Suberin Structure in Potato Periderm: Glycerol, Long-Chain Monomers, and Glyceryl and Feruloyl Dimers

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Suberin in extractive-free potato periderm amounts to ~25% determined by $NaOCH_3$ methanolysis. Monomeric composition is characterized by glycerol (20% of monomers), long-chain α,ω-diacids, ω-hydroxyacids, alkanoic acids, and alkan-1-ols, with predominance of octadec-9-enodioic acid and 18-hydroxyoctadec-9-enoic acid (39 and 15% of long-chain monomers, respectively). Aromatic hydroxycinnamyl monomers were also present (<1%). Partial depolymerization of potato periderm suberin using a $Ca(OH)_2$-catalyzed methanolysis solubilized ~10% of suberin aliphatics. GC-MS analysis showed the presence of monomers, dimers, and trimers (87, 12, and 1% of identified compounds, respectively). A total of 26 dimers were identified by EIMS: monoacylglyceryl esters of α,ω-diacids, ω-hydroxyacids, and alkanoic acids (with predominance of the 1- and 2-isomers of the monoacylglyceryl ester of the octadec-9-enodioic acid), as well as feruloyl esters of ω-hydroxyacids and alkan-1-ols and a small quantity of a monoferuloylglycerol. Following a discussion of suberin macromolecular structure, it is proposed that in suberized cell walls, the polyaliphatic polymers have a three-dimensional development ensured by glycerol and exist independently from the associated polyaromatics.

Keywords: *Suberin; Solanum tuberosum; potato periderm; monoacylglyceryl esters; feruloyl esters*

INTRODUCTION

Suberin is a major constituent of the cell walls of outer plant tissues, namely, secondary growth periderms, as well as interior tissues that function as isolation barriers. The specificity of suberin location relates to its function as a protective polymer involved in thermal insulation, in water-loss prevention, and as a barrier to pathogenic attack (Kolattukudy, 1977). Most of the studies on the composition and structure of suberin and associated materials have been made on the potato natural or wound-induced periderm [e.g., Kolattukudy and Agrawal (1974) and Stark and Garbow (1992)]. Other relevant works on suberin chemistry have been made on an important forest product, commercial cork [e.g., Marques et al. (1996) and Graça and Pereira (1997)], which is the outer bark of the cork oak (*Quercus suber* L.).

What is and what should be called suberin remains an open question. Suberin is known to include an aliphatic polyester part, normally quantified as "suberin content", after depolymerization by reactions that break ester bonds. The solubilized monomers after analysis by GC-MS show mixtures of aliphatic long-chain α,ω-diacids, ω-hydroxyacids, alkanoic acids, and *n*-alkanols (Holloway, 1983; Kolattukudy and Espelie, 1989). Varying quantities of aromatic materials are solubilized simultaneously with the above aliphatics and, except for small quantities of monomeric phenylpropenoids, are of unknown chemical nature (Riley and Kolattukudy, 1975; Borg-Olivier and Monties, 1993). Important quantities of non-depolymerized polyaromatics remain in the residual cell wall material. The macromolecular structure of these polyaliphatic and polyaromatic materials is not known, and it is not clear if they should be considered as one or two different polymers.

More recent results have brought a new insight into the possible structure of suberin and its relations with the other suberized cell wall components. Glycerol was found to be a major monomer of suberins, and dimers of glycerol esterified to all kinds of suberinic aliphatic acids present as monomers were also identified, giving body to the hypothesis that suberin is a glycerol-based polyester (Graça and Pereira, 1997, 1999). Dimers of ω-hydroxyacids were also found as esters of ferulic acid, showing one way to link aliphatics and aromatics (Graça and Pereira, 1998, 1999). Results from in situ analysis by solid-state NMR showed that aliphatics and aromatics occupied different areas in the suberized cells walls (Gil et al., 1997; Bernards and Lewis, 1998), thus allowing the existence of two independent polymers to be considered.

The suberin dimers that have been obtained so far were solubilized from bark corks, after a partial depolymerization methanolysis with calcium oxide as catalyst (Graça and Pereira, 1997, 1999). Here we apply a closely related procedure to obtain oligomers from potato periderm suberin to get a better understanding of the suberin polymer structure. The monomeric composition of potato periderm suberin was also quantified, using a technique that allows simultaneous analysis of glycerol and the long-chain aliphatics (Graça and Pereira, 2000).

MATERIALS AND METHODS

Potato Periderm. Potatoes (*Solanum tuberosum*) bought in a local grocery were boiled and peeled. The potato peel strips were thoroughly scraped of amilaceous tissue and oven-dried at 50 °C (~0.3% yield based on the initial wet weight). The

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dried potato peel was ground in a coffee mill and sieved, and the 40−60 mesh (0.25−0.42 mm) fraction was used for analysis. Extractives were removed by Soxhlet extraction successively with dichloromethane (8 h), ethanol (18 h), water (24 h), and methanol (18 h). The extracts were, respectively, 3.3, 7.1, 21.5, and 0.8% (total = 32.7%, based on the initial dry weight). The extracted material was dried before methanolysis reactions in a vacuum oven at 40 °C.

Analysis of Suberin Monomers. *$NaOCH_3$-Catalyzed Methanolysis*. The dry, extractive-free (three samples, ∼500 mg each) potato periderm was refluxed for 3 h in 50 mL of a 12 mmol L^{-1} solution of $NaOCH_3$ in methanol, prepared by dissolving metallic sodium in dry methanol (reagent grade, kept over 3 Å molecular sieve). The mixture was filtered through a 0.45 μm pore membrane filter, and the residue was washed with 25 mL of methanol and 25 mL of chloroform. Aliquots (2.5 mL) of the filtrate solution were taken for GC-MS and GC-FID analysis as described below. The residue was oven-dried and weighed. The methanolysis extract, corresponding to the "suberin content", determined by the mass loss, averaged 24.6% (extractive-free basis).

Quantitative Analysis. 1,12-Dodecanediol (11.29 mg) and 12-hydroxyoctadecanoic acid methyl ester (11.42 mg) were added in the methanolysis mixture as internal standards for the quantification of glycerol and long-chain monomers, respectively. Details on the calibration and response factors used for quantitative determinations of suberin monomers are described in Graça and Pereira (2000).

GC-MS and GC-FID Analyses. The aliquots taken from the methanolysate filtrates were concentrated to dryness under a flow of N_2 in a warm bath, further dried in a vacuum oven at 40 °C, and derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane in pirydine (1:1). After a 15 min staging in an oven at 60 °C, solutions were analyzed by GC-EIMS (Masslab Trio 1000) and GC-FID (HP 5890). Split injections were made in the same GC conditions in both analyses: column, DB5-MS (J&W), 60 m, 0.25 mm internal diameter, 0.25 μm film thickness; injector, 300 °C; initial oven temperature, 100 °C (5 min), 10 °C/min rate up to 240 °C, 2 °C/min rate up to 300 °C (15 min); FID temperature, 300 °C. EIMS conditions: source, 200 °C, ionization potential, 70 eV. Identification of monomer compounds was made through their EIMS spectra, from published and obtained spectra from model compounds, as described (Graça and Pereira, 1997).

Isolation of Octadec-9-ene-1,18-dioic Acid. Octadec-9-ene-1,18-dioic acid was obtained by preparative TLC from the methanolysis products of potato periderm, followed by hydrolysis to obtain the free acid. Methanolysis with sodium methoxide (3 g of Na in 500 mL of methanol) was carried out in ∼15 g of extractive-free potato periderm. After filtering, the methanolysate solution was acidified to pH 6, concentrated close to dryness, and partitioned in chloroform/water. The organic layer was washed two more times with water, concentrated, and rediluted with chloroform/methanol 7:3. This solution was applied to 0.5 mm silica TLC plates, developed with chloroform/ethyl acetate 8:2, and the band of the α,ω-diacids dimethyl esters (R_f ∼0.7) was scraped off and extracted (chloroform/methanol 2:1). Separation of the unsaturated octadec-9-ene-1,18-dioic acid dimethyl esters from their saturated counterparts was made by silver ion TLC (Nikolova-Damyanova, 1992). TLC plates (0.5 mm silica gel) were impregnated with silver by immersion in a solution of 20% silver nitrate. The solution of the α,ω-diacid methyl esters was applied to these plates, and after development with chloroform/ethyl acetate 100:5, pure octadec-9-ene-1,18-dioic acid dimethyl ester was recovered from a band at R_f 0.35 (a single peak in the GC-MS analysis). The recovered octadec-9-ene-1,18-dioic acid dimethyl ester was added to a 0.5 M solution of potassium hydroxide in 95% ethanol and stirred at 80 °C during 1.30 h. The mixture was acidified to pH 3.8 with 3 M HCl, evaporated, and partitioned in chloroform/water. The organic phase, after further washing, was dried over sodium sulfate. After solvent removal, 18 mg of octadec-9-ene-1,18-dioic acid [checked by GC-MS as its bis(trimethylsilyl)ester)] was obtained.

Position of Double Bond in the Octadec-9-ene-1,18-dioic Acid. Picolinyl and 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivatives were prepared from the isolated octadec-9-ene-1,18-dioic acid following the method of Gunstone (1997). The picolinyl derivative was synthesized from the acid chloride, prepared by adding 0.5 mL of oxalyl chloride to ∼1 mg of the diacid and letting it stand overnight. Excess reagent was evaporated under N_2, and 0.5 mL of a solution of 3-(hydroxymethyl)-pyridine (HMP) in dichloromethane (40 mg/2 mL) was added at 0 °C, left to warm to ambient temperature, and left for an additional 2 h. Excess solvent was evaporated under N_2 and the mixture derivatized with BSTFA/pyridine for GC-MS analysis. The DMOX derivative was prepared by adding 5 mg of 2-amino-2-methylpropanol to ∼1 mg of the free diacid and incubating the mixture for 5 h at 160 °C. After derivatization with BSTFA/pyridine, the mixture was analyzed by GC-MS.

Analysis of Suberin Dimers. *$Ca(OH)_2$-Catalyzed Methanolysis*. Dry, extractive-free potato periderm (three samples, 2.5−3.3 g) was mixed with dry calcium hydroxide (2:1, w/w) in powder form. Dry methanol (∼70 mL) was added, and the mixture was refluxed at 72 °C (heating provided by an oil bath) for 1 h, with stirring. The mixture was filtered hot (0.45 μm pore membrane) under slight vacuum, and the residue was washed with methanol and chloroform. The methanolysis filtrate was concentrated under reduced vacuum, further filtered through a 0.2 μm pore filter disk, and rediluted to a known volume. Aliquots from these solutions were taken for GC-MS analysis. Extracted material, quantified by the extrapolation of the weight of the dried aliquots, averaged 2.3% (extractive-free basis).

GC-MS Analysis. Aliquots taken from the methanolysate solutions were dried and derivatized as described above. The solutions prepared were analyzed by GC-EIMS, in split and splitless mode, in two different chromatographic conditions: (1) column, DB5-MS, 60 m, 0.25 mm internal diameter, 0.25 μm film thickness; injector, 325 °C; initial oven temperature, 100 °C (5 min), 10 °C/min rate up to 240 °C, 2 °C/min rate up to 300 °C, 1 °C/min up to 340 °C (10 min); (2) column, HT5 (SGE), 50 m, 0.33 mm internal diameter, 0.10 μm film thickness; injector, 325 °C; initial oven temperature, 100 °C (5 min), 8 °C/min rate up to 240 °C, 3 °C/min rate up to 325 °C (30 min). EIMS conditions were as described above.

$Ca(OH)_2$-Catalyzed Methanolysis of Model Compounds. $Ca(OH)_2$-catalyzed methanolysis was applied to several sets of model compounds, under the same conditions as described above, in duplicate. The weight of $Ca(OH)_2$ used in each case was half the weight of the total of other organic compounds present in the mixture: (1) 1-monostearoylglycerol (Sigma), 36 mg (0.1 mmol); (2) 2-monopalmitoylglycerol (Sigma), 26 mg (0.08 mmol); (3) 1-monostearoylglycerol, 72 mg (0.2 mmol), hexadecane-1,16-dioic acid (Tokyo Kasei), 29 mg (0.1 mmol), and eicosane-1,20-dioic acid dimethyl ester (Tokyo Kasei), 37 mg (0.1 mmol); (4) glycerol (Merck), 45 mg (0.5 mmol), hexadecane-1,16-dioic acid, 29 mg (0.1 mmol), and eicosane-1,20-dioic acid dimethyl ester, 37 mg (0.1 mmol). After methanolysis, the reaction mixtures were filtered and washed and aliquots were taken, dried, derivatized, and GC-MS-analyzed as described above. Quantitative data were determined from the areas of compound peaks in the EIMS ion chromatograms (TICs).

Synthesis of the 1-Monoferuloylglycerol. The synthesis was based on the procedure of Neises and Steglich (1978). Ferulic acid (Sigma), 20.99 mg (0.11 mmol), was dissolved in 250 μL of dichloromethane plus 250 μL of dimethylformamide. Under stirring, glycerol, 48.42 mg (0.53 mmol), and 50 μL of a solution of 4-(dimethylamino)pyridine (16.09 mg/mL of dichloromethane) were added. After 5 min, 400 μL of a solution of *N*,*N*′-dicyclohexylcarbodiimide (60.56 mg/mL of dichloromethane) was slowly added. After 2 h of reaction, the solution was filtered, an aliquot of 150 μL taken to dryness, and TMS derivatized as described and analyzed by GC-EIMS.

Table 1. Monomeric Composition of Potato Periderm Suberin, after Depolymerization by $NaOCH_3$-Catalyzed Methanolysis[a]

	mg/g
glycerol	220.3
coniferyl alcohol	1.9
ferulic acid	7.6
alkan-1-ols	**24.7**
octadecanol	3.4
docosanol	4.1
tricosanol	4.4
tetracosanol	4.5
hexacosanol	8.3
alkanoic acids	**84.1**
hexadecanoic acid	2.0
docosanoic acid	5.4
tetracosanoic acid	12.4
hexacosanoic acid	13.7
octacosanoic acid	38.5
nonacosanoic acid	4.3
tricontanoic acid	7.8
α,ω-diacids	**318.7**
hexadecanedioic acid	6.5
octadec-9-ene-1,18-dioic acid	300.8
9,10-dihydroxyoctadecanedioic acid	0.9
eicosanedioic acid	5.0
hexacosanedioic acid	5.5
ω-hydroxyacids	**148.6**
16-hydroxyhexadecanoic acid	13.1
18-hydroxyoctadec-9-enoic acid	120.8
9,10,18-trihydroxyoctadecanoic acid	0.3
22-hydroxydocosanoic acid	6.1
24-hydroxytetracosanoic acid	3.8
26-hydroxyhexacosanoic acid	2.2
28-hydroxyoctacosanoic acid	2.3
unidentified	194.1

[a] Compounds analyzed as methyl esters, trimethylsilyl ethers.

Table 2. Monomers and Oligomers from Potato Periderm Suberin, after Partial Depolymerization by $Ca(OH)_2$-Catalyzed Methanolysis

	mg/g[a]
monomers	
glycerol	431.7
aromatic monomers	2.9
18-hydroxyoctadec-9-enoic acid	97.5
octadec-9-ene-1,18-dioic acid	96.0
other aliphatic monomers	98.4
dimers	
monoacylglyceryl esters of alkanoic acids	
1-monodocosanoylglycerol	0.9
2-monodocosanoylglycerol	0.2
1-monotetracosanoylglycerol	1.9
2-monotetracosanoylglycerol	0.8
1-monohexacosanoylglycerol	2.5
2-monohexacosanoylglycerol	0.9
1-monooctacosanoylglycerol	2.1
2-monooctacosanoylglycerol	0.5
1-monotricontanoylglycerol	0.2
monoacylglyceryl esters of α,ω-diacids	
1-mono(octadecanedi-18-oic acid-1-oyl)glycerol	0.4
1-mono(octadec-9-enedi-18-oic acid-1-oyl)glycerol	61.0
2-mono(octadec-9-enedi-18-oic acid-1-oyl)glycerol	13.9
1-mono(tetracosanedi-24-oic acid-1-oyl)glycerol	0.6
2-mono(tetracosanedi-24-oic acid-1-oyl)glycerol	0.2
1-mono(hexacosanedi-26-oic acid-1-oyl)glycerol	0.2
monoacylglyceryl esters of ω-hydroxyacids	
2-mono(18-hydroxyoctadec-9-enoyl)glycerol	1.5
1-mono(18-hydroxyoctadec-9-enoyl)glycerol	4.0
1-mono(22-hydroxydocosanoyl)glycerol	0.2
1-mono(24-hydroxytetracosanoyl)glycerol	0.4
1-mono(26-hydroxyhexacosanoyl)glycerol	0.3
monoacylglyceryl ester of ferulic acid	
1-monoferuloylglycerol	0.7
feruloyl esters of alkan-1-ols	
hexadecanyl ferulate	0.6
octadecanyl ferulate	0.3
docosanyl ferulate	0.1
feruloyl esters of ω-hydroxyacids	
16-*O*-feruloyloxyhexadecanoic acid	0.1
18-*O*-feruloyloxyoctadec-9-enoic acid	4.8
trimers	
1,1′-diglycerol-octadec-9-ene-1,18-dioate	4.9
1,2′-diglycerol-octadec-9-ene-1,18-dioate	2.0
2,2′-diglycerol-octadec-9-ene-1,18-dioate	<0.1
unidentified	167.5

[a] Based in the integrated areas of the GC-MS reconstructed ion chromatogram. Compounds analyzed as methyl esters, trimethylsilyl ethers.

RESULTS AND DISCUSSION

Suberin Monomeric Composition. The $NaOCH_3$-catalyzed methanolysis solubilized ~25% of the extractive-free potato periderm. This value is close to others obtained for the quantification of the suberin content in this material, using different depolymerization techniques (Rodriguez-M. and Ribas-M., 1972; Kolattukudy and Agrawal, 1974; Brieskorn and Binnemann, 1975). Analysis of the methanolysis products (Table 1) showed the presence of the long-chain alkan-1-ols, alkanoic acids, ω-hydroxyacids, and α,ω-diacids, monomers also previously identified (Brieskorn and Binnemann, 1974; Kolattukudy and Agrawal, 1974; Holloway, 1983). In addition, glycerol was found in large quantities, accounting for ~20% of all monomers. Less than 1% of the mixture were hydroxycinnamyl aromatic monomers, namely, ferulic acid and coniferyl alcohol. As quantified by the calibrated internal standards, this mostly aliphatic monomer mixture accounted for ~74% of the mass loss after methanolysis.

The mixture of long-chain aliphatic acids is dominated by two mono-unsaturated acids, the octadec-9-ene-1,18-dioic acid and the 18-hydroxyoctadec-9-enoic acid, accounting for 39 and 15% of all monomers, respectively, excluding glycerol. The α,ω-diacids and ω-hydroxyacids are the main classes of acid compounds, accounting for 55 and 26% of the identified long-chain aliphatics, respectively. Alkanoic acids are present in a fairly high proportion compared to other suberins studied (Graça and Pereira, 2000) and have very long chain lengths, C28 being the more important homologue.

Earlier studies have shown that the position of the double bond in the two main monomers of potato suberin is midchain, at C-9 (Rodriguez-M. and Ribas-M., 1972; Kolattukudy and Agrawal, 1974). Although identification of monomers was made through their EIMS, it is known that the mass spectra of fatty acid methyl esters are little modified by changes in the position of the double bond along the hydrocarbon chain. The position of the double bond in the octadec-9-ene-1,18-dioic acid, isolated from the potato suberin methanolysis products, was checked after the synthesis of its picolinyl and DMOX derivatives. The latter are nitrogen-containing derivatives of the carboxyl group, which under electron-impact conditions produce fragment ions associated with each C−C cleavage along the chain, without migration of the double bonds (when present), thus allowing the determination of its position (Christie, 1997). Both derivatives confirmed the assignment of the

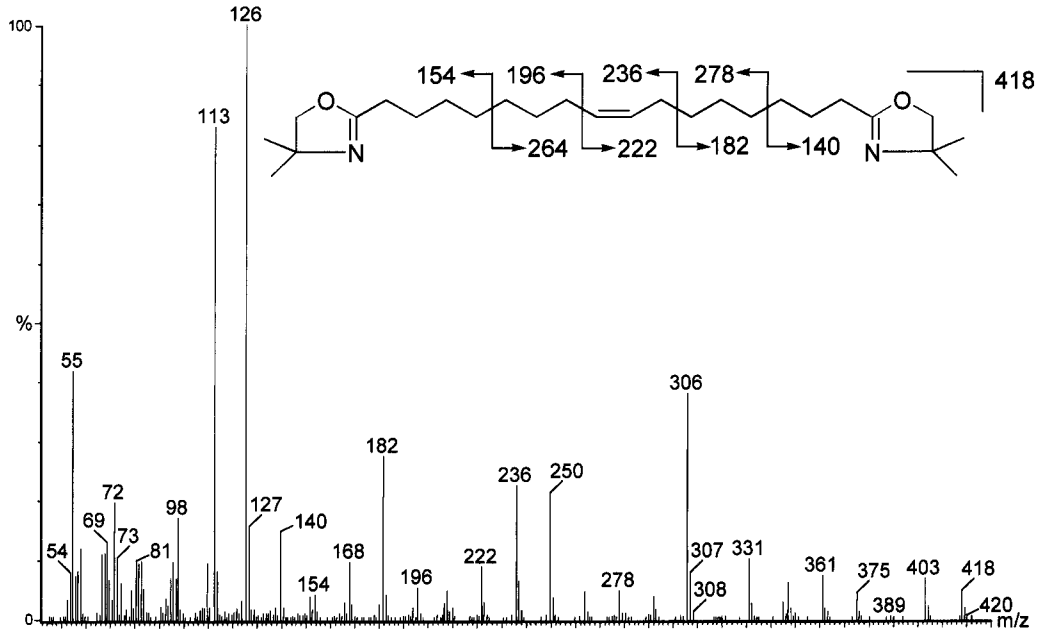

Figure 1. EI mass spectrum of the 2-alkenyl-4,4-dimethyloxazoline (DMOX) bis-derivative of the octadec-9-ene-1,18-dioic acid, isolated from the methanolysis products of the potato periderm suberin.

position of the double bond, and the mass spectrum of the DMOX derivative is presented in Figure 1.

The geometric isomerism of the two main monene acids was not studied here. The cis form was assigned before to both acids (Rodriguez-M. and Ribas-M., 1972). However, a poorly resolved division was observed here within the peak of each of the acids, with the same mass spectra. The proportion between the first eluted to the later eluted part of the peaks was roughly 4:1 in the octadec-9-ene-1,18-dioic acid and 2:1 in the 18-hydroxyoctadec-9-enoic acid. As the cis form precedes the trans form in the elution of unsaturated fatty acid methyl esters in nonpolar columns, the possible presence of both isomers should be considered here.

Suberin Partial Depolymerization. Partial depolymerization of bark suberins has been achieved before with a CaO-catalyzed methanolysis (Graça and Pereira, 1997, 1999). This technique allowed the depolymerization of between 5 and 10% of suberin aliphatics, of which about one-third were dimers. These dimers were mostly monoacylglyceryl esters of the long-chain aliphatic suberinic acids, namely, of α,ω-diacids, ω-hydroxyacids, and alkanoic acids. The acids appeared to be esterified in both assignable positions of glycerol, 1- and 2-, the first being predominant. The acids in the monoacylglyceryl esters appeared roughly in the same proportions as they appear as monomers after complete depolymerization of the suberins.

A similar procedure was used here, with $Ca(OH)_2$ instead of CaO as the catalyst of methanolysis reaction, which provided a higher yield of suberin oligomers. The $Ca(OH)_2$-catalyzed methanolysis solublized ~10% of the "total" suberin, as measured by the $NaOCH_3$-catalyzed methanolysis extracted materials. The GC-MS analysis of the mixture showed the presence of monomers, dimers, and trimers (Table 2). Unidentified compounds represented 17% of the mixture. Monomers accounted for most of all of the identified compounds, ~87%, of which about half was glycerol with the remainder being of the same type of aliphatic acids present after complete depolymerization. Oligomers represented 13% of identified compounds, mostly composed of dimers and a small quantity of trimers (25 and 2%, respectively, of identified compounds excluding glycerol).

Identification of the suberin dimers was made through their EIMS as previously discussed (Graça and Pereira, 1997, 1999). The mass spectra of two of the more significant dimers, 1-mono(octadec-9-enedi-18-oic acid-1-oyl)glycerol and 18-*O*-feruloyloxyoctadec-9-enoic acid, including some of their fragmentation patterns are presented in Figures 2 and 3. A total of 26 different dimers were identified, including monoacylglyceryl esters of the aliphatic acids, ferulates of ω-hydroxyacids and alkan-1-ols, and a small quantity of a monoacylglyceryl ester of ferulic acid (Table 2). The trimers were diglycerol diesters of the octadec-9-ene-1,18-dioic acid, the main acid monomer in the suberin of the potato periderm. Discussion of these trimers, namely, evidence regarding their identification, is presented elsewhere (Graça and Pereira, in press).

The $Ca(OH)_2$-catalyzed methanolysis procedure was applied to model compounds under the same conditions as for potato periderm, to ascertain the extent of partial hydrolysis of ester compounds and to check for the eventual occurrence of resynthesis of glyceryl esters from the depolymerized acids. The reaction conditions were applied independently to 1-monostearoylglycerol and 2-monohexadecanoylglycerol. In both cases only a partial methanolysis of the monoacylglycerol esters was observed, amounting to 65 and 57%, respectively, of transesterification to the methyl esters of the corresponding acids. However, interesterification was also observed in the remaining nonmethanolyzed monoacylglycerol ester, a situation known to occur in other reactions with acylglycerol esters (Sonnet, 1999). Migration of the acyl moiety in both directions between the 1- and 2-positions of glycerol occurred, with the move from the 2- to the 1-position predominating. After the $Ca(OH)_2$ methanolysis, ~10% of the 2-monostearoylglycerol was obtained from the initial 1-isomer, and ~95% of the 1-isomer was obtained from the initial 2-monohexadecanoylglycerol.

Glycerol and 1-monostearoylglycerol were also reacted independently under the $Ca(OH)_2$ methanolysis with

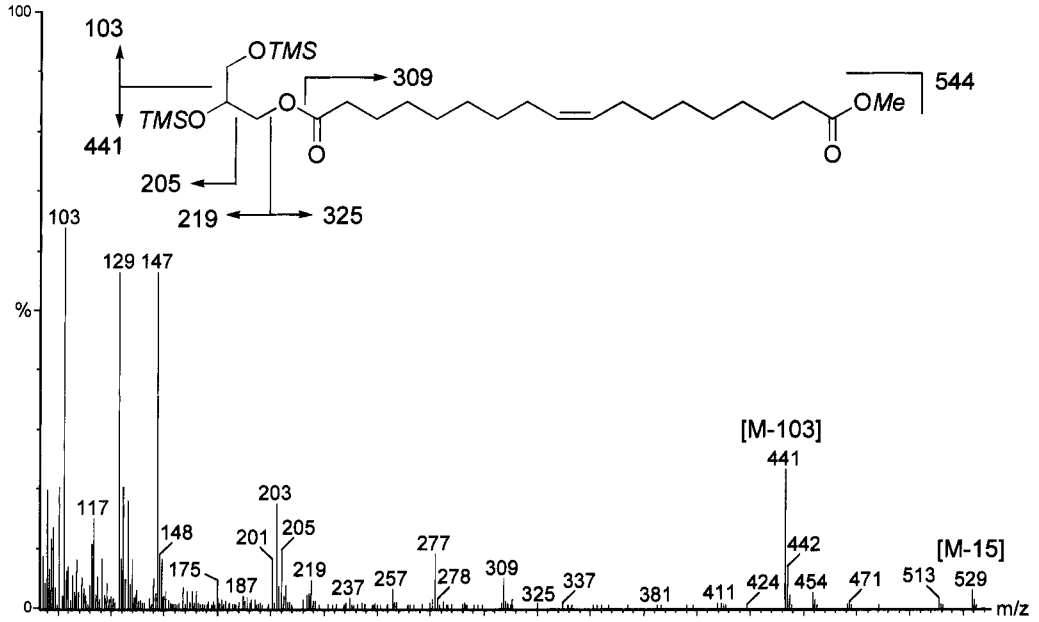

Figure 2. EI mass spectrum of the 1-mono(octadec-9-enedi-18-oic acid-1-oyl)glycerol, methyl ester, bis(trimethylsilyl) ether, isolated from the $Ca(OH)_2$-catalyzed partial methanolysis products of the potato periderm suberin.

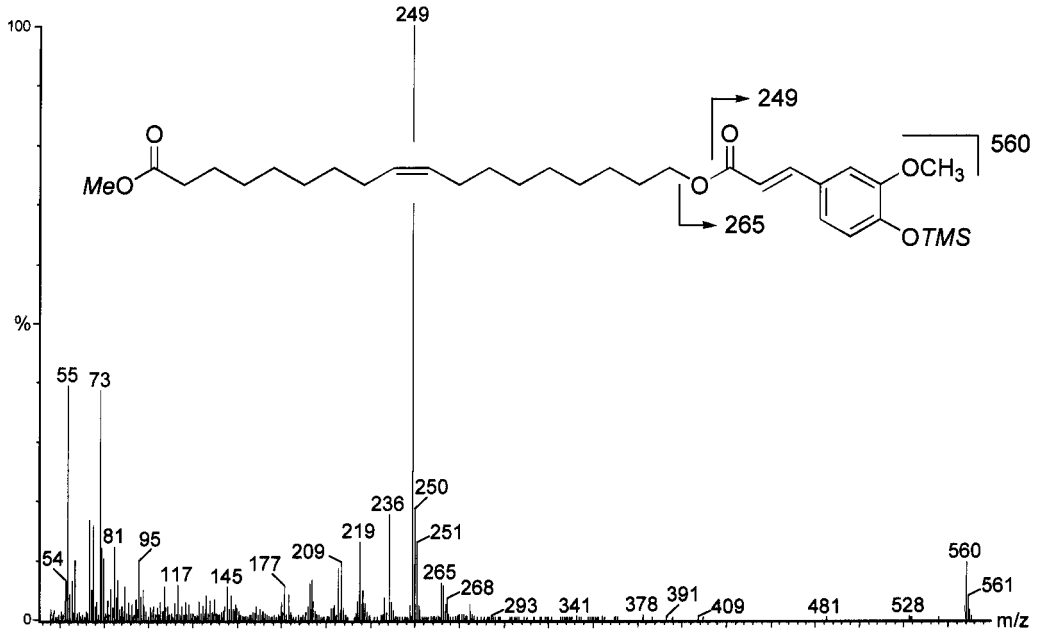

Figure 3. EI mass spectrum of the 18-*O*-feruloyloxyoctadec-9-enoic acid, methyl ester, trimethylsilyl ether, isolated from the $Ca(OH)_2$-catalyzed partial methanolysis products of the potato periderm suberin.

long-chain α,ω-diacids, as free acids and in the form of dimethyl esters. The acyl part of the monoacylglycerol, the free α,ω-diacid, and dimethylated α,ω-diacid were all different in order to be able to check for changes in positions. As expected under the conditions used, the synthesis of glycerol esters was not observed, nor was substitution of the acyl part of the monoacylglycerol by the non-glycerol-linked α,ω-diacids. This shows that the glycerol esters found after the partial methanolysis were originally present in the suberin polymer.

Suberin Dimers. Almost all of the suberinic acids of the potato periderm found as monomers were also found as monoacylglyceryl esters in the solubilized products of the $Ca(OH)_2$-catalyzed methanolysis (Table 2). About two-thirds of all identified dimers were monoacylglyceryl esters of α,ω-diacids, overwhelmingly dominated by the 1- and 2-isomers of octadec-9-ene-1,-18-dioic acid. Within each class of monoacylglycerols (e.g., alkanoic acids, α,ω-diacids, and ω-hydroxyacids), the proportion of each individual acid roughly followed the same proportion as for monomers after complete depolymerization of suberin. However, the ω-hydroxyacids were under-represented as monoacylglycerols, even after accounting for the ω-hydroxyacids that appeared as ferulate dimers (discussed below). We speculate that the position or chemical neighborhood of ω-hydroxyacids in the suberin polymer makes their ester linking to glycerol more susceptible to the methanolysis reaction used.

The most abundant monoacylglycerols appeared as 1- and 2-isomers, in a relative proportion of ~3−5:1. Because glycerol has two 1-positions (stereochemically distinct only when differently substituted) and has only one 2-position, the prevalence of 1-isomers is expected.

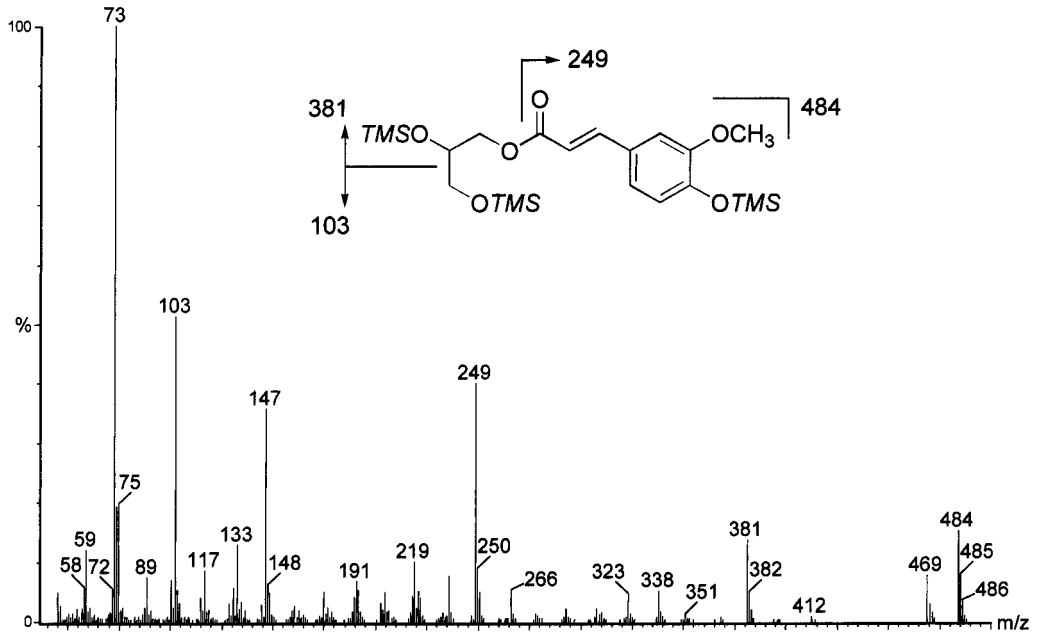

Figure 4. EI mass spectrum of the 1-monoferuloylglycerol, tris(trimethylsilyl) ether, isolated from the $Ca(OH)_2$-catalyzed partial methanolysis products of the potato periderm suberin.

However, care has to be taken in the interpretation of these results because reaction with model compounds has shown that acyl migration between the 1- and 2-positions of glycerol (with preferential migration to the 1-position) can occur under the conditions used.

The partial depolymerization of potato periderm suberin also yielded feruloyl esters of ω-hydroxyacids and alkan-1-ols (Table 2), as was the case for bark suberins previously studied (Graça and Pereira, 1998, 1999). The 18-hydroxyoctadec-9-enoic acid ester of ferulic acid was the most abundant ferulate (Figure 3). This 18-carbon acid is the most abundant ω-hydroxyacid and the second most abundant suberin aliphatic acid monomer. The peak of this ferulate in the GC-MS runs appears to be divided, presumably due to the four possible geometric isomers. Although the mass spectrum remains basically the same, important variations are observed in the abundance of some ions, namely, the feruloyl ion (*m*/*z* 249 as the TMS ether) and the molecular ion (*m*/*z* 560). The spectrum of the most abundant subpeak is presented (Figure 3) and represents the *trans*-ferulic acid−*cis*-18-hydroxyoctadec-9-enoic acid ester. Most of the ferulic acid found as monomer is in the trans form, which is easy to assign because the cis form exists in minor quantities and the two isomers are well separated chromatographically. As for the 18-hydroxyoctadec-9-enoic acid, the evidence available indicates that it will be mostly in the cis form, as discussed above.

A small quantity of another ester was found, with a mass spectrum showing characteristics of both a ferulate and a monoacylglycerol. After an open esterification synthesis between ferulic acid and an excess of glycerol, a GC-MS peak with the same mass spectrum was obtained (Figure 4), and the compound was identified as the 1-monoferuloylglycerol. Although the synthesis conditions used could produce both the 1- and 2-isomers, only one peak was obtained (45% yield based on the initial ferulic acid) and was attributed to the 1-isomer due to the presence of a significant ion at M − 103. We know that this ion is present in the TMS derivatives of 1-monoacylglycerols and absent in the corresponding 2-isomers (Myher et al., 1974).

Suberin Structure. The results previously obtained in bark corks and here in the potato peridem show that suberin is a glycerol-structured polymer. Already a significant number of suberin analyses have shown the presence of glycerol (Carvalho, 1993; Schmutz at al., 1993; Rosa and Pereira, 1994; Bento et al., 1998), and its quantity has been positively related with the suberization process (Moire at al., 1999). The role of glycerol in the macromolecular assembly of the suberin polymer seems now to be the interlinking between the long-chain acid monomers. As one of the main monomer families is the α,ω-diacids, their esterification to glycerol in both ends can form the backbone for growth of the macromolecular polyester structure. The actual finding of such diglycerol-α,ω-diacid diester trimers in the potato periderm suberin (Graça and Pereira, in press) gives substance to this hypothesis. The results of ^{13}C solid-state NMR studies in potato (Garbow et al., 1989; Stark and Garbow, 1992) and cork suberin (Gil et al., 1997) do not contradict the above hypothesis, showing carbons with chemical vicinities compatible with primary and secondary ester group positions.

Earlier assumptions for the macromolecular development of suberin involved the extensive cross-linking of the long-chain aliphatics with aromatic phenylpropenoids and the linear ω-hydroxyacid to ω-hydroxyacid interesterification (Kolattukudy, 1977). This hypothesis seems to be losing ground; direct evidence from solid state ^{13}C NMR shows that aliphatics and aromatics occupy separate domains in suberized cell walls (Gil et al., 1997; Bernards and Lewis, 1998). This supports earlier histological and ultrastructural observations that the suberin-containing cell walls have a multilayer structure of two materials of alternate dark−clear contrast (Sitte, 1962). Although glycerol has been found here, and in other suberins (Graça and Pereira, 1997, 1999), to be abundant enough to esterify all available carboxylic groups, there is no evidence to exclude other esters within the suberin polymer, such as the linear

interestérication of ω-hydroxyacids. The ω-hydroxyacid−ω-hydroxyacid interester structure was proposed to play a major role in cutins (Kolattukudy, 1977), and a linear ester dimer of the 16-hydroxyhexadecanoic acid has already been found in the tomato peel cutin, after a partial hydrolysis reaction (Osman et al., 1995).

Nevertheless, the linking points between the aliphatic polyester part in suberins and the polyaromatics are probably abundant. Solid state NMR has shown close proximity of acyl chains and phenolics in wounded potato tissue (Yan and Stark, 1998). Ferulic acid and other related small phenolics are always found in the suberin mixtures, as is also the case here in potato periderm. Ferulic acid was also found here esterified to the ω-hydroxyacids, as it has been before in two other suberins (Graça and Pereira, 1998, 1999). Because ω-hydroxyacids are esterified through their acid endgroup to glycerol and through their primary hydroxyl to ferulic acid, they may be the units in the suberin glycerol−aliphatic polyester especially involved in linking it to the surrounding polyaromatics. Such a trimeric diester, glycerol−ω-hydroxyacid−hydroxycinnamic acid (caffeic acid in this case) has been already identified in the extractives of green cotton fiber (Schmutz et al., 1994).

The linking between the glyceryl−aliphatic suberin polymer and the neighboring polyaromatics can also be assured by glycerol, by esterification to ferulic acid. The monoferuloylglycerol dimer here reported, although found in a small amount, opens the possibility for these interpolymer connections in the suberized cell walls.

The availability of ferulic acid to bridge the suberin aliphatics to the abundant polyphenolics in suberized cell walls can be questioned, due to the usually small quantities of ferulic acid obtained after suberin depolymerization. However, the major part of the ferulic acid necessary to fulfill this role can be part of the aromatic polymer itself and not accessible to solubilization by the ester-cleaving treatments used to depolymerize suberin. A polymer based in hydroxycinnamic acids in a lignin-like structure has even been proposed for the polyaromatics that accompany suberin aliphatics after a solid state ^{13}C NMR study in the potato wound suberin (Bernards et al., 1995; Bernards and Lewis, 1998).

Contradicting evidence remains regarding the nature and homogeneity of suberized cell wall polymeric aromatics. Several degradation reactions commonly used to study lignins were applied to suberized potato tissues, and the similarities with wood lignin led to the conclusion that the polyaromatics present had a "lignin-character" (Cottle and Kolattukudy, 1982; Walter and Schadel, 1983; Hammerschmidt, 1985; Borg-Olivier and Monties, 1993; Lapierre et al., 1996). In cork suberized cell walls, most of those polyaromatics are proposed to be identical to a wood guaiacyl lignin (Marques et al., 1994, 1996, 1999). Nevertheless, the total yield of phenolic units after the degradation reactions was much lower than the ones obtained from woods, and differences in the interunit ether and C−C bond patterns showed a more cross-linked structure (Lapierre et al., 1996; Marques et al., 1999).

Overall, chemical degradation studies, in situ solid ^{13}C NMR, and ultrastructural histology are concordant in that polyaromatics and polyaliphatics might constitute two different polymeric structures spatially separated in suberized cell walls. This agrees with our proposal that in the suberized cell walls the polyaliphatic polymer has a three-dimensional development ensured by glycerol and exists independently from the associated polyaromatics, to which they are peripherally bonded. In this case, we propose that the name suberin should be restricted to this glycerol-structured polyester.

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