Cork suberin as a new source of chemicals.
1. Isolation and chemical characterization of its composition

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Abstract

Extractive-free cork from *Quercus suber* L. was submitted to a solvolysis treatment with methanolic NaOH which yielded 37% (o.d. cork) of suberin. This mixture of compounds was thoroughly characterized by FTIR, ^{1}H- and ^{13}C-NMR, gas chromatography coupled with mass spectrometric (GC–MS) analysis, vapour pressure osmometry (VPO), mass spectrography (MS) and gel permeation chromatography (GPC). After derivatization, the main components of the volatile fraction, representing less than half of the total, were found to be ω-hydroxymonocarboxylates, α,ω-dicarboxylates, simple alkanoates and 1-alkanols, all with chain lengths ranging from C16 to C24. A second fraction, with an average molecular weight about three times higher, was detected by VPO, MS and GPC. The presence of this important fraction in cork suberin had not been recognized in earlier studies. Both fractions constitute interesting precursors for the elaboration of new materials. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cork, the outer bark of *Quercus suber* L., is composed of suberin, the main cork component contributing to about 40% of its dry weight, lignin (ca. 20%), polysaccharides (ca. 20%) and extractibles (ca. 15%) [1]. This chemical composition, together with its particular cellular structure [2], gives cork excellent barrier properties for polar liquids, heat and sound [3]. Its main applications are therefore in stoppers, corkboards for insulation and decorative uses, as well as composites. To our knowledge, cork is not exploited industrially as a source of chemicals.

The cork industry worldwide consumes annually ca 280 000 tons of cork. About 20–30% of the cork entering the mill is rejected, mainly as cork dust, a low granulometry fraction without industrial interest. The typical use for these wastes is combustion for energy production. It seems however, rather uneconomical to destroy in such a manner a product which we believe worthy of better exploitation. Indeed the unique properties of cork could be more rationally translated into novel added-value materials.

We recently started a research programme aimed at a better understanding of the composition and chemistry of cork [4–9] and at its potential use as a source of chemicals [7,10]. This programme also includes the development of original fractionation techniques for the cork components (N. Cordeiro, A. Gandini, C. Pascoal Neto, unpublished). Suberin, the major cork component, is receiving particular attention [7,10] and is being

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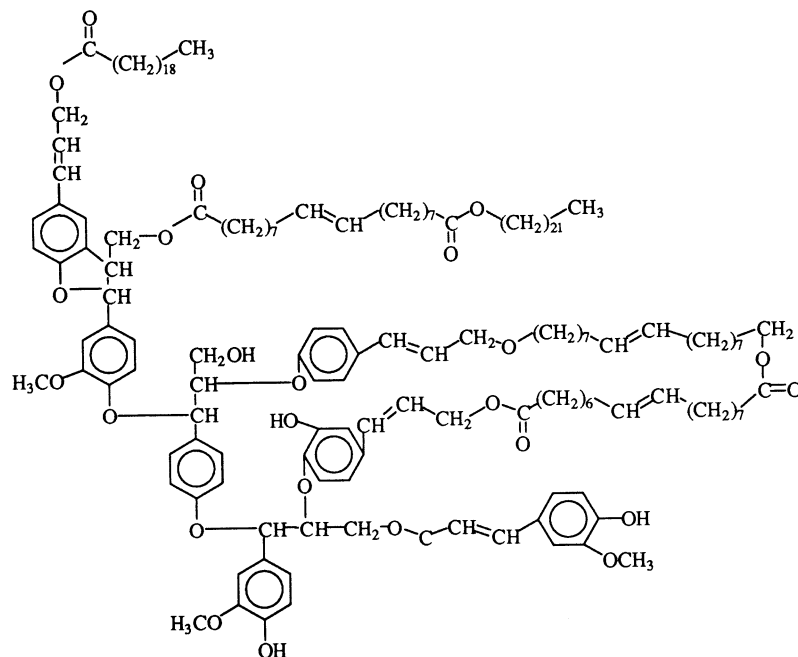
Scheme 1.

investigated as polyol in the synthesis of new polyurethanes [11].

The structure of suberin is not yet fully understood. Kolattukuddy [12] suggested that suberin from the potato periderm is an aliphatic polyester, similar to cutin, containing phenolic moieties such as lignin (Scheme 1). In situ suberin is a macromolecular network insoluble in all solvents. It can be de-crosslinked by chemical processes mostly based on the cleavage of ester bonds. Hydrolysis with aqueous alkali was used by the first cork chemists, but this procedure was later replaced by alkaline alcoholysis [13–18]. The products thus obtained were characterized by gas chromatography (GC) and gas chromatography coupled with mass spectrometric (GC–MS) techniques and found to consist mainly of 22-hydroxydocosanoic acid, 9,10-hydroxyoctadecanedioic acid, 18-hydroxyoctadecanoic acid, 9,10,18-trihydroxy-octadecanoic acid, 9-octadecenedioic acid, 9,10-epoxyoctadecanedioic acid, 18-hydroxy-9-octadecenoic acid and 18-hydroxy-9,10-epoxyoctadecanoic acid [14].

The present paper deals with the chemical characterization of suberin isolated by treating cork with an NaOH methanolic solution. To our knowledge, this constitutes the first thorough report on the chemical structure of the suberin from

Quercus suber L. The major features related to its physical properties have also been studied in this comprehensive investigation [7,10].

2. Experimental

2.1. Suberin extraction

Cork powder (60 mesh) was obtained by grinding and sieving high-quality reproduction cork kindly supplied by the Champcork Company. This powder was extracted in a soxhlet for 8 h sequentially with dichloromethane, ethanol and water. The total extracted material amounted to 19% w/w of the dry cork sample. The extractive-free cork powder was then dried to constant weight and submitted to alkaline methanolysis for 5 h at reflux with a 0.1 M NaOH methanolic solution using a solvent:cork ratio of 10:1 (v/w). The reaction products were filtered and the pH of the solution adjusted to 5–6. The latter was evaporated to dryness and the solid residue, suspended in water, was extracted with chloroform three times. The accumulated organic phases were dried over sodium sulphate and vacuum evaporated. The resulting paste-like residue will be called suberin hereafter.
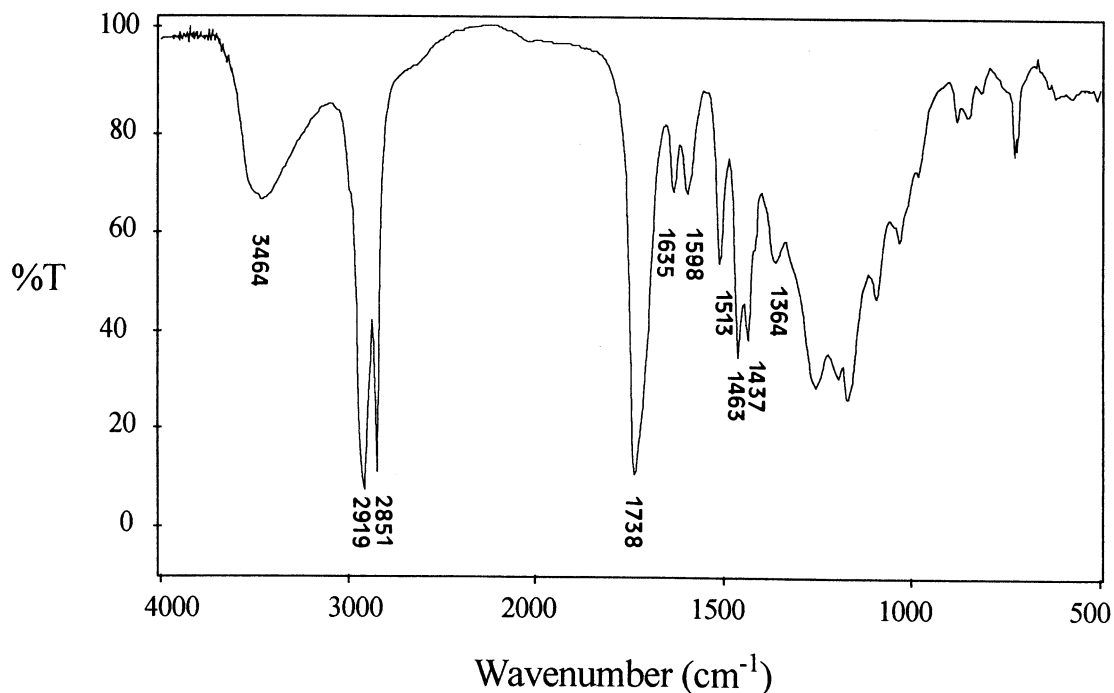
Fig. 1. FTIR spectrum of suberin.

2.2. Structural characterization

2.2.1. Spectroscopic and separative analyses

The FTIR spectra were recorded with a Perkin–Elmer PARAGON 1000 spectrophotometer by spreading suberin on NaCl disks.

^{1}H- and ^{13}C-NMR spectra were taken on a Brucker AMX-300 spectrometer working at ambient temperature. Suberin was dissolved in deuterated chloroform and tetramethylsilane (TMS) was used as an internal standard. The chemical shifts (δ) are given below in ppm downfield from TMS.

GC–MS analysis called upon a preliminary double derivatization of the suberin samples (30–40 mg) whose COOH groups were first converted into methyl esters with diazomethane and whose OH functions were then silylated with bis(trimethylsilyl)trifluoacetamide and trimethyl-chlorosilane according to a procedure described elsewhere [19]. These derivatized samples were analysed by GC–MS using a Hewlett Packard 5970 gas chromatograph, equipped with a Hewlett Packard MSD II detector, using a fused silica J&W DB-1 capillary column (30 m length, 0.32 mm i.d. and 25 μm film thickness). The temperature parameters used in these analy-

ses, conducted in a helium flow (35 cm/s) with a 1:100 split ratio, were: an initial temperature of 150°C; a heating rate of 4°C/min; a final temperature of 285°C; an injector temperature of 290°C and a detector temperature of 290°C.

The various components were identified by comparing their mass spectra with those from a library, with previously published data [19–21] and by a specific examination of their characteristic fragmentation patterns. Peak quantification was obtained using cholesterol as an internal standard. The calibration of the GC–MS involved the determination of the response factor of methyl linoleate relative to the internal standard.

2.2.2. Molecular weight distribution

Vapour pressure osmometry (VPO) was performed with a Knauer instrument using chloroform and tetrahydrofuran as solvents. Two standards were used for calibration, namely benzil as a primary standard and oleic acid as a secondary one.

The desorption chemical ionisation (DCI) mass spectra of underivatized suberin were taken with a Nermag R1010C spectrometer using ammonia as interacting gas.
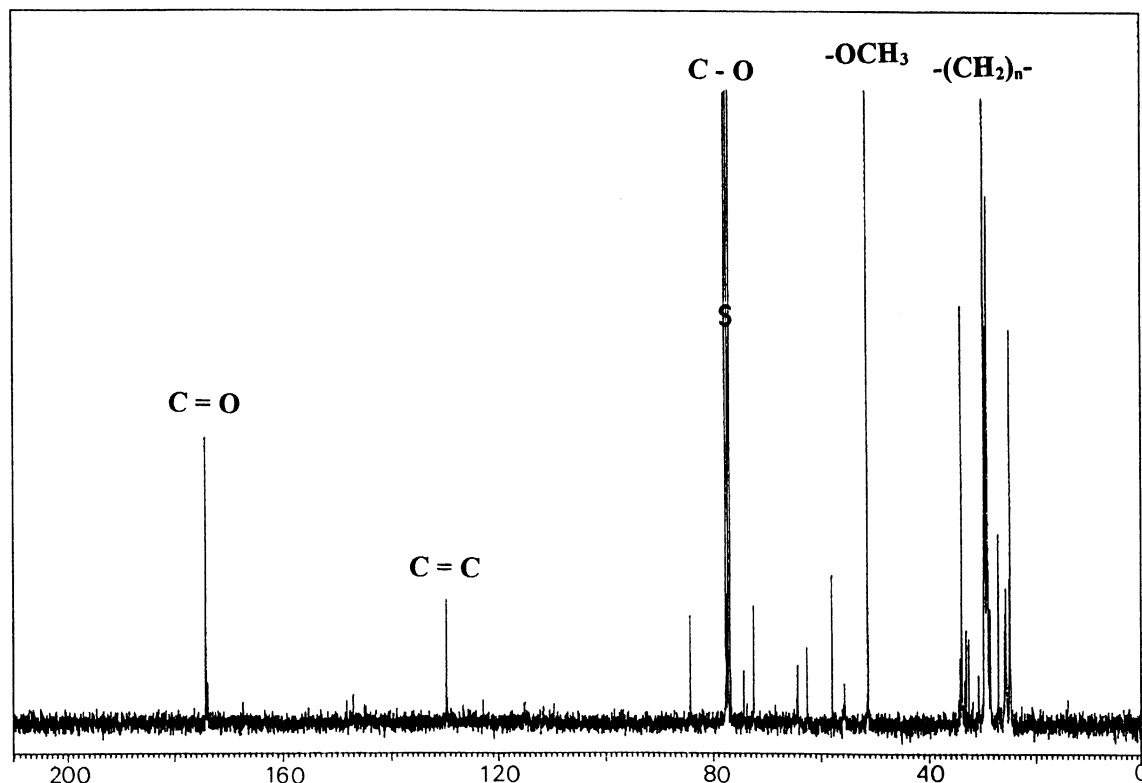
Fig. 2. ^{13}C-NMR spectrum of suberin in deuterated chloroform.

Gel permeation chromatography (GPC) was performed at 30°C with a Waters 150CV apparatus equipped with three Styragel columns (HR1, HR3 and HR4, 4.6×300 mm) and a differential refractometer detector. Tetrahydrofuran was used as the eluent, with a flow rate of 0.3 ml/min. The calibration curve was obtained with polystyrene standards.

2.2.3. Other analyses

Elemental analyses were conducted by the central analysis service of the National Research Council of France at Vernaison.

The hydroxy index or number (I_{OH}) is by definition the number of milligrams of potassium hydroxide equivalent to the hydroxy content of 1 g of the product (polyol). I_{OH} was determined following the ASTM D1638 standard, which consists of dissolving the polyol in pyridine, treating it with a known excess of phthalic anhydride under reflux for 1 h and back titrating the unreacted mixture of acid and anhydride. The titration of these solutions was carried out using an automatic potentiometric titrator (Mettler DL 21) equipped with a glass DG 111 electrode.

3. Results and discussion

3.1. Suberin extraction

The amount of suberin extracted by the 0.1 M NaOH methanolic solution was 370 mg/g of oven-dried cork powder. This corresponds to about 80% of the total suberin in cork, since the application of the quantitative method for suberin content [22] yielded 45% (o.d. cork). The elemental analysis of our suberin gave 68.00% C, 9.76% H and 20.66% O.

3.2. FTIR analysis

An FTIR spectrum of suberin is shown in Fig. 1. The strong absorption bands at 2919 (νass C–H) and 2851/cm (νs C–H), together with those at 1463, 1437 and 1364/cm (δ C–H), clearly indicated the prevalence of the aliphatic nature of suberin. The peaks at 3464 (ν O–H) and 1096/cm (δ O–H) indicated the presence of OH groups. Ester moieties, produced by the methanolysis of suberin chains at the corresponding ester functions (see Scheme 1), were charachterized by the strong bands at 1738 (ν C=O) and 1230/cm (ν C–O–C).
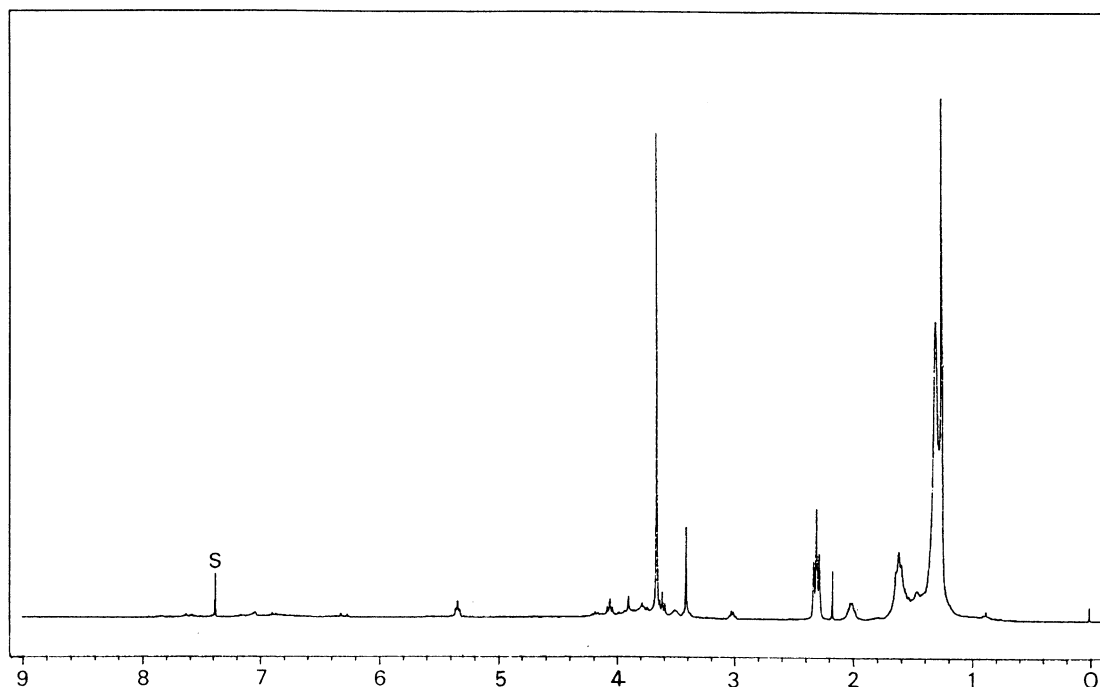
Fig. 3. ^{1}H-NMR spectrum of suberin in deuterated chloroform.

The band at 1635/cm may be attributed to C=C double bonds. The FTIR analysis therefore gave strong evidence in favour of a suberin structure dominated by aliphatic chains bearing some hydroxy and ester moieties.

3.3. ^{13}C-NMR analysis

A typical ^{13}C-NMR spectrum of suberin (Fig. 2) was characterized by the presence of two important groups of signals. The first, in the region of 23–35 ppm, was assigned to methylene carbons in different chemical environments. The second group, between 51 and 84 ppm, was attributed to carbon atoms bound to oxygen. The signal at 51 ppm dominates this region and corresponds to –OCH$_3$ groups of the methyl ester structures arising from the methanolysis.

The region between 100 and 160 ppm, corresponding to aromatic and unsaturated structures, presented only one signal at 130 ppm which was assigned to carbon atoms of alkenyl units. The signal at 174 ppm was attributed to ester carbonyl carbons. These features are in overall agreement with those previously reported for suberized potato cell wall [23] and for the suberin from beech bark [24]. They are also supported by recent results of the solid-state NMR of in situ cork suberin [8].

3.4. ^{1}H-NMR analysis

The characteristic ^{1}H-NMR spectrum of suberin (Fig. 3) was dominated by signals arising from the aliphatic methylenic protons in the region 1.2–2.2 ppm, representing some 75% of all protons. Signals between 2.2 and 2.6 ppm were attributed to methylene protons linked to carbonyl moieties of ester groups. Methoxy protons appeared in the range of 3.4–3.6 ppm and represented more than 10% of all protons. The weak resonances between 3.6 and 4.6 ppm (ca 6% of all protons) were assigned to protons attached to carbon atoms bearing OH groups, while alkenyl protons gave rise to the modest signal (2–3% of all protons) centered around 5.3 ppm.

The above spectroscopic evidence clearly indicates that, apart from the major and minor features exhibited by the various techniques, it seems entirely reasonable to conclude that aromatic moieties are for all purposes virtually absent from the structure of the suberin fragments isolated in this study. More specifically, the signals which could be attributed to these structures amounted to negligible contributions in the three spectra. The same can be said of COOH functions, although the sensitivity of these analytical tools towards these groups is less pronounced. Of course the lack of aromatic structures in our product does not imply
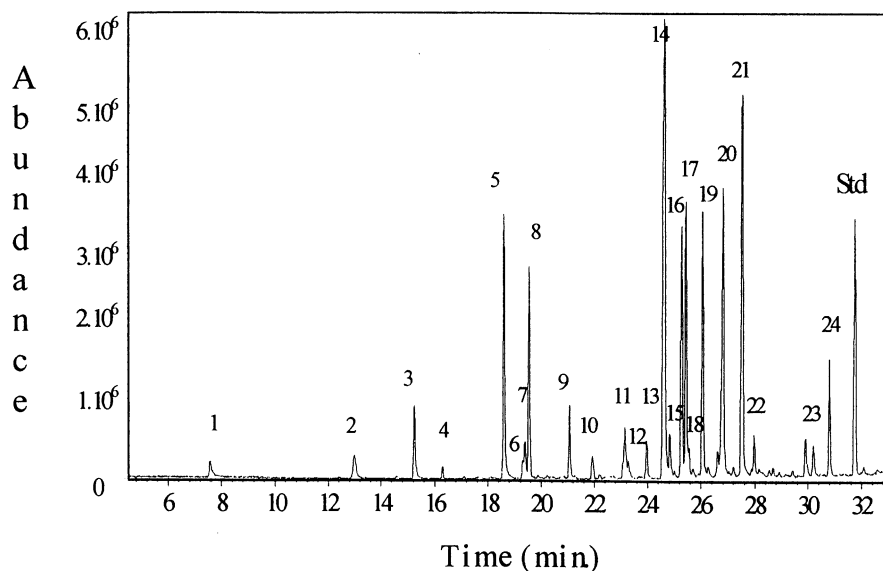
Fig. 4. Gas chromatogram of the methyl ester trimethylsilyl ether derivatives of suberin.

their absence in in situ suberin, but simply that the alkaline methanolysis used to split its network removed predominantly the aliphatic chains.

3.5. GC–MS analysis

Hydroxylated fatty acids and esters have low thermal stability and a very modest volatility associated with both the high molecular size and the intermolecular hydrogen bonding. Hence, GC–MS studies of these compounds are usually carried out using the corresponding methyl esters trimethylsilyl ethers [17]. As shown above, most of the carboxylic moieties of our suberin were already in the form of methyl esters, as indeed expected from the very nature of the chemical treatment, viz. methanolysis. However we decided to carry out both derivatizations to avoid volatilization problems associated with the presence of sporadic COOH groups.

A typical chromatogram of these suberin derivatives is shown in Fig. 4. The identification and quantification of each component is summarized in Table 1. More than 90% of the detected components could be identified. They corresponded mainly to methyl esters of aliphatic hydroxyacids with chain lengths ranging between C16 and C24 and smaller amounts of non-hydroxylated homologues, long-chain al-

cohols and neutral constituents. The relevant components, arranged by structural family are given below.

The primary alcohols docosanol and tetracosanol which represented about 2% of the total.

The saturated C18, C20, C22 and C24 monocarboxylates constituted ca 5%.

The α,ω-dicarboxylic structures were considerably more abundant with more than 20% of the identified components with a saturated (docosane-1,22-dioate; **20**; Table 1) and an unsaturated member (octadec-9-ene-1,18-dioate (**5**)) being the major components of this group.
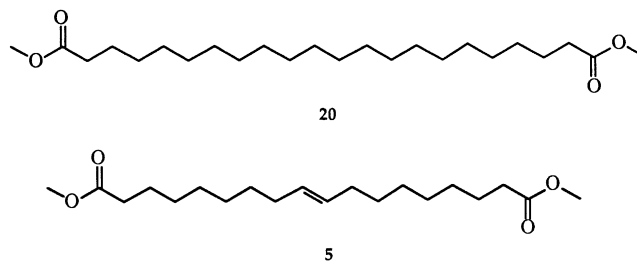
The ω-hydroxymonocarboxylates represented the dominant group with a contribution higher than one quarter of the total. Within this family, the major component was 22-hydroxydocosanoate (**21**), followed by the saturated (**12**) and unsaturated C18 (**8**), as well as the C16, C20 and C24 homologues.

21

12

8
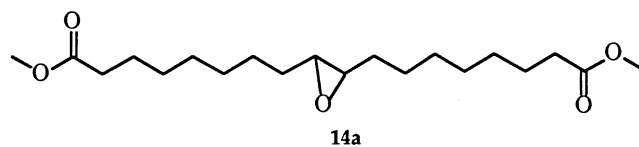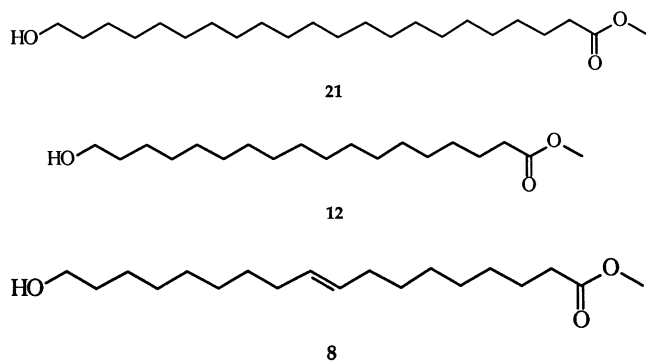
10-methoxy-9-hydroxyoctadecane-1,18-dioate (**14**), the methoxyhydrin artefact from 9,10-epoxy-octadecane-1,18-dioate (**14a**)[19], was the dominant constituent representing about 20% of the total identified suberin components.

Table 1
Composition of the low molecular-weight volatile fraction of suberin from *Quercus suber* L

Peak no.	Composition (w/w %)	Compound (detected as methyl ester trimethylsilyl ether)
1	0.4–1.1	Ferulic acid
2	1.1–2.0	Octadecanoic acid
3	1.9–2.5	Hexadecane-1,16-diacid
4	0.3–0.7	16-hydroxyhexadecanoic acid
5	6.6–7.5	Octated-9-ene-1,18-dioic acid
6	0.3–0.6	Octatedecane-1,18-dioic acid
7	0.8–1.5	10,16-dihydroxyhexadecanoic acid
8	5.2–6.3	18-hydroxyoctadec-9-enoic acid
9	1.6–2.0	Docosanoic acid
10	0.6–1.2	Docosanol
11	1.0–1.9	Eicosanoic 1,20-dioic acid
12	0.5–0.7	18-hydroxyoctadecanoic acid
13	0.7–1.6	20-hydroxyeicosanoic acid
14	18.4–21.5	10-methoxy-9-hydroxyoctadecane-1,18-dioic acid[a]
15	1.2–1.6	Tetracosanoic acid
16	7.0–8.0	10-methoxy-9,18-dihydroxyoctadecanoic acid[b]
17	6.4–8.0	9,10-dihydroxyoctadecane-1,18-dioic acid
18	0.7–1.2	Tetracosanol
19	6.6–7.4	9,10,18-trihydroxyoctadecanoic acid
20	10.0–10.6	Docosane-1,22-dioic acid
21	13.7–17.1	22-hydroxydocosanoic acid
22	0.5–1.2	9,10-dihydroxyeicosane-1,20-dioic acid
23	0.6–1.3	Tetracosane-1,24-dioic acid
24	2.6–3.3	24-hydroxytetracosanoic acid

[a] Methoxyhydrin artefacts from 9,10-epoxy-octadecane-1,18-dioic acid.
[b] Methoxyhydrin artefacts from 9,10-epoxy-18-hydroxyoctadecanoic acid.

14a

Other hydroxycarboxylates identified in this analysis included 9,10,18-trihydroxyoctadecanoate (**19**); 9,10-dihydroxyoctadecane-1,18-dioate (**17**); and minor amounts of 10,16-dihydroxyhexadecanoate and 9,10-dihydroxyeicosane-1,20-dioate, which made up more than 15%.

19

17
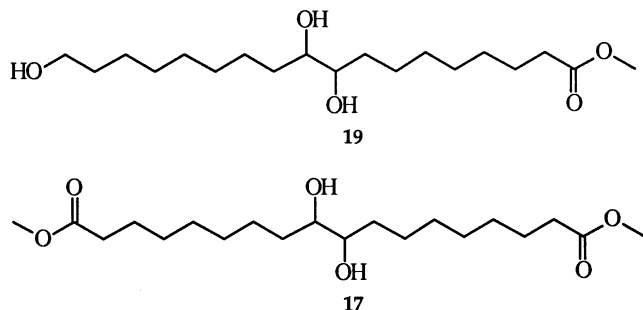
Very small amounts of ferulate were also found in our suberin samples. This explains the trace signals of aromatic structures detected by FTIR, ^{13}C- and ^{1}H-NMR analysis.

Many of the components characterized here represent interesting structures in terms of potential polycondensation monomers, viz. all molecular species bearing at least two reactive (OH, COOR) moieties. Indeed, we have already made use of this potential in a preliminary study on the synthesis of suberin-based polyurethanes [11]. This investigation is being pursued and will also include polyesters and polyethers.

The present results generally agree with those obtained by other authors working with the suberin of *Quercus suber* L., barks from other wood species and tuber tissues [14–16] [19,25,26]. The differences observed from one study to another can be attributed to the different suberin solvolysis and extraction techniques used and to structural variabilities among species.

However, a significant quantitative difference in the total yield of volatile products was observed between our work and a previous study [14]. In fact, according to our results, the yield of the detected components (total chromatogram) corresponded to only about 40% of the amount of suberin injected. This result suggests that there was a large fraction of suberin of high molecular weight, which was not volatile and therefore went undetected. The same behaviour was observed with suberin samples characterized both before
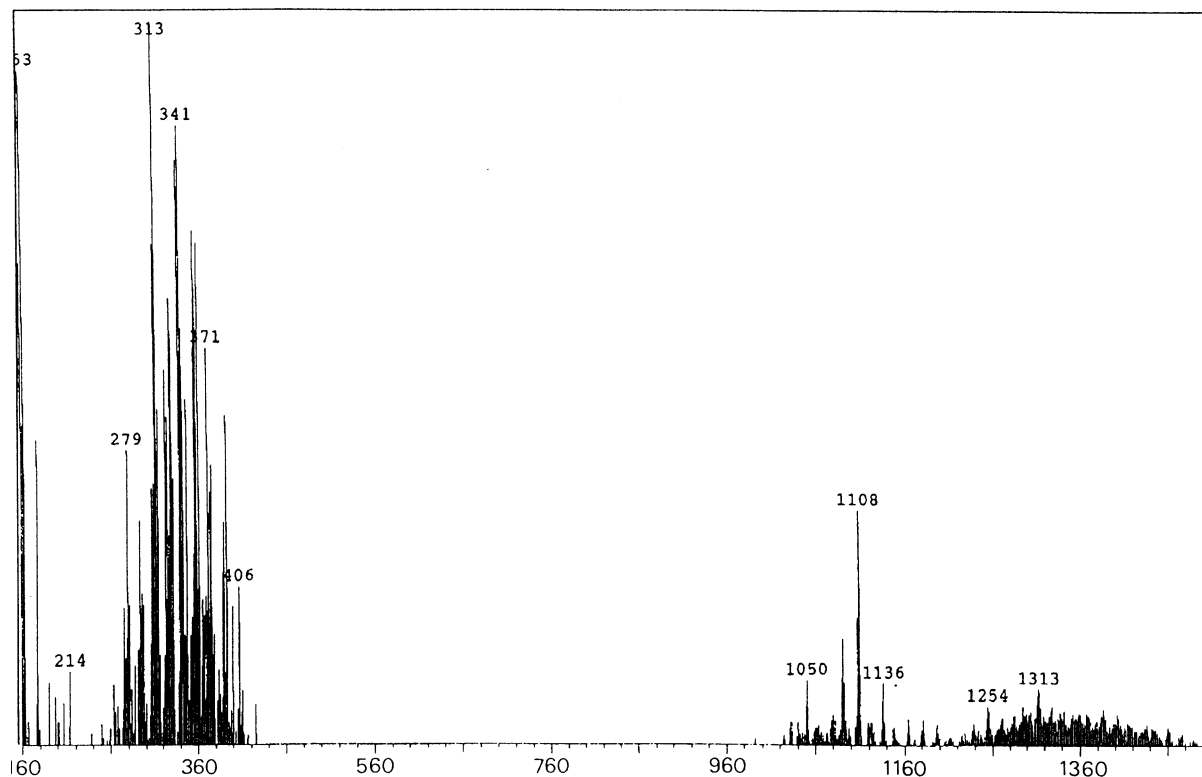
Fig. 5. DCI–MS spectrum of suberin.

and after evaporation to dryness (see Section 2) and with samples isolated by the standard quantitative suberin isolation method used for cork analysis [22]. This strongly suggests that the presence of a high molecular weight non-volatile fraction in suberin is not the result of either side reactions induced by temperature (recondensation as with, e.g. lignins) or to an incomplete solvolytic breakdown.

Recent results indeed suggest the existence of an insoluble, non-hydrolyzable aliphatic biomacromolecule called 'suberan', in the periderm tissue of some angiosperm species [27]. For the suberin of *Quercus suber* L., such a component has never been reported. In order to verify the presence of a high molecular weight fraction in our suberin samples, we submitted them to VPO, MS and GPC analysis.

3.6. VPO analysis

The number average molecular weight (Mn) of suberin, determined by VPO was 800 ± 30. This result strongly supports the existence of a high molecular-weight fraction in suberin.

3.7. MS analysis

The DCI–MS spectrum of suberin (Fig. 5) shows two groups of peaks:
- A group corresponding to a fraction made up of components with molecular masses between 260 and 410 which corresponded to the volatile fraction detected by GC–MS after the double derivatization (Table 1).
- A second set of compounds with molecular weights ranging from 1000 to 1500 which again clearly confirms the presence of a high molecular weight fraction in suberin.

3.8. GPC analysis

A typical molecular weight distribution (MWD) of our suberin samples is shown in the GPC tracing given in Fig. 6. The first strong peak in this chromatogram indicates the existence of a low molecular-weight fraction (nominally Mn = 550) of low dispersity, whereas the rest corresponds to a higher molecular weight (nominally centred around Mn = 2000) and possessing a much wider distribution. Although the quantitative aspect re-
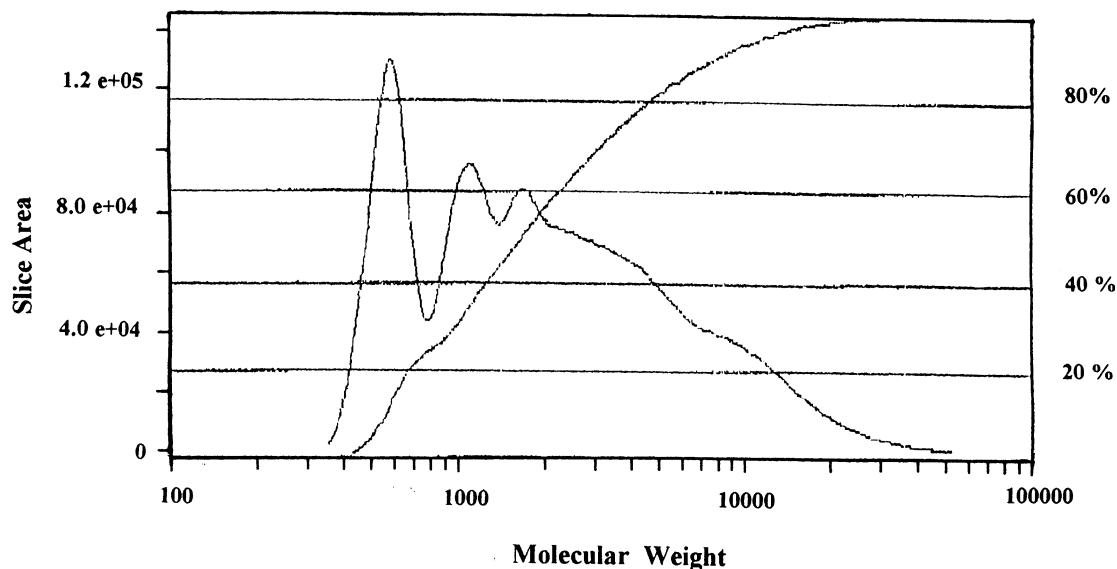
Fig. 6. GPC of suberin.

lated to these results is certainly incorrect because of the major difference between the structures of suberin and polystyrene (used for calibration), the polymodal character of the MWD again indicates the existence of major families of components in suberin extracted from cork.

The hydroxy number of our overall suberin was found to be 160 ± 5. With an Mn from VPO data of 800, this gives an average functionality of 2.3 OH groups per molecule. The volatile fraction displayed an average molecular weight lower than 400 and from its composition in Table 1, an average OH functionality just higher than unity. It follows that the non-volatile fraction must contain molecules with non-negligible hydroxy contents, namely an average of more than two. Our ongoing study on polyurethanes prepared using suberin samples and diisocyanates confirms this conclusion. Indeed, the fraction of crosslinked products in these polycondensations is systematically higher than 50%, which, in conjunction with the low OH functionality of the shorter molecules in suberin, implies that the larger ones have a higher aptitude to give branching, i.e. more than two OH groups per unit.

groups (mainly OH and carboxylates) and unsaturations. However, the most relevant aspect of this study was the discovery of the existence of a fraction possessing a substantially higher molecular weight, not reported in previous studies of the suberin from the solvolysis of *Quercus suber* L. A more thorough investigation of this interesting feature is in progress in order to clarify the chemical nature of this fraction and its specific properties. The knowledge gathered in this study on suberin opens new perspectives for the future utilization of this unique renewable resource in added-value applications.

Acknowledgements

4. Conclusion

The methanolysis of cork suberin yielded a complex mixture of structures in which long aliphatic chains dominated, but which also bore polar

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