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SUBERINS OF *MALUS PUMILA* STEM AND ROOT CORKS

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Key Word Index—*Malus pumila*; Rosaceae; apple; stem cork; root cork; suberins; monomeric composition; fatty and hydroxy-fatty acids; epoxides.

Abstract—The suberin contents of the isolated superficial cork layers of *Malus pumila* stems and root ranged from 15 to 35% of the dry weight. The qualitative composition of the aliphatic monomers obtained after alkaline depolymerization of the extractive-free corks was similar but some quantitative differences were found according to cultivar and age of the cork layer. 1-Alkanols (mainly 22:0, 24:0 and 26:0), alkanoic acids (mainly 22:0 and 24:0), α, ω-alkanedioic acids (mainly 16:0, 18:1 (9) and 18:0) and ω-hydroxyalkanoic acids (mainly 18:1 (9) and 22:0) were major constituents of all the samples examined and together they comprised 40–50% of the total monomeric mixture. The remainder was composed mainly of 9,10-epoxy-18-hydroxy- and 9,10,18-trihydroxyoctadecanoic acids. The corresponding dibasic acids, 9,10-epoxy- and 9,10-dihydroxyoctadecane-1,18-dioic, were minor components as were C_{16} and C_{18} dihydroxyalkanoic acids (mainly 10,16-dihydroxyhexadecanoic and 10,18-dihydroxyoctadecanoic acids, respectively). The root suberin differed from that of the stem in containing larger amounts of 9,10-epoxy-18-hydroxyoctadecanoic and 18-hydroxyoctadec-9-enoic acids.

INTRODUCTION

Although the monomeric composition of the cutins occurring in different organs of various *Malus pumila* cultivars has been established (leaves and fruits [1] and refs. cited therein; stigma and petals [2]; seed coat [3]), and some studies made of their biosynthesis [4–6], nothing is known about the chemical nature of the suberins of this plant. These related polyester lipid substances are found in both aerial and subterranean parts of the plant. Whereas cutins are associated almost exclusively with the cuticular membrane on the outer epidermal wall of the aerial tissues, suberins are the characteristic structural components of the walls of the cork cells (phellem) which comprise the superficial periderm layers of the stem and root. The periderm thus replaces the cutin-containing cuticular membrane as the protective layer on tissues that undergo secondary thickening but it is also associated with the formation of russet on the fruit [7, 8]. In addition, polymeric compounds with similar histochemical and ultrastructural properties to those of suberin are present in the internal tissues of the primary root in the walls of the endodermal cells [9].

In this paper the monomeric composition of the suberins occurring in the superficial cork layers of the stem and root of *M. pumila* is described and the influence of age is briefly examined for the stem of one cultivar. The results are compared with those obtained previously in our laboratory for apple leaf and fruit cutins.

RESULTS AND DISCUSSION

The main components of the six samples of isolated superficial cork layers of *M. pumila* examined were those soluble in organic solvents and water, suberin and orange-brown coloured phenolic material soluble in aqueous alkali after desuberization (Table 1). In the stem corks there were approximately equal proportions of each. Substantial amounts of wax-like chloroform-soluble material (15–25% dry wt) were present in the stem corks but in the root cork the methanol-soluble fraction was the principal soluble material. Although the various soluble fractions were not examined in detail, friedelin was identified as the major component of the wax fraction in all cork samples. This pentacyclic triterpenone is of common occurrence in the bark (which includes the cork layer) of a wide range of species (reviews [10, 11]) including members of the Rosaceae. The long chain 1-alkanols most characteristic of the suberin fraction of *M. pumila* cork cells (see below) were present only in trace quantities in the corresponding wax fractions.

An approximate value for the proportion of the phenolic fraction in corks can be obtained from the difference between the total loss of weight from alkaline treatment and the suberin content [12]. For the *Malus* stem corks this was *ca* 35% of the dry wt (Table 1) but was considerably more for the roots (*ca* 60% dry wt). Comparable amounts of similar materials have been previously recorded in the stem corks in other species [12, 13]. Although the origin of this fraction is not clear it is unlikely to be lignin or part

Table 1. Analysis of *Malus pumila* cork layers (% dry wt)

	Cox's Orange Pippin			Fortune	Laxton's Superb	MM 106 rootstock
	1-year-old	2-year-old	3-year-old	15-year-old	15-year-old	3-year-old
$CHCl_3$ extractive*	14.9	17.1	18.2	25.3	20.0	4.8
MeOH extractive*	6.4	5.7	6.1	3.8	3.6	11.7
H_2O extractive*	6.8	6.2	8.7	1.3	6.0	0.2
Suberin†	35.4	33.1	28.3	28.9	31.3	14.9
Residue‡	3.1	5.4	2.5	1.4	8.7	8.0
Total loss of wt from alkaline treatment	68.8	65.6	64.5	68.2	61.7	75.3

*Exhaustive extraction of powdered cork under reflux conditions.

†Determined from weight of Et_2O-soluble monomers obtained after depolymerization of extractive-free cork with NaOMe–MeOH solution.

‡Weight of material remaining after treatment of extractive-free cork with NaOMe–MeOH solution followed by aq. KOH solution.

of the suberin biopolymer *per se* as suggested by Kolattukudy [14, 15]. The yellow colour of *Malus* cork cell walls visible in the light microscope suggest that part is derived from the wall but the bulk probably originates from the highly pigmented inclusions that fill the lumina of many of the cells. The latter are only released from within the cork cells following desuberization of the walls. Using the analytical procedures described in the present work, lignin if present would be found in the final residue (Table 1).

The suberin contents of the *Malus* corks examined ranged from 15 to 35%, with the root containing the least (Table 1). For stems there was little variation with cultivar or age, a 1-year-old cork layer having a similar amount of suberin (*ca* 30% dry wt) to that of one aged 15 years. The qualitative composition of the suberin monomers obtained after alkaline depolymerization of the extractive-free corks was also similar. All were complex mixtures of various classes of long-chain aliphatic compounds, the major classes being 1-alkanols, alkanoic, α,ω-alkanedioic, ω-hydroxyalkanoic, monohydroxyepoxyalkanoic and trihydroxyalkanoic acids (Table 2). Epoxyalkanedioic, dihydroxyalkanoic and dihydroxyalkanedioic acids were the minor classes. In these mixtures the monomers were predominantly even-carbon numbered, ranging from C_{16} to C_{28}, with C_{18} compounds comprising more than 60% of the total. Much smaller amounts of C_{16} compounds (2.5–7% total) occurred but substantial quantities (22–33% total) of monomers greater than C_{18} were present, particularly C_{22} (10–18% total) and C_{24} (6–11% total). The overall carbon number distribution for the 3-year-old stem suberin from the Cox's Orange Pippin cultivar is shown in Fig. 1.

Compounds greater than C_{18} in chain length, however, were confined to the 1-alkanol, alkanoic, α, ω-alkanedioic and ω-hydroxyalkanoic acid fractions (Table 3). These simple mono- and disubstituted compounds together comprise 40–50% of the total monomers in *Malus* suberins although the root suberin differed from that of the stem in possessing a greater relative proportion of ω-hydroxyalkanoic acids. The major homologues in the 1-alkanol fractions of all the cork samples analysed were 22:0, 24:0 and 26:0; in the alkanoic acid fractions 20:0, 22:0 and 24:0; in the α, ω-alkanedioic acid fractions 16:0, 18:1 (9) and 18:0; and in the ω-hydroxyalkanoic acid fractions 18:1 (9) and 22:0 (Table 3).

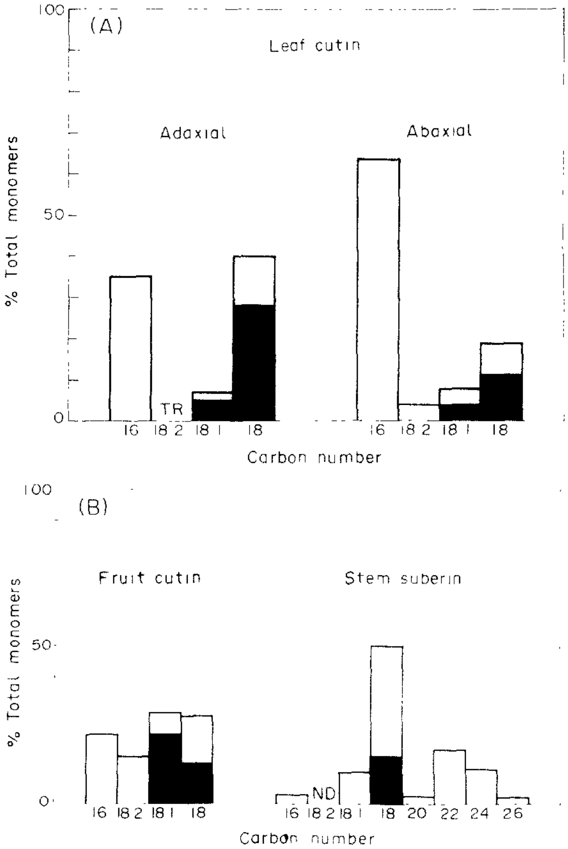

Fig. 1. Chain length distribution of the principal monomers present in the lipid polymers from different regions of *Malus pumila* cv. Cox's Orange Pippin (leaf and fruit data from Ref. [1]). Solid bars represent epoxide contents for each chain length; TR trace, ND not detected.

Table 2. Monomeric composition of suberins from cork layers of *Malus pumila* expressed as a percentage of the total depolymerization products

Classes	Cox's Orange Pippin 1-year-old	Cox's Orange Pippin 2-year-old	Cox's Orange Pippin 3-year-old	Fortune 15-year-old	Laxton's Superb 15-year-old	MM 106 rootstock 3-year-old
1-Alkanols (C_{18}–C_{28})*	9.5	12.5	12.9	12.5	9.5	8.9
Alkanoic acids (C_{16}–C_{26})†	8.1	9.3	10.1	8.8	10.4	3.7
α, ω-Alkanedioic acids (C_{16}–C_{24})†	12.1	8.8	7.6	4.4	4.7	8.8
ω-Hydroxyalkanoic acids (C_{16}–C_{26})‡	17.8	18.8	19.3	16.8	13.9	25.0
Epoxyalkanedioic acids§						
9, 10-Epoxyoctadecane-1, 18-dioic	3.8	3.7	3.0	3.0	0.9	2.9
Monohydroxyepoxyalkanoic acids§						
9, 10-Epoxy-18-hydroxyoctadecanoic	16.0	15.4	11.5	13.6	2.8	25.9
Dihydroxyalkanoic acids‡						
9, 10-Dihydroxyhexadecanoic	0.8	0.1	0.1	0.1	tr	0.4
10, 16-Dihydroxyhexadecanoic‖	2.0	0.4	0.1			0.2
9, 18-Dihydroxyoctadecanoic	0.3	0.5	1.5	0.6	0.1	2.8
10, 18-Dihydroxyoctadecanoic‖	0.7	1.1	0.7	0.3		1.2
Dihydroxyalkanedioic acids‡						
9, 10-Dihydroxyoctadecane-1, 18-dioic	5.4	5.3	5.5	5.7	8.8	1.6
Trihydroxyalkanoic acids‡						
9, 10, 18-Trihydroxyoctadecanoic	19.2	22.1	24.8	23.6	38.8	14.4
Unidentified	4.3	2.0	2.9	10.6	10.1	4.2

*Determined by GC as corresponding TMSi ethers.

†Determined by GC as corresponding methyl esters.

‡Determined by GC as corresponding methyl ester TMSi ethers.

§Determined by GC as corresponding methoxyhydrin methyl ester TMSi ethers.

‖Not resolved by GC, approximate isomer content determined from GC/MS.

tr, Less than 0.05%.

Table 3. Homologue composition (%) of 1-alkanol, alkanoic, α, ω-alkanedioic and ω-hydroxyalkanoic acid fractions of the suberins from cork layers of *Malus pumila*

	Cox's Orange Pippin			Fortune	Laxton's Superb	MM 106 rootstock
Fraction*	1-year-old	2-year-old	3-year-old	15-year-old	15-year-old	3-year-old
1-Alkanols						
18:0	tr†	0.6	0.5	0.6	tr	tr
20:0	1.9	2.1	1.6	2.0	1.0	1.3
22:0	52.0	41.3	38.3	45.1	31.6	25.0
24:0	40.2	38.9	42.5	35.1	36.7	36.9
26:0	5.9	16.6	16.6	16.1	27.6	32.3
28:0	tr	0.5	0.5	1.1	3.1	4.5
Alkanoic acids						
16:0	2.3	1.9	1.9	6.6	2.7	3.4
18:0	1.1	0.9	1.2	1.5	0.8	2.3
20:0	8.2	5.0	4.0	2.4	2.7	3.8
22:0	64.7	57.9	54.4	46.8	47.9	51.1
24:0	22.7	32.8	36.6	39.5	43.0	37.3
26:0	1.0	1.5	1.9	3.2	2.9	2.1
α, ω-Alkanedioic acids						
16:0	23.5	23.0	24.3	24.9	43.4	30.5
18:1 (9)	39.8	41.0	38.3	39.8	5.6	43.6
18:0	29.0	27.6	28.0	28.0	38.5	20.1
20:0	4.4	4.1	3.8	4.0	2.7	2.2
22:0	3.3	4.1	5.0	3.3	8.3	3.3
24:0	tr	0.2	0.6	tr	1.5	0.3
ω-Hydroxyalkanoic acids						
16:0	8.4	4.2	3.9	3.5	7.1	6.3
18:1 (9)	39.0	41.3	36.1	22.8	7.0	56.5
18:0	6.5	3.8	3.5	3.4	4.6	2.4
20:0	10.2	8.8	8.1	8.5	9.8	5.6
22:0	31.5	35.7	38.8	47.6	55.7	24.1
24:0	4.4	6.2	9.6	13.9	15.8	5.1
26:0	tr	—	—	tr	—	—

*Odd-carbon numbered homologues present in trace amounts in all fractions.

†tr, Less than 0.01%.

For details of GC derivatization consult Table 2.

Thus, there does not appear to be a simple biogenetic relationship between any of these suberin monomer classes in *M. pumila*. However, some quantitative differences in the homologue composition within the four fractions were apparent between the various *Malus* samples. In Cox's Orange Pippin stem there was an increase in the relative amounts of 1-hexacosanol, tetracosanoic, 22-hydroxydocosanoic and 24-hydroxytetracosanoic acids with an age increase of 1–3 years. Also, the older stem corks from the Fortune and Laxton's Superb cultivars contained higher proportions of the 22:0 and 24:0 and lesser amounts of the 18:1 (9) ω-hydroxyalkanoic acids than the younger Cox's Orange Pippin samples. 18-Hydroxyoctadec-9-enoic and 22-hydroxydocosanoic acids comprised *ca* 14 and 6%, respectively, of the total *Malus* root suberin monomers.

The *M. pumila* suberins also contained variable but substantial quantities of C_{18} epoxy monomers and their corresponding *vic*-diol analogues (Table 2). The greatest epoxide content was found in the root (*ca* 40% total monomers) with smaller values for the stem especially the Laxton's Superb cultivar (only *ca* 3% total monomers). The principal epoxy compound was 9,10-epoxy-18-hydroxyoctadecanoic acid; the corresponding dibasic acid, 9,10-epoxyoctadecane-1,18-dioic, was a minor component of all *Malus* suberins examined. Both epoxy compounds were accompanied by their hydration products, 9,10,18-trihydroxyoctadecanoic and 9,10-dihydroxyoctadecane-1,18-dioic acids, the former being a major constituent of all samples, particularly the Laxton's Superb stem suberin where it comprised *ca* 40% of the total monomers. Much smaller amounts (*ca* 2–6% total monomers) of the *vic*-diolalkanedioic acid were present.

Positional isomers of C_{16} and C_{18} dihydroxyalkanoic acids were also identified as minor components of all the *Malus* suberins. In both cases the major isomer had the secondary hydroxyl group in the 10-position (Table 2). Microscopical examination of the youngest sample of cork isolated from Cox's Orange Pippin stem suggested that the dihydroxyhexadecanoic acids are likely to originate in part from the remnants of the cuticle which was observed on the outside of the cork layer. However, a cuticle could not be detected on the older stem cork samples or on that from the root.

The suberin composition of *M. pumila* cork layers most resembles those of commercial cork (*Quercus suber*) [12, 16, 17] and *Betula pendula* [12, 16], two species also characterized by a high content of C_{18} epoxy and *vic*-diol acid monomers. However, such C_{18} monomers are only minor components of the suberins of other species, such as that of *Solanum tuberosum* tuber [18, 19], of stems of *Ribes* species [13] and of the roots of *Daucus carota*, *Pastinaca sativa*, *Brassica napobrassica*, *Brassica rapa*, *Beta vulgaris* and *Ipomoea batatas* [20]. α, ω-Alkanedioic- and ω-hydroxyalkanoic acids predominate in these plants, together with significant proportions of alkanoic acids and 1-alkanols.

The cutins and suberins on the different organs of *M. pumila* are clearly biochemically related since many of the classes of monomeric constituents are common to both; all contain 9, 10-epoxy-18-hydroxy- and 9, 10, 18-trihydroxyoctadecanoic acids. The main difference between the suberins and cutins is the presence of a substantial proportion of monomers greater than C_{18} in chain length (Fig. 1), particularly 1-alkanols, alkanoic- and ω-hydroxyalkanoic acids, the first class of compound, however, being absent from the cutins. On the other hand, the dihydroxyhexadecanoic acids (mainly 10, 16-dihydroxy) which predominate in the leaf cutins [1], especially that on the abaxial surface, are minor components of the suberins, whereas the mono- and di-unsaturated C_{18} acids, chiefly 18-hydroxyoctadeca-9,12-dienoic [21] and 9,10-epoxy-18-hydroxyoctadec-12-enoic [1], most characteristic of the fruit cutins are absent from the suberins. Interestingly dihydroxyoctadecanoic acids are minor constituents of both the cutins and suberins of *M. pumila*; these compounds have also been identified in one other suberin in *Betula pendula* [12]. In *M. pumila* there would appear to be a much closer resemblance between the two groups of lipid biopolymer than that reported for other species [22–26] where marked qualitative as well as quantitative differences occur between the two.

EXPERIMENTAL

Plant material. Superficial cork layers were mechanically removed from 1, 2 and 3-year-old branches of mature Cox's Orange Pippin apple trees growing in the orchards at Long Ashton Research Station. The samples were collected at the end of the growing season when the fruits were ripe. Strips of outer bark were removed with a machete from near the base of the main trunk of orchard trees of Laxton's Superb and Fortune *ca* 15-years-old and the cork layer detached from the rest of the bark by soaking in a soln of ammonium oxalate–oxalic acid [27]. Cork from MM 106 apple rootstock was prepared in a similar manner from 3-year-old trees grown outdoors in containers of sand. Microscopical examination of all samples confirmed that only the periderm layer had been isolated and that the main components were phellem (cork) cells. Prior to analysis the pieces of cork isolated from each source were dried and ground to a fine powder in a Wiley mill. The powdered corks were exhaustively extracted under reflux conditions with $CHCl_3$ followed by MeOH and then H_2O.

Analysis of soluble fractions. TLC patterns of the $CHCl_3$-soluble fractions obtained from the *Malus* cork samples were similar and all showed one major spot (R_f 0.23) and four minor spots (Si gel G; C_6H_6). The major compound was isolated by prep. TLC and identified as friedelin by mp, TLC, GC (Dexsil 300 and SE 30) and MS comparison with an authentic sample (supplied by Dr. M. Sainsbury, University of Bath). The MeOH and H_2O-soluble cork extractives were not examined further.

Suberin analysis. Extractive-free cork samples (*ca* 50 mg dry wt) were depolymerized for *ca* 24 hr in sealed Pyrex tubes using solns of NaOMe–MeOH [28] at 70° and aq. KOH [12] at 100°. Suberin contents were assessed from the wt of Et_2O-soluble monomers recovered from each sample. The qualitative and quantitative composition of the monomeric mixtures was determined using the TLC, prep. TLC, GC and GC/MS methods previously described [1, 12, 16, 28]. All epoxide components were estimated as their corresponding methoxyhydrin derivatives [16]; these compounds are only generated during the depolymerization with NaOMe–MeOH.

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