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Composition of Lipid-derived Polymers from Different Anatomical Regions of Several Plant Species[1]

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ABSTRACT

The composition of the aliphatics of the protective cuticular polymers from different anatomical regions from several plant species was determined by combined gas-liquid chromatography and mass spectrometry of the depolymerization products derived from the polymers. The polymer from the aerial parts of *Vicia faba* showed similar composition; dihydroxypalmitic acid was the major (>85%) component of the cutin covering leaves, petioles, flower petals and stem with smaller amounts of palmitic acid and ω-hydroxy palmitic acid. On the other hand, the chief components of the polymer from the tap root were ω-hydroxy $C_{16:0}$ and $C_{18:1}$ acids and/or the corresponding dicarboxylic acids. The positional isomer composition of the dihydroxy C_{16} acids was shown to be dependent upon anatomical location, developmental stage, and light. Apple cutin from rapidly expanding organs (flower petal and stigma) was shown to contain predominately C_{16} family acids whereas the C_{18} family dominated in cutin of slower growing organs (leaf and fruit). The composition of the aliphatic components of cutin found in the seed coats of pea, corn, barley, and lettuce was found to be similar to that of the cuticular polymer of the leaves in each species.

Most chemical investigations on the lipid-derived protective polymeric materials of plants have been limited to fruits and leaves (19, 20) and in a few cases to underground storage tissues (15) and barks (9, 10). Lipid-like staining layers in various parts of plants have been observed ultrastructurally and called cutin, suberin, or even "cutin-suberin like" (22, 24–26). However, the chemical composition of most such layers remains unknown with a few exceptions (6, 7). In the absence of adequate chemical information, cutin and suberin cannot be defined by means of their chemical composition although tentative definitions have been proposed on the basis of the limited amount of available information (16). To test the validity of such tentative definitions, much more information concerning the chemical composition of the lipid-derived polymers from different anatomical regions of plants is needed. In this paper we report the composition of the aliphatic components of polymers from different anatomical regions of plants belonging to six genera.

MATERIALS AND METHODS

Seeds of corn (*Zea mays* L. cv. Golden Cross, Burpee Seed Co.), barley (*Hordeum vulgare* L.), pea (*Pisum sativum* L. cv. Frosty, Gurney Seed Co.), and lettuce (*Lactuca sativa* L. cv. Grand Rapids, Burpee) were utilized in this study. Plants were grown in a 1:1:1 mixture of peat moss, Perlite, and sand under natural light in the greenhouse. Seeds of corn, barley, and peas were soaked (2 h) in distilled H_2O and the seed coats were individually removed and cleaned. Since the seed coats of lettuce seeds could not be readily isolated, the whole seeds were autoclaved at 110 C and 15 p.s.i. for 15 min and used for analysis. The four tissue samples were extracted in a Soxhlet extractor with $CHCl_3$ and then with CH_3OH for 48 h each. After air-drying, the tissues were ground in a Wiley mill (40 mesh) and treated with cellulase and pectinase as described before (28). The tissue residues were filtered, air-dried, ground in a Wig-L-Bug amalgamator (Crescent Dental Manufacturing Co., Chicago, Ill.) and the resulting powders were subjected to two further extractions in a Soxhlet extractor with $CHCl_3$ and CH_3OH for 72 h each. The cutin-containing residues were obtained from the leaves of the same species by thorough extraction of the leaves with methanol and a 2:1 mixture of chloroform and methanol. The remaining residue was finely powdered and extracted in a Soxhlet extractor with solvents as indicated above. Portions of the dry powder were subjected to depolymerization with $LiAlH_4$ and $LiAlD_4$ as described before (28). Each analysis was repeated at least two times.

Cutin from the mature fruit and fully expanded leaves of apple (*Malus pumila* L. cv. Golden Delicious) was isolated as described before (28). The flower petals and stigma from mature flowers were homogenized and extracted and the final residue, containing cutin, was subjected to depolymerization as described before (18).

V. faba leaves of appropriate size were obtained and the cutin-containing polymeric material was isolated and subjected to reductive depolymerization as described before (18).

Leaf petiole, stem from the second internode, stem from the soil level to the seed, and the tap root were excised from *V. faba* plants grown to maturity (bloom) under artificial light at (≃800 μE) in a 1:1:1 mixture of peat moss, Vermiculite, and sand. The tissues were frozen, lyophilized, ground in a Wiley mill (No. 60 mesh screen) and powdered as above. The powder was extracted with methanol followed by methanol-chloroform (1:1, v/v) and subsequently subjected to extraction in a Soxhlet extractor with chloroform for 24 h. A portion of the final residue (0.5 g each) was subjected to reductive depolymerization and analysis of products by combined GLC-MS (28).

Approximately 100 *V. faba* seeds were soaked in tap water for 3 h and subsequently planted in two flats of moist Vermiculite. The flats were placed in the dark for 7 days after which time one flat was placed in the light (fluorescent light, ≃800 μE) while the other remained in the dark. The plants were allowed to grow under these conditions for another 5 days after which time a 3-cm section from above the first node and the two largest leaves from each plant were excised. The tissues were placed immediately in chloroform-methanol (2:1, v/v), washed and soaked with fresh solvent for 18 h. They were then extracted in a Soxhlet extractor

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with chloroform for 24 h and the residue was powdered with a mortar and pestle. Portions (≃200 mg) of the powder were subjected to reductive depolymerization with $LiAlD_4$ and products were subjected to TLC and combined GLC-MS. The relative proportion of 9- and 10-hydroxy and ω-oxo components were estimated from the relative intensities of α-cleavage ions as described before (13).

The products of reductive depolymerization ($LiAlH_4$ in tetrahydrofuran) recovered by extraction with $CHCl_3$ were subjected to TLC with 1-mm layers of Silica Gel G and ethyl ether-hexane-methanol (8:2:1, v/v). Unless otherwise specified, all components which moved from the origin were recovered by elution with a 2:1 mixture of chloroform and methanol. The material thus recovered was free of polar impurities and was considered to be the aliphatic components of lipid-derived polymers contained in the insoluble plant residue. When the C_{16} triol derived from *V. faba* leaves of different developmental stages was measured this triol was recovered and subjected to combined GLC-MS and the positional isomer composition was determined from the relative intensities of α-cleavage ions as described before (13). All of the depolymerization products were analyzed and identified by combined GLC-MS using diagnostic ions discussed elsewhere (28).

RESULTS AND DISCUSSION

Composition of the Lipid-derived Polymers from Different Organs of *V. faba*. The chemical composition of the cutin from leaves and flower petals of *V. faba* has been examined previously (17, 18). To determine whether the composition of the polymer on the petiole and stem of this plant is similar to that of the leaf, the polymeric materials from segments of these organs were subjected to hydrogenolysis and the reduced aliphatic products were examined by combined GLC-MS. In both cases C_{16} triol was the major (86–88%) component and thus dihydroxyhexadecanoic acid was shown to be the major monomer of the polymer covering these organs just as previously observed with leaves and flower petals. Small amounts of C_{16}-α,ω-diol (<4%) originating from ω-hydroxyhexadecanoic acid and fatty alcohols (<10%) derived from fatty acids were also found. The cutin layer, possibly with some attached epidermal cells, was detached from lyophilized stem sections and this layer was isolated and subjected to hydrogenolysis followed by GLC-MS analysis of the products. The results were identical to those obtained with the entire stem segments. Thus, it is unlikely that noncutin components from the internal portion of the stem contributed significantly to the products mentioned above. These results indicate that all of the aerial surfaces of *V. faba* are covered with cutin of a very similar, if not identical, composition.

The mass spectra of the C_{16} triols mentioned above showed that the α-cleavage ions (produced by cleavage of the carbon chain at either side of the mid-chain trimethylsiloxy function) were at m/e 275, 289, 303, and 317 indicating that the triol fraction was a mixture of hexadecane-1,7,16-triol and hexadecane-1,8,16-triol derived from 10,16- and 9,16-dihydroxypalmitic acid, respectively. The relative intensities of these α-cleavage ions were used to estimate the proportion of the 9-hydroxy and 10-hydroxy isomers present in the original polymer as described previously (13). Cutin from leaves and flower petals contained <10% 9-hydroxy isomer of the dihydroxy C_{16} acid whereas petiole showed a slightly higher (≃15%) content of the 9-hydroxy compound. Since stem cutin contained a significantly higher proportion (25–30%) of the 9-hydroxy isomer, we determined the isomer composition of the dihydroxypalmitate from the cutin of the aerial portion of the stem and from the cutin of the underground portion of the stem (Fig. 1). The former contained 25% 9-hydroxy isomer (represented by the α-cleavage ions at m/e 289 and 303) whereas the latter contained 55% 9-hydroxy isomer. The reason for this remarkable difference in the positional isomer composition of the dihydroxy acids isolated from the same organ grown above and below the soil line is not understood.

Based on the observation that exogenous [1-^{14}C]palmitic acid was incorporated into dihydroxy C_{16} acid only by the shoot and not by the roots of germinating *V. faba*, it appeared that dihydroxy C_{16} acid was not a significant component of the polymer of the root (13). However, the chemical composition of the root polymer is not known. TLC examination of the hydrogenolysis products of the polymeric material from the tap roots of *V. faba* showed a major alkane-α,ω-diol fraction with little indication of any of the more polar polyhydroxyalkanes. Combined GLC-MS examination of the aliphatic components of the hydrogenolysate showed that the composition of the root polymer was quite different from that of the stem polymer (Fig. 2). The major components of the hydrogenolysate of the former were identified as hexadecane-1,16-diol (62%) and octadecene-1,18-diol (37%) with smaller amounts (<1%) of C_{17} and C_{19} alcohols, whereas hexadecane triol was the dominant (≃90%) component of the hydrogenolysate of the stem polymer. As the dominance of ω-hydroxy acids and dicarboxylic

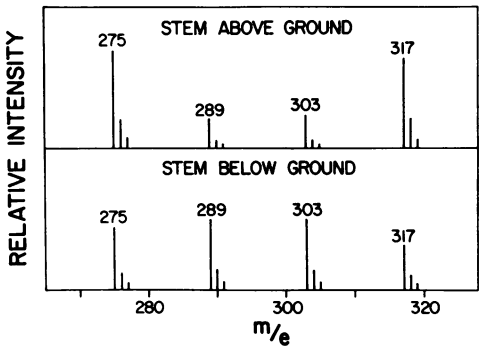

FIG. 1. Partial mass spectra of trimethylsilyl derivatives of C_{16} triols released by hydrogenolysis of cutin polymers from the above ground and below ground portions of *V. faba* stem.

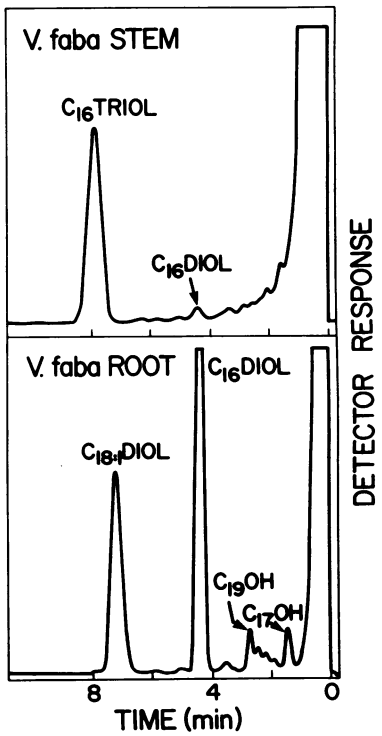

FIG. 2. GLC of trimethylsilyl ethers of aliphatic monomers obtained by hydrogenolysis of polymers from stem and tap root of *V. faba*. Experimental conditions were described previously (13).

acids is a characteristic feature of suberin-type polymers (16) the present results show that the root polymer is a typical suberin-type polymer.

Accumulation of 9,16- and 10,16-Dihydroxy C_{16} Acids in *V. faba* Leaves during Development. Our previous studies suggested that the proportion of the 9-hydroxy isomer of the dihydroxy C_{16} acid decreased from ≃35% to <10% as very young leaves of *V. faba* matured (13). Because of the present findings that the positional isomer composition of the stem below ground and above ground is different, we attempted to determine the factors which might contribute to the positional isomer composition. First, we determined the changes in the relative amounts of 9-hydroxy and 10-hydroxy isomers generated during the expansion of *V. faba* leaves by combined GLC-MS of the hydrogenolysis products of the polymeric material. The total amount of the dihydroxy acid increased slowly until the leaf area reached about 12 cm^2 and subsequently there was a rapid increase in this acid until the leaf reached about 25 cm^2 (Fig. 3). The quantity of 9-hydroxy isomer increased much more slowly than that of the 10-hydroxy isomer and it leveled off much earlier than that of the 10-hydroxy isomer. As a result the proportion of the 9-hydroxy isomer decreased as the leaf expanded.

Effect of Light on Positional Isomer Composition of the Dihydroxy C_{16} Acid of *V. faba* Cutin. The higher proportion of the 9-hydroxy isomer (noted above) found in the very young leaves and the underground part of stem of *V. faba* might be explained as being due to the lack of photosynthetic processes. To test this possibility, *V. faba* was grown in the dark for 1 week and then the effect of light on the positional isomer composition was determined. After 1 week of growth in the dark the stem contained a much higher proportion of 9-hydroxy isomer than that found in the leaf (Table I). After 5 days of further growth in the dark the isomer composition of the leaf did not change but there was a significant increase in the 9-hydroxy isomer content of the stem. A substantial amount of 16-oxo-9-hydroxy C_{16} acid was also present. On the other hand the dihydroxy C_{16} acid from the plants which were grown in the light during the 5-day period showed quite a different isomer composition which was very much like that of the leaf with <20% 9-hydroxy isomer. In all cases dihydroxy C_{16} acid was the major cutin component (>80%). These results raise the possibility that 10-hydroxy isomer content is somehow related to light. The previous observation that the 9-hydroxy isomer fraction contains a high proportion of 16-oxo compound (13) was verified by the present results and the dark-grown stem as well as the stem below ground contained substantial proportions of the 16-oxo compound (Table I). It appears possible that the 9-hydroxy isomer and 10-hydroxy isomer are generated by two different hydroxylating enzyme systems and that the 10-hydroxylating enzyme might be somehow induced by the presence of light.

Composition of the Cutin from Fruit, Leaf, Flower Petal, and Stigma of Apple. On the basis of the known composition of cutin from several types of plants, it was suggested that cutin of fast growing plants contains mainly the C_{16} family of monomers whereas slower growing plants with a thicker cuticle contain both the C_{16} and C_{18} families of monomers (19). If this tentative generalization is valid, rapidly growing organs of a plant could show a higher content of the C_{16} family of monomers than that found in the slower growing organs of the same plant. To test this possibility, we compared the composition of cutin from the fruit, leaf, flower petal, and stigma of apple. TLC of the hydrogenolysates showed that fruit cutin had the highest amount of the C_{18} family of components followed by the leaf cutin whereas the cutin of flower parts contained very much less of the C_{18} family of monomers (Fig. 4). This dramatic difference in composition was

Table I. *Effect of Light on Positional Isomer Composition of Midchain Hydroxylated C_{16} Acids in Cutin from Stem and from Leaf of V. faba Seedlings*

Growth Conditions	% Composition			
	9-Hydroxy-		10-Hydroxy-	
	ω-oxo	ω-hydroxy	ω-oxo	ω-hydroxy
Stem, 7 days dark	1.5	35.7	6.3	56.5
Leaf, 7 days dark	1.3	18.7	1.6	80.6
Stem, 7 days dark + 5 days dark	22.8	31.5	5.5	40.2
Leaf, 7 days dark + 5 days dark	1.6	18.5	1.6	78.3
Stem, 7 days dark + 5 days light	3.4	16.4	2.4	77.8
Leaf, 7 days dark + 5 days light	2.1	13.2		84.7
Stem grown below ground[a]	19.4	55.2	4.3	21.1

[a] Stem was from mature plants (in bloom).

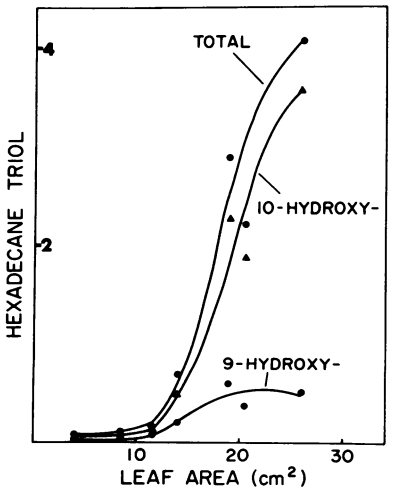

FIG. 3. Age-dependent changes in amount and in positional isomer composition of hexadecane triol fraction from hydrogenolysate of *V. faba* leaf cutin. The amount of total triol was determined by GLC and is represented by arbitrary units; positional isomer composition was estimated by MS (13).

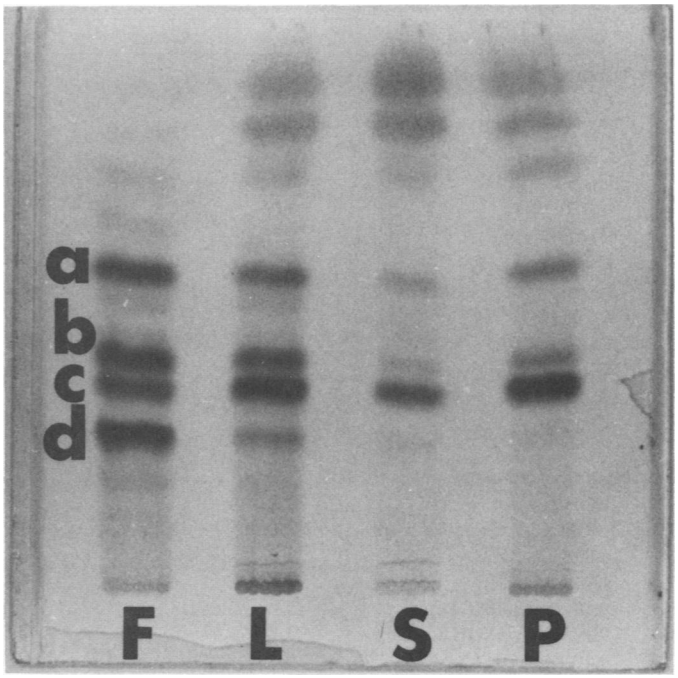

FIG. 4. TLC of aliphatic monomers released by hydrogenolysis of cuticular polymer from fruit (F), leaf (L), stigma (S), and flower petal (P) of apple. a, Alkane-α,-ω-diols; b, C_{18} triols; c, C_{16} triols; d, C_{18} tetraols.

confirmed by GLC-MS analyses of the hydrogenolysates. The C_{18}-hydroxylated components comprised 73% of the fruit cutin monomers and in the leaf cutin such components constituted 45% of the monomers, as previously observed (11). In the flower parts, however, these C_{18} monomers comprised only 12 to 14% (Table II). Thus, the C_{18} monomer content of the cutin reflected the rate of expansion of the various organs and supports the generalization that in fast growing plants and organs, cutin is composed of chiefly the C_{16} monomers. These results demonstrate that the chemical composition of cutin from one organ can be quite different from that of another organ of the same plant.

The positional isomer composition of the dihydroxy C_{16} acid fraction showed only small variation among the organs of apple which were examined. The dihydroxy acid from fruit, leaf, flower petal, and stigma contained 28, 27, 25, and 21% 9-hydroxy isomer, respectively (as judged by the relative intensities of the α-cleavage ions from the C_{16} triol). A more detailed analysis (28) of the C_{16} triol derived from $LiAlD_4$ treatment of the stigma polymer showed that 10-hydroxy, 9-hydroxy and 8-hydroxy isomers constituted 74, 20 and 7% of the dihydroxy acid respectively.

Table II. *Per Cent Composition of Aliphatic Monomers Released by Hydrogenolysis of Cutin from Fruit, Leaf, Flower Petal, and Stigma of Golden Delicious Apple Plants*

T indicates that the component was detectable but could not be measured.

	Fruit	Leaf	Flower Petal	Stigma
C_{16} alcohol	0.3	1.6	3.3	2.4
C_{18} alcohol	0.7	0.7	1.3	2.2
C_{16} diol	4.9	4.9	6.4	4.5
C_{18} diol	9.7	T	T	T
C_{16} triol	21.2	57.6	77.1	76.8
$C_{18:1}$ triol	14.6	8.2	7.8	5.7
$C_{18:0}$ triol	10.0	16.7	4.2	3.3
$C_{18:1}$ tetraol	9.4	2.4	T	3.0
$C_{18:0}$ tetraol	29.2	8.0	T	2.1

Composition of the Lipid-derived Polymer from Seed Coats and Leaves of Pea, Corn, Barley, and Lettuce. The seed coats of plants have been described as containing one or more cuticular layers (2, 4, 5, 21, 23). Little is known about the chemical composition of such layers, which develop in such internal parts of plants. However these individual cuticular layers cannot be readily isolated in pure form for chemical analysis. In this case, the seed coats were physically separated from the embryo. The insoluble polymeric residue, remaining after thorough solvent extraction and enzymic removal of the carbohydrates from the powdered seed coat, was subjected to depolymerization and the resulting monomers were analyzed by combined GLC-MS. In every case ω-hydroxylated products constituted the major class of monomers (Table III). C_{16} to C_{22} fatty alcohols and fatty acids were common components but in most cases they constituted relatively small proportions of the monomers. $C_{16:0}$ and $C_{18:1}$ ω-hydroxy acids were significant components in most cases although the predominance of ω-hydroxyoleic acid was observed only in the case of corn in which this acid was the most abundant monomer in both the seed coat and the leaf. ω-Hydroxy-9,10-epoxy C_{18} acid was present in most cases although in pea and corn seed coats this acid was a minor component, whereas in barley this epoxy acid constituted the most dominant monomer in both the seed coat and the leaf. Trihydroxy C_{18} acids, although present in most cases, were not the dominant component in any case. In general, the composition of the aliphatic components of the polymer in the seed coat is quite similar to that of the cuticular polymer of the leaf although some relatively small differences were observed.

The chemical composition of the aliphatic components of the polymeric materials strongly suggests that the seed coats of the plants examined contained cutin. Earlier reports have mentioned the presence of suberin in the internal layers of seed coats (1, 12, 27). Additionally, it has been proposed that the hilum (8) and the pigment strand of grass seeds (3, 29) are suberized. Typical aliphatic monomers of suberin include very long chain (C_{16}–C_{28})

Table III. *Per Cent Composition of Aliphatic Monomers from Cutin of Seed Coats and Leaves of Pea, Corn, Barley, and Lettuce*

Component	Pea		Corn		Barley		Lettuce	
	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed[b]	Leaf
Alcohols								
C_{16}	1.9	8.2	0.3	0.3	0.2	1.3	0.6	2.8
C_{17}[a]	0.8	19.4		4.0	7.8	6.9	8.2	5.1
$C_{18:1}$	0.5		0.2	0.8				
C_{18}	0.3		0.4	0.9			0.9	1.0
$C_{19:1}$[a]	0.06	1.8			3.5	2.1	2.1	2.0
C_{19}[a]	0.3	5.9		1.4	3.9	1.5	1.3	1.3
C_{20}	0.3	0.2	0.3	0.2	0.1	0.05		0.2
C_{22}	0.04	0.1	0.2	0.4		1.4		0.4
Fatty acids								
C_{16}	1.8	1.4	0.2	1.3	0.7	0.8	0.3	2.2
$C_{18:1}$	0.6		0.1	0.7				
C_{18}	0.4		0.3	0.6			0.6	0.8
C_{20}	0.5	0.5	0.4	0.2	0.2	0.05		0.3
C_{22}	0.1	0.7	1.8	0.2		0.2		0.2
Hydroxylated acids								
ωOH C_{16}	22.3	3.8	5.7	0.7	1.6	1.0	1.6	3.2
diOH C_{16}	59.1	37.2	20.0	13.7	16.2	20.1	8.1	26.9
ωOH $C_{18:1}$			51.2	31.7	11.2	4.4	15.8	4.8
Unknown C_{18}	0.3		4.4	17.9	1.4	9.9		
9,10 epoxy-18OH $C_{18:1}$							2.5	13.6
9,10 epoxy-18OH C_{18}	3.1		2.6	18.5	30.2	33.8	19.0	10.6
9,10,18 triOH $C_{18:1}$	6.0		0.4			7.8	8.3	2.9
9,10,18 triOH C_{18}	0.5	3.7	6.9	2.7	9.6	2.8	2.2	8.7

[a] Tentatively identified as a 2-ol on the basis of mass spectral data.

[b] Values obtained from analysis of whole seed.

Table IV. *Positional Isomer Composition of Dihydroxypalmitic Acid from Seed Coat and Leaf Cutin of Pea, Corn, Barley, and Lettuce*

	7,16-	8,16-	9,16-	10,16-
Pea				
Seed	7	14	40	39
Leaf	2	18	70	10
Corn				
Seed	7	19	28	46
Leaf	3	14	75	7
Barley				
Seed	9	38	42	11
Leaf	11	41	36	12
Lettuce				
Seed[a]		15	35	50
Leaf	3	7	68	22

[a] Values obtained from analysis of whole seed.

ω-hydroxy acids and the corresponding dicarboxylic acids, as well as C_{16}–C_{28} alcohols and fatty acids (15). The data in Table III give no indication of the presence of a suberin-type polymer in the seed coat. Since the insoluble polymeric material used in this study could have contained more than one type of polymer, possibly originating from different regions of the seed coat, the possibility of the presence of small suberized regions in the seed coat cannot be ruled out. It seems clear that the major aliphatic polymer of the seed coat is cutin.

Since the positional isomer composition of dihydroxy C_{16} acid appeared to show some variation within the same plant, it appeared possible that seed coat and leaf cuticles might show different positional isomer composition. With the exception of barley, significant differences in the positional isomer composition were observed (Table IV) and in all cases the seed coat contained a higher proportion of the 10,16-dihydroxy isomer than that found in the leaf. Based on previous results that the positional isomer composition of the dihydroxy C_{16} acid in the leaf cutin was quite similar to that in the tuber suberin of potato, it appeared that the isomer composition might be a characteristic feature of a species (14, 16) but the present results show that it is not necessarily so. However, the factors which control the positional isomer composition are not understood.

CONCLUSION

The monomer composition of the cuticular polymer in the aerial parts of *V. faba* is quite similar whereas in apple different organs showed different monomer compositions. It appears possible that in fast growing plants with relatively simple monomer composition (only C_{16} family) all aerial organs would show similar composition. On the other hand in plants with a more complex cutin, containing both the C_{16} and the C_{18} family of monomers, the composition would probably show organ-dependent variation. Although the factors which affect cutin composition have not been examined previously, the present results suggest that cutin composition could depend on the organ, growth condition, and the physiological state of the plant. Therefore, such factors should be carefully considered in attempts to use cutin composition for chemotaxonomic purposes.

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LITERATURE CITED

1. Ballard LAT 1973 Physical barriers to germination. Seed Sci Technol 1: 285–303
2. Bradbury D, MM MacMasters, IM Cull 1956 Structure of the mature wheat kernel. II. Microscopic structure of pericarp, seed coat, and other coverings of the endosperm and germ of hard red winter wheat. Cereal Chem 33: 342–360
3. Briggs DE 1974 Hydrocarbons, phenols and sterols of the testa and pigment strand in the grain of *Hordeum distichon*. Phytochemistry 13: 987–996
4. Collins EJ 1918 The structure of the integumentary system of the barley grain in relation to localized water absorption and semi-permeability. Ann Bot 32: 381–414
5. Esau K 1977 Anatomy of Seed Plants, Ed. 2. John Wiley & Sons, New York, pp 455–473
6. Espelie KE, PE Kolattukudy 1979 Composition of the aliphatic components of suberin of the endodermal fraction from the first internode of etiolated *Sorghum* seedlings. Plant Physiol 63: 433–435
7. Espelie KE, PE Kolattukudy 1979 Composition of the aliphatic components of suberin from the bundle sheaths of *Zea mays* leaves. Plant Sci Lett 15: 225–230
8. Giles KL, HCM Bassett, JD Eastin 1975 The structure and ontogeny of the hilum region in *Sorghum bicolor*. Aust J Bot 23: 795–802
9. Holloway PJ 1972 The composition of suberin from the corks of *Quercus suber* L. and *Betula pendula* Roth. Chem Phys Lipids 9: 158–170
10. Holloway PJ 1972 The suberin composition of the cork layers from some *Ribes* species. Chem Phys Lipids 9: 171–179
11. Holloway PJ 1973 Cutins of *Malus pumila* fruits and leaves. Phytochemistry 12: 2913–2922
12. Johann H 1942 Origin of the suberized semipermeable membrane in the caryopsis of maize. J Agric Res 64: 275–282
13. Kolattukudy PE 1974 Biosynthesis of a hydroxy fatty acid polymer, cutin. Identification and biosynthesis of 16-oxo-9- or 10-hydroxy palmitic acid, a novel compound in *Vicia faba*. Biochemistry 13: 1354–1363
14. Kolattukudy PE 1975 Biochemistry of cutin, suberin, and waxes, the lipid barriers on plants. *In* T Galliard, EI Mercer, eds, Recent Progress in the Chemistry and Biochemistry of Lipids. Academic Press, London, pp 203–246
15. Kolattukudy PE 1978 Chemistry and biochemistry of the aliphatic components of suberin. *In* G Kahl, ed, Biochemistry of Wounded Plant Tissues. Walter de Gruyter, Berlin, pp 43–84
16. Kolattukudy PE, VP Agrawal 1974 Structure and composition of the aliphatic components of potato tuber skin. Lipids 9: 682–691
17. Kolattukudy PE, R Croteau, L Brown 1974 Hydroxylation of palmitic acid and decarboxylation of C_{26}, C_{30}, and C_{32} acids in *Vicia faba* flowers. Plant Physiol 54: 670–677
18. Kolattukudy PE, TJ Walton 1972 Structure and biosynthesis of the hydroxy fatty acids of cutin in *Vicia faba* leaves. Biochemistry 11: 1897–1907
19. Kolattukudy PE, TJ Walton 1972 The biochemistry of plant cuticular lipids. Prog Chem Fats Other Lipids 13: 119–175
20. Martin JT, BE Juniper 1970 The Cuticles of Plants. St Martins Press, New York
21. Morrison IN 1975 Ultrastructure of the cuticular membranes of the developing wheat grain. Can J Bot 53: 2077–2087
22. O'Brien TP, DJ Carr 1970 A suberized layer in the cell walls of the bundle sheaths of grasses. Aust J Biol Sci 23: 275–287
23. Sanders EH 1955 Developmental morphology of the kernel in grain *Sorghum*. Cereal Chem 32: 12–25
24. Scott FM 1964 Lipid deposition in intercellular space. Nature 203: 164–165
25. Scott FM 1966 Cell wall surface of the higher plants. Nature 210: 1015–1017
26. Scott FM, BG Bystrom 1970 Mucilaginous idioblasts in okra, *Hibiscus esculentus* L. *In* NKB Robson, DF Cutler, M Gregory, eds, New Research in Plant Anatomy. Academic Press, London, pp 15–24
27. Scott FM, BG Bystrom, E. Bowler 1962 *Cercidium floridium* seed coat, light and electron microscopy study. Am J Bot 49: 821–833
28. Walton TJ, PE Kolattukudy 1972 Determination of the structure of cutin monomers by a novel depolymerization procedure and combined gas chromatography and mass spectrometry. Biochemistry 11: 1885–1897
29. Zee S-Y, TP O'Brien 1970 Studies on the ontogeny of the pigment strand in the caryopsis of wheat. Aust J Biol Sci 23: 1153–1171