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LIPIDS AND HYDROXYCINNAMIC ACIDS IN CELL WALLS OF *ERIOPHORUM VAGINATUM*

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Key Word Index—*Eriophorum vaginatum*; Cyperaceae; cutin; suberin; cell wall; decay.

Abstract—The contents of aliphatic and aromatic compounds in alkaline hydrolysates from cell wall preparations of leaves of *Eriophorum vaginatum* varied between 27 and 10 mg/g dry wt depending on the age of the tissue. The amounts were highest in living green leaves at the end of the growth period and lowest in highly decayed leaf remains (mainly leaf bases) located below the peat surface. Living roots contained less alkaline hydrolysis products than the green leaves, and the decaying roots even less. The absolute and relative amounts of individual compounds differed in the hydrolysis products from mature and senescent leaves and leaves at various stages of decomposition. This suggests that the compounds are liberated from the cell walls or transformed to other constituents less susceptible to alkaline hydrolysis at different rates during tissue decay. Those that appear to be lost most rapidly are hydroxycinnamic acids (*p*-coumaric and ferulic acids), 9,10-epoxy-18-hydroxyoctadecanoic and 9,16- and 10,16-dihydroxyhexadecanoic acids. Compounds that are lost less rapidly are C_{18} ω-hydroxy acids and certain long chain α,ω-dicarboxylic and fatty acids, those lost most slowly are very long chain (C_{22}–C_{28}) ω-hydroxy acids. In roots also, both the absolute and relative amounts of very long chain ω-hydroxy acids were highest in the decaying cell walls.

INTRODUCTION

Polymeric lipids form thick layers on epidermal leaf cells and herbaceous stems (cutin) and inside the primary cell walls (suberin) of stem bark cells, root hypodermis and endodermis cells in all vascular plants [1, 2] and in the mestome sheath cell walls of grass leaves [3, 4]. They are also cell wall constituents in lower plants such as mosses [5–7].

Polymeric lipids consist of interesterified aliphatic monomers, ω-hydroxy acids, α,ω-dicarboxylic acids, fatty acids and fatty alcohols. These monomers occur in both cutins and suberins. Suberins are, however, generally characterized by long chain (C_{18}–C_{28}) ω-hydroxy and α,ω-dicarboxylic acids, whereas the cutin monomers mostly have shorter carbon chains (C_{16}–C_{18}) and are always highly oxygenated [1, 8, 9]. In addition, cutins may contain small amounts of aromatic constituents, hydroxycinnamic acids, and the proportions of such acids in suberins may be as high as 50% of the hydrolysis products [1, 9].

Polymeric lipids are resistant to decay and erosion [10, 11] and, thus, probably contribute to soil formation through their slow decomposition. Accumulation of ω-hydroxy acids occurs in slowly decaying shoots of *Sphagnum* mosses [12, 13], the primary peat formers throughout the world [14]. These acids are also found in *Sphagnum* peat [15, 16]. Whether such accumulation is a general feature of peat-forming plants is not yet known. Therefore, we studied the age-dependent changes of polymeric lipids in *Eriophorum vaginatum*, a higher plant, whose structure remains recognizable in peat for long periods, thereby contributing to the formation of *Eriophorum* peat [14].

RESULTS AND DISCUSSION

Aliphatic monomers of leaves

The total amounts of alkaline hydrolysis products from *E. vaginatum* leaf cell wall preparations varied with leaf age (Table 1), being highest in green leaves collected in October (26.7 mg/g dry cell wall preparation), when they were *ca* 1.5-fold the amounts in the green leaves collected in June. Slightly less than 50% of the hydrolysis products were aliphatic monomers (the rest being aromatic compounds) and accordingly their amount was highest at the end of the growth period. The increase during the growth period was mainly due to an increase in the typical major cutin monomers (9,10-epoxy-18-hydroxyoctadecanoic and 9,16- and 10,16-dihydroxyhexadecanoic acids) and also to an increase in 18-hydroxyoctadec-9-enoic acid. This result agrees with the report of active cutin synthesis during the maturation of the tissues [1]. However, the increases may also indicate activation of the synthesis of polymeric lipids by the cold weather at the end of the growth period [4, 17].

The total amount of alkaline hydrolysis products in *E. vaginatum* leaves was considerably smaller in senescent and blackened decaying leaves collected on the peat surface (18.4 and 17.7 mg/g cell wall preparation, respectively) than in the green leaves in October, and smaller still (10.1 mg) in the leaf remains gathered from 15–30 cm below the peat surface (Table 1). The percentage of aliphatic monomers, however, increased with increased tissue age and destruction, being 44% in senescent leaves, 53% in blackened decaying leaves and 58% in the leaf remains. Apparently, the aliphatic monomers were more decay-resistant than the aromatic compounds.

Table 1. Amounts ($\mu g \pm$ s.e./g dry cell-wall preparation) of polymerized lipid monomers and hydroxycinnamic acids in leaves of *E. vaginatum*

Compounds	Tissue stage 1	2	3	4	5
Epoxyhydroxy acids					
9,10-Epoxy-18-hydroxyoctadecanoic*	2740±140	4690±318	1470±183	630±51	190±21
Hydroxy acids	3260±92	5120±366	4330±112	5930±479	4340±501
16-Hydroxyhexadecanoic	80±5	160±19	120±5	170±34	110±11
18-Hydroxyoctadec-9-enoic	800±34	1810±141	2170±98	2780±273	1290±153
9,16- and 10,16-Dihydroxyhexadecanoic†	1220±59	2070±194	650±74	350±39	240±35
20-Hydroxyeicosanoic	70±18	120±10	20±10	50±7	40±4
9,10,18-Trihydroxy-octadecanoic‡	920±44	790±5	450±28	400±60	580±87
22-Hydroxydocosanoic	50±4	70±4	190±28	450±52	560±18
24-Hydroxytetracosanoic	70±2	50±2	260±43	630±27	530±43
26-Hydroxyhexacosanoic	50±3	50±4	280±59	650±46	620±92
28-Hydroxyoctadecanoic	+	+	190±38	450±26	370±71
Dicarboxylic acids	250±25	370±31	720±44	970±57	290±44
9-Octadecene-1,18-dioic	90±8	190±13	550±45	810±54	170±26
Octadecane-1,18-dioic	160±5	180±19	120±9	80±7	70±6
Eicosane-1,20-dioic	+	+	+	+	+
Docosane-1,22-dioic	+	+	+	+	+
Tetracosane-1,24-dioic	+	+	20±3	40±2	20±4
Hexacosane-1,26-dioic	+	+	+	+	30±8
Octacosane-1,28-dioic	+	+	30±3	40±1	+
Fatty acids	1190±98	370±19	800±86	1200±146	560±51
Unsaturated C_{18}	920±67	230±12	340±49	590±119	110±9
Octadecanoic	50±2	60±4	50±8	50±18	30±8
Eicosanoic	+	+	+	+	+
Docosanoic	130±35	30±9	40±4	60±5	60±13
Tetracosanoic	+	+	90±6	110±8	90±10
Hexacosanoic	40±6	50±3	140±15	180±12	160±22
Octacosanoic	50±11	+	90±9	170±17	110±15
Dotriacontanoic	+	+	50±11	40±12	+
Fatty alcohols	90±14	110±12	80±11	110±17	100±27
Octadecanol	50±11	20±4	+	40±20	60±14
Eicosanol	+	+	+	+	+
Docosanol	+	30±2	30±5	50±3	20±7
Tetracosanol	+	+	+	+	+
Hexacosanol	40±7	60±8	50±10	20±5	20±1
Octacosanol	+	+	+	+	+
Unknown	650±38	1550±112	610±26	1090±43	390±41
Total aliphatic compounds	8180±295	12210±770	8040±82	9430±410	5860±674
Hydroxycinnamic acids	10980±657	14520±1855	10420±1650	8380±224	4200±483
p-Coumaric	8070±830	10090±1580	7120±1180	5660±90	2940±356
Ferulic	2910±245	4440±278	3300±471	2720±135	1260±129

+ = <20 μg/g dry cell-wall preparation. (1) Mature green leaves collected in June; (2) mature green leaves collected in October; (3) senescent yellow leaves; (4) decaying blackened leaves; (5) blackened leaf remains from below the peat surface. The data are the means of 3 independent replicates.

* The data are the sums of 9,10-epoxy-18-hydroxy acid, the hydrolysis products 9-ethoxy-10,18-dihydroxyoctadecanoic and 10-ethoxy-9,18-dihydroxyoctadecanoic acids and the silylation products 9-chloro-10,18-dihydroxyoctadecanoic and 10-chloro-9,18-dihydroxyoctadecanoic acids.

† Probably contains small amounts of 7,10- and 8,10-isomers of dihydroxyhexadecanoic acid.

‡ Some of the 9,10,18-trihydroxyoctadecanoic acid is formed as a hydrolysis product of 9,10-epoxy-18-hydroxyoctadecanoic acid.

The individual cutin monomers appeared to be lost at different rates. The amount of cutin acids was particularly low in the senescent and decaying leaves. The absolute amount of the epoxyhydroxy acid decreased strongly with ageing and decay of the tissue, which resulted in a strong decrease in the relative amounts, as well: 33–38% of the total aliphatic monomers in the green tissue, 18% in senescent leaves, 7% in blackened leaves and 3% in the leaf remains. Schmidt and Schönherr [18] have observed that eopxyhydroxy acids in ageing plants form new, probably ether, bonds that are resistant to alkaline hydrolysis. Thus, the decrease in the amount of epoxyhydroxy acid in senescent and decaying leaves of *E. vaginatum* may be due to a synthesis of new, resistant compounds in the cell walls. The amount of trihydroxy acid was also highest in the green leaves, but the age-dependent decrease was not as drastic as for epoxyhydroxy acids. This most probably indicates that part of the measured amount of this acid originates from epoxyhydroxy acid released by the relatively strong alkaline hydrolysis conditions used in our investigations [19]. The natural trihydroxy acid content of *E. vaginatum* leaves is thus masked by formation of the trihydroxy acid from epoxyhydroxy acid, and the amounts given for epoxyhydroxy acid in Table 1 should be somewhat higher, and those for trihydroxy acid somewhat lower. The age-dependent decrease of another major cutin acid, dihydroxyhexadecanoic acid, was as drastic as that observed for the epoxyhydroxy acid. Thus, the age-dependent pattern in *E. vaginatum* is the exact opposite of that in decaying *Sphagnum* shoots where the amounts of dihydroxyhexadecanoic and trihydroxyoctadecanoic acids (no epoxyhydroxy acid was present) increased with tissue age and decay [12, 13].

In constrast to the decrease in the amount of cutin acids during senescence and decay of *E. vaginatum* leaves, a clear increase is evident in the amount of long chain ω-hydroxy acids (Table 1). They made up 30% of all aliphatic compounds in the green leaves, 63% in blackened leaves and 72% in the leaf remains. This increase is due not only to the decrease in the amount of cutin acids, but also to an increase in the absolute amounts of long chain ω-hydroxy acids. It indicates activated synthesis of new long chain ω-hydroxy acids during tissue senescence, and/or that other constituents are liberated more rapidly than long chain ω-hydroxy acids from the polymer by peat micro-organisms during tissue decay.

A clear sequence in susceptibility to decay is also evident within the long chain ω-hydroxy acids (Table 1). The amount of 18-hydroxyoctadec-9-enoic acid was highest in blackened leaves, but decreased with further destruction of the leaves. The amounts of very long chain (C_{22}–C_{28}) ω-hydroxy acids were highest in both weakly and more strongly decomposed leaves, probably indicating a high resistance to decay. A similar age-dependent change in the polymeric lipid pattern has been observed in peat-forming *Sphagnum* mosses [12, 13]. The increases in *E. vaginatum* leaves were less marked, however, and occurred only during senescence and at an early stage of tissue decay. Further tissue destruction did not lead to such accumulation of long chain ω-hydroxy acids as in *Sphagnum*.

Slight age-dependent changes were also found in the amounts of minor aliphatic monomer classes, α,ω-dicarboxylic and fatty acids. Their amounts were highest in senescent and blackened leaves, but decreased with further tissue destruction. These changes are attributable to 9-octadecene-1,18-dioic acid and unsaturated C_{18} fatty acids. Fatty alcohols were found in small amounts in leaves of all ages. In *Sphagnum* mosses the amount of 9-octadecene-1,18-dioic acid increased with increasing shoot age and decay and a smaller increase was evident in the amounts of other long chain α,ω-dicarboxylic acids and long chain fatty alcohols [12, 13].

Aliphatic monomers of roots

In roots of *E. vaginatum* the total amounts of both the alkaline hydrolysis products and aliphatic monomers were lower than those in the leaves (cf. Tables 2 and 1). Furthermore, the cell walls of roots differ from those of leaves in the absence of one aliphatic monomer class, epoxyhydroxy acids. The largest aliphatic monomer group in the roots was the hydroxy acids, which contributed up to 40% of all compounds (5.1 mg/g dry cell wall preparation) in new roots. The amounts of di- and trihydroxy acids, however, were very small. As in leaves, the minor aliphatic monomer classes of roots were α,ω-dicarboxylic acids, fatty acids and fatty alcohols. The relative amounts of long chain (C_{20} or longer) monomers were higher, however, in roots than in leaves.

The total amount of alkaline hydrolysis products was lower in the old blackened roots than in the living roots, (Table 2). The lower value was in part due to a lower content of ω-hydroxy acids and to a lesser extent to α,ω-dicarboxylic and fatty acids. Certain monomers were mainly responsible for these losses: 18-hydroxyoctadec-9-enoic acid, 9-octadecene-1,18-dioic acid and unsaturated fatty acids. These losses were counterbalanced, however, by a slight increase in the amount of very long chain (C_{22}–C_{26}) ω-hydroxy acids. Thus, in roots as in leaves the very long chain ω-hydroxy acids were apparently most decay resistant.

Aromatic compounds

Hydrolysis products of cell wall preparations of both green leaves and young roots of *E. vaginatum* also contained aromatic hydroxycinnamic acids, *p*-coumaric and ferulic acids (Tables 1 and 2), these making up 52–58% and 44% of the total products in living leaves and roots, respectively. In leaves collected in October, the amount was *ca* 1.5-fold that of leaves collected in June. The decrease evident from the very beginning of the decay process in the absolute amounts of the two hydroxycinnamic acids, and in their amounts in relation to the aliphatic monomers suggests more rapid liberation of hydroxycinnamic acids than aliphatic monomers. The involvement of the hydroxycinnamic acids with the lipid polymer skeleton of the *E. vaginatum* cell wall is uncertain, however, in view of the methods used in this investigation [1]. Therefore, the losses in the amount of hydroxycinnamic acids are not necessarily indicative of cutin or suberin decay.

In conclusion, the various alkaline hydrolysis products from *E. vaginatum* leaves appear to be released from the cell walls or transformed in chemical structure and susceptibility to alkaline hydrolysis at different rates during the cell wall decay. The compounds lost most rapidly are aromatic acids, and epoxyhydroxy and dihydroxy acids, those lost less rapidly are C_{18} ω-hydroxy acids and certain long chain α,ω-dicarboxylic and fatty acids and those lost least slowly are C_{22}–C_{28} ω-hydroxy acids.

Table 2. Amounts ($\mu g \pm$ s.e./g dry cell-wall preparation) of polymerized lipid monomers and hydroxy cinnamic acids in roots of *E. vaginatum*

Compounds	Tissue stage 1	Tissue stage 2
Hydroxy acids	5100±220	4240±167
14-Hydroxytetradecanoic	80±32	+
16-Hydroxyhexadecanoic	180±14	230±10
18-Hydroxyoctadec-9-enoic	3470±201	2170±192
9,16- and 10,16-Dihydroxy-hexadecanoic*	20±3	90±15
20-Hydroxyeicosanoic	20±4	50±14
9,10,18-Trihydroxyoctadecanoic	20±2	80±12
22-Hydroxydocosanoic	110±20	180±15
24-Hydroxytetracosanoic	190±10	320±37
26-Hydroxyhexacosanoic	590±6	700±88
28-Hydroxyoctacosanoic	420±28	420±60
Dicarboxylic acids	1100±69	720±41
Hexadecane-1,16-dioic	10±1	20±2
9-Octadecene-1,18-dioic	840±51	460±13
Octadecane-1,18-dioic	+	+
Eicosane-1,20-dioic†	100±5	150±23
Docosane-1,22-dioic	+	+
Tetracosane-1,24-dioic	40±5	+
Hexacosane-1,26-dioic	100±18	90±6
Fatty acids	1290±23	960±55
Hexadecanoic	70±15	40±1
Unsaturated C_{18}	280±18	140±22
Octadecanoic	+	+
Eicosanoic	+	+
Docosanoic	80±15	50±4
Tetracosanoic	180±7	170±13
Hexacosanoic	420±8	350±36
Octacosanoic	260±9	210±23
Fatty alcohols	20±1	50±6
Octadecanol	+	+
Eicosanol	+	+
Docosanol	+	+
Tetracosanol	+	+
Hexacosanol	20±1	30±5
Octacosanol	+	20±3
Total aliphatic compounds	7510±403	5970±528
Hydroxycinnamic acids	5690±426	2340±44
p-Coumaric	3340±234	1210±125
Ferulic	2350±217	1130±92

+ = <20 μg/g dry cell-wall preparation. (1) Young white roots; (2) decaying blackened roots. The data are the means of 3 independent replicates.

*May contain small amounts of 18-hydroxyoctadecanoic acid.

†May contain small amounts of 9,10-epoxy-18-hydroxy-octadecanoic acid.

EXPERIMENTAL

E. vaginatum L. samples were collected from the Karevansuo bog in SW-Finland at the beginning of June, during flowering, and in the middle of October, soon after the first frosts. Samples consisted of mature green leaves, yellow senescent leaves and leaves representing different stages of decay, viz. blackened leaves of less advanced stage from the peat surface and leaf remains (mainly leaf bases) of advanced stage from below the peat surface at a depth of 15–30 cm. Root samples, young white roots and old blackened roots, were also collected in October.

Cell wall fragments, free of MeOH, $CHCl_3$–MeOH, $CHCl_3$ and petrol sol. lipids, were submitted to alkaline hydrolysis (1 M KOH in 90% EtOH at 75° for 3 hr) under N_2. After acidification [19] the products were methylated and silylated and subsequently analysed by GC/MS [6, 12]. The quantitative calculations were based on peak areas relative to 5 α-cholestane and Me heptadecanoate, int stds for aliphatic monomers and hydroxycinnamic acids, respectively.

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