Phytochemistry, Vol. 22, No. 2, pp. 495–502, 1983.
Printed in Great Britain.

0031–9422/83/020495–08$03.00/0

SOME VARIATIONS IN THE COMPOSITION OF SUBERIN FROM THE CORK LAYERS OF HIGHER PLANTS

PETER J. HOLLOWAY

Long Ashton Research Station, University of Bristol, Bristol BS19 9AF, U.K.

(*Received* 10 *June* 1982)

Key Word Index—Higher plants; cork; suberin; monomeric composition; 1-alkanols; alkanoic acids; α,ω-alkanedioic acids; ω-hydroxyalkanoic acids; epoxy- and hydroxyalkanoic acids; epoxy- and hydroxyalkanedioic acids.

Abstract—The monomeric composition of the suberins from 16 species of higher plants was determined by chromatographic methods following depolymerization of the isolated extractive-free cork layers with sodium methoxide–methanol. 1-Alkanols (mainly C_{18}–C_{28}), alkanoic (mainly C_{16}–C_{30}), α,ω-alkanedioic (mainly C_{16}–C_{24}), ω-hydroxyalkanoic (mainly C_{16}–C_{26}), dihydroxyhexadecanoic (mainly 10,16-dihydroxy- and 9,16-dihydroxyhexadecanoic), monohydroxyepoxyalkanoic (9,10-epoxy-18-hydroxyoctadecanoic), trihydroxyalkanoic (9,10,18-trihydroxyoctadecanoic), epoxyalkanedioic (9,10-epoxyoctadecane-1,18-dioic) and dihydroxyalkanedioic (9,10-dihydroxyoctadecane-1,18-dioic) acids were detected in all species. The suberins differed from one another mainly in the relative proportions of these monomer classes and in the homologue content of their 1-alkanol, alkanoic, α,ω-alkanedioic and ω-hydroxyalkanoic acid fractions. C_{18} epoxy and vic-diol monomers were major components (32–59%) of half of the suberins examined (*Quercus robur*, *Q. ilex*, *Q. suber*, *Fagus sylvatica*, *Castanea sativa*, *Betula pendula*, *Acer griseum*, *Fraxinus excelsior*) whereas ω-hydroxyalkanoic and α,ω-alkanedioic acids predominated in those that contained smaller quantities of such polar C_{18} monomers (*Acer pseudoplatanus*, *Ribes nigrum*, *Euonymus alatus*, *Populus tremula*, *Solanum tuberosum*, *Sambucus nigra*, *Laburnum anagyroides*, *Cupressus leylandii*). All species, however, contained substantial amounts (14–55%) of ω-hydroxyalkanoic acids, the most common homologues being 18:1 (9) and 22:0. The dominant α,ω-alkanedioic acid homologues were 16:0 and 18:1 (9), whereas 22:0, 24:0 and 26:0, and 20:0, 22:0 and 24:0 were usually the principal homologues in the 1-alkanol and alkanoic acid fractions, respectively. The most diagnostic feature of the suberins examined was the presence of monomers greater than C_{18} in chain length; most of the C_{16} and C_{18} monomers identified in the suberins also occur in plant cutins emphasizing the close chemical similarity between the two anatomical groups of lipid biopolymer.

INTRODUCTION

Many higher plants synthesize two unique groups of insoluble high MW lipid polyesters, cutins and suberins, usually distinguished from one another simply on the basis of the tissues in which they occur in the plant. Thus, cutins refer only to the structural polymers of cuticular membranes, the non-living extracellular layers present on the outer epidermal cell walls of aerial parts, particularly leaves and fruits. Suberins, on the other hand, are the names given to the various lipid polymers that characterize the secondary walls of cork cells (phellem) found mainly in the superficial periderm layers of both aerial and subterranean organs exhibiting secondary thickening, especially stems and roots. However, suberin, or suberin-like materials, are also described as structural components of the cell walls of other specialized internal tissues, such as the endodermis in roots [1–3], in some stems and in bundle sheaths of certain leaves [4–6], as well as the hypodermis of roots [7–9] and the crystal sheaths of idioblasts [10–12].

Although cutins and suberins occur in morphologically distinct tissues, they possess similar histochemical properties, have some ultrastructural features in common, and are clearly chemically related since they yield mixtures of various long chain substituted aliphatic monomers after de-esterification, several of which are common to both. However, the general chemical distinction between the two groups of biopolymer is not clear, even though their composition may be different for a given species, e.g. *Solanum tuberosum*, tuber periderm, leaf cuticle [13]; *Ribes grossularia*, stem periderm [14], leaf cuticle [15]; *Zea mays*, bundle sheath endodermis, leaf cuticle [16]; *Sorghum bicolor*, bundle sheath endodermis, stem cuticle [17]; *Citrus paradisi*, inner seed coat, leaf cuticle, fruit cuticle [18]; *Malus pumila*, leaf cuticle, fruit cuticle, stem periderm, root periderm [19]. Whilst criteria for the chemical characterization and classification of cutins have been reliably established from the examination of more than 50 species (for review see ref. [20]) those for suberins have not been adequately documented in a similar manner. This is reflected in the number of modifications made by Kolattukudy and co-workers (e.g. refs. [16, 18, 21–24]), to his original proposals for a chemical definition of suberin [25].

The object of the present work was to determine some more basic criteria to clarify the chemical nature of suberin. This was achieved by the compositional analysis of the monomers obtained from the depolymerization of the polymers in a range of higher plant species. For analysis the superficial cork layer was isolated from all the 16 plants studied so that there could be no doubt about

the identity of the biopolymer being examined. The results obtained are then examined in the light of the main proposals made by Kolattukudy for the classification of a lipid polymer as a suberin,* viz. that the monomers contain: (1) a substantial proportion of α,ω-alkanedioic and/or ω-hydroxyalkanoic acids (C_{16}–C_{24}); (2) significant amounts of alkanoic acids and 1-alkanols (C_{20}–C_{26}); and (3) usually small quantities of the substituted C_{16} and C_{18} monomers most characteristic of cutins, namely 9,16- and 10,16-dihydroxyhexadecanoic acids and 9,10-epoxy-18-hydroxy- and 9,10,18-trihydroxyoctadecanoic acids.

RESULTS AND DISCUSSION

The suberin contents of the cork layers of the 16 species examined ranged from 8 to 60% of the weight of the isolated extractive-free cork (see Experimental). The greatest amounts of suberin were found in the cork layers of *Laburnum anagyroides* and *Betula pendula*, and the least in those of the cork 'wings' of *Euonymus alatus* and young *Solanum tuberosum* tubers. All the corks yielded complex mixtures of various classes of long-chain aliphatic monomers on depolymerization with sodium methoxide–methanol. 1-Alkanols, alkanoic, α,ω-alkanedioic, ω-hydroxyalkanoic, dihydroxyalkanoic, monohydroxyepoxyalkanoic, trihydroxyalkanoic, epoxyalkanedioic and dihydroxyalkanedioic acids were detected in all species (Table 1). Wide variations occurred in the relative proportions of the above monomer classes between the various suberins but all contained substantial amounts of ω-hydroxyalkanoic acids. In *Ribes nigrum*, *Euonymus alatus*, *Populus tremula*, *Sambucus nigra*, *Laburnum anagyroides*, *Cupressus leylandii*, *Quercus suber*, *Acer griseum* and *Acer pseudoplatanus* this class of compound comprised 40–50% of the total monomeric mixture. However, several of the suberins also contained significant quantities (20–30% of total monomers) of α,ω-alkanedioic acids (*Ribes nigrum*, *Euonymus alatus*, *Populus tremula*, *Solanum tuberosum*, *Sambucus nigra*, *Laburnum anagyroides* and *Cupressus leylandii*) whilst others comprised up to 55% of monohydroxyepoxy- and trihydroxyalkanoic acids (*Quercus robur*, *Quercus ilex*, *Fagus sylvatica* and *Castanea sativa*). Epoxy- and dihydroxyalkanedioic acids were important components only of *Quercus suber*, *Acer griseum* and *Fraxinus excelsior*. 1-Alkanols, alkanoic and dihydroxyalkanoic acids were the minor classes of all the suberins, the largest amounts of 1-alkanols being present in *Solanum tuberosum* and *Fraxinus excelsior* (11 and 9.5% of total monomers, respectively), those of alkanoic acids in *Acer griseum* and *Cupressus leylandii* (11 and 9% of total monomers, respectively) and those of dihydroxyalkanoic acids in *Laburnum anagyroides* and *Betula pendula* (9 and 4% of total monomers, respectively).

The qualitative and quantitative composition of the various monomer classes present in the depolymerization products of the suberins was made by GC and GC/MS analysis after their initial separation by prep. TLC on silica gel. A wide range of homologues was present in the 1-alkanol, alkanoic, α,ω-alkanedioic and ω-hydroxyalkanoic acid fractions; in all species these were predominantly even-numbered carbon ranging between C_{16} and C_{32} (Table 2). In the 1-alkanol fractions, 22:0 was often the main compound but higher or lower homologues were also predominant in some species, e.g. 18:0 and 20:0 in *Sambucus nigra* and *Cupressus leylandii*, 24:0 in *Acer pseudoplatanus*, *Quercus ilex* and *Castanea sativa* and 26:0 and 28:0 in *Fraxinus excelsior*, *Euonymus alatus* and *Solanum tuberosum*. *Betula pendula* was unusual in containing very small amounts of 1-alkanols with only a trace of 22:0 being detected.

A similar overall chain-length distribution to that found in the 1-alkanols occurred in the alkanoic acid fractions with the major components usually being either 20:0 (*Acer pseudoplatanus* and *Ribes nigrum*) or 22:0 (*Sambucus nigra*, *Fagus sylvatica*, *Laburnum anagyroides* and *Acer griseum*) or 24:0 (*Quercus ilex* and *Euonymus alatus*). However, in *Betula pendula* there was a substantial proportion of 16:0 and 26:0, of 26:0 in *Quercus suber*, *Quercus ilex*, *Quercus robur and Euonymus alatus*, and of 26:0 and 28:0 in *Fraxinus excelsior* and *Solanum tuberosum*.

The α,ω-alkanedioic and ω-hydroxyalkanoic fractions of all species differed mainly from the corresponding 1-alkanol and alkanoic fractions in containing 18:1 (9) monounsaturated homologues (Table 2). The most common α,ω-alkanedioic acids were 16:0 (up to 14% of total monomers) and 18:1 (9) (up to 32% of total monomers) but higher homologues comprised a substantial proportion in some species, e.g. *Cupressus leylandii* and *Ribes nigrum* (18:0), *Betula pendula* (20:0 and 22:0) and *Quercus suber* (22:0). *Solanum tuberosum* was exceptional in containing essentially all 18:1 (9).

A much wider variation occurred in the homologue content of the ω-hydroxyalkanoic acid fractions of the suberins examined. Although 18:1 (9) and 22:0 (both up to *ca* 25% of total monomers) were important constituents of all, considerable proportions of 16:0 were present in *Sambucus nigra*, *Acer pseudoplatanus*, *Cupressus leylandii*, *Laburnum anagyroides*, *Castanea sativa and Populus tremula*, of 20:0 in *Acer pseudoplatanus*, *Betula pendula* and *Ribes nigrum* and of 24:0 in *Quercus suber*, *Quercus ilex* and *Euonymus alatus*. A significant proportion of ω-hydroxyalkanoic acids greater than 24:0 in chain length was detected in *Fraxinus excelsior* and *Solanum tuberosum*.

Surprisingly there appeared to be little chemical relationship between any of the simple mono- and di-substituted suberin monomers for a given species (Table 2); there was some resemblance in the chain length distribution of the homologues between the 1-alkanol and alkanoic acid fractions of *Quercus ilex*, *Fagus sylvatica*, *Castanea sativa* and *Quercus suber* and between the α,ω-alkanedioic and ω-hydroxyalkanoic acid fractions of *Solanum tuberosum* and *Populus tremula*. These differences of composition in turn suggest that for the four classes of monomer there is probably a separate biosynthetic pathway within the cork cell.

The two epoxy and the corresponding two vic-diol acid

*In this paper, as in those of previous publications from this laboratory, suberin is regarded as a predominantly aliphatic material by analogy with cutin. Kolattukudy [25] has also proposed that a high content of phenolic materials is another feature which distinguishes a suberin from a cutin. However, such compounds whilst present are obtained from cork cell or similar suberin-containing tissue preparations; a pure suberin polymer has not at the present time been isolated in a pure state. Therefore, further work is necessary to establish whether or not there is a direct involvement of these phenolic compounds with the suberin polyester *per se*.

Table 1. Composition (% total monomers) of the suberins from the cork layers of 16 higher plants

Species	1-Alkanols* (C_{18}–C_{32})	Acids							
						Octadecanoic		Octadecane-1,18-dioic	
		Alkanoic† (C_{16}–C_{32})	α,ω-Alkanedioic† (C_{16}–C_{32})	ω-Hydroxyalkanoic‡ (C_{16}–C_{32})	Dihydroxy-hexadecanoic‡	9,10-Epoxy-18-hydroxy§	9,10,18-Trihydroxy‡	9,10-Epoxy§	9,10-Dihydroxy‡
Quercus robur	2.3	2.0	4.7	21.3	3.7	22.7	25.3	3.5	6.9
Quercus ilex	5.6	6.0	2.6	23.9	0.7	31.4	16.6	4.4	3.5
Fagus sylvatica	6.8	4.4	8.4	18.7	0.8	28.7	26.4	0.5	3.0
Castanea sativa	3.9	1.1	1.7	13.4	2.2	12.8	31.9	3.1	5.1
Betula pendula	TR	TR	8.0	21.6	4.2	15.9	29.1	TR	TR
Quercus suber	2.3	1.3	9.0	40.7	TR	15.3	5.4	15.9	7.8
Acer griseum	2.0	10.7	5.9	39.6	0.3	2.0	10.9	2.9	17.1
Fraxinus excelsior	9.5	7.9	15.2	24.3	TR	2.9	4.5	11.3	13.6
Acer pseudoplatanus	4.7	2.8	17.0	45.3	2.8	2.1	3.5	1.3	2.2
Ribes nigrum‖	6.2	4.0	20.2	49.7	2.9	1.2	1.6	TR	TR
Euonymus alatus	1.5	6.2	24.0	61.2	0.8	1.0	1.6	TR	TR
Populus tremula	2.3	6.2	27.1	54.5	1.9	0.5	1.1	TR	0.7
Solanum tuberosum	11.1	8.2	32.7	31.7	TR	0.5	TR	TR	1.5
Sambucus nigra	6.1	6.7	23.7	50.5	0.6	0.3	0.8	0.6	0.3
Laburnum anagyroides	1.5	1.5	21.7	48.6	9.4	0.3	1.2	TR	0.1
Cupressus leylandii	3.6	9.4	31.5	51.2	0.4	0.1	TR	TR	0.1

*Determined by GC analysis of corresponding TMSi ether derivatives.
†Determined by GC analysis of corresponding Me ester derivatives.
‡Determined by GC analysis of corresponding Me ester TMSi ether derivatives.
§Determined by GC analysis of corresponding methoxyhydrin Me ester TMSi ether derivatives.
‖Also contains 9.6% of α,ω-alkanediols; homologue composition (%) 18:0 (13.1), 20:0 (70.9), 22:0 (14.0) and 24:0 (2.0).
TR, trace.

Table 2. Composition of the 1-alkanol, alkanoic, α,ω-alkanedioic and ω-hydroxyalkanoic acid fractions from the cork suberins of 16 higher plants

Species/fraction	Homologue composition of fraction (%)									
	16:0	18:1 (9)	18:0	20:0	22:0	24:0	26:0	28:0	30:0	32:0
Quercus robur										
1-Alkanols	TR	ND	15.9	29.5	24.7	22.0	7.9	TR	ND	ND
Alkanoic acids	1.9	ND	1.7	5.4	42.7	22.1	13.7	9.9	2.6	ND
α,ω-Alkanedioic acids	49.4	22.7	8.0	6.4	9.9	3.6	TR	ND	ND	ND
ω-Hydroxyalkanoic acids	9.6	37.3	1.1	6.4	35.7	9.9	TR	ND	ND	ND
Quercus ilex										
1-Alkanols	ND	ND	1.5	8.5	30.1	45.2	14.3	0.4	ND	ND
Alkanoic acids	1.5	ND	1.0	6.2	25.8	48.2	16.0	1.1	0.2	ND
α,ω-Alkanedioic acids	27.6	36.7	9.3	9.2	11.4	5.0	1.8	TR	ND	ND
ω-Hydroxyalkanoic acids	5.6	30.6	1.5	8.5	24.1	21.1	8.5	TR	ND	ND
Fagus sylvatica										
1-Alkanols	ND	ND	TR	1.1	65.4	30.5	3.0	ND	ND	ND
Alkanoic acids	0.8	ND	0.4	8.1	66.5	23.0	1.2	TR	ND	ND
α,ω-Alkanedioic acids	64.4	11.2	12.5	6.8	4.3	0.8	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	17.9	7.2	2.5	13.6	48.5	10.3	TR	ND	ND	ND
Castanea sativa										
1-Alkanols	ND	ND	3.2	6.8	41.9	43.4	4.7	ND	ND	ND
Alkanoic acids	3.0	ND	1.6	8.9	53.5	30.7	2.2	TR	ND	ND
α,ω-Alkanedioic acids	62.9	5.0	12.7	9.9	7.3	2.2	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	20.6	33.3	2.8	10.8	27.0	5.5	ND	ND	ND	ND
Betula pendula										
1-Alkanols	ND	ND	ND	ND	TR	ND	ND	ND	ND	ND
Alkanoic acids	22.0	ND	TR	17.0	34.2	15.0	11.8	TR	ND	ND
α,ω-Alkanedioic acids	6.8	7.2	15.2	18.8	51.7	0.3	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	1.9	11.6	1.7	19.6	64.7	0.5	ND	ND	ND	ND
Quercus suber										
1-Alkanols	ND	ND	2.6	8.6	54.3	26.5	8.0	TR	ND	ND
Alkanoic acids	7.6	ND	1.9	3.2	40.9	35.1	10.4	0.9	ND	ND
α,ω-Alkanedioic acids	14.1	31.4	3.6	6.8	35.6	7.9	0.5	ND	ND	ND
ω-Hydroxyalkanoic acids	1.9	28.1	0.4	3.8	48.5	16.3	1.0	ND	ND	ND
Acer griseum										
1-Alkanols	ND	ND	ND	TR	23.0	34.1	39.5	3.4	TR	ND
Alkanoic acids	0.5	ND	0.3	9.8	67.9	19.8	1.7	TR	ND	ND
α,ω-Alkanedioic acids	26.3	39.0	5.6	7.2	18.5	3.4	TR	ND	ND	ND
ω-Hydroxyalkanoic acids	2.7	16.7	0.5	8.5	59.6	11.8	0.2	ND	ND	ND
Fraxinus excelsior										
1-Alkanols	ND	ND	ND	0.7	31.8	10.1	26.6	29.8	1.0	TR
Alkanoic acids	2.3	ND	0.7	3.2	14.3	21.6	23.3	26.7	7.9	TR
α,ω-Alkanedioic acids	10.6	78.8	5.1	2.5	1.2	1.3	0.5	TR	ND	ND
ω-Hydroxyalkanoic acids	2.8	63.1	0.2	3.2	8.5	10.9	7.4	3.9	TR	ND
Acer pseudoplatanus										
1-Alkanols	ND	ND	TR	17.0	16.9	50.5	15.6	TR	ND	ND
Alkanoic acids	5.9	ND	2.0	51.0	35.2	4.0	1.8	TR	ND	ND
α,ω-Alkanedioic acids	73.3	11.5	7.9	3.1	4.2	TR	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	29.6	15.0	4.5	24.5	25.5	0.9	TR	ND	ND	ND
Ribes nigrum										
1-Alkanols	TR	ND	1.9	34.0	59.3	4.0	0.8	ND	ND	ND
Alkanoic acids	4.9	ND	1.1	47.2	38.2	8.6	TR	ND	ND	ND
α,ω-Alkanedioic acids	33.6	42.1	14.0	8.2	2.1	TR	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	18.6	39.3	4.7	25.5	11.5	0.4	ND	ND	ND	ND
Euonymus alatus										
1-Alkanols	ND	ND	10.5	22.8	33.7	6.7	14.6	11.7	TR	ND
Alkanoic acids	9.9	ND	3.1	16.9	27.9	30.2	11.4	0.6	TR	ND
α,ω-Alkanedioic acids	27.7	46.0	5.0	4.8	8.3	7.7	0.5	TR	ND	ND
ω-Hydroxyalkanoic acids	4.2	32.1	0.8	6.3	29.9	23.0	3.7	TR	ND	ND
Populus tremula										
1-Alkanols	ND	ND	4.1	12.4	41.2	18.1	24.2	TR	ND	ND
Alkanoic acids	0.7	ND	TR	7.3	50.7	37.9	3.4	TR	ND	ND
α,ω-Alkanedioic acids	49.0	40.2	6.5	1.2	2.6	0.5	ND	ND	ND	ND

Table 2—*contd.*

Species/fraction	Homologue composition of fraction (%)									
	16:0	18:1 (9)	18:0	20:0	22:0	24:0	26:0	28:0	30:0	32:0
ω-Hydroxyalkanoic acids	40.1	39.4	1.1	2.3	12.7	4.4	TR	ND	ND	ND
Solanum tuberosum										
1-Alkanols	ND	ND	4.1	1.4	40.0	18.1	13.4	23.0	ND	ND
Alkanoic acids	1.4	ND	1.2	1.7	9.6	20.1	29.2	31.1	5.7	TR
α,ω-Alkanedioic acids	1.2	98.2	0.2	TR	0.1	0.2	0.1	ND	ND	ND
ω-Hydroxyalkanoic acids	0.7	76.6	5.0	0.2	4.3	6.2	4.8	2.2	ND	ND
Sambucus nigra										
1-Alkanols	TR	ND	20.2	42.7	37.1	TR	TR	ND	ND	ND
Alkanoic acids	TR	ND	TR	18.1	77.4	4.5	TR	ND	ND	ND
α,ω-Alkanedioic acids	39.5	47.3	4.2	1.2	7.8	TR	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	20.3	44.2	0.9	6.0	28.0	0.6	TR	ND	ND	ND
Laburnum anagyroides										
1-Alkanols	ND	ND	2.6	7.5	84.1	2.7	3.1	ND	ND	ND
Alkanoic acids	2.1	ND	0.9	24.9	68.6	2.4	1.1	ND	ND	ND
α,ω-Alkanedioic acids	43.5	35.5	10.4	2.3	8.0	0.3	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	31.1	51.6	2.9	2.9	11.2	0.3	ND	ND	ND	ND
Cupressus leylandii										
1-Alkanols	TR	ND	34.4	21.3	35.5	1.1	7.7	ND	ND	ND
Alkanoic acids	1.5	ND	2.2	28.4	49.8	17.0	1.1	ND	ND	ND
α,ω-Alkanedioic acids	15.4	54.6	16.5	9.6	3.7	0.2	ND	ND	ND	ND
ω-Hydroxyalkanoic acids	25.0	34.2	15.2	18.0	7.2	0.4	TR	ND	ND	ND

For details of GC analysis consult Table 1. TR, trace; ND, not detected.

classes of the 16 suberins were exclusively C_{18} in chain length and the monomers were 9,10-epoxy-18-hydroxyoctadecanoic (**1**) and 9,10,18-trihydroxyoctadecanoic (**2**), and 9,10-epoxyoctadecane-1,18-dioic (**3**) and 9,10-dihydroxyoctadecane-1,18-dioic (**4**). In half of the plant suberins examined such compounds comprised a major proportion of the total depolymerization products (Table 1); they were particularly prominent in members of the Fagaceae (*Quercus*, *Fagus* and *Castanea*). The relative amounts of **3** and **4** were usually much less than those of **1** and **2** except in *Quercus suber* (**1**+**2** = 20.7%, **3**+**4** = 23.7% of total monomers), *Acer griseum* (**1**+**2** = 12.9%, **3**+**4** = 20% of total monomers) and *Fraxinus excelsior* (**1**+**2** = 7.4%, **3**+**4** = 24.9% of total monomers). For **1** and **2** in some of the suberins there was more of the epoxide than the vic-diol (*Quercus ilex*), in others more of the vic-diol than the epoxide (*Castanea sativa* and *Acer griseum*) as well as some containing *ca* equal proportions of each (*Quercus robur* and *Fagus sylvatica*). The maximum epoxide content was found in the suberin of *Quercus ilex* (*ca* 35% of total monomers). Compounds **1** and **2** are common constituents of plant cutins where they may occur in equally large amounts [20]; **3** and **4** are found much less frequently in such biopolymers.

Positional isomers of C_{16} dihydroxyalkanoic acids were identified in all species (Table 1), the major compounds being 10,16-dihydroxy- and 9,16-dihydroxyhexadecanoic acids with much smaller amounts of the corresponding 8,16- and 7,16-isomers. These acids are ubiquitous in plant cutins [20] and their presence in suberins, albeit as minor components, is curious. In some of the species, especially those where the cork cambium (phellogen) arises in the sub-epidermis or cortex of the stem, the compounds might originate in part from persistent remnants of the cuticular membrane which originally covered the young stem. Such a situation would probably be more apparent in any samples aged between 1 and 3 years but detailed microscopical examination was not carried out to confirm this point. Nevertheless the presence of a cuticle cannot explain the occurrence of small amounts of dihydroxyhexadecanoic acids in the thick and extensively developed cork layers of *Quercus suber*. Dihydroxyoctadecanoic acids (mainly 10,18-dihydroxy-; 0.9% of total monomers) were also confirmed in *Betula pendula* [26] and trace amounts of the same compounds were tentatively identified in the suberins of *Sambucus nigra*, *Acer pseudoplatanus*, *Quercus suber*, *Quercus ilex*, *Laburnum anagyroides*, *Quercus robur* and *Castanea sativa*.

Another type of monomer more characteristic of plant cutins [20], monohydroxyalkanedioic acids, was also identified in all the suberins except those from *Cupressus leylandii* and *Solanum tuberosum*. 7-Hydroxy- and 8-hydroxyhexadecane-1,16-dioic acids together comprised 9.1% of the total monomers of *Acer pseudoplatanus*, 3.7% of those from *Castanea sativa*, 2.3% of those from *Quercus suber* and 1.9% of those from *Fagus sylvatica*; only trace amounts of the acids occurred in the other suberins. The same compounds have been previously reported as minor polar constituents of the suberins in the roots of *Ipomoea batata* and *Beta vulgaris* [27]. In some of the species examined in the present work these alkanedioic acids might represent the oxidation products derived from the corresponding dihydroxyhexadecanoic acids present in the remains of the cuticle on the isolated cork layer preparation. As found in a recent analysis [28], the presence of 9-hydroxyoctadecane-1,18-dioic acid in the suberin of *Quercus suber* [29] was not confirmed.

Small amounts of simple phenolic acids were found in

the depolymerization products of a few of the species. Since the corresponding methyl esters were obtained with sodium methoxide–methanol such compounds were originally in an esterified form in the cork layer preparations. However, it is not possible to say from these results whether they are esterified to the aliphatic components of the suberin or not. Ferulic acid was the main phenolic compound present and it comprised *ca* 1.5% of the total depolymerization products from *Acer pseudoplatanus*, *Quercus suber*, *Populus tremula*, *Acer griseum* and *Solanum tuberosum*.

A series of compounds with GC R_t values much greater than those of the usual suberin aliphatic constituents were also present in the depolymerization products obtained from the cork layers of *Betula pendula* and from all the species belonging to the Fagaceae. Although not investigated in detail by mass spectrometry they could be isolated by prep. TLC in the same fraction as that containing the 1-alkanols. Therefore, they are probably pentacyclic triterpenols. Since such compounds are common constituents of the soluble wax fraction of cork cells [19] they could possibly have arisen, in the present work, from inadequate removal of such wax during extraction of the cork samples prior to depolymerization.

Amongst the other unidentified monomers most of the suberins contained variable, but small amounts (less than 5% of total monomers), of the same two compounds but with the relative proportions between them varying with species. The content of these constituents was always greatest in those suberins which lacked epoxide and vic-diol monomers especially *Sambucus nigra*, *Laburnum anagyroides* and *Populus tremula*. The compounds had similar TLC (unresolved), GC (resolved) and mass spectral properties, which suggested that they were both monounsaturated dihydroxyoctadecanoic acids. Their GC/MS were typical of long chain methyl ester TMSi ethers [20] showing $[M-15]^+$ (m/z 457), $[M-31]^+$ (m/z 441) and $[M-47]^+$ (m/z 425) ions at the high mass end. Mid-chain fragment ions (max. rel. int. 10–20%) were observed at m/z 271, 285, 315 and 329, indicating the existence of a number of positional isomers; the two compounds differed from one another only in the relative intensities of these four ions. Interestingly, identical components comprise a substantial proportion of the sodium methoxide–methanol depolymerization products of *Zea mays* leaf cutin [Holloway, P. J., unpublished results] and a similar C_{18} compound has also been described, but not fully characterized, in the same cutin following $LiAlH_4$–$LiAlD_4$ depolymerization [16]. Further work is in progress in our laboratory to determine the precise structure of these novel cutin and suberin monomers.

The composition of the 16 suberins described in the present work was complex and, consequently, there was no monomer, or group of monomers, that was particularly characteristic of all species. The analyses of the suberins of *Betula pendula*, *Quercus suber*, *Ribes nigrum* and *Solanum tuberosum* were in general agreement with published information [14, 26, 28, 30–32]; these species were included so that a direct comparison could be made with the suberins of the previously uninvestigated species. *Solanum tuberosum* tubers have an unusual suberin composition with a very high content of 18:1 (9) monomers; *Ribes* species are also exceptional in containing an additional monomer class of α,ω-alkanediols. Although the qualitative composition of the suberins examined was similar, they could be roughly divided into two main chemical groups according to the relative amounts of the more polar C_{18} epoxy and vic-diol monomers present. The species are arranged in approximate order of decreasing content of such monomers in Table 1. In the suberins that contain small amounts of polar C_{18} monomers there is a predominance of ω-hydroxyalkanoic and α,ω-alkanedioic acids. This type of suberin has been described previously in the exodermis of six root vegetables [27], in the inner seed coat of grapefruit [18] and is now confirmed in several stem periderms. The present survey has clearly established that suberins with a high content of polar C_{18} monomers are not unusual and must, therefore, be included in any general scheme of classification. It is already apparent that suberin chemistry can have only a very limited application to problems concerned with chemotaxonomy because widely different species may have a similar composition.

The most diagnostic feature of all the suberins was the presence of detectable and sometimes substantial amounts of monomers greater than C_{18} in chain length (Table 3), especially C_{22} compounds, with some species also containing significant quantities of C_{20} (*Acer pseudoplatanus*, *Cupressus leylandii* and *Ribes nigrum*) or C_{24} compounds (*Quercus suber*, *Quercus ilex* and *Euonymus alatus*). These monomers are simple saturated 1-alkanols, alkanoic, α,ω-alkanedioic and ω-hydroxyalkanoic acids. Nearly all of the C_{16} and C_{18} monomers identified in the suberins, however, have been reported before in plant cutins [20], emphasizing the close chemical similarity between the two groups of biopolymers. Unlike cutins [20] a definite classification according to chain length cannot be proposed for suberins at the present time.

Even in the limited survey of plant suberins carried out in the present work exceptions were found to most of the criteria that have been proposed by Kolattukudy in order to identify a lipid polymer as a suberin (see Introduction). Not all of them contained substantial proportions of α,ω-alkanedioic acids, e.g. *Castanea sativa* (1.7% of total monomers) and *Quercus ilex* (2.6% of total monomers), although ω-hydroxyalkanoic acids were important components of most. Likewise, significant quantities of 1-alkanols and alkanoic acids were not always detected, e.g. in *Betula pendula* and *Laburnum anagyroides*. Most important of all was the presence, often as major components of the suberins, of the substituted C_{16} and C_{18} monomers most characteristic of cutins. Clearly further work is needed on other species before a precise chemical definition of suberin can be made.

EXPERIMENTAL

Plant material. Quercus suber L. was a commercial sample of cork kindly supplied by the Southern Cork Co., Crawley, U.K. All other samples were collected in the field from trees or shrubs growing in the vicinity of the Research Station. Naturally exfoliating cork layers were detached from the main trunks of *Betula pendula* Roth. and *Acer griseum*, and from branches of *Ribes nigrum* L. Mechanical removal was used to obtain the superficial cork layers from 2-year-old branches of *Laburnum anagyroides* Medic., *Quercus robur* L. and *Populus tremula* L., and that from 3-year-old branches of *Castanea sativa* Mill. and *Sambucus nigra* L. Strips of outer bark were cut from 2–4-year-old branches of *Fagus sylvatica* L., *Quercus ilex* L., *Cupressus leylandii*, *Acer pseudoplatanus* L. and *Fraxinus excelsior* L. and

Table 3. Overall chain length distribution of the principal monomers from the suberins of 16 higher plants

Species	Total monomers (%)									
	16:0	18:1 (9)	18:0	20:0	22:0	24:0	26:0	28:0	30:0	32:0
Quercus robur	11.0	9.5	62.7	2.6	10.0	3.4	0.6	0.2	TR	ND
Quercus ilex	3.0	8.7	59.8	3.3	9.8	11.2	4.1	0.1	TR	ND
Fagus sylvatica	10.7	2.6	60.7	3.7	17.0	5.1	0.2	TR	ND	ND
Castanea sativa	11.9	5.6	65.7	2.4	7.3	3.4	3.7	TR	ND	ND
Betula pendula	6.4	3.9	59.6	7.2	22.7	0.2	TR	ND	ND	ND
Quercus suber	2.3	14.6	46.0	2.4	25.3	8.6	0.8	TR	ND	ND
Acer griseum	4.6	9.6	36.0	5.2	35.0	8.3	1.2	0.1	TR	ND
Fraxinus excelsior	2.8	30.6	37.2	1.7	7.1	6.2	7.0	6.6	0.8	TR
Acer pseudoplatanus	40.8	9.4	15.9	14.8	15.1	3.1	0.9	TR	ND	ND
Ribes nigrum	21.4	28.7	9.7	26.0	13.1	1.0	0.1	ND	ND	ND
Euonymus alatus	11.6	32.5	4.9	6.6	22.7	18.1	3.4	0.2	TR	ND
Populus tremula	39.3	34.3	5.0	2.5	12.4	5.7	0.8	ND	ND	ND
Solanum tuberosum	0.8	65.9	4.9	0.5	7.7	6.6	6.3	6.8	0.5	ND
Sambucus nigra	22.5	37.4	5.3	8.0	26.2	0.6	TR	ND	ND	ND
Laburnum anagyroides	40.3	38.9	6.3	2.9	11.3	0.3	TR	ND	ND	ND
Cupressus leylandii	18.9	36.0	15.2	16.3	11.2	2.0	0.4	ND	ND	TR

TR, trace; ND, not detected.

soaked in ammonium oxalate–oxalic acid soln [33] until the cork layers could be easily separated from the rest of the bark tissue. A similar separation method was used for the periderm of young tubers of *Solanum tuberosum* L. cv Whites using 2 cm^2 discs punched out from the outer skin with a cork borer. The 'cork wings' which occur naturally on branches of *Euonymus alatus* were broken off and used directly as the source of suberin. After separation, all isolated cork layer preparations were dried, ground to a fine powder in a Wiley mill and then exhaustively extracted with boiling $CHCl_3$ followed by boiling MeOH.

Suberin analysis. Depolymerization of each extractive-free cork layer preparation was carried out with 0.5 M NaOMe in dry MeOH in the presence of MeOAc as described previously [34]. Suberin contents were determined from the wt of Et_2O-soluble monomers recovered and were as follows (mean of three determinations, % of extractive-free cork preparation): *Laburnum anagyroides*, 61.7; *Betula pendula*, 58.6; *Fagus sylvatica*, 48.3; *Quercus suber*, 43.3; *Castanea sativa*, 43.2; *Quercus robur*, 39.7; *Populus tremula*, 37.9; *Cupressus leylandii*, 27.5; *Acer pseudoplatanus*, 26.6; *Acer griseum* 26.1; *Quercus ilex*, 24.9; *Fraxinus excelsior*, 22.1; *Sambucus nigra*, 21.7; *Ribes nigrum*, 21.1; *Solanum tuberosum*, 12.1 and *Euonymus alatus*, 8.0. The monomeric composition of the suberin depolymerization products was determined using published TLC (Si gel), prep. TLC (Si gel), GC (SE30 and OV17) and GC/MS methods [20, 26, 30, 34, 35]. Identification of monomers was made by direct chromatographic and MS comparison with authentic compounds. GC was used for quantitation and peak areas were measured by electronic integration. Values quoted in Tables 1–3 are the mean of at least two separate GC determinations; the s.d.s were less than 5% for all species.

Acknowledgements—Long Ashton Research Station is a state-aided institute of the Agricultural Research Council to whom I am grateful.

REFERENCES

1. Kuo, J. and Cambridge, M. L. (1978) *Aquatic Botany* **5**, 191.
2. Mackenzie, K. A. D. (1979) *Protoplasma* **100**, 21.
3. Scott, M. G. and Peterson, R. L. (1979) *Can. J. Botany* **57**, 1040.
4. O'Brien, T. P. and Kuo, J. (1975) *Aust. J. Botany* **23**, 783.
5. Evert, R. F., Eschrich, W. and Heyser, W. (1977) *Planta* **136**, 77.
6. Eleftheriou, E. P. and Tsekos, I. (1979) *Protoplasma* **100**, 139.
7. Olesen, P. (1978) *Protoplasma* **94**, 325.
8. Peterson, C. A., Peterson, R. L. and Robards, A. W. (1978) *Protoplasma* **96**, 1.
9. Robards, A. W., Clarkson, D. T. and Sanderson, J. (1979) *Protoplasma* **101**, 331.
10. Wattendorff, J. and Schmid, H. (1973) *Z. Pflanzenphysiol.* **68**, 422.
11. Wattendorff, J. (1976) *Planta* **128**, 163.
12. Wattendorff, J. (1978) *Protoplasma* **95**, 193.
13. Brieskorn, C. H. and Binneman, P. H. (1975) *Phytochemistry* **14**, 1363.
14. Holloway, P. J. (1972) *Chem. Phys. Lipids* **9**, 171.
15. Holloway, P. J., Baker, E. A. and Martin, J. T. (1972) *An. Quim.* **68**, 905.
16. Espelie, K. E. and Kolattukudy, P. E. (1979) *Plant Sci. Letters* **15**, 225.
17. Espelie, K. E. and Kolattukudy, P. E. (1979) *Plant Physiol.* **63**, 433.
18. Espelie, K. E., Davis, R. W. and Kolattukudy, P. E. (1980) *Planta* **149**, 498.
19. Holloway, P. J. (1982) *Phytochemistry* **21**, 2517.
20. Holloway, P. J. (1982) in *The Plant Cuticle* (Cutler, D. F., Alvin, K. L. and Price, C. E., eds.) Linnean Society Symposium No. 10, p. 45. Academic Press, London.
21. Kolattukudy, P. E. (1977) in *The Structure, Biosynthesis, and Degradation of Wood* (Loewus, F. A. and Runeckles, V. C., eds.) p. 185. Plenum Press, New York.
22. Kolattukudy, P. E. (1978) in *Biochemistry of Wounded Plant Tissues* (Kahl, G., ed.) p. 43. De Gruyter, Berlin.
23. Kolattukudy, P. E. (1980) *Science* **208**, 990.
24. Kolattukudy, P. E. (1981) *Annu. Rev. Plant Physiol.* **32**, 539.
25. Kolattukudy, P. E. (1975) in *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (Galliard, T. and Mercer, E. I., eds.) p. 203. Academic Press, London.

26. Holloway, P. J. (1972) *Chem. Phys. Lipids* **9**, 158.
27. Kolattukudy, P. E., Kronman, K. and Poulose, A. J. (1975) *Plant Physiol.* **55**, 567.
28. Arno, M., Serra, M. C. and Seoane, E. (1981) *An. Quim.* **77C**, 82.
29. Gonzalez-Gonzalez, A., Sola, J., Iglesias-Martin, F., Rivas-Paris, V. and Ribas-Marques, I. (1968) *Quim. Ind.* **15**, 139.
30. Holloway, P. J. and Deas, A. H. B. (1973) *Phytochemistry* **12**, 1721.
31. Brieskorn, C. H. and Binneman, P. H. (1974) *Z. Lebensm. Unters.-Forsch.* **154**, 213.
32. Kolattukudy, P. E. and Agrawal, V. P. (1974) *Lipids* **9**, 682.
33. Huelin, F. E. and Gallop, R. A. (1951) *Aust. J. Sci. Res. Ser. B.* **4**, 526.
34. Holloway, P. J., Brown, G. A. and Wattendorff, J. (1981) *J. Exp. Botany* **32**, 1051.
35. Holloway, P. J. (1973) *Phytochemistry* **12**, 2913.