Linear Aliphatic Dimeric Esters from Cork Suberin

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Received February 24, 2006; Revised Manuscript Received April 20, 2006

Cork suberin was partially depolymerized by methanolysis catalyzed by calcium hydroxide. Analysis by GC-MS of the methanolysate showed suberin monomers, including glycerol and long-chain α,ω-diacids and ω-hydroxyacids. ESI-MS analysis of the methanolysate showed, besides the aliphatic monomers, suberin oligomers, including linear dimeric esters of α,ω-diacids and ω-hydroxyacids. Two types of dimeric esters were identified: a α,ω-diacid linked to a ω-hydroxyacid and two inter-linked ω-hydroxyacids. The α,ω-diacids and ω-hydroxyacids found as monomer residues in the dimeric esters were mainly the C18 monomers with midchain substituents. The identification of these dimeric esters was based in their CID-MS/MS spectra and confirmed after synthesis of model compounds. The occurrence of inter-esterified long-chain monomers in suberin brings a new insight in the understanding of the polyester structure of this biopolymer.

Introduction

Plant's outer tissues have to ensure protection from environmental aggressions. For this purpose, they developed specialized cell walls with a resistant polymer as its main structural component, suberin. Suberin gives insulating properties to suberized cells and allows the control of exchanges with the environment.[1] The outer bark of the cork-oak tree (*Quercus suber* L.) is an exceptionally suberin-rich tissue, known as commercial cork. Cork is harvested every 9 to 10 years in a sustainable basis and has a set of valuable properties making it sought out for technologically demanding uses.[2] Cork cell walls have a suberin content of 50%, and its unique properties as a material have been ascribed to the presence of this biopolymer.[3]

Suberin is a complex polyester composed by aliphatic long-chain α,ω-diacids and ω-hydroxyacids, presumably reticulated with glycerol.[4] These aliphatic long-chain monomers have either saturated chains or are functionalized at midchain with an unsaturation, epoxide or diol group. The relative abundance of α,ω-diacids and ω-hydroxyacids and the proportion of midchain substituted monomers are variable in suberin from different plant sources.[5]

Up till now, little is known about the macromolecular structure of suberin. Small oligomers with two and three ester-linked monomers have been identified after partial methanolysis of suberin and have given some information on how monomers are linked in the polymer.[4] The dimeric structures found so far include glycerol esterified to α,ω-diacids, to ω-hydroxyacids, and to monoacids in the form of monoacylglycerols, and the trimeric structures include α,ω-diacids linked at both ends to glycerol units.[6] Also, dimeric and trimeric structures including ferulic acid linked to ω-hydroxyacids and the latter esterified to glycerol have been found in suberin.[7]

In suberized cell walls, significant amounts of lignin-related polyaromatics are associated with the aliphatic suberin.[8] Based on this evidence, tentative models were proposed for the suberin structure, including polyaliphatic and polyaromatic domains.[9] In one of these models, it was proposed that α,ω-diacids and ω-hydroxyacids are linked together sequentially, forming linear aliphatic esters.[9a] The existence of such structures has long been hypothesized but never proved. Here we present evidence of its occurrence in cork suberin. Linear dimeric esters were obtained after partial methanolysis of cork suberin and were identified by electrospray-MS/MS. Model compounds were synthesized to confirm the MS/MS spectra. The relevance of these linear dimeric esters for the suberin macromolecular structure is discussed.

Experimental Section

Cork Powder Material. Cork from *Quercus suber* L. was obtained as cork powder from the granulated Portuguese cork industry. The cork powder was sieved and the 40−60 mesh (0.25−0.425 mm) granulometric fraction recovered. This cork powder was thoroughly extracted sequentially with dichloromethane (6 h), ethanol (12 h), water (24 h), and methanol (12 h), removing approximately 15% of the initial dry mass. The extracted cork powder was air-dried and used as such for methanolysis reactions.

Partial Depolymerization of Cork Suberin. A total of 3.0 g of extracted cork powder were mixed with 1.5 g of powdered calcium hydroxide, suspended in 100 mL of methanol and refluxed for 6 h, with stirring. The methanolysis mixture was filtered in a 0.45 μm PTFE filter and washed with small portions of dichloromethane and methanol. Aliquots of the partial methanolysis filtrate were taken for GC-MS analysis, ESI-MS analysis, and gravimetric determination of extracted material.

GC-MS Analysis. Aliquots taken from the cork suberin partial methanolysate were evaporated with a nitrogen flow, taken to dryness under vacuum, and derivatized with *N*,*N*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)/pyridine 1:1 (40 μL of the derivatizing mixture/mg of dry weight). The derivatized solution was injected in a GC-MS (Agilent 5973 MSD) with the following GC conditions: DB5-MS column (60 m × 0.25 mm ID × 0.25 μm film thickness), injector 320 °C, oven temperature program, 100 °C (5 min), rate of 8 °C/min up to 250 °C, rate of 2.5 °C/min up to 320 °C (20 min). The MS source was kept at 220 °C and the electron impact mass spectra (EIMS) taken at 70 eV of energy.

Electrospray(ESI)-MS/MS Analysis. Solutions for ESI-MS analysis were prepared after diluting the aliquots of the cork suberin partial methanolysate in methanol/chloroform 3:1 to a concentration of ca. 50

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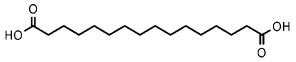
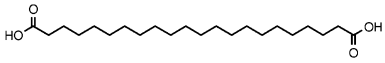
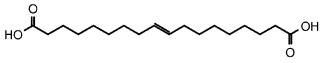
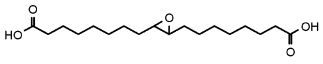
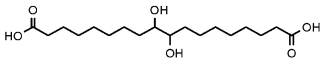
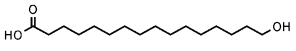
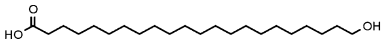
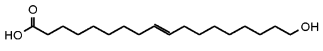
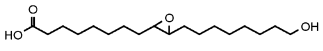
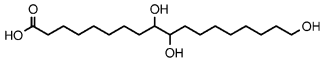
10.1021/bm060174u CCC: $33.50 © 2006 American Chemical Society
Published on Web 05/24/2006

Table 1. Chemical Structure, Systematic Name, and Abbreviation of the Monomers Found as Part of Cork Suberin Dimeric Esters

Molecular structure	Systematic name	Abbreviation
α,ω-diacids		
	hexadecane-1,16-dioic acid	Di16
	docosane-1,22-dioic acid	Di22
	octadec-9-ene-1,18-dioic acid	Di18:1
	9-epoxyoctadecane-1,18-dioic acid	Di18Epox
	9,10-dihydroxyoctadecane-1,18-dioic acid	Di18Diol
ω-hydroxyacids		
	16-hydroxyhexadecanoic acid	Hid16
	22-hydroxydocosanoic acid	Hid22
	18-hydroxyoctadec-9-enoic acid	Hid18:1
	9-epoxy-18-hydroxyoctadecanoic acid	Hid18Epox
	9,10,18-trihydroxyoctadecanoic acid	Hid18Diol

ng/μl. Solutions with different ionizing promoters were prepared with concentrations of respectively 0.2% ammonium hydroxide, 0.2% formic acid, and 2 mM lithium acetate. These solutions were injected in the electrospray source of a triple quadrupole (Quattro LC, Micromass) with an infusion pump at a flow rate of 10 μL/min. The source block temperature was kept at 80 °C and the dessolvation temperature of nitrogen flow at 150 °C. Voltage in the source sampling cone was varied to maximize peak intensity between 40 and 90 V. Collision induced dissociation (CID) MS/MS of the selected parent ions was done with argon as the collision gas with a pressure of ca. 0.5 bar. Daughter-ions MS/MS spectra were obtained with voltages in the collision cell of 20−60 V. ESI-MS/MS of the synthesized dimeric esters was carried out in similar analytical conditions.

Synthesis of Model Compounds. *Total Depolymerization of Cork Suberin.* A total of 29 g of extracted cork powder was suspended in 1500 mL of 60 mM sodium methoxide. The mixture, heated in an oil bath at 72 °C, was refluxed for 3 h with stirring. The methanolysis mixture was filtered in a glass G3 filter and the residue washed with dichloromethane and methanol. The solution was acidified to pH 6 with 0.1 M sulfuric acid in methanol and evaporated under reduced pressure, close to dryness. This residue (ca. 15 g) was partitioned in 200 mL of dichloromethane and 200 mL of water, and the organic phase was further washed twice with 200 mL of water. The organic phase was dried over sodium sulfate anhydrous, filtered in a glass G3 filter, and further filtered in a 1 μm PTFE filter. Aliquots were taken for quantitative determination and GC-MS analysis.

Isolation and Purification of Monomers. The organic phase from the methanolysate was applied to a silica solid-phase extraction column (15.5 cm × 3.8 cm) and sequentially eluted with dichloromethane and dichloromethane/2-propanol in the following proportions: 95:5, 92.5:7.5, and 9:1. Fractions enriched in octadec-9-ene-1,18-dioic acid dimethyl ester (Di18:1 2me), 18-hydroxyoctadec-9-enoic acid methyl ester (Hid18:1 me), 9-epoxyoctadecane-1,18-dioic acid dimethyl ester (Di18Epox 2me), 9-epoxy-1,18-dioic acid methyl ester (Hid18Epox me), and 22-hydroxydocosanoic acid methyl ester (Hid22 me) were recovered.

These monomers were purified by preparative TLC Silica separations, as follows. The above enriched fractions were applied to 0.5 mm plates and developed with chloroform/ethyl acetate 7:3. Three fractions were recovered, including the following: (1) Di18:1 2me and Di18Epox 2me (Rf 0.80, 580 mg); (2) Hid22 me and Hid18:1 me (Rf 0.53, 1450 mg); (3) Hid18:1 me and Hid18Epox me (Rf 0.43, 735 mg). From fraction (1), 70 mg of Di18:1 2me (Rf 0.47) and 110 mg of Di18Epox 2me (Rf 0.40) were obtained after elution with chloroform. From fraction (2), 260 mg of Hid22 me (Rf 0.43) and 130 mg of Hid18:1 me (Rf 0.25) were obtained after elution with chloroform/ethyl acetate 7:3 in Ag/Silica TLC plates. From fraction (3), 170 mg of Hid18Epox me (Rf 0.27) were obtained after elution with chloroform/ethyl acetate 9:1. Di18:1, Di18Epox, and Hid18:1 were obtained as free acids after hydrolysis with 0.5 M potassium hydroxide in ethanol/water 95:5.

Synthesis of Dimeric Esters. Hid18Epox_Di18:1. To 12.8 μmol (4.2 mg) of Hid18Epox me was added 8.5 μmol (2.7 mg) of Di18:1 dissolved in 3500 μL of dichloromethane. To this solution were added 340 μL (12.9 μmol in dichloromethane) of 1,3-dicyclohexylcarbodiimide (DCC) and 34 μL (0.35 μmol in dichloromethane) of 4-(dimethylamino)pyridine (DMAP). The reaction was kept at room temperature, for 30 h, with stirring in a closed vial purged with N_2 (adapted from ref 10). The free carboxylic acid end-groups of the synthesized dimeric esters were then methylated by adding 1 mL of methanol, and stirring for 1 h. These same reaction conditions were used for the synthesis of the following dimeric esters. *Hid18:1_Di18:1*: Hid18:1 me (12.8 μmol, 4.0 mg); Di18:1 (8.5 μmol, 2.7 mg); DCC (340 μL, 12.9 μmol); DMAP (34 μL, 0.35 μmol). *Hid18Epox_Di18Epox*: Hid18Epox me (10.2 μmol, 3.3 mg); Di18Epox (6.8 μmol, 2.2 mg); DCC (270 μL, 10.2 μmol); DMAP (27 μL, 0.28 μmol). *Hid18:1_Di18Epox*: Hid18:1 me (10.2 μmol, 3.2 mg); Di18Epox (6.8 μmol, 2.2 mg); DCC (270 μL, 10.2 μmol); DMAP (27 μL, 0.28 μmol). *Hid18:1_Hid18:1*: Hid18:1 me (12.5 μmol, 3.9 mg); Hid18:1 (10.0 μmol, 3.0 mg); DCC (330 μL, 12.5 μmol); DMAP (33 μL, 0.34 μmol). *Hid16_Hid16*: 16-hydrohyhexadecanoic acid methyl ester (purchased from TCI, methylated with diazomethane) (15.0 μmol, 4.3 mg); 16-hydroxyhexadecanoic acid (TCI) (12.0 μmol, 3.3 mg); DCC (395 μL, 15.0 μmol); DMAP (40 μL, 0.41 μmol). The synthesis mixtures were filtered in 0.2 μm PTFE filters and aliquots from the filtrate solutions were taken for ESI-MS analysis.

All solvents were of HPLC grade. Methanol used in the methanolysis reactions was dried over molecular sieve.

Results and Discussion

Partial Depolymerization of Cork Suberin. Cork suberin was partiallly depolymerized by methanolysis catalyzed by calcium hydroxide. This partial methanolysis solubilized ca. 10% of the extractive-free initial cork material. When cork suberin is completely depolymerized, yields of ca. 50% are obtained.[11] This means that the calcium hydroxide-catalyzed partial methanolysis extracted only one-fifth of cork suberin. The analysis by GC-MS of the partial methanolysate showed mostly the monomers that comprise cork suberin, with glycerol (ca. 80%), long-chain α,ω-diacids (8%), ω-hydroxyacids (4%), and monoacids (1%). In this partial depolymerization of cork suberin, glycerol is preferentially solubilized compared to the long-chain acid monomers. In the complete depolymerization of cork suberin, glycerol accounts for only 14% of all monomers.[11]

The analysis of the cork suberin partial methanolysate by ESI-MS showed, besides the monomers observed in the GC-MS analysis, a mixture of compounds with molecular masses compatible with dimeric and trimeric oligomers of interesterified suberin monomers. These oligomers were detected in the electrospray analysis as M + H, M + Na, and M + Li adducts, depending on the ionizing agents added. Most of the MS/MS analysis for the identification of these ester oligomers were carried out in the M + Li adducts, as discussed below. Among the oligomers detected, two types of linear aliphatic esters of long-chain monomers were identified: a ω-hydroxyacid linearly linked to a α,ω-diacid and two ω-hydroxyacids linked "head-to-tail". Besides these dimeric oligomers, masses corresponding to trimeric linear di-esters of two ω-hydroxyacids and one α,ω-diacid were also present in the ESI-MS ion chromatograms and are presently under study.

Linear Dimeric Esters. Table 1 presents the molecular structure of the monomers found as moieties in the linear dimeric esters, together with their systematic name and abbreviation used throughout the text to identify them. Table 2 lists the identified linear dimeric esters in cork suberin partial methanolysate. An example of an ESI-MS ion chromatogram of the lithiated adducts of these dimeric esters is presented in Figure 1.

The identification of these compounds was based in their ESI-MS/MS spectra as discussed below. Some of the dimeric esters were synthesized to prove their structure and to assign the fragmentation patterns of their MS/MS spectra. The synthesized structures were the 18-hydroxyoctadec-9-enoic acid esterified to the 9-epoxyoctadecane-1,18-dioic acid [Hid18:1_Di18Epox, Figure 2a], the 9-epoxy-18-hydroxyoctadecanoic acid with the octadec-9-ene-1,18-dioic acid [Hid18Epox_Di18:1, Figure 2b], the 9-epoxy-18-hydroxyoctadecanoic acid with the 9-epoxyoctadecane-1,18-dioic acid [Hid18Epox_Di18Epox, Figure 2d], the di(18-hydroxyoctadec-9-enoic acid) [Hid18:1_Hid18:1, Figure 3b], and the di(16-hydroxyhexadecanoic acid) [Hid16_Hid16].

The carboxylic acid end groups of the dimeric esters solubilized from cork suberin were found as methyl esters due to the methanolysis procedure used in the depolymerization reaction. Identically, in the synthesized dimeric esters, these same acid end groups were prepared as methyl esters.

Some of the mass peaks in the ion chromatograms of the cork suberin partial methanolisate corresponded to two dimeric esters with the same molecular mass (Figure 1, Table 2). For instance, the MS/MS spectrum of the ion of *m*/*z* 643.5 (Figure 2c) was shown to be a mixture of two dimeric esters, Hid18:1_Di18Epox and Hid18Epox_Di18:1. These two dimeric esters were separately synthesized and their MS/MS spectrum obtained: Hid18:1_Di18Epox (Figure 2a) and Hid18Epox_Di18:1 (Figure 2b). A similar approach was used in the identification of the other pairs of isomers of dimeric esters, as also illustrated in Figure 3, panels a−c.

Table 2. Linear Dimeric Esters, ω-Hydroxyacid−α,ω-Diacid, and ω-Hydroxyacid−ω-Hydroxyacid, Found in the Partial Methanolysis Products of Cork Suberin

molecular mass (monoisotopic)	linear dimeric ester[a]	relative intensity[b]
592.5	Hid18:1_Hid18:1	24
594.5	Hid16_Di18:1	27
	Hid18:1_Di16	
608.5	Hid18Epox_Hid18:1	33
	Hid18:1_Hid18Epox	
610.5	Hid16_Di18Epox	28
	Hid18Epox_Di16	
620.5	Hid18:1_Di18:1	38
624.5	Hid18Epox_Hid18Epox	26
626.5	Hid18Diol_Hid18:1	25
	Hid18:1_Hid18Diol	
636.5	Hid18Epox_Di18:1	88
	Hid18:1_Di18Epox	
650.6	Hid18:1_Hid22	27
	Hid22_Hid18:1	
652.5	Hid18Epox_Di18Epox	100
654.5	Hid18:1_Di18Diol	59
	Hid18Diol_Di18:1	
666.6	Hid8Epox_Hid22	30
	Hid22_Hid18Epox	
670.5	Hid18Diol_Di18Epox	56
	Hid18Epox_Di18Diol	
678.6	Hid22_Di18:1	32
	Hid18:1_Di22	
684.6	Hid22_Hid18Diol	41
	Hid18Diol_Hid22	
688.5	Hid18Diol_Di18Diol	39
694.6	Hid22_Di18Epox	37
	Hid18Epox_Di22	
708.7	Hid22_Hid22	22
712.6	Hid18Diol_Di22	30
	Hid22_Di18Diol	

[a] Key to monomer abbreviation in Table 1. [b] Based on the mass peaks intensity in the ESI-MS total ion chromatogram (M + Li adducts).

The relative abundance of the dimeric esters solubilized from cork suberin can be estimated by the intensity of their mass peaks in the ion chromatograms. The ω-hydroxyacid−α,ω-diacid dimeric esters were more abundant than the ω-hydroxyacid−ω-hydroxyacid dimeric esters (Figure 1, Table 2). However, as in most cases, each mass peak corresponded to two isomers, the quantitative estimation of each individual dimeric ester is difficult. Care should also be taken in this semiquantitative approach due to the eventual different ionizability of the dimeric esters in the ESI conditions.

The monomers found as part of the dimeric esters are the main ones obtained when the complete depolymerization of cork suberin is carried out,[11] namely, the 9-epoxyoctadecane-1,18-dioic acid (Di18Epox), the 9-epoxy-18-hydroxyoctadecanoic acid (Hid18Epox), the octadec-9-ene-1,18-dioic acid (Di18:1), the 18-hydroxyoctadec-9-enoic acid (Hid18:1), the 9,10-dihydroxyoctadecane-1,18-dioic acid (Di18Diol), the 9,10,18-trihydroxyoctadecanoic acid (Hid18Diol), the 22-hydroxydocosanoic acid (Hid22), the docosane-1,22-dioic acid (Di22), the 16-hydroxyhexadecanoic acid (Hid16), and the hexadecane-

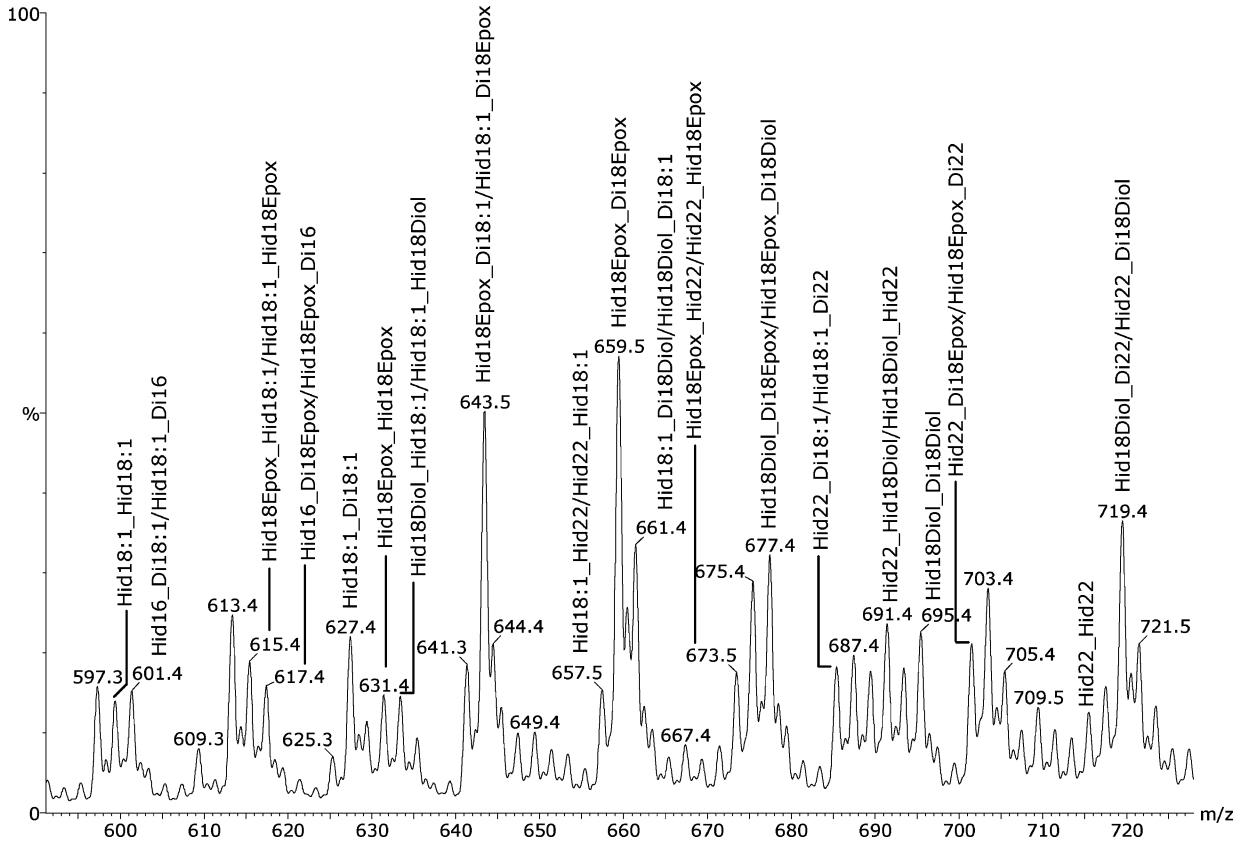


Figure 1. ESI-MS ion chromatogram of the lithiated adducts (M + Li) of the dimeric esters found after the partial depolymerization of cork suberin. Key to monomer abbreviation in Table 1.

1,16-dioic acid (Di16). The relative abundance of these monomers in the dimeric structures follow the order of their relative abundance as monomers after complete depolymerization, with the exception of the saturated chain acids. This applies particularly to the 22-hydroxydocosanoic acid (Hid22), which is commonly one of the most important monomers in cork suberin,[11] and is apparently underrepresented within the dimeric structures found. The epoxy and unsaturated ω-hydroxyacids and α,ω-diacids are the prevalent monomers in the dimeric esters. The ester of the 9-epoxy-18-hydroxyoctadecanoic acid with the 9-epoxyoctadecane-1,18-dioic acid [Hid18Epox_Di18Epox] is the most abundant dimeric ester in the partial methanolizate of cork suberin (Figure 1, Table 2).

Electrospray-MS/MS Analysis. Cork suberin partial methanolysis products and the synthesized dimeric esters were identified by ESI-MS/MS. Electrospray analysis was done after adding ionization promoters to the respective solutions. The acid formic-added solutions, analyzed in positive ion mode, showed mostly M + Na and M + K adduct ions. The ammonium hydroxide-added solutions showed, in positive mode, protonated (M + H), and ammoniated (M + NH_4) ions. In negative mode, the dimeric esters ionized very poorly. The lithium acetate-added solutions gave comparatively intense M + Li cation adducts for most of the molecules analyzed. Good fragmentation under CID-MS/MS conditions was obtained from M + NH_4 and M + Li parent ions, producing MS/MS spectra with informative daughter ions. MS/MS of M + Na ions of the molecules analyzed afforded no useful MS/MS fragmentation in the low energy CID conditions used.

MS/MS spectra from M + H and M + NH_4 parent ions of the dimeric esters showed intense fragmentation. These MS/MS spectra included ions assignable to each of the ester-linked monomers, as well as alkyl ion series. However, the diagnostic ions assignable to each monomer residue, showed intense secondary fragmentation with successive losses of 18 and 32 amu, corresponding to nominal neutral losses of water and methanol. This made the MS/MS spectra of the M + H and the M + NH_4 adducts somehow difficult to interpret. A simpler fragmentation pattern is present in the MS/MS spectra of the lithiated adducts. The daughter ion spectra of the M + Li adducts are dominated by the fragments derived from each of the two monomer moieties, with little secondary fragmentation. The fragment ions in these MS/MS spectra are both diagnostic and easy to assign. Therefore, the discussion and the identification of the dimeric esters was based in the ESI-MS/MS analysis of their lithiated adducts.

The MS/MS spectra of the lithiated adducts of some of the ω-hydroxyacid−α,ω-diacid dimeric esters from cork suberin and synthesized are shown in Figure 2. The MS/MS spectra of the lithium adducts of the ω-hydroxyacid−α,ω-diacid esters show diagnostic ions assignable to the two monomers present in the dimeric structure. The α,ω-diacid moiety gives rise to an ion, corresponding to the regenerated acid plus lithium (*m*/*z* 349, Figure 2, panels a, c, and d; *m*/*z* 333, Figure 2, panels b and c). The ω-hydroxyacid monomer residue originates an intense ion, after a loss of water (18 amu) from the monomer in its free form, plus lithium (*m*/*z* 317, Figure 2, panels b−d). However, when the ω-hydroxyacid has no secondary substituted oxygens, which is the case of the unsaturated ω-hydroxyacid (Figure 2a), this ion is very weak or absent. The presence of secondary oxygens in the middle of the hydrocarbon chain (epoxide or *vic*-diol group) leads to secondary fragments due to losses of 18 and 32 amu from the main fragment ions (Figure 2, panels a, c, and d).

An interesting and diagnostic ion, although of small intensity, is derived from the cleavage of the oxirane ring of the epoxide

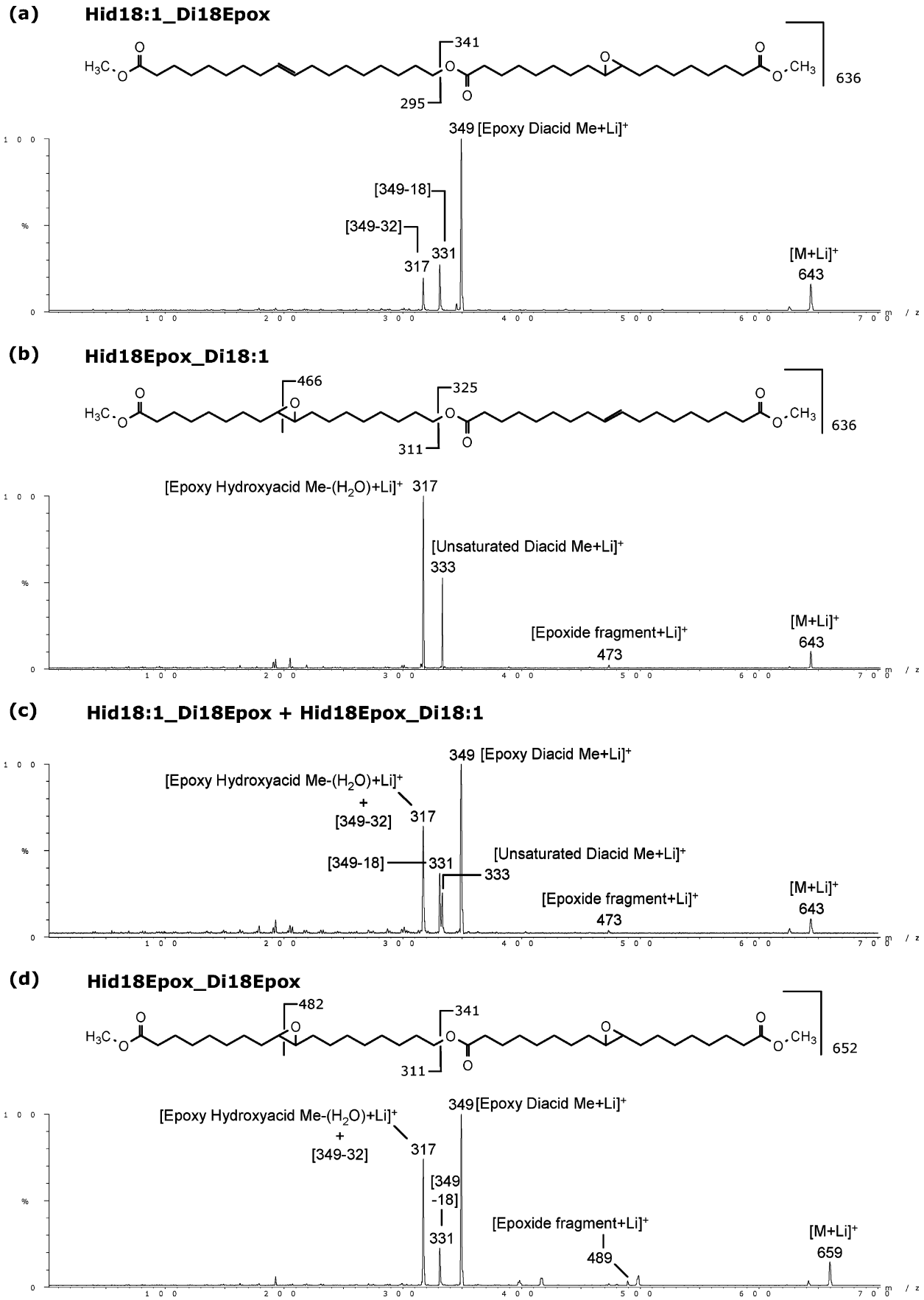


Figure 2. ESI-MS/MS spectra of the lithiated adducts of linear aliphatic dimeric esters ω-hydroxyacid-α,ω-diacid: (a) Hid18:1_Di18Epox (synthesized): 18-hydroxyoctadec-9-enoic acid (methyl ester) esterified to the 9-epoxyoctadecane-1,18-dioic acid (methyl ester); (b) Hid18Epox_Di18:1 (synthesized): 9-epoxy-18-hydroxyoctadecanoic acid (methyl ester) esterified to the octadec-9-ene-1,18-dioic acid (methyl ester); (c) mixture of Hid18:1_Di18Epox and Hid18Epox_Di18:1 (from cork suberin); (d) Hid18Epox_Di18Epox (from cork suberin): 9-epoxy-18-hydroxyoctadecanoic acid (methyl ester) esterified to the 9-epoxyoctadecane-1,18-dioic acid (methyl ester).

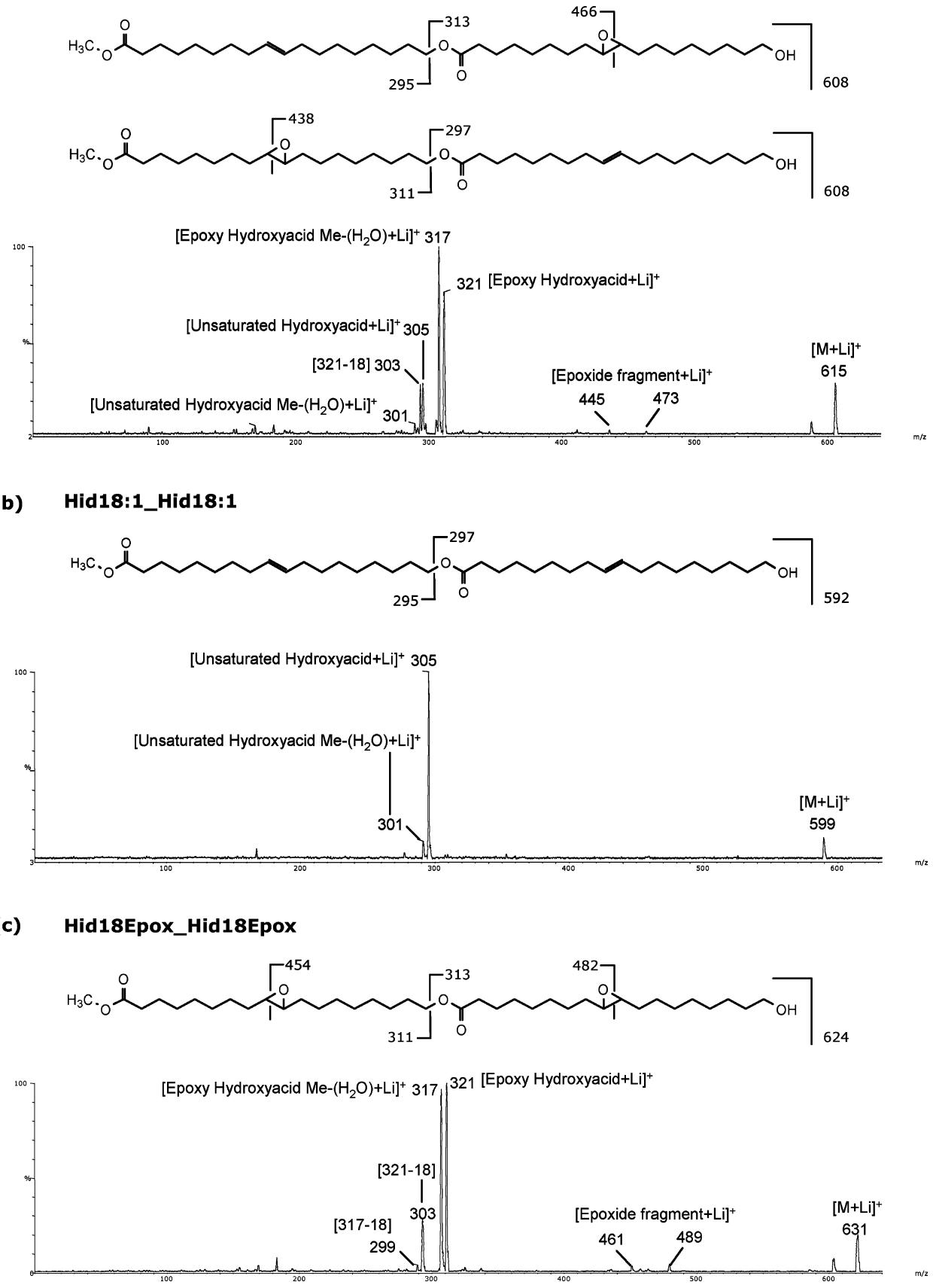


Figure 3. ESI-MS/MS spectra of the lithiated adducts of linear aliphatic dimeric esters ω-hydroxyacid-ω-hydroxyacid: (a) mixture of Hid18:1_Hid18Epox (18-hydroxyoctadec-9-enoic acid (methyl ester) esterified to 9-epoxy-18-hydroxyoctadecanoic acid), and Hid18Epox_Hid18:1 (9-epoxy-18-hydroxyoctadecanoic acid (methyl ester) esterified to 18-hydroxyoctadec-9-enoic acid), from cork suberin; (b) Hid18:1_Hid18:1 (synthesized): 18-hydroxyoctadec-9-enoic acid (methyl ester) esterified to 18-hydroxyoctadec-9-enoic acid; (c) Hid18Epox_Hid18Epox (from cork suberin): 9-epoxy-18-hydroxyoctadecanoic acid (methyl ester) esterified to 9-epoxy-18-hydroxyoctadecanoic acid.

group, when the latter is present either in the ω-hydroxyacid or α,ω-diacid monomer residues. Two of the bonds of the oxirane ring are apparently broken, producing a fragment which includes the moiety of the molecule that keeps the oxygen atom, which is further lithiated (*m*/*z* 473 in Figure 2b and *m*/*z* 489 in Figure 2d).

The MS/MS spectra of the lithium adducts of the ω-hydroxyacid−ω-hydroxyacid dimeric esters (Figure 3) showed similar fragmentation patterns as those discussed above for the ω-hydroxyacid−α,ω-diacid dimeric esters. Both ω-hydroxyacid monomer residues give rise to diagnostic fragment ions. The ω-hydroxyacid that has the acyl group in the ester bond originates an intense lithiated ion, illustrated by *m*/*z* 321 (Figure 3, panels a and c) and *m*/*z* 305 (Figure 3, panels a and b). On the other hand, the methylated ω-hydroxyacid moiety that gives the hydroxyl group to the dimeric ester originates an ion, after a nominal loss of water, plus lithium. This ion is intense if the ω-hydroxyacid residue has secondary-substituted oxygens (*m*/*z* 317 in Figure 3, panels a and c) but weak if the ω-hydroxyacid has a saturated or unsaturated chain (*m*/*z* 301 in Figure 3, panels a and b). The ion resulting from the fragmentation of the epoxide group is also present in the ω-hydroxyacid−ω-hydroxyacid dimeric esters. After addition of lithium, it gives rise to the small ions of *m*/*z* 445 and 473 in the MS/MS spectrum of the mixture of Hid18Epox_Hid18:1 and Hid18:1_Hid18Epox (Figure 3a) and the ions of *m*/*z* 461 e 489 in the MS/MS spectrum of Hid18Epox_Hid18Epox (Figure 3c). These characteristic epoxide-derived ions, although of small intensity, can be important in the interpretation of the MS/MS spectra of these compounds.

Cork Suberin Polyester Structure. Suberin is known to be a polyester biopolymer. Upon depolymerization, it releases a mixture of mostly aliphatic monomers with carboxylic acid and hydroxyl groups. The main monomers are glycerol and long-chain ω-hydroxyacids and α,ω-diacids. Small quantities of long-chain *n*-alkanoic acids and *n*-alkanols are also solubilized after suberin depolymerization.[5] In cork suberin, the more abundant ω-hydroxyacids and α,ω-diacids have chain lengths of 18C and 22C. The 22C monomers have saturated hydrocarbon chains and the 18C have substituent groups at midchain. The latter include the ω-hydroxyacids and α,ω-diacids with either an unsaturation, an epoxide group, or *vic*-diol substitution.[11] The relative proportion of each family of monomers, the dominating chain-lengths and the type and degree of substitution in the aliphatic chains, are quite variable among suberins from different plant origins.[5] The suberin polyester is also known to have extensive linkages to polyaromatics in suberized cell walls.[7,8] In fact, some authors regard "suberin" as the overall blend of the aliphatic polyester and the associated polyaromatics.[12] The discussion below will be, however, centered in the polyester aliphatic structure and suberin will be interpreted as such.

How the suberin monomers are inter-linked to build its polymeric structure has long been argued. Early works have shown that long-chain ω-hydroxyacids and α,ω-diacids were suberin monomers.[13] With the development of the GC-MS technique, the knowledge about the monomeric composition of suberin was highly improved.[14] These results led to the first tentative model for the suberin structure.[15] In this model, the linkage between the long-chain ω-hydroxyacids and α,ω-diacids was suggested. More recently, after the finding that glycerol was a major monomer in suberin, new models were proposed for the biopolymer where glycerol plays a relevant role in the polyester structure.[12] Syntheses of suberin model compounds based in glycerol, ω-hydroxyacids, and α,ω-diacids have been done, and their capacity to physically aggregate is shown.[16]

Actual knowledge about how suberin monomers are linked is based in the oligomeric structures found so far. These oligomers were obtained after the partial depolymerization of suberin and include glycerol linked to long-chain monomers in the form of monacylglycerols[4] and α,ω-diacids linked in both acid groups to glycerol in the form of diglycerol−alkenodioates.[6] In this work, we have shown the existence in cork suberin of linearly-linked long-chain monomers. Two types of dimeric esters were found: ω-hydroxyacid−α,ω-diacid and ω-hydroxyacid−ω-hydroxyacid. Linearly ester-linked ω-hydroxyacids at their primary groups are known from other natural origins. Oligomers with up to five ω-hydroxyacids have been reported in cutins, solubilized after partial hydrolysis.[17] Polyhydroxyalkanoates (PHAs), based in short-chain ω-hydroxyacids, are produced by microorganisms and have been intensively studied.[18]

The building of the suberin polyester structure will have to be based in the monomers with two or more functional groups. These include the bifunctional ω-hydroxyacids and α,ω-diacids and the tri-hydroxylated glycerol. Based on the monomer composition and the oligomers found so far, including the linear dimeric esters discussed here, two main building blocks are apparent in suberin: the glycerol−α,ω-diacid-glycerol and the ω-hydroxyacids linked head-to-tail in a linear structure. The former can ensure the cross-linking of the macromolecule through the polyfunctionality of glycerol and the latter can make concatenated chains of monomers grow. α,ω-Diacids were also found as part of the linear dimeric esters, but they cannot ensure the growing of linear chains and need to be further esterified to a hydroxyl group either from glycerol or from a ω-hydroxyacid.

The monomers found as part of the dimeric esters were mostly the ones with substituents at midchain. Saturated chain monomers, like 22-hydroxydocosanoic acid, were less represented in these dimeric esters comparatively to their importance as individual monomers in cork suberin. The 22-hydroxydocosanoic acid has been found esterified through its primary hydroxyl group to ferulic acid and further to glycerol.[7] The very long-chain saturated monomers, like the 22-hydroxydocosanoic acid, through its esterification to ferulic acid, may have a preferential role in linking the polyaliphatic suberin to polyaromatics in suberized cell walls.

More work is needed in order to achieve a better understanding of this biopolymer. The partial depolymerization of suberin and the analysis of the solubilized oligomers by ESI-MS/MS already gave new information on how suberin monomers are linked. More oligomeric structures are currently under study and expected to be elucidated.

Acknowledgment. This work was supported by the Portuguese MCES FCT Grants QUI/33411 and AGR/46419 and is part of the activities of the Centro de Estudos Florestais (BioPol Group). We also acknowledge Corticeira Amorim SA for providing the cork powders.

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BM060174U