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Suberin: A promising renewable resource for novel macromolecular materials

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Abstract

Suberin, an aliphatic-aromatic cross-linked natural polymer present in the outer tissues of numerous vegetable species, is discussed in terms of (i) its occurrence, particularly where it dominates the bark composition of some trees, (ii) its macromolecular structure and positioning within the cell wall, (iii) its controlled chemical splicing (depolymerization through ester cleavage), (iv) the qualitative and quantitative composition of the ensuing monomeric fragments, and (v) the exploitation of this mixture of monomers in macromolecular science, both as a possible functional additive and as a source of novel materials. The presence of terminal carboxylic and hydroxy groups and of side hydroxy and epoxy moieties on the long chains of suberin "monomers" makes them particularly suited as building blocks for polymers with original architectures and interesting properties.

Keywords: Suberin; Cork; Long-chain aliphatic compounds; Hydroxyacids; Dicarboxylic acids; Polyurethanes

Contents

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1. Introduction

Suberin is a natural aliphatic–aromatic cross-linked polyester, almost ubiquitous in the vegetable realm, albeit in very variable proportions. It is mostly found in the cell walls of normal and wounded external tissues of aerial and/or subterranean parts of plants where it plays the fundamental role of a protective barrier between the organism and its environment [1–4]. In higher plants, suberin, organized in a characteristic lamellar structure, is one of the main components of the outer bark cell walls.

As in the case of lignin, there is no unique chemical "structure" of suberin (as opposed, e.g. to cellulose or natural rubber), since its constitutive moieties can vary appreciably both in their specific nature and relative abundance within the macromolecular network.

The main components of the aliphatic domains of suberin are ω-hydroxyfatty acids, α-, ω-dicarboxylic acids and homologous mid-chain di-hydroxy or epoxy derivatives, whereas the aromatic domains are dominated by variously substituted phenolic moieties [1–11]. Although the suberin "monomer unit" composition is relatively well known for many species, its detailed macromolecular structure (i.e. the precise assembly of the units in the network) and its association to other cell wall biopolymers are still not completely understood.

The availability of hydroxyfatty acids in nature is concentrated in specific plant seed oils such as *Ricinus communis* (castor oil) or *Lesquerella spp*, cutin (an extracellular aliphatic polyester covering most of the aerial surfaces of plants) [12] and, particularly, in suberin [13]. On the other hand, epoxy derivatives of fatty acids are present in significant amounts almost exclusively in the suberized cell walls of plant periderms and tree bark tissues [1–3].

The foreseeable depletion of fossil resources and the need for sustainable development are driving both the scientific community and industry to look for alternative (renewable) resources for the production of energy and chemical commodities. Thus, for example, the implementation of the biorefinery concept in agroforest-based activities and the concomitant need to upgrade the by-products generated in the processing of agricultural and forest products, represent a clear response to this situation [14]. These growing concerns have also been the object of thorough appraisals by governments and international institutions, with the very important result that the funding for basic and applied research in the various relevant areas has been increasing dramatically in the last few years.

Forest-related industries produce huge amounts of barks that represent a potential source of green chemicals [15,16] but which, at present, are mainly burned for energy production. Among bark components the suberin hydroxy and epoxy derivatives of fatty acids, some of which are relatively rare in nature, may constitute interesting chemical precursors for many applications.

This brief review deals with the essential literature on suberin bioavailability, structure and composition, with the specific purpose of emphasizing its potential (modestly exploited thus far) as a precursor to original macromolecular materials, particularly in terms of its long-chain aliphatic units.

2. Natural occurrence

It is practically impossible to estimate the real content of suberin in suberized plant tissues because of its complex macromolecular nature and the structural similarity between the suberin aromatic domains and lignin [1–5]. Typically, the analysis of suberin containing substrates involves a preliminary solvent extraction of low molecular weight components, followed by the chemical scission of the various ester moieties in the network and the isolation as well as the qualitative and quantitative characterization, of the ensuing fragments [2].

The outer bark of higher plants and tuber periderms constitute the major sources of suberin in nature (Table 1). Its content and composition in outer barks is quite variable, depending on the wood species and the isolation method used. In hardwoods of industrial relevance, suberin represents typically between 20% and 50% of the extractive-free bark weight. The industrial transformation of such woods (papermaking, construction, furniture, etc.) generates enormous amounts of outer barks as by-product. Several examples show the relevance of this point. Thus *Betula pendula* (birch), one of the most important industrial hardwood species in Northern European countries, is used predominantly for pulp and paper production. A birch kraft pulp mill, with a typical yearly pulp production of 400,000 ton, generates about 28,000 ton of outer bark, corresponding to a potential annual production of about 8000 ton of suberin "aliphatic monomers" [17]. Yet another

Table 1
Relative abundance of aliphatic suberin in the extractive-free outer bark of some higher plants and periderm of *Solanum tuberosum* (*—includes extractives)

Species	Suberin		
	%	Isolation method[a]	Ref.
Laburnum anagyroides	61.7	0.5 M MeONa in MeOH (1)	[27]
Fagus sylvatica	48.3		
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		
Euonymus alatus	8.0		
Pseudotuga menziesii	53.0*	0.02–0.03 M MeONa in MeOH (2)	[33]
Betula pendula	58.6	0.5 M MeONa in MeOH(1)	[27]
	51.0	1.3 M MeONa in MeOH(1)	[47]
	32.2*	0.5 M KOH in EtOH/H_2O(9:1, v/v)(3)	[17]
	49.3	0.5 M KOH in EtOH/H_2O (9:1, v/v)(3)	[48]
	46.0	96% H2SO4 in MeOH (1/9, v/v)(4)	[48]
Quercus suber	43.3	0.5 M NaOMe in MeOH(1)	[27]
	37.8–41.2*	3% MeONa in MeOH(2)	[39]
	37.0*	0.1 M NaOH in MeOH(2)	[54]
	60.0*	0.02–0.03 M MeONa in MeOH(2)	[33]
	62	3% MeONa in MeOH	[43]
	40.0–45.0*	3% MeONa in MeOH(2)	[30]
	54–56	1–3% MeONa in MeOH	[41]
Solanum tuberosum	12.1	0.5 M NaOMe in MeOH(1)	[27]
	25*	0.0012 M NaOMe in MeOH(5)	[35]

[a]Preliminary sequential boiling solvent extraction: (1) $CHCl_3$ + MeOH; (2) CH_2Cl_2 + EtOH + water; (3) no solvent extraction; (4) acetone; (5) CH_2Cl_2 + EtOH + water + MeOH.

interesting example of a potential industrial source of "suberin monomers" is of course the cork industry in the Mediterranean region [18]. Portugal produces about 185,000 ton/year of cork [19], viz. more than 50% of the world production. Cork, the outer bark of *Quercus suber*, is mainly used for the production of cork stoppers as well as agglomerates and composites for thermal and acoustic insulation. These industrial processes generate substantial amounts of cork powder, whose average particle size is too low for the manufacturing of agglomerates. This by-product is presently burned to produce energy, but, with an estimated production of 40,000 ton/year in Portugal [20], it could represent a yearly source of more than 16,000 ton of suberin.

Periderms of tubers such as potatoes (*Solanum tuberosum*), show a suberin content as high as 30% (w/w) (Table 1). Suberin is also present in the roots of plants such as *Oryza sativa* [21], *Zea mays* [21,22] and *R. communis* [23], among others, tobacco (*Nicotina tabacum*) cells [24], soybean (*Glycine max*) seedlings [25], green cotton (*Gossypium hirsutum*) [26] and many other plant tissues [1–3,27]. Many of these tissues, such as periderms from tubers, can be isolated as by-products in agro-food industries, thus representing yet another potential industrial source of suberin monomers.

Table 1 summarizes some relevant data concerning the importance of the aliphatic suberin contents in barks and periderms.

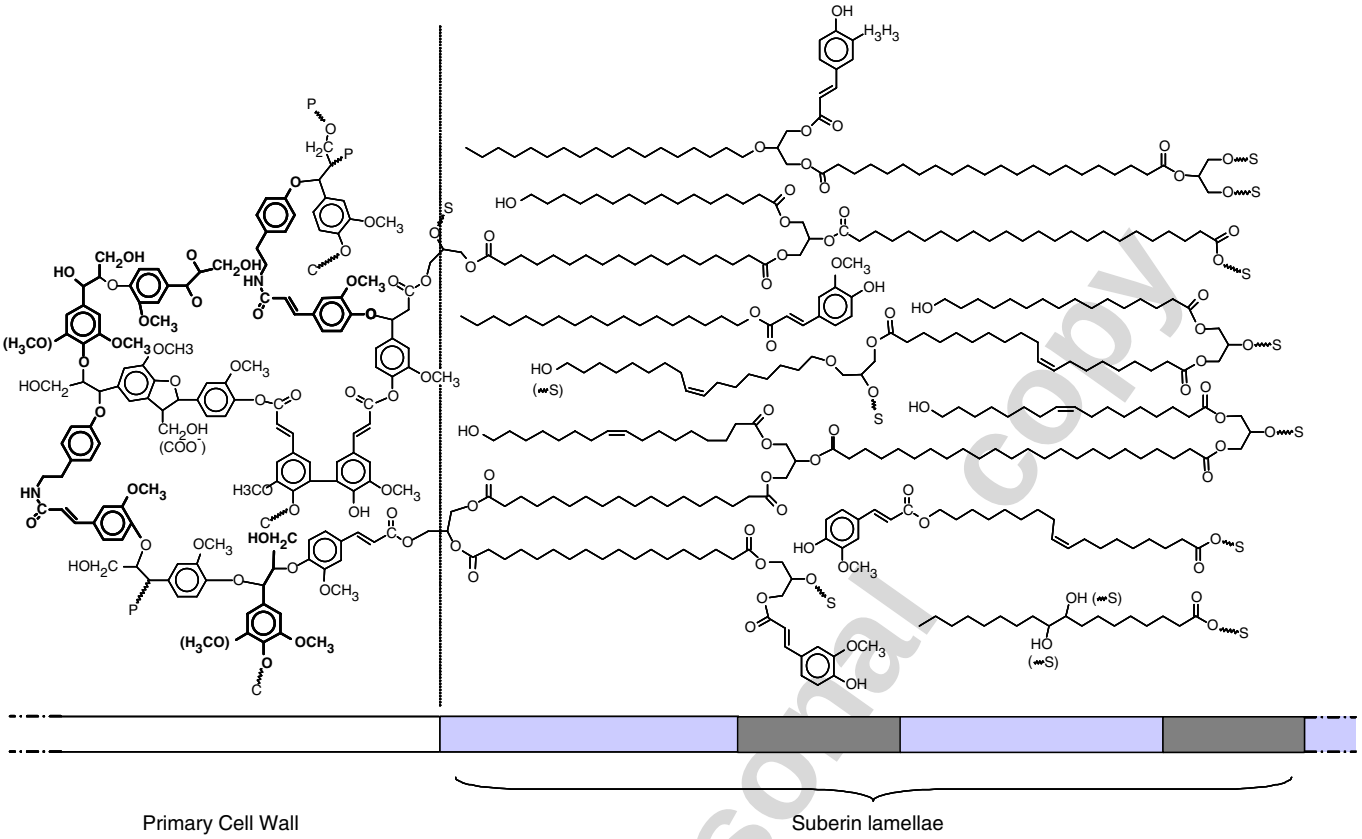

Fig. 1. The suberin model proposed by Bernards [4]. C: carbohydrate, P: phenolic, S: suberin (reprinted with permission from NRC Research Press).

3. Macromolecular structure

Suberized plant cells show secondary walls with a typical lamellar structure where the aromatic and aliphatic domains of suberin are heterogeneously distributed. Several models attempting to describe the macromolecular structure of suberin and the assembly of its macromolecular components in suberized cell walls have been proposed in the last few decades [2,4,28–30]. However, the macromolecular architecture of the two domains, their spatial distribution in the lamellar structure, as well as the interaction of suberin with other cell wall components, namely lignin and polysaccharides, remain a matter of debate. Recently, Bernards [4] reviewed the state of the art in this context and put forward an updated model for the suberin macromolecular architecture in suberized potato cell walls [4] (Fig. 1). The aliphatic domains of suberin (situated in the secondary cell walls, see Fig. 1) are made up of branched polyester macromolecules mainly composed of long-chain hydroxylated fatty acid moieties (see Section 4 for monomer composition), similarly to those of cutin [12]. Glycerol was earlier detected in suberin depolymerization extracts [1–3], but was only recently shown to be an essential structural building block of this natural polymer [5,31–35].

The nature of the aromatic domains of suberin are much more complex than that of its aliphatic counterparts. Solid-state NMR studies on molecular dynamics of cork [28,30] and potato cell wall components [36–38], supported by chemical analysis results, suggested the existence of two distinct aromatic domains in suberized cell walls (Fig. 1). The first, lying inside the aliphatic domains, consists mainly of hydroxycinnamates esterified with glycerol or ω-hydroxyfatty acids (Fig. 1). The second is a lignin-like polymer, (indeed hard to distinguish from lignin), spatially segregated from aliphatic suberin, sits in the primary cell walls (Fig. 1) and is composed of cross-linked hydroxycinnamic acid-based moieties, including amides, covalently bound to aliphatic suberin, either by ester (Fig. 1) or ether linkages [10,28,30]. The existence of ether or ester bonds between polysaccharides and this lignin-like

polymer, or directly between polysaccharides and aliphatic suberin, has also been suggested [28,30,38]. This lignin-like suberin fraction, at least in the case of *Q. suber* cork cells, is embedded (not spatially segregated) in the lignin-carbohydrate matrix of the primary cell wall [28,30].

The nature of lamellae of suberized cell walls has also attracted the attention of many researchers. Following previous findings [28,30,36–38] and recent molecular dynamics studies [28,30], lamellae (Fig. 1) correspond most likely to layers of esterified aliphatic moieties with low molecular mobility, stacked in a relatively ordered arrangement, alternating with layers rich in esterified coumarates and glycerol (and, probably, waxes), which display a much higher molecular mobility [4,28,30]. In the case of *Q. suber* cork, the presence of a crystalline aliphatic suberin fraction in aliphatic lamellae of suberized cells was clearly demonstrated [28,30].

4. Monomer composition through ester cleavage

4.1. Depolymerization methods

The analysis of the monomer composition resulting from the chemical cleavage of suberin native structure is an essential step both for the detailed chemical characterization of this natural material and for the development of applications for its components. Being essentially an insoluble three-dimensional polyester network, most degradative techniques are based on simple ester cleavage reactions, namely hydrolysis, trans-esterification or reductive cleavage. The first studies on the monomer composition of suberin were published before the middle of the last century ([1–3] and references therein), but the detailed characterization of suberin's cleavage products was only possible (after suitable chemical derivatization) when high-resolution gas chromatography coupled with mass spectrometry (GC–MS) became a routine analytical technique.

The most common procedure used for suberin monomer preparation is ester cleavage through alkaline methanolysis [30–35,39–48], although studies involving specific reagents have also been carried out, to confirm the position and functionalization of hydroxy groups [49,50] and to distinguish between free and esterified carboxylic groups [51].

Methanolic sodium methoxide (NaOMe) is the most frequently used reagent, whereas calcium oxide (CaO) in methanol has been selected to generate very mild ester cleavage conditions [31–35] to induce the *partial* cleavage of the suberin structure for structural elucidation purposes, as discussed below.

Alkaline methanolysis with NaOMe has shown to be the least harsh depolymerization procedure to determine the full suberin monomer composition [17,30,44–48] and has, therefore, been used as a reference method in most published studies. In this context, epoxy moieties can be detected as such or in the form of methoxyhydrins, whereas in an aqueous alcoholic medium, such moieties are converted to *vic*-diol structures. It was however demonstrated that epoxides can be preserved in alkaline hydrolysis (using solutions of KOH in ethanol with a few percent of water), provided short reaction times are used [48].

Complete depolymerization of suberin is normally achieved by treating it with refluxing methanol, containing 3% of NaOMe, for about 3 h (e.g. [30,41]). However, when the reaction is carried out using KOH in ethanol:water 9:1 v:v, total depolymerization occurs within 15 min, provided particles below 20 mesh are used [48]. It has also been claimed that full depolymerization can be achieved under much milder methanolysis conditions [33]. However, it is generally recognized that such conditions only lead to *partial* depolymerization, resulting in decreasing extraction yields and in the preferential removal of certain groups of monomers, such as alkanoic acids and α,ω-diacids, whereas ω-hydroxiacids are more resistant to cleavage [30,41]. The advantage of applying this milder process is instead related to a better understanding of the in situ suberin structure, because it leads to the formation of intermediate feruloyl ester and acylglicerol type oligomeric structures [31–35,39,41] (mainly with ω-hydroxyacids) which do not resist the more severe methanolysis conditions.

Interestingly, the suberin composition can also be accessed by Flash Pyrolysis-GC–MS (Py-GC/MS) in the presence of tetramethylammonium hydroxide [52,53], a very versatile procedure providing the relative proportion of monomers, but not the percentage of suberin within the analysed substrate.

4.2. Monomer composition of suberin

Table 2 contains a collection of quantitative results related to suberin composition published in the last several years, selected among the most

Table 2
Relative abundance of aliphatic suberin monomers from the extractive-free outer bark of some higher plants

Source	*Q. suber*								*B. pendula*		St[a]	Pm[b]
Ref	[30]	[33]	[41]	[42]	[43]	[52][c]	[53]	[54]	[17][c]	[48][c]	[35]	[35]
Aliphatic alcohols	4.7	0.4	2.2	4.5	8.3	1.0	1.8	1.8	0	0	3.1	0.6
C(16:0)	0.2	—	—	—	—	—	—	—	—	—	—	—
C(18:0)	0.3	0	—	—	—	—	—	—	—	—	0.4	0.1
C(18:1)	0.2	—	—	—	—	—	—	—	—	—	—	—
C(20:0)	0.5	0.1		0.3	0.5	—	0.1	—	—	—	—	0.1
C(22:0)	2.0	0.2	1.7	3.2	3.1	—	0.7	0.9	—	—	0.5	0.3
C(23:0)	—	—	—	—	—	—	0	—	—	—	0.5	—
C(24:0)	1.0	0.1	0.5	0.5	4.3	—	0.9	0.9	—	—	0.6	0.1
C(26:0)	0.5	—	—	0.5	0.4	—	0.2	—	—	—	1.0	
Fatty acids	14.9	1.0	2.5	4.9	14.1	8.0	4.2	4.7	12.3	7.4	10.4	5.2
C(12:0)	—	—	—	—	—	—	0	—	—	—	—	—
C(16:0)	0.2	0	—	0.5	—	—	0.1	—	—	—	0.2	0.1
di(OH)-C(16:0)	0.9	—	—	—	—	—	—	—	—	—	—	—
C18:0)	0	0	—	—	—	—	0	1.5	—	—	—	0.1
C18:1)	0	—	—	—	1.8	—	0	—	—	—	—	—
9,10-di(OH)-C(18:0)	1.3	—	—	—	6.6	2.0		—	—	—	—	—
9,10-epoxi-C(18:0)	—	—	—	—	2.2	—	—	—	—	—	—	—
C(20:0)	0	0.1	—	0.3	0.2	—	—	—	—	—	—	0.8
di-OH-C(20:0)	10.1	—	—	—	—	—	—	—	—	—	—	—
C(22:0)	1.3	0.9	2.0	1.7	1.5	—	2.5	1.8	—	—	0.7	2.6
C(24:0)	1.1	—	0.5	0.5	0.4	—	1.5	1.4	—	—	1.5	1.4
C(26:0)	—	—	—	2.0	1.4	—	0.1	—	—	—	1.7	0.2
C(28:0)	—	—	—	—	—	—	—	—	—	—	4.8	—
C(29:0)	—	—	—	—	—	—	—	—	—	—	0.5	—
C(30:0)	—	—	—	—	—	—	—	—	—	—	1.0	
ω-Hydroxfatty acids	51.5	26.3	36.0	44.0	46.5	58.0	61.7	40.8	76.7	79.7	18.4	11.4
C(16:0)	1.1	0.4	0	0.6	0.8	—	1.2	0.5	—	—	1.6	4.3
9,16di-OH(C16:0)	—	—	—	—	—	—	—	—	3.7	3.2	—	—
C(18:0)	0	0.1	—	0.4	0.3	—	0	0.6	—	—	—	0.9
C(18:1)	8.8	5.4	11.1	9.7	10.3	—	18.2	5.7	11.1	12.2	15.0	1.7
9,10-epoxy-C(18:0)	0.8	7.3	5.5	2.1	1.8	5.0	3.2	—	37.0	39.2	—	—
9,10-di(OH)-C(18:0)	12.7	2.2	—	10.0	10.6	3.0	4.8	7.0	8.6	8.4	0	0.2
9,10-(OH,OMe)-C(18:0)	3.8	—	—	—	—	—	—	7.5	—	—	—	—
C(20:0)	2.2	0.5	0.7	0.9	0.9	—	0	1.2	2.8	2.8	—	2.2
C(20:1)	1.2	—	—	0.9	—	—	—	—	—	—	—	—
C(21:0)	0.0	—	—	—	—	—	—	—	—	—	—	—
C(22:0)	16.3	7.9	17.4	11.7	13.4	—	28.6	15.4	13.6	13.9	0.8	1.7
C(23:0)	0	—	—	—	—	—	—	—	—	—	—	—
C(24:0)	4.6	2.4	1.3	3.1	3.2	—	5.8	2.9	—	—	0.5	0.4
C(26:0)	—	0.1		4.4	5.2	—	0	—	—	—	0.3	
C(28:0)	—	—	—	—	—	—	—	—	—	—	0.3	
α, ω-dicarboxylic acids	27.6	45.5	53.3	18.6	6.1	22.0	21.3	48.8	10.4	12.9	39.5	39.9
C(16:0)	1.6	2.0	2.2	—	0.5	—	3.1	2.2	—	—	0.8	18.7
C(18:0)	0.5	0.5	—	—	—	—	—	0.5	0.9	—	—	5.0
C(18:1)	4.1	6.2	7.7	2.1	—	—	9.1	7.1	3.4	4.7	37.3	9.1
9,10-epoxy-(C18:0)	0.9	22.9	37.8	3.1	—	4.0	0.9	—	—	—	—	—
9,10-di(OH)-C(18:0)	5.0	7.7	2.5	6.8	—	0.0	1.3	7.2	—	—	0.1	1.6
9,10-(OH,OMe)-C(18:0)	4.4	—	—	—	—	—	—	20.0	—	—	—	—
C(20:0)	2.6	1.0	1.1	4.9	3.8	—	1.2	1.5	—	—	0.6	3.9
C(20:1)	0.3	—	—	—	—	—	—	—	—	—	—	—
C(22:0)	7.1	4.5	1.7	1.5	1.5	—	5.6	10.3	6.1	8.2		1.6
C(24:0)	1.1	0.7	0.3	0.3	0.3	—	0.5	—	—	—	—	—
C(26:0)	—	—	—	—	—	—	—	—	—	—	0.7	—

Table 2 (*continued*)

Source	*Q. suber*								*B. pendula*		St[a]	Pm[b]
Ref	[30]	[33]	[41]	[42]	[43]	[52][c]	[53]	[54]	[17][c]	[48][c]	[35]	[35]
Aromatic	1.3	1.1	3.9	6.6	7.9		0.1	0.8	0	0	1.2	0.7
Quinic acid	—	0.1	—	—	—	—	0	—	—	—	—	—
Conyferyl alcohol	—	0.3	—	—	—	—	0	—	—	—	0.2	0.2
Ferulic acid	1.3	0.5	3.9	6.6	7.9	6.0	0.1	0.8	—	—	0.9	0.2
Vanillin	—	0.1	—	—	—	—	0	—	—	—	—	0.2
3,4-di-hidroxibenzoic acid	—	0.1	—	—	—	—	0	—	—	—	—	0.1
Others	—	11.4	—	—	—	—	—	—	—	—	0	13.0

The notation "0" means that the compound was only detected in trace amounts.

[a] *St*: *Solanum tuberosum*.

[b] *Pm*: *Pseudotsuga menziesii*.

[c] Although average values were given in these publications for each family of suberin components, the individual abundances of certain components were not reported.

detailed studies applied to important suberin sources (*Q. suber* cork, *B. pendula* outer bark and *S. tuberosum* periderm) and *Pseudotsuga menziesii*. Since the various authors followed different analytical methodologies and ways of expressing their results, the figures in the table should be read with care, despite the fact that they all provide good indications of the relative abundance of the suberin components. We endeavoured to rearrange the published data in order to present them on the same basis (relative abundance of each component). In some instances the results of several samples are shown as average values. The structures of representative elements of each group are shown in Fig. 2.

In addition to the high variability of suberin contents referred to above, the monomer composition of suberin also shows a significant qualitative and quantitative variability, as highlighted in Table 2. The total amount of monomers detected, relative to the mass of depolymerized suberin, is seldom provided, but values between 27% and 74% for *Q. suber* cork [30,33,54] and around 60% for potato periderm [33,35] have been published, which clearly means that a non-negligible percentage of suberin is frequently *not detected* by GC–MS analysis (see below).

The relative abundance of each family (fatty acids, ω-hydroxyfatty acids, α-, ω-dicarboxylic acids, aliphatic alcohols and aromatic acids) and of individual components (Table 2) shows a very high variability.

Figures for aliphatic alcohols range between 0.4% and 8.3%. This group is mainly composed of saturated even C-numbered chains, ranging from C16 to C26, with C20, C22, C24, followed by C26, as the most frequently found components. References to odd C-numbered and unsaturated structures are very scarce.

Alkanoic acids represent between 1% and 15% of suberin monomers. This fraction is mainly composed of saturated even C-numbered homologues, ranging most commonly from C16 to C26. References to saturated C12 and C28–C30 monomers, as well as to unsaturated C18 structures, were also found. The most abundant saturated alkanoic acids are the C22–C24 homologues, followed by C16 and C20. Mid-chain dihydroxy and epoxy derivatives of C18 alkanoic acids were occasionally detected in significant amounts, but the dihydroxy derivatives of C16 and C20 were rarely reported.

ω-Hydroxyalkanoic acids are generally the most abundant group of components, representing between 11.4% and 62.4% of suberin monomers. Even C-numbered chains between C16 and C26 are frequently found, and among them the C(22:0) and C(18:1) are clearly dominant, whereas in the previous groups, unsaturated structures were not frequent. The mid-chain dihydroxy and epoxy derivatives of C18 ω-hydroxyacid are also abundant and frequent, sometimes together with the mid-chain *vic*-hydroxymethoxy derivative resulting from the opening of the epoxy moiety. Finally, the saturated odd C-numbered components C21 and C23 together with C28, are seldom reported.

α, ω-Alkanedioic acids are generally the second most abundant group of components, representing between 6.1% and 45.5% of suberin monomers.

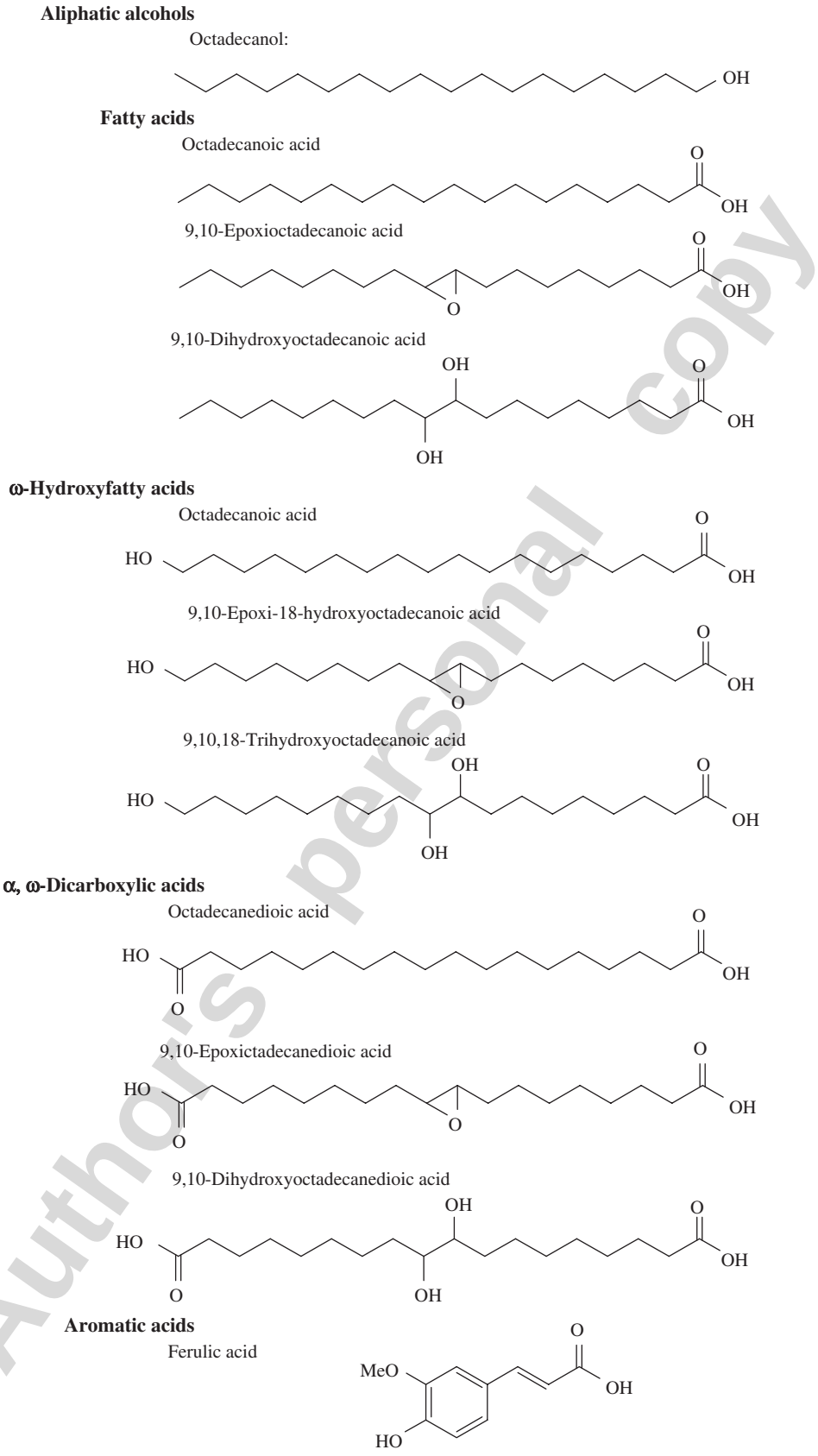

Fig. 2. Representative structures of monomeric components resulting from suberin depolimerization.

This group is mainly composed of saturated even C-numbered chains comprised between C16 and C24 (C26 is seldom reported). Again, the unsaturated C18 homologue is frequently reported.

As detailed above, most of the aliphatic suberin monomers are carboxylic acids (56.5–94%) and most of them bear at least one aliphatic OH functionality (13.6–69.8%). In general, the C18 components are clearly dominant, followed by C22 homologues. Among the C18 components, the prevalent structures are mid-chain unsaturated or dihydroxy derivatives, and, in some cases, the mid-chain epoxydes or the corresponding methoxyhydrines.

Concerning the aromatic fraction of suberin, ferulic acid is the compound most frequently detected, but other structures, like *p*-coumaric, caffeic, sinapic, 4-hydroxybenzoic, 3,4-dihydroxybenzoic and 4-hydroxy-3-methoxybenzoic acids have also been reported. In addition, aromatic alcohols, like *p*-coumaroyl, coniferyl and sinapyl alcohols were occasionally found as suberin fragments [26,30,32,33,35,43,54–56].

Glycerol has been recognized as a suberin component [26,55,56], and some authors reported that it represents up to 20% of suberin in potato periderm [35], 26% in *P. menziessi* outer bark [33] and 14% in *Q. suber* cork [33].

The presence of the glycerol moiety in suberin was confirmed by methanolysis, using calcium oxide as a base. Under these mild conditions, a number of acylglycerol and feruloylacyl derivatives [31–35], resulting from the partial cleavage of the suberin network, were identified, thus confirming unambiguously the importance of glycerol and ferulic acid as key suberin building blocks [4,56]. However, some of the higher figures for glycerol content mentioned above [33,35] seem to be excessively high, in the light of the most reliable suberin structure models recently put forward (Fig. 1) [4].

The presence of a high molecular weight fraction in some suberin depolimerization mixtures from *Q. suber* cork, obtained when mild cleavage conditions were used [31–35], was clearly attributed to the presence of ω-hydroxyacid oligomers. However, a high molecular weight fraction was also detected in significant amounts, even when more severe methanolysis conditions were used [30,54]. It is most unlikely that in these instances, such fraction would be composed of oligomers of ω-hydroxyacids resulting either from incomplete depolymerization or from recondensation reactions [54]. It can be speculated, therefore, that this high molecular weight suberin fraction may in fact be composed of suberan like structures. Suberan is a non-hydrolysable highly aliphatic macromolecule, commonly found in the periderm tissue of some angiosperm species [57], whose inertness justifies its detection in forest soils and fossilized samples [58,59]. The presence of these peculiar components in extracted suberin may contribute to explain the low detection yields on the GC–MS analysis of suberin samples referred to above [30,54].

5. Physical properties of depolymerized suberin

Although the composition of the aliphatic suberin fraction has attracted much attention from several laboratories, as discussed in the previous section, the physical properties of the ensuing mixtures of monomeric components, hereby denoted "*dep-suberin*", were only assessed in our comprehensive investigation of this remarkable material. The samples studied were obtained by alkaline methanolysis (0.1 M NaOH methanolic solution) of cork from *Q. suber* L. and had an opaque pasty consistency [54,60]. Under the conditions used for the depolymerization, trans-esterification predominated over alkaline hydrolysis (traces of water always present) and most of the carboxylic acid functions were, therefore, converted to the corresponding methyl esters.

Given the predominance of long aliphatic chains in most of its components, which indeed imparts to cork its well known and largely exploited hydrophobic character, it seemed interesting to assess the surface properties of *dep-suberin*. A thorough study was therefore carried out using several complementary techniques [60]. The surface energy of the solid (pasty) *dep-suberin* at 25 °C, determined from contact angle measurements with liquids of different polarity and applying the Owens–Wendt approach, was 42 mJ m^{-2}, with a polar component of about 4 mJ m^{-2}. Measurements of the surface tension of the liquid samples at 50–110 °C, gave a linear variation of γ with temperature, with an extrapolated value of 37 mJ m^{-2} at 25 °C. This difference was attributed to the microcrystalline character of the solid sample (see below), associated with a higher cohesive energy and, hence, a higher surface energy. Since a mixture of alkanes with the same range of chain lengths as the *dep-suberin* components would display a surface energy close to 28 mJ m^{-2}, it follows that (i) some of the polar

groups in those components were present on the *dep-suberin* surface, as confirmed by the modest, but non-negligible, polar contribution to the surface energy, and (ii) some intermolecular interactions, mostly through hydrogen bonding, induced an increase in cohesive energy, compared with purely dispersive alkane structures, as suggested by the correspondingly higher γ_d values obtained by both contact angle and inverse chromatography [60]. Notwithstanding these fine-tuned considerations, *dep-suberin* must be considered as a rather non-polar material with surface properties that resemble those of its cork precursor, whose reported values of surface energy range between 30 and 40 mJ m^{-2} [61].

The DSC tracings of *dep-suberin* (see Fig. 3) showed that annealing a molten sample in liquid nitrogen produced an amorphous material with a glass transition temperature of ~−50 °C, which crystallized when brought to about 30 °C [62]. The melting temperature of the microcrystalline phase was centred at ~40 °C (broad endothermic peak). These observations were confirmed by optical microscopy observations, carried out with reproducible temperature cycles between −20 and 80 °C [62]. A quantitative assessment of the birefringence (Fig. 4) showed a constant maximum value (heating cycle) up to ~0 °C, followed by a gradual decrease to zero birefringence at ~50 °C. The cooling cycle reproduced the same features in reverse. The images captured in this context showed dense microcrystalline domains within an amorphous matrix [62].

Given the broad temperature range associated with the melting or forming of these crystalline phases, and the very small size of the crystals, it seems likely that the *dep-suberin* components more apt to crystallize, because of their suitable structures, do so on an individual basis, at their respective freezing temperature, when the liquid mixture is slowly cooled down. The result is therefore a set of microcrystals, each member belonging to a given *dep-suberin* component. Interestingly, the fact of having a rather complex mixture of compounds does not hinder the individual crystallization of some of them, most probably because the major driving force is associated with the ease of self-assembly among their *long and linear* aliphatic sequences.

The characteristic whitish and pasty appearance of these *dep-suberin* samples at room temperature reflects, therefore, the combination of a viscous liquid containing a substantial proportion of microcrystals.

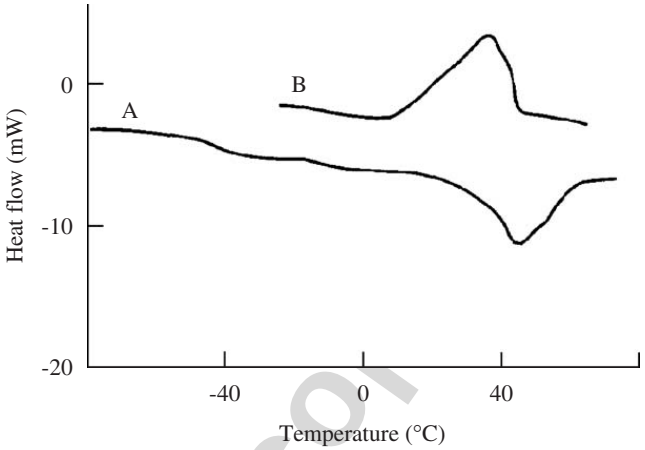

Fig. 3. DSC thermograms of suberin. (A) Heating and (B) cooling (reprinted with permission from Elsevier).

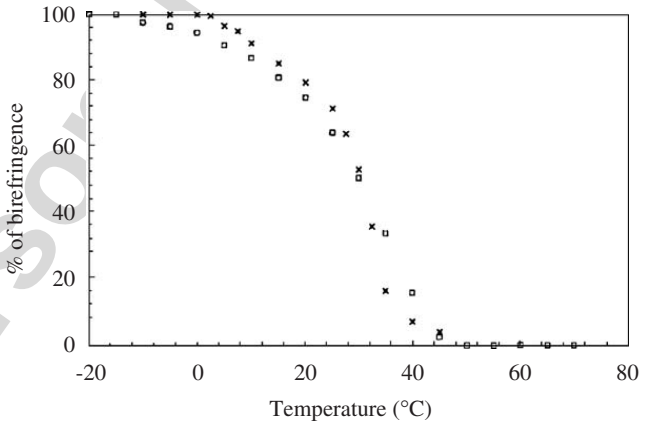

Fig. 4. Melting (x) and recrystallization [□] of suberin, as observed by the change in birefringence intensity, as a function of temperature (the 100% birefringence refers to the *maximum extent* of crystallization and *not* to the actual percentage of the crystalline phase) (reprinted with permission from Elsevier).

The densities of these *dep-suberin* samples were surprisingly high, viz. ca. 1.08 at room temperature and above unity up to ~55 °C [62], compared with those of alkanes of similar chain length, which are about 0.8 at room temperature. This clearly confirmed the existence of additional intermolecular interactions through hydrogen bonding from the OH groups borne by the different monomeric structures (see previous section). Indeed, fatty acid esters, as well as fatty alcohols and diols, have densities close to those measured for *dep-suberin* in this work [62].

The TGA of *dep-suberin* in a nitrogen atmosphere [62] showed a good thermal stability up to ~280 °C, followed by a progressive weight loss, reaching a plateau at about 80% volatilization at 470 °C and leaving a carbonaceous residue.

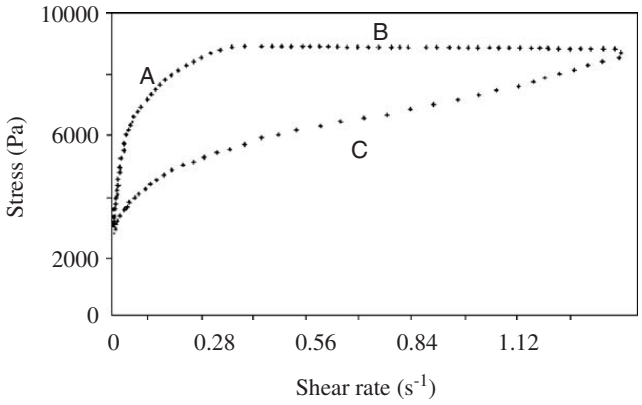

Fig. 5. A Typical rheogram of suberin at 20 °C. (A) Increasing stress; (B) constant stress; C: Decreasing stress (reprinted with permission from Elsevier).

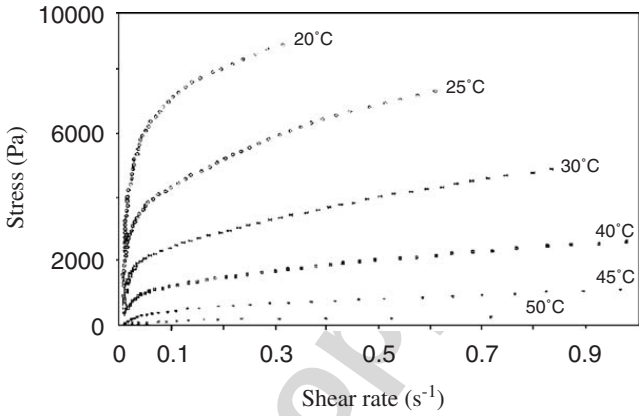

Fig. 6. Rheograms of suberin at different temperatures (increasing stress mode) (reprinted with permission from Elsevier).

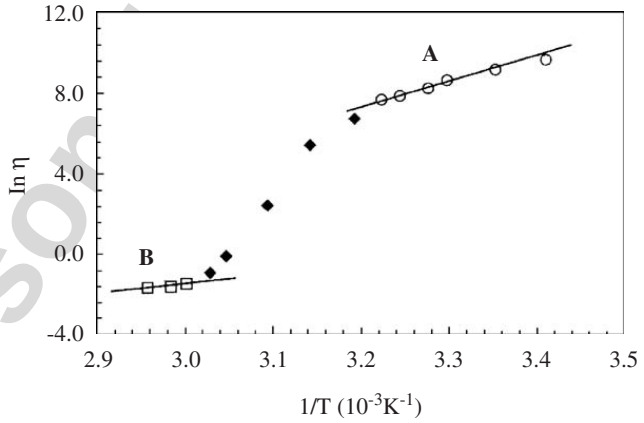

Fig. 7. Eyring plot related to the viscous flow of suberin (reprinted with permission from Elsevier).

The rheological properties of *dep-suberin* at room temperature were typical of a plastic response, with an important yield-stress value and a thixotropic behaviour, as shown in Fig. 5 [62,63]. These features are usually associated with either intermolecular or interphase shear-induced destructuration (or both), followed by a time-dependent restructuration at rest. Since *dep-suberin* was associated with both intermolecular association through hydrogen bonding and the existence of a liquid/crystal interphase at room temperature, its rheological study was extended to higher temperatures. The extent of yield stress decreased drastically as the temperature was raised and indeed vanished at 50 °C, i.e. when all the microcrystals had melted. Moreover, the rheogram at this temperature became linear, viz liquid *dep-suberin* displayed a Newtonian behaviour. These observations, displayed in Fig. 6, revealed that the major cause of its plastic behaviour at room temperature was the heterogeneous nature of *dep-suberin* and the consequent strong interfacial interactions between the liquid and the microcrystals.

The actual values of viscosity varied dramatically with temperature, going from 14,000 to 0.18 Pa.s between 20 and 65 °C [63]. The corresponding Eyring plot [63] showed three distinct regimes (Fig. 7): (i) below 37 °C, the presence of the microcrystalline phase induced a high value of the flow activation energy ($E_a = 88\,kJ\,mol^{-1}$); (ii) above 55 °C, where the sample was a homogeneous liquid, E_a dropped to $34\,kJ\,mol^{-1}$; (iii) a transition zone between these two temperatures, reflecting the progressive melting of the microcrystals, which gave rise to a continuous change in the substrate solid–liquid contents and physical consistency.

Tack measurements [63] showed that the dynamic resistance of *dep-suberin* to film splitting decreased, as expected, with both increasing temperature and increasing shear rate. The temperature effect reflected mostly the melting of the crystalline phase, since the drop in tack was quite drastic between 30 and 50 °C (the melting range). All tack values were constant with respect to time in experiments lasting up to 20 min.

6. Application in macromolecular materials

To the best of our knowledge, the only *dep-suberin* which has been the object of studies related to its use in macromolecular materials, whether as an additive or as a reactive monomer mixture, is that extracted from *Q. suber* L. These few investigations are discussed below.

6.1. Dep-suberin as a functional additive

The microcrystalline character of *dep-suberin*, described in the previous section, prompted us to examine its possible role as an additive to offset printing inks, in replacement of other waxy materials like PTFE oligomers [63]. Two reference inks were employed for this study, namely a typical vegetable oil-based commercial ink and a waterless ink containing petroleum-based diluents, to both of which *dep-suberin* was added in proportions of 2–10 w/w%. The characterization of these formulations included the determination of tack and viscosity, as well as printing tests. The presence of *dep-suberin* in the waterless ink only affected its bulk properties, by stabilising the tack value with time and inducing a modest decrease of viscosity (with 10% *dep-suberin*), without any detectable modification of the surface properties. This suggested that the hydrocarbon diluent of that ink acted as a good solvent for the *dep-suberin*, which, therefore, did not migrate to the surface of the printed film. With the more conventional vegetable-oil ink, *dep-suberin* induced a significant decrease in tack, small changes in viscosity and a two-fold decrease in the gloss of a printed film containing 10% of *dep-suberin*. The latter result clearly showed that at least part of the crystalline components of *dep-suberin* were not dissolved in the ink medium and could, therefore, migrate to the surface to produce the desired change in its optical properties.

6.2. The oxypropylation of cork

Although, strictly speaking, this topic does not deal with suberin as such, but rather with one of its major natural substrates, we deemed it appropriate to include it in this review because the working hypothesis applies equally well to the suberin monomer mixture. Indeed, the oxypropylation of natural polymers has been applied successfully to a host of OH-bearing natural polymers, like cellulose, starch, chitosan, lignin and more complex agricultural by-products, such as sugar-beet pulp [64]. In all instances, a nucleophilic catalyst (strong Brønsted bases like KOH work best) is used to deprotonate some of the substrate's OH groups and thus generate oxianions, which initiate the anionic polymerization of propylene oxide (PO), thereby inserting polyPO grafts onto the starting macromolecule. This reaction typically transforms the solid powder of the natural polymeric material into a viscous liquid polyol bearing as many OH groups as the initial substrate, since the oxypropylation is simply a "chain extension" process. This branching mechanism is always accompanied by some PO homopolymerization, which produces oligomeric diols. Fig. 8 provides a schematic view of the process, which requires typically temperatures above 150 °C and thus maximum PO pressures of 12–15 bar.

Cork powder was oxypropylated under these conditions [64] and the ensuing polyol fully characterized in terms of structure, homopolymer content, solubility, OH index and viscosity. The latter two parameters proved to be entirely comparable with those of commercial counterparts used in the manufacture of polyurethane materials. A study was, therefore, conducted [65] on the reactivity of the polyol mixture, as obtained from the oxypropylation process, towards various diisocyanates and on the structure and properties of the ensuing polyurethanes.

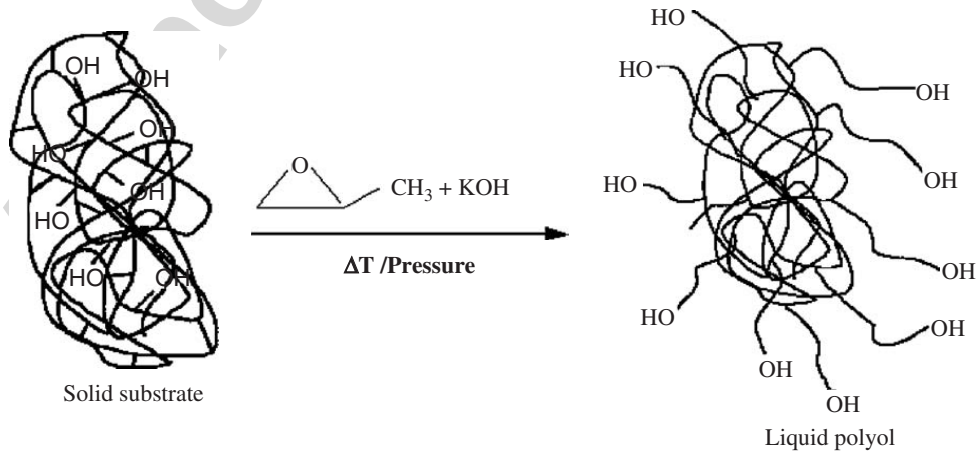

Fig. 8. Schematic view of the oxypropylation of OH-bearing macromolecular materials.

This ongoing investigation is a good example of the interest in exploiting renewable resources. In this instance, cork powder is a cheap commodity arising as a by-product of the manufacturing of cork artefacts and is potentially available in large quantities. Instead of burning it (its present fate), it can be readily converted into a novel material in the form of a polyol macromonomer, suitable for the preparation of polyurethanes. The same strategy applies equally well to other suberin-rich tree barks, such as that of the *Betula* species, separated in huge amounts, as a side-product, in the pulp and paper industry.

6.3. Polymers from suberin monomers

Little has been published on the use of the suberin depolymerization products as monomers for the synthesis of novel macromolecular materials. Our work has so far been concentrated on polyurethanes, using the mixture of aliphatic monomers in their methyl ester form, arising from the methanolysis procedure used to cleave the suberin ester moieties [54].

In a preliminary study [66], the kinetics of urethane formation was followed by FTIR spectroscopy using an aliphatic and an aromatic monoisocyanate and their homologous di-isocyanate. Both the model reactions and the polymer synthesis gave a clean-cut second-order behaviour, indicating that the hydroxyl groups borne by the suberin monomers displayed a conventional aliphatic–OH reactivity.

The following investigation [67] concentrated on the polymerization conditions and the thorough characterization of the ensuing polyurethanes, prepared using both aliphatic and aromatic di-isocyanates. When the initial [NCO]/[OH] molar ratio was unity, all the polymers gave ~30% of soluble material, the rest being a cross-linked product. This systematic result suggested that, on the one hand, some of the suberin monomers had a functionality higher than two, thus promoting a non-linear polycondensation leading to ~70% of gel, and, on the other hand, mono-functional components must have been present in the monomer mixture, which played the role of chain-growth terminators, giving rise to the sol fraction. This conclusion was corroborated by the fact that the FTIR spectra of both fractions were practically identical, as shown in Fig. 9, suggesting that the solubility/insolubility factor was not based on differences in the polymer chemical structure, but instead on its macromolecular architecture.

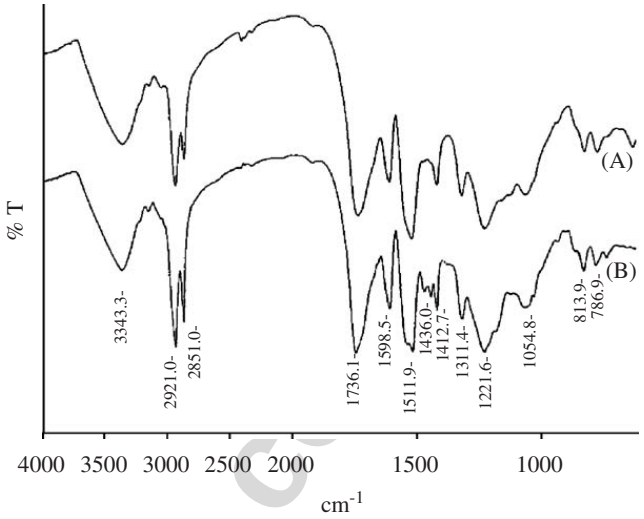

Fig. 9. FTIR spectra of a polyurethane prepared from suberin and MDI-2.0 with $[NCO]_0/[OH]_0 = 1$. (A) Insoluble fraction and (B) soluble fraction (reprinted with permission from Elsevier).

The Tg of these polyurethanes [66] followed classical trends in that, for the networks, the use of aromatic diisocyanates resulted in high values (~100 °C) associated with the stiffness of their moieties, whereas with the aliphatic counterparts, values around room temperature indicated much higher chain flexibility. The Tg's of the soluble fractions were much lower than those of their corresponding cross-linked materials, which is in tune with the presence of very mobile open-ended branches, generated by the insertion of monofunctional monomers into the polymer structure.

Benitez et al. [68], recently reported the synthesis of a polyester resembling cutin, a natural polymer whose structure is close to that of aliphatic suberin [12], by a circular approach, which consisted in depolymerizing cutin through ester cleavage and then submitting the ensuing monomer mixture to a chemical polyesterification process. The cross-linked material they obtained displayed, as one would indeed expect, very similar spectroscopic features compared with those of the starting cutin. In a subsequent study in the same vein [69], glycerol derivatives of mono- and di-carboxylic acids, whose structure simulated those present in both suberin and cutin, were prepared and characterized in an effort to simulate the biological synthesis of those natural polymers and exploit their peculiar properties, particularly, their tendency to form supramolecular assemblies.

To the best of our knowledge, there are no other publications dealing with suberin-based synthetic polymers.

7. Conclusions and perspectives

The main purpose of this short report is to bring to the attention of the polymer community the interest in considering suberin, a cheap renewable resource potentially available in very large amounts, as a valuable precursor to novel macromolecular materials. Given the structure of its aliphatic components, polyesters and polyurethanes seem to be the obvious structures to be sought, and the long alkane chains borne by the suberin monomers ought to be considered as its peculiar feature in terms of the repercussion on the properties of the ensuing polymers.

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