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Chemical Composition of Hypodermal and Endodermal Cell Walls and Xylem Vessels Isolated from *Clivia miniata*[1]

Identification of the Biopolymers Lignin and Suberin

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The occurrence of the biopolymers lignin and suberin was investigated with hypodermal (HCW) and endodermal cell walls (ECW) and xylem vessels (XV) isolated from *Clivia miniata* Reg. roots. Both biopolymers were detected in HCW and ECW, whereas in XV, typical aliphatic suberin monomers were missing and only representative lignin monomers such as guaiacyl (G) and syringyl (S) units could be detected. The absolute amounts of lignin were about one order of magnitude higher compared with suberin in both HCW and ECW. The ratios of the two aromatic lignin units (G/S) decreased from 39 in XV and 10 in HCW to about 1 in ECW, indicating significant differences in lignin structure and function between the three investigated samples. Additionally, compared with the detectable lignin-derived aromatic units G and S, significantly higher amounts of esterified *p*-coumaric acid-derived aromatic monomers were obtained with HCW, but not with ECW. This is interpreted as a functional adaption of HCW toward pathogen defense at the root/soil interface. The final aim of this study was to provide a thorough chemical characterization of the composition of HCW, ECW, and XV, which in turn will form the basis for a better understanding of the relevant barriers toward the passive, radial, and apoplastic diffusion of solutes from the soil across the root cortex into the root cylinder.

The possible role of the root endodermis as an apoplasmatic transport barrier has been studied extensively (Clarkson, 1991). These transport-limiting properties are essentially based on the chemical composition and structure of ECW. As concluded from indirect and sometimes nonspecific histochemical methods, the chemical nature of ECW is reported to be cutin-, suberin-, and/or lignin-like (Priestley and Rhodes, 1926; van Fleet, 1961; Wilson and Peterson, 1983). To date, only a few attempts have been made to provide a detailed chemical characterization of ECW. Whereas one investigation described the presence of suberin in the Casparian strip of etiolated stems of *Sorghum bicolor* (Espelie and Kolattukudy, 1979), it has been shown that the ECW of *Clivia miniata* roots contain lignified anticlinal walls (Schreiber et al., 1994; Schreiber, 1996).

Both lignified and suberized cell walls represent a characteristic feature of plant tissues associated with specialized physiological functions. Lignin is a complex and highly variable biopolymer derived from oxidative polymerization of the cinnamyl alcohols *p*-coumaryl, coniferyl, and sinapyl alcohol (Freudenberg, 1965; Campbell and Sederoff, 1996). The content of the three monomeric units can vary considerably: gymnosperm (softwood) lignin essentially consists of G, dicotyledon angiosperm (hardwood) lignin is composed of G and S, and monocotyledon angiosperm (grass) lignin represents a mixture of H, G, and S (Higuchi et al., 1967; Nimz, 1974; Boudet et al., 1995). In terms of functionality, lignin is reported to provide mechanical stability (Monties, 1989) and to form one of several plant responses toward the defense of pathogens (Lange et al., 1995).

The biopolymer suberin consists of an aliphatic and an aromatic domain (Kolattukudy, 1984). The major aliphatic constituents are esterified ω-hydroxy fatty acids and 1,ω-dicarboxylic acids. However, structural knowledge of the aromatic part is not well established (Schmutz et al., 1996). Although it has been reported that esterified hydroxycinnamic acids are the major constitutive components (Bernards et al., 1995), a lignin-like structure has also been postulated for the polyaromatic domain (Kolattukudy, 1980; Lapierre et al., 1996). Suberin is known to act as a barrier toward the movement of water and solutes and toward microbial attack (Kolattukudy, 1984).

Thus, on the basis of their functional and structural properties, both polymers, lignin and suberin, might occur in the ECW of plant roots. Additionally, HCW might also be composed of these two types of polymers, because the role of the hypodermis as a primary apoplastic barrier toward the passive radial diffusion of solutes into the root has been discussed recently (Frensch et al., 1996). XV of

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Abbreviations: ECW, endodermal cell walls; G, guaiacyl units; H, hydroxyphenyl units; HCW, hypodermal cell walls; S, syringyl units; XV, xylem vessels.

the central cylinder of the root were also investigated, because there is no doubt about their lignification and they therefore serve as an internal standard for lignin. The aim of the present work was to provide a thorough chemical characterization of ECW, HCW, and XV isolated from *C. miniata* Reg. roots. Knowledge of the chemical composition will finally help in understanding the function and importance of these different root tissues as an apoplastic barrier toward the passive radial diffusion of solutes from the soil across the root cortex into the central cylinder of the root.

MATERIALS AND METHODS

Isolation and Purification of Cell Walls

Cell wall samples were obtained according to the method described in detail by Schreiber et al. (1994). Roots were sampled from full-grown plants of *Clivia miniata* Reg. growing in the greenhouse of the Botanical Garden in Würzburg. The hypodermis was carefully removed from pieces of roots (50 mm in length) without damaging the central cylinder. Hypodermal tissues and the central cylinders were incubated at room temperature in a solution of cellulase (Onozuka R-10, Serva, Heidelberg, Germany) and pectinase (Macerozyme R-10, Serva) dissolved in 10^{-2} M citric buffer (pH 3.0). Sodium azide (final concentration 10^{-3} M) was added to prevent microbial growth. After about 1 to 2 weeks, enzymatic digestion was complete and the nondegradable cell wall fraction could be isolated mechanically. ECW were separated from the XV using two forceps and the aid of a binocular microscope (model SZ 30, Olympus, Hamburg, Germany). Isolated cell wall materials were extracted with a mixture of chloroform and methanol (1:1, v/v) at 50°C for 3 h, oven-dried at 50°C, and stored at room temperature over silica gel.

Chemical Degradation Methods

Thioacidolysis

Thioacidolysis was carried out using the method described by Rolando et al. (1992). The sample was added to a mixture of BF_3 etherate (Merck, Darmstadt, Germany), ethanethiol (Fluka, Neu-Ulm, Germany), and dioxane, stirred for 4 h at 100°C, and extracted with $CHCl_3$ containing dotriacontane (Fluka) as an internal standard.

Desulfurization

Desulfurization was performed basically following the method of Lapierre et al. (1991). An aliquot of the thioacidolysate $CHCl_3$ solution was evaporated to dryness and treated for 4 h with Raney-Nickel aqueous slurry (Fluka) at 80°C and extracted with $CHCl_3$.

Cupric Oxide Oxidation

Alkaline cupric oxide oxidation was performed according to the method described by Chen (1992). The sample was added to a solution of $CuSO_4$ in 3 M NaOH and heated for 2.5 h to 170°C and extracted with $CHCl_3$ containing dotriacontane as an internal standard.

Treatment with BF_3/MeOH

BF_3/MeOH treatment was carried out basically according to the method of Kolattukudy and Agrawal (1974). The sample was kept at 70°C for 24 h in a 10% BF_3/MeOH solution (Fluka) and extracted with $CHCl_3$ containing dotriacontane as an internal standard.

Chromatographic Analysis of the Reaction Products

Chloroform solutions were concentrated and treated with 30 μL of pyridine and 30 μL of *N,N*-bis-trimethylsilyltrifluoroacetamide (Machery-Nagel, Düren, Germany) at 70°C for 30 min to convert free hydroxyl and carboxyl groups into their corresponding trimethylsilyl derivatives. Quantitative analysis was carried out by injecting 1 μL of the silylated samples into a gas chromatograph (model 5890 Series II, Hewlett-Packard) equipped with a flame ionization detector. Qualitative analyses were obtained by injecting 1 μL into the gas chromatograph combined with a quadrupole mass selective detector (model 5971A, Hewlett-Packard). Mass spectra were recorded at 70 eV. Gas chromatographs were equipped with fused silica capillary columns having an i.d. of 0.32 mm and a film thickness of 0.1 mm (DB-1, Fisons, Folsom, CA).

Degradation products were identified by comparison with reference spectra from literature data (Kolattukudy and Agrawal, 1974; Holloway, 1982; Goni and Hedges, 1990, 1992; Lapierre et al., 1991; Rolando et al., 1992) or by structure elucidation from fragmentational pattern. Whenever possible, Fourier transform IR spectra (model 5965B IR detector, Hewlett-Packard) were used to confirm the proposed structures. Adequate correction factors were determined for aromatic and aliphatic monomers using commercially available standard substances. Because of the inavailability of dimeric standards, correction factors for dimeric degradation products were set equal to 1 according to the method of Lapierre et al. (1991). Reproducibility of the applied chemical degradation methods was estimated for cupric oxide oxidation of HCW. The analysis of three independent samples of HCW resulted in a coefficient of variation of 10%.

RESULTS

Aromatic Monomers Obtained by Thioacidolysis

The thioacidolysis reaction was performed by treating the samples with ethanethiol and BF_3 etherate (Fig. 1A). The main degradation products from lignins were mono-

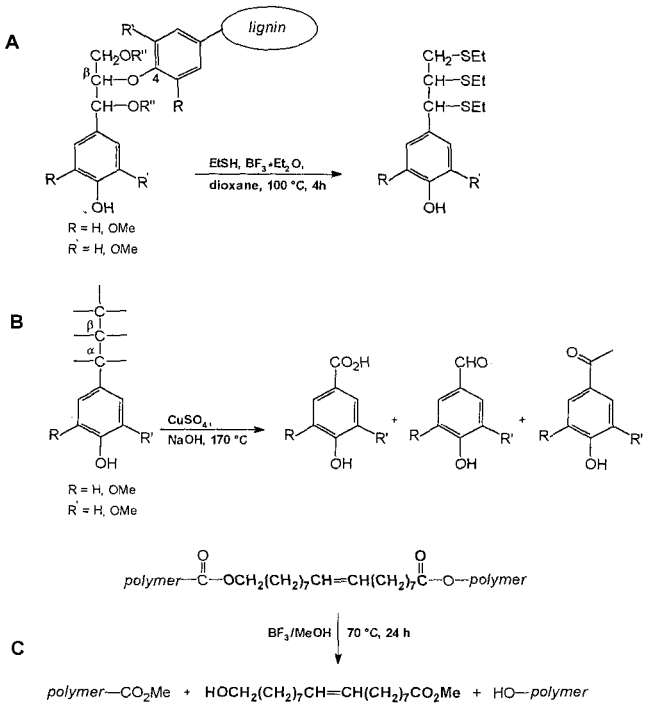


Figure 1. Reaction mechanisms of the three applied degradation methods. A, Origin and main monomeric products obtained after thioacidolysis of lignified cell walls. Predominantly aryl-glycerol-β-arylether linkages (β-O-4) are cleaved. *p*-Hydroxyphenyl unit (H): R = R' = H; guaiacyl unit (G): R = H and R' = OMe; syringyl unit (S): R = R' = OMe. B, The three dominant, monomeric reaction products obtained after cupric oxide oxidation of lignified cell walls. C, Conversion of oleic acid esterified in a polymer to the resulting methylester of the released acid as an example for the transesterification reaction by BF_3/MeOH.

meric trithioethylated aromatic compounds (Fig. 2), which were derived from G (*1*) and S (*5*) and in minor amounts from H (*8*) (see Fig. 1A). ECW released approximately the same number of G and S, whereas in XV and HCW, G clearly predominated over S (Fig. 2). Quantitatively, the sum of G and S decreased from XV (7.6%) over ECW (4.6%) to HCW (2.4%) (Fig. 2). In addition, HCW showed significant amounts (5.7%) of H-derived monomers (Fig. 2). Because the trithioethylated compound (*8*) was only a minor component (0.5%), the most abundant monomers were derived from *p*-coumarates (*9*) and (*10*) (Fig. 2).

Aromatic Monomers Obtained by Cupric Oxide Oxidation

Degradation of lignified tissue by alkaline cupric oxide oxidation, which is based on the oxidative cleavage of the propanoid side chain at α- or β-positions (Fig. 1B), released aromatic carboxy acids, aldehydes, or acetyl derivatives of the three lignin precursors H, G, and S (Fig. 3). Results obtained with cupric oxide oxidation (Fig. 3) were quantitatively and qualitatively in good agreement with thioacidolysis (Fig. 2).

Aromatic Monomers Obtained by Treatment with BF_3/MeOH

After treatment with BF_3/MeOH (Fig. 1C), aromatic monomers were released from the three investigated samples (Fig. 4). High amounts of esterified coumaric acid methyl ester (*34*) (4.7%) and the corresponding Michael addition product (*35*) (0.3%) were released from HCW (Fig. 4). To a much lesser extent, the three samples showed the presence of other ester-bound aromatic compounds, namely ferulic acid (*25*), vanillin (*24*), vanillic acid (*23*), syringaldehyde (*30*), and syringic acid (*29*) (Fig. 4). In relatively small amounts, lignin-related aromatic degradation products mainly consisting of G (*21*, *22*) and S (*26*, *27*, *28*) were obtained after BF_3/MeOH treatment. They were identified according to their mass spectrometric fragmentation patterns (Fig. 5). IR spectra gave additional evidence for the proposed structures (Table I). Compared with thioacidolysis (Fig. 2) and cupric oxide oxidation (Fig. 3), the quantitative yield of lignin-derived degradation products from BF_3/MeOH treatment (Fig. 4) was low (0.5%). S were cleaved more readily then G.

Aromatic Dimers Obtained by Thioacidolysis

Desulfurization of the reaction mixture with Raney-Nickel aqueous slurry after thioacidolysis (Fig. 1A) al-

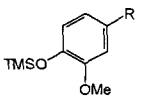
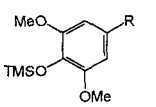
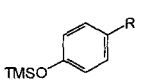
aromatic cores	side chains R		HCW (µg mg^{-1})	ECW (µg mg^{-1})	XV (µg mg^{-1})
guaiacyl	CHSEt-CHSEt-CH_2SEt	(1)	16.4	20.1	59.7
	CH_2-CHSEt-CH(SEt)$_2$	(2)	1.8	2.6	5.9
	CH_2-CH(SEt)$_2$	(3)	1.4	2.2	3.2
	CHSEt-CH_2-CH_2SEt	(4)	1.9	---	---
	total		**21.5**	**24.9**	**74.2**
syringyl	CHSEt-CHSEt-CH_2SEt	(5)	1.6	17.1	1.9
	CH_2-CHSEt-CH(SEt)$_2$	(6)	0.5	2.6	---
	CH_2-CH(SEt)$_2$	(7)	---	1.7	---
	total		**2.1**	**21.2**	**1.9**
total G + S			**23.6**	**46.1**	**76.1**
ratio G/S			**10.2**	**1.2**	**39.1**
p-hydroxyphenyl	CHSEt-CHSEt-CH_2SEt	(8)	4.6	---	---
	CH=CH-CO_2TMS	(9)	32.5	---	---
	CHSEt-CH_2-CO_2TMS	(10)	19.9	---	---
	total		**57.0**	---	---

Figure 2. Monomeric aromatic compounds obtained after thioacidolysis of HCW, ECW, and XV from *C. miniata* roots. The trimethylsilyl (TMS)-derivatized aromatic cores of the three lignin units G, S, and H with the respective thioethylated and TMS-derivatized side chains (R) are given to identify the different detected compounds.

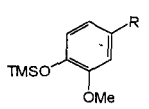
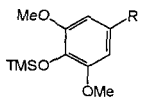
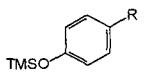
aromatic cores	side chains R		HCW	ECW	XV
			(μg mg^{-1})	(μg mg^{-1})	(μg mg^{-1})
guaiacyl	CO_2TMS	(11)	1.4	30.6	8.1
	CHO	(12)	12.8	---	42.2
	$COCH_3$	(13)	1.6	1.3	6.0
	total		**15.8**	**31.9**	**56.3**
syringyl	CO_2TMS	(14)	1.6	24.6	---
	CHO	(15)	0.5	---	1.4
	$COCH_3$	(16)	---	1.2	---
	total		**2.1**	**25.8**	**1.4**
total G + S			**17.9**	**57.7**	**57.7**
ratio G/S			**7.5**	**1.2**	**38.8**
p-hydroxyphenyl	CO_2TMS	(17)	0.1	---	---
	CHO	(18)	7.2	---	---
	$COCH_3$	(19)	1.4	---	---
	$CH{=}CH{-}CO_2TMS$	(20)	72.0	---	---
	total		**80.7**	---	---

Figure 3. Monomeric aromatic compounds obtained after alkaline cupric oxide oxidation of HCW, ECW, and XV from *C. miniata* roots. The trimethylsilyl (TMS)-derivatized aromatic cores of the three lignin units G, S, and H with the respective TMS-derivatized side chains (R) are given to identify the different detected compounds.

lowed the quantitation of dimeric lignin-derived compounds by GC due to the greater volatility of the desulfurized dimers (Fig. 6). Structural components of the dimers recovered from ECW were G (*36, 37, 38, 43, 45*) and S (*39, 40, 44*), whereas XV solely consisted of G. In HCW, G dominated, but one dimer consisting of G and H (*42*) was also present. From a quantitative point of view, most dimers were released from XV (3.2%) and ECW (2.5%), whereas HCW tissue had the lowest content of lignin-derived dimers (0.4%) (Fig. 6).

Aromatic Dimers Obtained by Cupric Oxide Oxidation

Lignin-derived dimeric degradation products were also detectable after alkaline cupric oxide oxidation (Fig. 1B). Again, ECW released G/G (*49, 50, 51, 55, 58*) and G/S dimers (*56*), XV were solely composed of G/G (*47-50, 52, 53, 55, 57*) dimers, and HCW released H/G (*46, 54*) and G/G dimers (Fig. 7). Quantitatively, XV released more dimeric compounds (1.1%) than ECW (0.3%) or HCW (0.3%) (Fig. 7).

Aliphatic Monomers Obtained by Treatment with BF_3/MeOH

Besides aromatic degradation products, esterified long-chain fatty acid derivatives typical of suberin, i.e. ω-hydroxyacids and α,ω-dicarboxylic acids, were released from the wall samples by treatment with BF_3/MeOH (Fig. 1C). Significant amounts of 18-hydroxy-octadec-9-enoic acid and octadec-9-ene-1,18-dioic acid were detectable in HCW and ECW, respectively (Fig. 8A). Additionally, ECW showed smaller amounts of 16-hydroxyhexadecanoic acid and a distribution of diacids, with an even number of C-atoms reaching from C_{16} to C_{22} (Fig. 8A). XV only released minor amounts of typical suberin acids (Fig. 8A).

Aliphatic Monomers Obtained by Cupric Oxide Oxidation

Aliphatic monomers were also obtained after cupric oxide oxidation (Fig. 1B), because the alkaline conditions led to a saponification of suberin by NaOH. Qualitative and quantitative composition of released monomers (Fig. 8B) were in good agreement with results obtained after BF_3/MeOH treatment (Fig. 8A). However, cupric oxide oxidation led to a complete oxidation of ω-hydroxyacids to 1,ω-dicarboxylic acids in the case of the ECW (Fig. 8B).

DISCUSSION

It was the aim of this investigation to identify and characterize the biopolymers lignin and suberin in HCW, ECW,

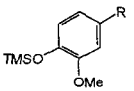
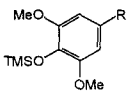
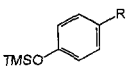
aromatic cores	side chains R		HCW	ECW	XV
			(μg mg^{-1})	(μg mg^{-1})	(μg mg^{-1})
guaiacyl	$CH(OMe){-}CH(OMe)_2$	(21)	---	0.2	0.7
	$CH(OMe){-}CO{-}CH_3$	(22)	---	---	0.4
	CO_2Me	(23)	0.5	0.7	1.0
	CHO	(24)	0.5	0.3	0.7
	$CH{=}CH{-}CO_2Me$	(25)	0.4	0.7	0.3
	related guaiacyl compounds		---	0.4	1.2
	total		**1.4**	**2.3**	**4.3**
syringyl	$CH(OMe){-}CH(OMe)_2$	(26)	---	1.6	0.4
	$CH(OMe){-}CO{-}CH_3$	(27)	---	0.7	0.1
	$CH(OMe){-}CH(OMe){-}CH_2OTMS$	(28)	---	2.3	0.5
	CO_2Me	(29)	---	0.9	0.2
	CHO	(30)	---	0.7	---
	related syringyl compounds		---	1.2	0.2
	total		---	**7.4**	**1.4**
total G + S			**1.4**	**10.7**	**5.7**
ratio G/S			---	**0.2**	**3.1**
p-hydroxyphenyl	$CH_2{-}CH(OMe){-}CH(OMe)_2$	(31)	0.3	---	---
	CO_2Me	(32)	0.3	---	---
	CHO	(33)	0.5	---	---
	$CH{=}CH{-}CO_2Me$	(34)	47.0	0.2	0.4
	$CH(OMe){-}CH_2{-}CO_2Me$	(35)	3.1	---	---
	total		**51.2**	**0.2**	**0.4**

Figure 4. Monomeric aromatic compounds obtained after treatment of HCW, ECW, and XV from *C. miniata* roots by BF_3/MeOH. The trimethylsilyl (TMS)-derivatized aromatic cores of the three lignin units G, S, and H with the respective methylated and TMS-derivatized side chains (R) are given to identify the different detected compounds.

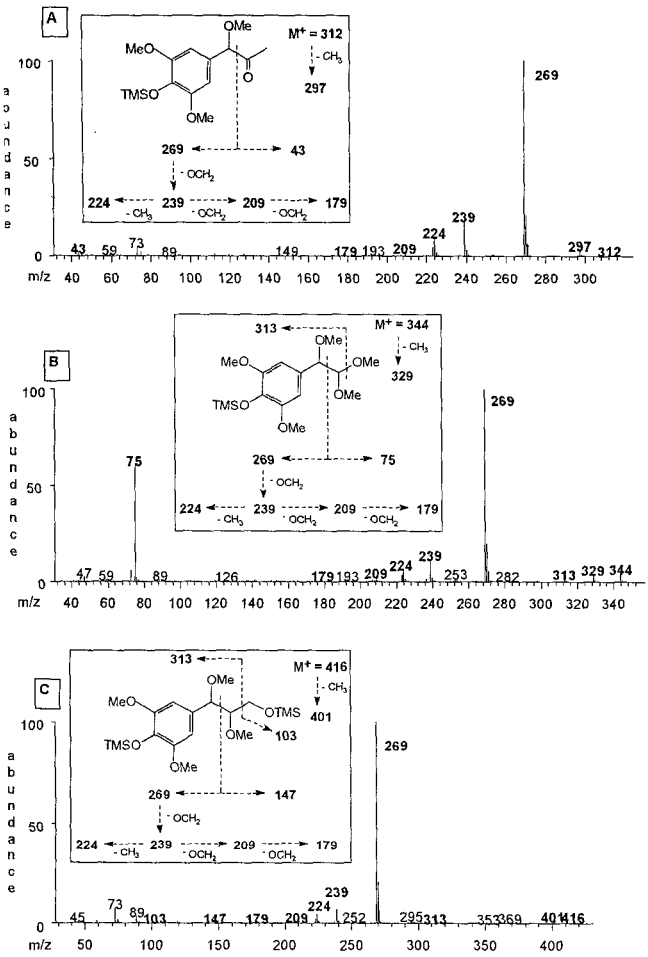


Figure 5. Mass spectra and interpretation of the fragmentation pattern of three syringyl-derived aromatic compounds obtained after treatment by BF_3/MeOH. A, Syringyl-derived lignin monomer with a molar mass of 312 carrying one methoxy group in the ethanoid side chain. B, Syringyl-derived lignin monomer with a molar mass of 344 carrying two methoxy groups in the propanoid side chain. C, Syringyl-derived lignin monomer with a molar mass of 416 carrying two methoxy groups in the propanoid side chain. The boxes in the mass spectra give the molecular structures of the compounds derived from their mass spectra and their fragmentation patterns.

and XV isolated from *C. miniata* roots. To accomplish this goal, three independent degradation methods, thioacidolysis, cupric oxide oxidation, and BF_3/MeOH treatment, which are generally used for the analysis of lignin and suberin (Pepper et al., 1967; Kolattukudy and Agrawal, 1974; Goni and Hedges, 1990; Chen, 1992; Rolando et al., 1992), were applied to the three samples. Therefore, the following discussion will basically focus on three aspects: (a) The different methods used in this investigation will be compared and evaluated; (b) the qualitative and quantitative chemical composition of the three investigated cell wall samples will be compared and discussed; and (c) some hypothesis concerning the biological functions of the chemical composition of the various polymers detected in the different investigated cell wall samples will be formulated.

Evaluation of the Applied Methods

In the past, various degradative chemical methods have been developed for investigation of different plant biopolymers. For the identification and chemical characterization of lignin, thioacidolysis (Fig. 1A; Rolando et al., 1992) and alkaline cupric oxide oxidation (Fig. 1B; Pepper et al., 1967; Chen, 1992) have been used. These methods provide knowledge about the monomeric composition of lignin (Figs. 2 and 3), but they also give structural information about interunitary linkages, because dimers can also be obtained with these methods (Figs. 6 and 7; Lapierre et al., 1991; Goni and Hedges, 1992). Comparing the quality and quantity of lignin-derived monomeric degradation products, thioacidolysis and alkaline cupric oxide oxidation gave similar results (Figs. 2 and 3). This illustrates that both methods are suited for the chemical characterization of the lignins under examination. The quantitative agreement of both methods further suggests that similar bonding patterns in the biopolymer are degraded. However, in contrast to cupric oxide oxidation (Fig. 3), thioacidolysis leads to degradation products retaining features of side chain functionality (Fig. 2) and therefore provides additional structural information (Boudet et al., 1995).

With the identified dimeric degradation products, a good qualitative agreement between both methods was also evident (Figs. 6 and 7). However, a reliable quantitative description of dimeric reaction products was difficult (Figs. 6 and 7) because GC response factors have only been attainable for the monomers. This helps to explain the quantitative differences between dimers obtained with thioacidolysis and those obtained with cupric oxide oxidation.

The monomeric composition of suberin can be characterized by acid- or base-catalyzed trans- or deesterification reactions (Kolattukudy et al., 1975) or by cupric oxide oxidation (Goni and Hedges, 1990). In the case of suberin, both treatment with BF_3/MeOH (Fig. 8A) and cupric oxide oxidation (Fig. 8B) provided comparable information. However, a serious disadvantage of cupric oxide oxidation was the partial oxidation of the ω-hydroxyacids to diacids, which occurred with ECW (Fig. 8B) if the reaction was not strictly performed under conditions excluding oxygen.

It is an important observation that different methods such as thioacidolysis and cupric oxide oxidation, in the case of the aromatic lignin monomers and dimers, and treatment with BF_3/MeOH and cupric oxide oxidation, in the case of aliphatic suberin monomers, provide similar results. The methods of choice for a thorough characterization of potentially suberized or lignified tissue should be a combination of BF_3/MeOH treatment and thioacidolysis with subsequent desulfurization due to the retainment of the functionalities of the aliphatic suberin monomers and the side chains of the aromatic lignin monomers and dimers. On the other hand, alkaline cupric oxide oxidation has the striking advantage that samples can be tested for lignin and suberin simultaneously, in only one degradative reaction. Thus, for a qualitative investigation simply establishing the existence of the two biopolymers lignin and suberin, cupric oxide oxidation could be the method of choice; aromatic lignin monomers and dimers and esteri-

Table I. *IR spectral data of the three S-derived aromatic compounds shown in Figure 5, A, B, and C*

Wave numbers (ν cm^{-1}) of the three different compounds are given with their respective intensities in parentheses. w, Weak; m, middle; s, strong; vs, very strong; sh, shoulder.

Vibration Mode	Wave Numbers		
	Compound A (*27*)	Compound B (*26*)	Compound C (*28*)
		ν cm^{-1}	
ν (C-H) aromatic	3000 (w)	3000 (w)	3000 (w)
ν (C-H) aliphatic	2945 (m)	2945 (m)	2965 (m), 2940 (m)
ν (C-H), OCH_3	2840 (m)	2840 (m)	2835 (w)
ν (C=O)	1730 (m)	–[a]	–
ν (C=C) aromatic	1585 (m), 1505 (s)	1585 (w), 1505 (m)	1585 (w), 1500 (s)
δ (C-H), CH_2	1465 (sh)	1460 (w)	1465 (w)
δ (C-H), OCH_3	1420 (m)	1420 (w)	1420 (w)
δ (C-H), CH_3 and	1340 (s)	1335 (m)	1330 (m)
δ (C-H), $COCH_3$ (*27*)	–	–	–
ν (C-O)	1255 (s)	1250 (m), 1190 (w)	1255 (s)
ν (C-O), OCH_3	1115 (s)	1120 (vs)	1115 (vs)
ν (Si-O)	920 (s), 850 (m)	920 (m), 850 (m)	920 (s), 845 (s)

[a] —, Wave numbers have not been detected.

fied aromatic and aliphatic suberin monomers can be detected simultaneously.

Nevertheless, it has to be mentioned that only about 10% of the total amount of isolated cell wall material was characterized by GC following degradation. There are two possible reasons for this observation. First, the applied methods are not able to cleave carbon-carbon bonds occurring in lignins, and degraded units larger than dimers are not volatile enough to be detected by GC. Second, polar degradation products such as amino acids or carbohydrates are lost during workup procedures and cannot be identified. Therefore, other methods have to be used in future studies to prove the existence of proteins and nondigested carbohydrates in cell wall isolates.

Comparison of the Investigated Cell Wall Samples

A qualitative consideration of the identified lignins (Figs. 2 and 3) reveals that ECW (G- and S-rich), XV (G-rich), and HCW lignin (H- and G-rich) resemble hardwood, softwood, and grass lignins, respectively, in their monomeric composition (Boudet et al., 1995). The distribution of H, G, and S in the identified dimers confirms the above classification (Figs. 6 and 7). This allows the conclusion that those regions of the lignin polymer that are not accessible to sufficient chemical degradation will presumably contain similar ratios of lignin units, which is the case with the monomers and the dimers identified in this study. Thus, our findings suggest that the chemical nature of the constituent units in a special lignin can be appreciated by the recovered monomers. Furthermore, it is important to realize that similar and constant ratios of H, G, and S were found with several independent depolymerization methods (thioacidolysis, cupric oxide oxidation, and desulfurization) with all three investigated samples HCW, ECW, and XV (Figs. 2, 3, 6, and 7). A quantitative comparison of the three samples under investigation shows that the total amounts of detected lignin (i.e. the sum of released monomers and dimers of one degradative method; Figs. 2, 3, 6, and 7) decreased from XV (7–11%) over ECW (6–7%) to HCW (2–3%). Thus, using the *C. miniata* roots investigated in this study as an example, the nature of lignins in different tissues of the same species (HCW, ECW, and XV) or even of the same plant organ (the root) shows considerable variation.

Although treatment with BF_3/MeOH is an established method for the degradation of ester-linked polymers, it would not appear to be suitable for the degradation of lignin. However, aromatic degradation products bearing methoxyl groups in their side chains could be identified after BF_3/MeOH treatment (Fig. 4), indicating that weak ether bonds in the lignin polymer were also cleaved by this method. One remarkable feature of these compounds was the presence of various methoxyl substituents in their propanoid or ethanoid side chains (Fig. 4). The mass spectra of S-derived compounds were dominated by an intensive base peak at m/z 269 resulting from α-cleavage adjacent to a methoxyl group in α-position of the side chain (Fig. 5) generating a stabilized benzylic cation. Accordingly, G derivatives carrying only one methoxy group in their aromatic rings showed a base peak at m/z 239 (spectra not shown). Another characteristic feature of the spectra were subsequent losses of formaldehyde, indicating the existence of aromatic and benzylic methoxyl groups (Fig. 5). IR spectra (Table I) gave further convincing evidence for the proposed molecular structures (Figs. 4 and 5). For example, the keto-function in compound A is confirmed by a carbonyl band at 1730 cm^{-1} (Table I). Furthermore, a strong band at 1115 to 1120 cm^{-1}, which can be assigned to the C-O stretching mode in methoxyl groups, had the lowest relative intensity in A (*27*), medium relative intensity in C (*28*), and the highest relative intensity in B (*26*), supporting the existence of three, four, and five methoxyl groups, respectively (Table I). Therefore, results presented here provide for the first time to our knowledge convincing evidence that aromatic lignin

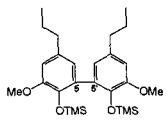
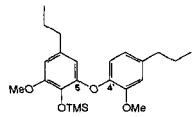
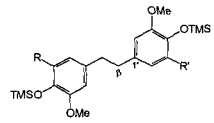
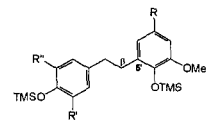
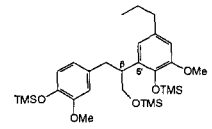
aromatic dimers	side chains		HCW (μg mg^{-1})	ECW (μg mg^{-1})	XV (μg mg^{-1})
		(36)	1.2	4.9	7.3
		(37)	1.4	2.1	3.3
	R=R'=H	(38)	0.5	3.9	6.5
	R=H, R'=OMe	(39)	---	2.1	---
	R=R'=OMe	(40)	---	3.2	---
	R=Et, R'=OMe, R''=H	(41)	---	---	1.8
	R=Pr, R'=R''=H	(42)	0.3	---	---
	R'=OMe, R''=H	(43)	0.7	4.7	13.5
	R'=R''=OMe	(44)	---	2.4	---
		(45)	---	1.5	---
total			**4.1**	**24.8**	**32.4**

Figure 6. Dimeric aromatic compounds obtained after thioacidolysis and subsequent Raney-Nickel desulfurization of HCW, ECW, and XV from *C. miniata* roots. The trimethylsilyl (TMS)-derivatized aromatic cores of the dimers with the respective functional moieties of the side chains (R and R') are given to identify the different detected compounds. Et, Ethyl; Pr, Propyl.

monomers can also be released from the polymer by treatment with BF_3/MeOH.

Dimeric degradation products contain valuable information about interunitary linkages between the different lignin precursors, H, G, and S (Figs. 6 and 7). Dimers obtained after thioacidolysis by desulfurization of the reaction mixture with Raney-Nickel aqueous slurry showed ether linkages (5-O-4') and carbon-carbon linkages (5–5', β-1', β-5') (Fig. 6), which are typical bonding patterns in lignin (Adler, 1977; Higuchi, 1981; Lapierre, 1991). With cupric oxide oxidation (Fig. 7) interunitary bonding patterns were 5–5', 5-O-4', β-1', α-1', and α-5' (Fig. 7), the three former linkages also occurring with dimers obtained in thioacidolysis (Fig. 6). If aliphatic bridges between aromatic nuclei are present similar to those in β-1'-, α-1'-, and α-5'-substructures, their carbon atoms are oxidized during cupric oxide oxidation, forming carbonyl functions (Fig. 7). Furthermore, during cupric oxide oxidation nonbridging side chains of the dimers, i.e. in the 5–5'- and 5-O-4'-compounds (Figs. 6 and 7), are cleaved in the α- or β-position to yield carboxylic acids, aldehydes, or acetofunctions (Fig. 7), as observed in monomeric degradation products (Fig. 3).

Comparing the interunitary linkages found in the lignins of the different root cell wall samples no "tissue-specifity" was detectable. It can be stated that β-O-4, which is cleaved by the degradative reactions, 5–5', 5-O-4', β-1', β-5', α-1', and α-5' linkages, occurred in all tissues under examination (Figs. 6 and 7). The aromatic polymer fraction showing these chemical bonding patterns should be designated as lignin. In contrast, HCW contained 5 to 7% of esterified coumaric acid and, in minor amounts, ferulic acid (Fig. 4). These ester-bound aromatics might be of suberin origin (Riley and Kolattukudy, 1975; Bernards et al., 1995). The consequences of this interpretation would be that the presence of suberin is strongly associated with the presence of lignin, which itself could serve as a matrix for the suberin components (Kolattukudy, 1984). Furthermore, suberin may or may not have an aromatic domain. In our study, the first type of suberin would be realized in HCW, the latter in ECW. However, it must be added that *p*-coumaric acid derivatives may also be associated with polysaccharides and lignins, as was demonstrated with maize (Ralph et al., 1994). Looking more closely at the quantitative occurrence of the aliphatic suberin components it is obvious that they decreased from ECW (1%) over HCW (0, 6%) to XV (0–0, 2%) (Fig. 8).

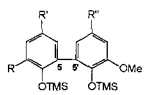
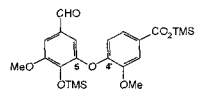
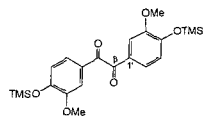
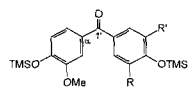
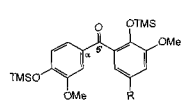
aromatic dimers	side chains		HCW (μg mg^{-1})	ECW (μg mg^{-1})	XV (μg mg^{-1})
	R=H, R'=R''=CHO	(46)	0.3	---	---
	R=OMe, R'=R''=CHO	(47)	1.0	---	3.2
	R'=CHO, R''=$COCH_3$	(48)	0.3	---	1.5
	R'=CHO, R''=CO_2TMS	(49)	0.4	0.2	1.8
	R'=$COCH_3$, R''=CO_2TMS	(50)	---	0.7	1.3
	R'=R''=CO_2TMS	(51)	---	0.6	---
		(52)	0.4	---	0.9
		(53)	---	---	0.3
	R=R'=H	(54)	0.1	---	---
	R=H, R'=OMe	(55)	0.1	0.2	0.3
	R=R'=OMe	(56)	---	0.4	---
	R=CHO	(57)	0.4	---	1.3
	R=CO_2TMS	(58)	---	0.7	---
total			**3.0**	**2.8**	**10.6**

Figure 7. Dimeric aromatic compounds obtained after alkaline cupric oxide oxidation of HCW, ECW, and XV from *C. miniata* roots. The trimethylsilyl (TMS)-derivatized aromatic cores of the dimers with the respective TMS-derivatized, functional moieties of the side chains (R and R') are given to identify the different detected compounds.

Biological Relevance of the Results

According to Kroemer (1903), the root endodermis can pass through three consecutive stages. The primary endodermis is characterized by a thickening of the anticlinal cell walls known as Casparian strips. In the secondary developmental stage, a suberin lamella is deposited onto the inner surface of the cell wall. Further deposition of lignified cell wall material onto the suberin lamella occurs in the tertiary stage. Recently, it has been shown by scanning electron microscopy (Schreiber et al., 1994) that the root endodermis of *C. miniata* remains predominantly in the primary stage of development. After enzymatic isolation, most of the periclinal walls were digested, whereas anticlinal walls restricted enzymatic degradation. Only small amounts of periclinal walls from older parts of the roots also restricted enzymatic digestion. Thus, the isolated ECW used in this study can be regarded as predominantly pure Casparian strips.

Recently, the occurrence of lignin in Casparian bands isolated from *C. miniata* has been described, but information on the occurrence of suberin was still missing (Schreiber, 1996). This question can now be answered positively, because long-chain hydroxy- and dicarboxylic acids, which are typical suberin components (Kolattukudy, 1984), have been detected as constituents of *C. miniata* Casparian bands (Fig. 8). Therefore, direct chemical evidence is provided that, in addition to lignin, suberin is also part of the cell wall polymer forming the Casparian strip in roots of *C. miniata*. However, the quantitative amount of aliphatic suberin components detected in the Casparian strips is only about 1% (Fig. 8). This is much lower compared with the sum of the identified lignin-derived degradation products (see Figs. 2, 3, 4, 6, and 7), which amounts to nearly 10%. Taking into account that some parts of the lignin material could not be depolymerized sufficiently for detection by GC, lignin is clearly dominant in Casparian strips, as was indicated recently (Schreiber et al., 1994; Schreiber, 1996).

The differences in ECW and HCW suberin are evident (Fig. 8). This might be interpreted as a consequence of their physiological function. As indicated previously, the root endodermis is thought to act as an efficient barrier toward apoplastic transport (Clarkson, 1991). The detected long-chain acid derivatives might play the key role concerning these transport-limiting properties, either as a direct barrier or indirectly as a matrix for associated waxes (Soliday et al., 1979). In contrast to the endodermis, the hypodermis is in direct contact with the rhizosphere. Thus, aside from possible barrier properties, the hypodermis might provide a protective function against pathogenic organisms. Only a few pathogens are able to penetrate suberized tissue, a process that involves enzymatic degradation of the polymer by means of esterases (Kolattukudy, 1984). In this context, it is argued that covalently bound hydroxycinnamic acids, which are reported to have antimicrobial effects, play an important role in the resistance against microbial attack (Friend, 1981; Nicholson and Hammerschmidt, 1992). If the corresponding free hydroxycinnamic acids are released by an esterase activity of the attacking microorganisms, they could develop their antimicrobial effects. Thus, the high content of esterified *p*-coumaric acid in the hypodermis in contrast to the endodermis could be related to its role in pathogen defense.

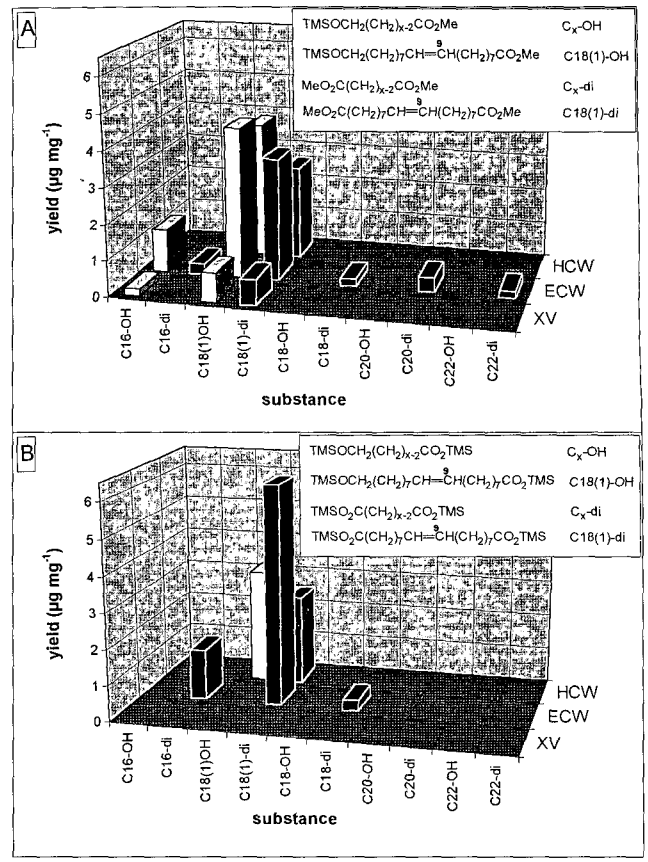


Figure 8. Aliphatic suberin monomers obtained from isolated cell wall samples. A, Aliphatic long-chain acid derivatives obtained after treatment of HCW, ECW, and XV by BF_3/MeOH. B, Aliphatic long-chain acid derivatives obtained after cupric oxide oxidation of HCW, ECW, and XV. The boxes in the upper right corner of the figures (A) and (B) give the structural formulas of the respective trimethylsilyl derivatives (TMS) of the different detected substance classes: C_x-OH, ω-hydroxy acid; $C_{18}(1)$-OH, unsaturated ω-hydroxy acid; C_x-di, α,ω-dicarboxylic acid; $C_{18}(1)$-di, unsaturated α,ω-dicarboxylic acid.

CONCLUSION

Results presented here form an important basis for future investigations concerning the structure and function of the root endodermis. Aside from ECW in their primary state of development it will be necessary to investigate ECW in their secondary and tertiary states of development. This will allow a comparison of the chemical composition of the three different developmental states and help to elucidate their role as apoplastic barriers. Additionally, the hypodermis of the root must also be investigated, which will help to evaluate and compare transport properties of the hypodermis versus the endodermis. Future investigations will have to combine investigations on the chemical composition of the

endodermis with experiments focusing on the radial transport in roots. On the basis of this integrated approach, it will finally be possible to understand the apoplastic root transport on the basis of the chemical composition of the relevant apoplastic barriers in the roots.

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