; 2, ferulic acid; 3, palmitic acid; 4, oleic acid; 5, stearic acid; 6, unknown (*m/z* 306); 7, stigmastatriene; 8, campesterol; 9, stigmasterol; 10, sitosterol.

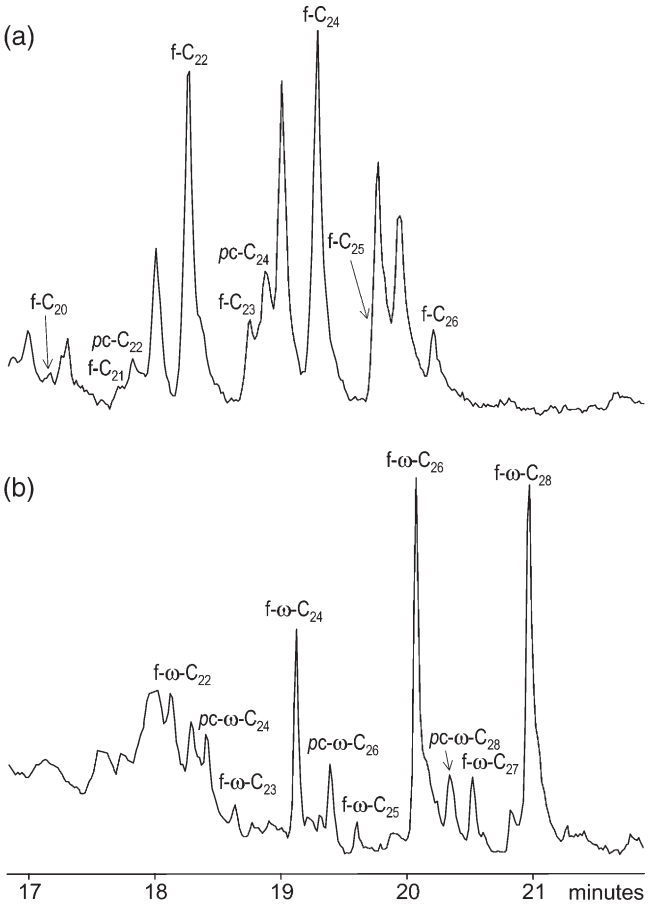

Figure 3. Partial chromatograms of the SPE fractions: (a) fraction eluted with chloroform showing the series of *n*-alkyl ferulates and *n*-alkyl *p*-coumarates and (b) fraction eluted with diethyl ether/acetic acid (98:2) showing the series of feruloyl and *p*-coumaroyl esters of ω-hydroxyacids. Labels: f-C_n, indicates the *trans*-*n*-alkyl ferulates; f-ω-C_n, indicates the *trans*-feruloyl esters of ω-hydroxyfatty acids; *p*c-C_n, indicates the *trans*-*n*-alkyl *p*-coumarates; *p*c-ω-C_n, indicates the *trans*-*p*-coumaroyl esters of ω-hydroxyfatty acids.

of the underivatized lipophilic extracts from abaca fibers. Several peaks corresponding to esters of ferulic acid with long-chain fatty alcohols and ω-hydroxyfatty acids were clearly observed in the high-temperature region of the chromatogram. *p*-Coumarate esters of fatty alcohols and ω-hydroxyfatty acids could also be detected although in minor amounts. In order to concentrate these compounds in homologous series, the lipophilic extracts were subjected to fractionation using a SPE protocol, as described above. The GC/MS analysis of the fractions isolated after SPE (Fig. 3) revealed the presence of long-chain esters of ferulic and *p*-coumaric acids in the chloroform-eluted fraction, and similar acidic esters in the SPE fraction eluted with diethyl ether/acetic acid (98:2). The composition, abundance and distribution of the different series of alkylhydroxycinnamate esters present in the leaf fibers of abaca are shown in Fig. 4.

n-Alkyl ferulates and *p*-coumarates

The series of *n*-alkyl *trans*-ferulates was observed in the range from C_{20} to C_{28}, with both even and odd carbon atom number homologues; C_{22} and C_{24} were the most prominent. In most

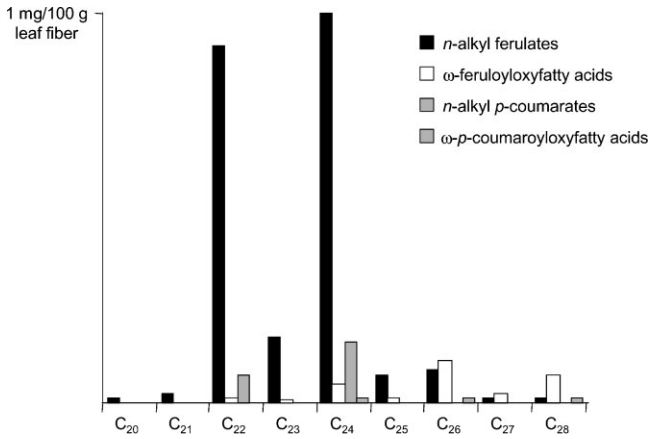

Figure 4. Distribution of the different series of *p*-hydroxycinnamate esters identified in abaca leaf fibers. The vertical axis shows the abundances of the compounds as mg/100 g fiber.

softwood and hardwood species in which ferulic acid esters have been reported, C_{22} and C_{24} fatty alcohols were the dominant components esterifed to ferulic acid with minor amounts of C_{16}, C_{18}, C_{20}, C_{26} and C_{28}.[1] *n*-Alkyl ferulates with odd numbers of carbon atoms have rarely been reported.[5]

The mass spectrum of a selected *n*-alkyl ferulate (*trans*-tetracosanyl ferulate) is shown in Fig. 5. The mass spectrum of this compound is characterized by an abundant molecular

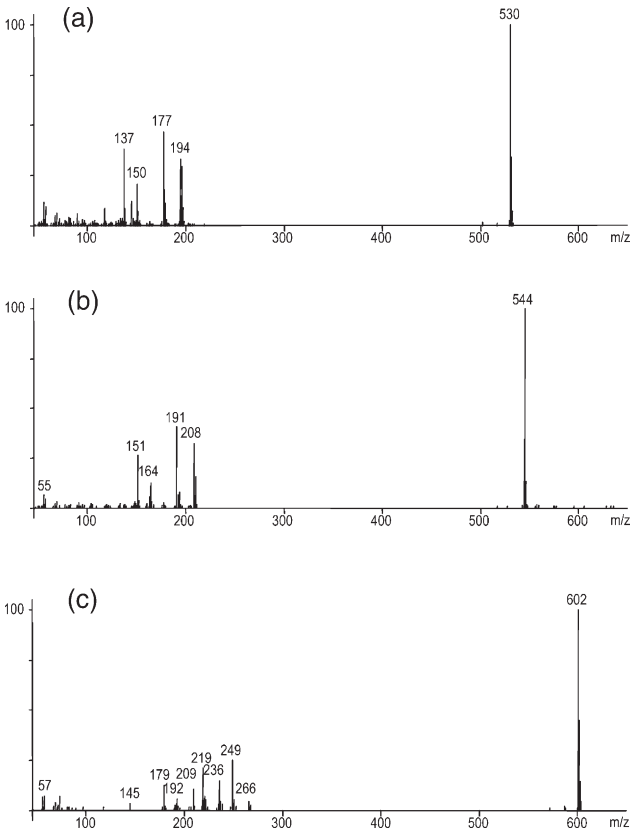

Figure 5. Mass spectra of *trans*-tetracosanyl ferulate: (a) underivatized, (b) methyl derivative, and (c) TMS ether derivative.

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Table 1. Molecular ions in EI-MS analysis of *n*-alkyl ferulates and *n*-alkyl *p*-coumarates identified in abaca leaf fibers

		m/z of molecular ion $[M]^{+\cdot}$ (relative abundance)		
	n-Fatty alcohol moiety	Underivatized	Methylated	TMS ether
n-Alkyl ferulates				
trans-Eicosanyl ferulate	C_{20}	474 (100)	488 (100)	546 (100)
trans-Heneicosanyl ferulate	C_{21}	488 (100)	502 (100)	560 (100)
trans-Docosanyl ferulate	C_{22}	502 (100)	516 (100)	574 (100)
trans-Tricosanyl ferulate	C_{23}	516 (100)	530 (100)	588 (100)
trans-Tetracosanyl ferulate	C_{24}	530 (100)	544 (100)	602 (100)
trans-Pentacosanyl ferulate	C_{25}	544 (100)	558 (100)	616 (100)
trans-Hexacosanyl ferulate	C_{26}	558 (100)	572 (90)	630 (100)
trans-Heptacosanyl ferulate	C_{27}	572 (100)	586 (100)	644 (100)
trans-Octacosanyl ferulate	C_{28}	586 (100)	600 (100)	658*
n-Alkyl *p*-coumarates				
trans-Docosanyl *p*-coumarate	C_{22}	472 (15)	486 (35)	544 (70)
trans-Tetracosanyl *p*-coumarate	C_{24}	500 (15)	514 (30)	572 (70)

* Out of the MS instrument range.

ion that is also the base peak, at *m/z* 530. The molecular ions of the other compounds in the series of *n*-alkyl ferulates identified in abaca fibers are listed in Table 1. Abundant fragments at *m/z* 137, 150, 177 and 194, arising from the ferulic acid moiety, are also present in the mass spectra. The ion at *m/z* 194 corresponds to ferulic acid while the ion at *m/z* 177 derives from the ferulate moiety by simple homolytic cleavage of the ferulate ester bond. The identities of this series of compounds were also confirmed by the formation of the methyl and/or trimethylsilyl (TMS) ether derivatives.

The formation of the methyl derivatives of *n*-alkyl ferulates introduced one methyl group into the phenolic alcohol. The EI-MS fragmentations of the methyl derivatives gave mass spectra similar to those of the underivatized ferulates but with ions at *m/z* values 14 higher (Fig. 5(b), Table 1). The mass spectrum is characterized by an abundant molecular ion that is usually the base peak, and fragments at *m/z* 151, 191 and 208, arising from the methylated ferulic acid moiety. The ions at *m/z* 208 and 191 correspond respectively to methylated ferulic acid and the feruloyl fragment already seen at *m/z* 194 and 177 in the underivatized compounds. To our knowledge this is the first report of the mass spectrum of a methyl derivative of intact *n*-alkyl ferulates.

The mass spectra of the TMS ether derivatives (Fig. 5(c), Table 1) confirmed the assignments of the compounds achieved by MS analysis of the underivatized and methylated fractions. The molecular ions of the TMS ether derivatives are the base peaks of the mass spectra. The TMS group is not lost readily from the phenolic groups, and hence the ferulic acid fragment ions are shifted to higher masses by the expected 72 Da to *m/z* 249, corresponding to the TMS ether of the feruloyl fragment which must arise in an manner analogous to that of the corresponding ion at *m/z* 177 of the underivatized alkyl ferulate. The ion corresponding to the TMS ether of ferulic acid was also evident at *m/z* 266. The abundant ion at *m/z* 236 has been rationalized based on the elimination of ethane from ferulic acid TMS ether to give a bridged dimethylsilyl ether.[4] A similar mass spectrum has been reported for the TMS ether of docosanyl ferulate[8] although it showed the ion at *m/z* 249 as the base peak, whereas, in the present case, the molecular ion is the base

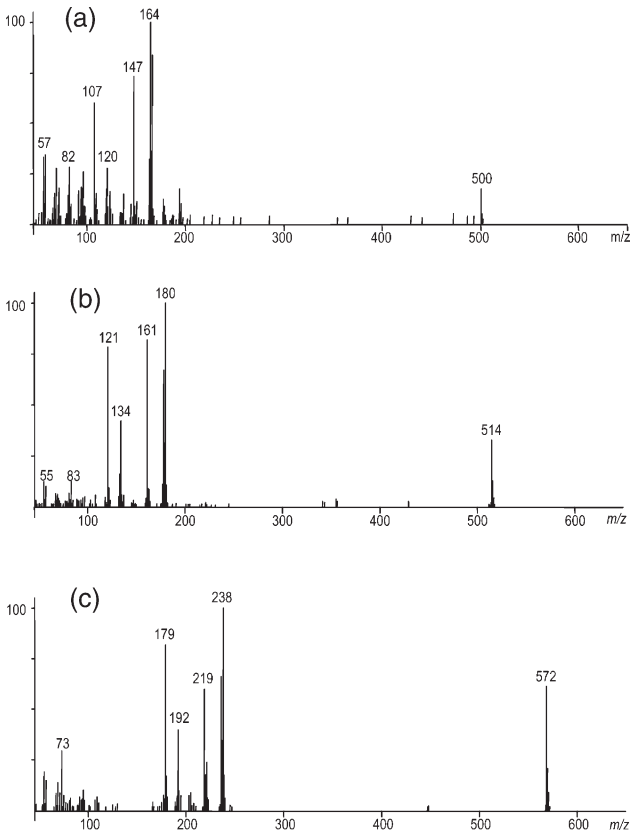

Figure 6. Mass spectra of *trans*-tetracosanyl *p*-coumarate: (a) underivatized, (b) methyl derivative, and (c) TMS ether derivative.

peak. Probably this is due, at least in part, to the differences in the instruments used, quadrupole[8] or ion trap in this case.

A series of *n*-alkyl *trans*-*p*-coumarates could also be detected, although in minor amounts. This series included only the C_{22} and C_{24} alkyl moieties, with the latter being the most prominent. The mass spectrum of a representative compound (*trans*-tetracosanyl *p*-coumarate) is shown in Fig. 6. The mass spectra of the *n*-alkyl *p*-coumarates are similar to those of the *n*-alkyl ferulates but the molecular ions are not the base peaks. The mass spectra are characterized by

the molecular ions at *m/z* 472 and 500 (Table 1), corresponding to docosanyl and tetracosanyl *p*-coumarates, respectively, and abundant fragments at *m/z* 107, 120, 147, 164, and 166 (arising from the *p*-coumaric acid moiety). The ion at *m/z* 164, which is the base peak, corresponds to *p*-coumaric acid, while the ion at *m/z* 147 derives from the *p*-coumarate moiety by simple homolytic cleavage of the *p*-coumarate ester bond. The identities of these compounds were also confirmed by the formation of the corresponding methyl or TMS ether derivatives. The formation of the methyl derivatives of *n*-alkyl *p*-coumarates introduced one methyl group into the phenolic alcohol. EI-MS of the methyl derivative gave mass spectra similar to those of the underivatized *p*-coumarates with mass peaks 14 Da higher (Fig. 6(b), Table 1). The mass spectra are characterized by the molecular ions and fragments at *m/z* 121, 134, 161, 178, and 180 (the latter being the base peak), arising from the methylated *p*-coumaric acid moiety. The ions at *m/z* 178 and 161 correspond respectively to the methylated *p*-coumaric acid and the *p*-coumaroyl fragment already observed at *m/z* 164 and 147 for the underivatized compounds. The mass spectra of the TMS ether derivatives (Fig. 6(c), Table 1) are characterized by an intense molecular ion (which is not the base peak, contrary to what occurred with the ferulic acid counterparts, Table 1). As was also observed for the TMS ether derivatives of the alkyl ferulates, the fragments from the *p*-coumaric acid moiety are shifted up by 72 Da, producing the fragments at *m/z* 179, 192, 219, 236, and 238.

The series of *n*-alkyl ferulates and *p*-coumarates identified occurred mostly in the *trans*-form, although some minor amounts of the *cis*-isomer were also observed at lower retention times. Although *trans*-compounds are usually the natural ones, some *cis*-compounds have been unambiguously identified and reported in some plants.[12]

Feruloyl and *p*-coumaroyl esters of *ω*-hydroxyfatty acids

A series of compounds, with mass spectra related to those of the *n*-alkyl *trans*-ferulates and *trans*-*p*-coumarates, was present in the high-temperature region of the chromatograms of abaca fiber extract. These compounds were concentrated in the acidic SPE fraction, indicating the presence of free carboxylic groups, and were identified as feruloyl and *p*-coumaroyl esters of *ω*-hydroxyfatty acids from the mass spectra of underivatized and derivatized (methyl) forms. The TMS ether derivatives could not be studied because the molecular ions fall out of the mass range of the instrument used (maximum at *m/z* 650).

The feruloyl ester-linked *ω*-hydroxyfatty acids identified ranged from C_{22} to C_{28}, the C_{24}, C_{26} and C_{28} homologues being the most abundant. The odd carbon number members of the series were also present but in minor amounts. Feruloyl esters of *ω*-hydroxyfatty acids have been rarely reported in plants. A series of acidic esters derived from C_{22} to C_{29} *ω*-hydroxy fatty acids and *trans*- and *cis*-ferulic acids was reported for the first time in *Virola* species.[10] Small amounts of feruloyoxydocosanoic acid and feruloyloxyhexacosanoic acid were also recently reported in *Eucalyptus globulus* wood.[8]

The mass spectrum of a representative member of this series (*trans*-feruloyloxyhexacosanoic acid) is shown in Fig. 7.

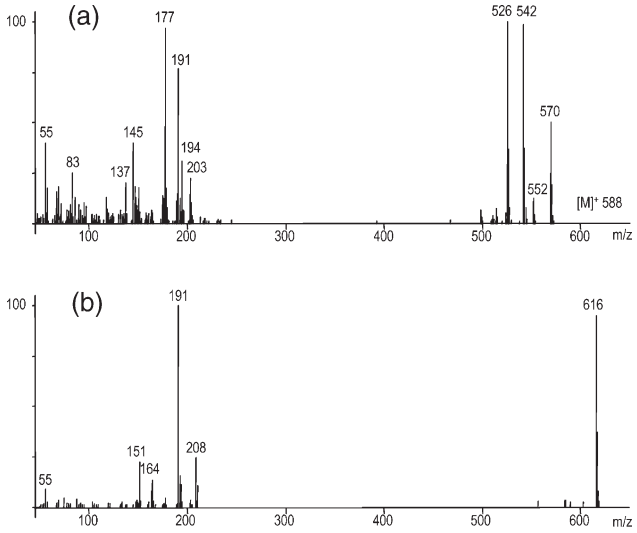

Figure 7. Mass spectra of *trans*-feruloyloxyhexacosanoic acid: (a) underivatized and (b) methyl derivative.

The molecular ions were not present in the mass spectra; however, the molecular masses could be readily determined from the intense peaks at $[M-18]^+$, $[M-46]^+$ and $[M-62]^+$ (Table 2), which corresponded to losses of water and the carboxylic group. The mass spectral fragmentations of these compounds also yielded characteristic fragment peaks at *m/z* 145, 177, 191, 194, and 203, similar to those found in the mass spectra of alkylferulates, and arising from the ferulic acid moiety. This series of compounds was present in the *trans*-form, although minor peaks corresponding to the *cis*-isomers also appeared in the chromatogram.

The presence of the phenolic and carboxylic groups was confirmed by forming the methyl derivatives. Two methyl groups were introduced and therefore the mass spectra of the methyl derivatives, also shown in Fig. 7, contain the corresponding molecular ions at 28 Da higher than the molecular ions of the underivatized compounds (Table 2). The mass spectra of the methyl derivatives were characterized by the fragments at *m/z* 191 (base peak) and *m/z* 151, 164, and 208, arising from the methylated ferulic acid moiety, and similar to the fragmentation pattern of the methyl derivatives of the alkylferulates.

A series of *p*-coumaroyl esters of *ω*-hydroxyfatty acids could also be identified in minor amounts in abaca leaf fibers. To the best of our knowledge, this is the first report of the occurrence of this series in plants. This series was observed in the range from C_{24} to C_{28}, with only the even carbon atom members. The mass spectrum of a representative member of the series (*trans*-*p*-coumaroyloxyoctacosanoic acid) is shown in Fig. 8. As occurs with the corresponding feruloyl esters of *ω*-hydroxyfatty acids, the mass spectra of *p*-coumaroyl esters of *ω*-hydroxyfatty acids do not include the molecular ions, but the molecular masses could be easily determined from the $[M-18]^+$ fragments; $[M-46]^+$ and $[M-62]^+$ are also observed as in the mass spectra of feruloyl esters of *ω*-hydroxyfatty acids. The rest of the spectrum is characterized by a base peak at *m/z* 147 and fragments at *m/z* 120, 161, and 164 from the *p*-coumaric acid moiety, similar to that of *n*-alkyl *p*-coumarates. The identity of this series of compounds was

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Table 2. EI-MS analysis of the ω-carboxyalkyl ferulates and ω-carboxyalkyl *p*-coumarates identified in abaca leaf fibers

	ω-Hydroxyfatty acid moiety	Underivatized		Methylated
		$[M]^{+\cdot}$	$[M-18]^{+\cdot}$	$[M]^{+\cdot}$
ω-Carboxyalkyl ferulates				
trans-Feruloyloxydocosanoic acid	C_{22}	532 (0)	514 (45)	560 (80)
trans-Feruloyloxytricosanoic acid	C_{23}	546 (0)	528 (40)	574 (100)
trans-Feruloyloxytetracosanoic acid	C_{24}	560 (0)	542 (50)	588 (90)
trans-Feruloyloxypentacosanoic acid	C_{25}	574 (0)	556 (50)	602 (90)
trans-Feruloyloxyhexacosanoic acid	C_{26}	588 (0)	570 (50)	616 (100)
trans-Feruloyloxyheptacosanoic acid	C_{27}	602 (0)	584 (50)	630 (80)
trans-Feruloyloxyoctacosanoic acid	C_{28}	616 (0)	598 (50)	644 (100)
ω-Carboxyalkyl *p*-coumarates				
trans-*p*-Coumaroyloxytetracosanoic acid	C_{24}	530 (0)	512 (25)	558 (5)
trans-*p*-Coumaroyloxyhexacosanoic acid	C_{26}	558 (0)	540 (30)	586 (5)
trans-*p*-Coumaroyloxyoctacosanoic acid	C_{28}	586 (0)	568 (30)	614 (8)

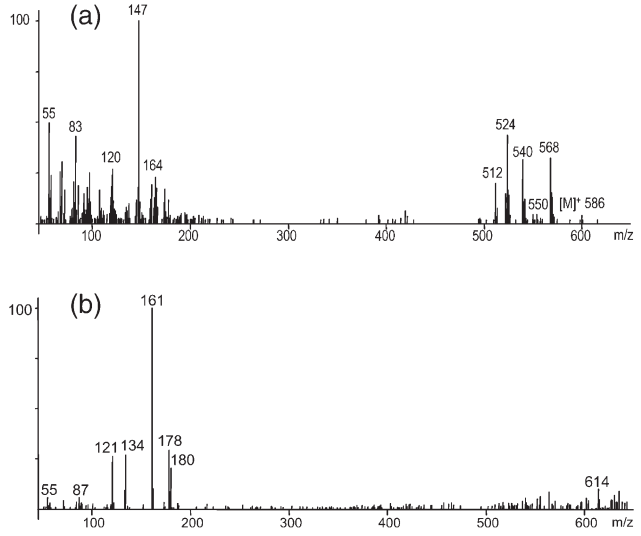

Figure 8. Mass spectra of *trans*-*p*-coumaroyloxyoctacosanoic acid: (a) underivatized and (b) methyl derivative.

also confirmed by forming the methyl derivatives; two methyl groups were introduced. The mass spectrum of the methyl derivative of *trans*-*p*-coumaroyloxyoctacosanoic acid is shown in Fig. 8(b); the molecular ion is present at low abundance (Table 2) and corresponds to the addition of 28 Da to the molecular ions of the underivatized compounds. The mass spectrum is characterized by a base peak at *m*/*z* 161 and abundant fragments at *m*/*z* 121, 134, 178, and 180 arising from the methylated *p*-coumaroyl moiety, similar to the fragmentation pattern of *n*-alkyl *p*-coumarates.

CONCLUSIONS

A comprehensive report of mass spectra of *p*-hydroxycinnamate esters (ferulates and *p*-coumarates) with long-chain fatty alcohols and ω-hydroxyfatty acids is presented. The structures of the different series can be successfully deduced from the mass spectra of either the underivatized forms or their methyl or TMS derivatives. To our knowledge this is the first time that these phenolic compounds have been reported in abaca (*Musa textilis*) and is also the first time that the series of *p*-coumarates of ω-hydroxyfatty acids has been reported in plants.

Acknowledgements

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