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Short Note

Products of the Permanganate Oxidation of Cork, Desuberized Cork, Suberin and Lignin from *Quercus suber* L.

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Introduction

Suberin and lignin are the main components of cork, contributing to its dry weight with about 40 % and 22 %, respectively (Pereira 1988).

Suberin has been previously assumed to be composed of aliphatic hydroxyacids polyester domains covalently bound to aromatic domains (Kolattukudy 1980). Whereas the composition of the aliphatic moieties of suberin has been characterised using alkaline hydrolysis or alcoholysis depolymerization methods (Arno *et al.* 1981; Holloway 1983), the structure of its aromatic domains has not been elucidated. Aliphatic suberin extracts obtained by alkaline treatment of several tree barks have been shown to contain small amounts of aromatic moieties (Ekman 1983). However, in cork, the aromatic fraction of suberin extracts has not yet been characterised in detail. In the few reported attempts to extract lignin from cork, a mixture of a phenolic polymer and suberinic aliphatic acids was obtained (Marques *et al.* 1994). A guaiacyl type lignin has been also isolated from saponified cork and characterised by FTIR and Py-GC-FID (Marques *et al.* 1996). Recently, we have isolated from cork a lignin-like polymer which was characterised by ^{13}C NMR, FTIR and nitrobenzene oxidation (Pascoal Neto *et al.* 1996). The ^{13}C NMR structural characterisation of this polymer indicated that it is covalently bound to the aliphatic suberin moities and showed structural differences with respect to typical lignins. These differences are significant enough to suggest that this phenolic polymer is not a typical lignin but rather a lignin-like polymer (Pascoal Neto *et al.* 1996). To our knowledge little else is known about the *in situ* structure of the lignin of cork and the ambiguity between lignin and the aromatic domain of suberin is still a matter of debate.

The permanganate oxidation method is a suitable technique to investigate the structure and proportions of the so-called condensed and non-condensed units in both *in situ* and isolated lignins (Chen 1988). Although only phenolic phenylpropane units (25–35 % of the total units in typical lignins) protected towards the oxidation by methylation are accessible to this analysis, it has been assumed that they reflect the structural tendencies of the lignin as a whole (Chen 1988; Gellerstedt 1992). This technique has not been applied before to the characterisation of the lignin and/or the aromatic domain of suberin of cork.

In this work, samples of untreated cork, partially and completely desuberized cork, methanolysis extracts (suberin) and the previously isolated cork lignin-like polymer (Pascoal Neto *et al.* 1996) were submitted to permanganate oxidation (Gellerstedt 1992) followed by the analysis of the obtained aromatic oxidation products by GC and GC-MS.

Material and Methods

Extractive free cork powder (> 40 mesh) obtained from high quality cork boards, was used in this study. The extraction was carried out in a soxhlet apparatus using sequentially dichloromethane, ethanol and water (8 hours for each solvent). Desuberization was carried out with $NaOCH_3$ methanolic solutions according to a previously described method (Pereira 1988) using $NaOCH_3$ concentrations ranging from 0.01 to 3.00 %. The extractive-free cork powder was submitted to reflux (3 h) in dry MeOH containing $NaOCH_3$. The reaction products were filtered and the pH adjusted to 5–6. The latter was evaporated to dryness and the solid residue, suspended in water, was extracted with CH_2Cl_2 three times. The accumulated organic phases were dried over Na_2SO_4 and vacuum evaporated. The resulting paste-like residue was called suberin. At least three desuberization experiments were carried out for each $NaOCH_3$ concentration. The cork lignin-like polymer was isolated by a mild organosolv treatment [EtOH/H_2O 50 : 50 (v/v) solution containing 0.10 M acetic acid at 160 °C, during 4 h] following a procedure we reported recently (Pascoal Neto *et al.* 1996). The permanganate oxidation of samples was carried out based on a method described elsewhere (Gellerstedt 1992). The methylated oxidation products were identified by GC-MS using a VG Autospec-Q spectrometer and assessed quantitatively by GC using a Varian Star 3400CX chromatograph equipped with a fused silica capillary column DB-5 (J & W Scientific, Inc., 30 m × 0.25 mm i.d.; 0.25 µm film thickness) and operated at 150–260 °C with a heating rate of 5 °C/min. Injector and detector temperatures were held at 220 °C and 270 °C, respectively. Peaks representing more than 90 % of the chromatogram area were identified. Pyromellitic acid tetramethyl ester was used as internal standard.

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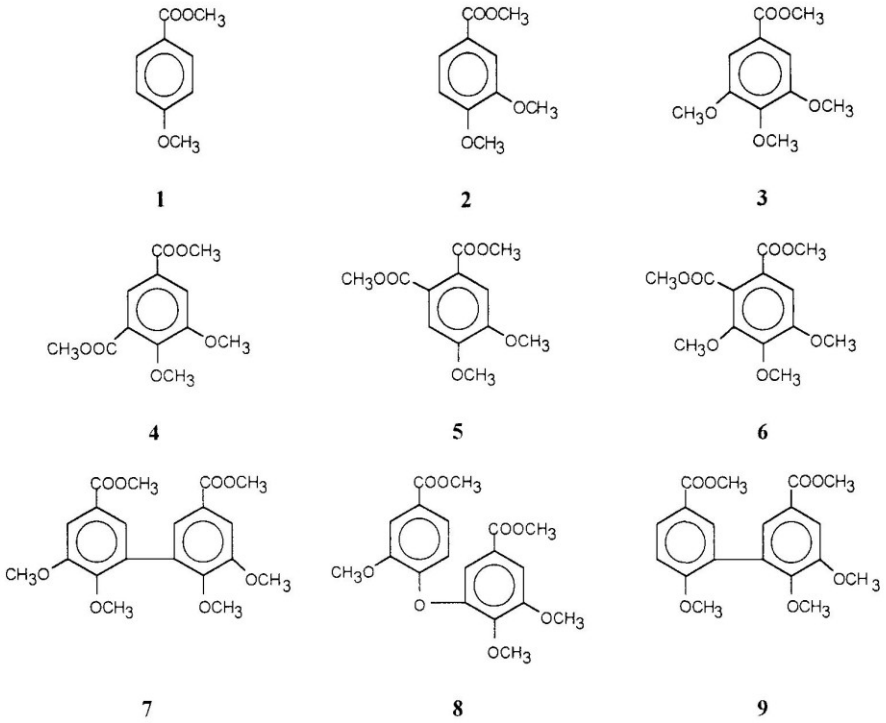

Fig. 1. Aromatic carboxylic acid methyl esters obtained by the permanganate oxidation of cork and cork components.

Results and Discussion

The yields of suberin extraction (o.d. cork) with different $NaOCH_3$ concentrations are given in Table 1. $NaOCH_3$ concentrations between 0.01 and 0.03 % were used in order to obtain partially desuberized cork samples and to investigate the effect of desuberization on the aromatics removal. A 3 % $NaOCH_3$ solution was also used, since it is the concentration of the methanolic solution used for quantitative suberin extraction in a proposed scheme of cork analysis (Pereira 1988).

The isolated lignin-like polymer studied was extracted from cork by a mild organosolv treatment with 2.6 % yield (o.d. cork) (Pascoal Neto *et al.* 1996). For simplicity we will refer to this polymer as "isolated lignin" in the text below.

The permanganate oxidation of cork, desuberized cork, suberin and isolated lignin, followed by analysis of the oxidation products by GC and GC-MS, allowed the identification of 9 different aromatic carboxylic acid methyl esters (Fig. 1). Products **1–3** arise from the oxidation of non-condensed hydroxyphenyl- (H), guaiacyl- (G) and syringylpropane (S) units, respectively, whereas products **4–9** arise from the so-called condensed units. The yields of permanganate oxidation products from cork, desuberized cork and isolated lignin were in the range of 5 to 10 % (o.d. lignin) and of 2 to 4 % (o.d. extract) in the suberin extracts.

Table 1. Yields of extracted suberin (o.d. cork) with different concentrations of methanolic $NaOCH_3$.

$NaOCH_3$ in methanol % (w/w)	Yield of extracted suberin % (o.d. cork)
0.01	3.6
0.02	22.1
0.03	43.8
3.00	42.3

Table 2 shows that the *in situ* cork lignin is a guaiacyl-type lignin with approximate H:G:S proportion of 3:93:4. The H:G:S proportion as well as the type and frequencies of occurrence of the obtained oxidation products for the isolated lignin are quite similar to those obtained for untreated cork lignin. This support the idea that the isolated lignin is representative of the aromatic fraction of cork and, consequently, suggest that the aromatic domain of suberin is a phenolic polymer with a lignin-like structure. The frequencies of occurrence of condensed units of the *in situ* cork lignin are very close to those found in common hardwood and softwood *in situ* lignins (Chen 1988; Gellerstedt 1992). The isolated lignin yields higher proportions of oxidation products **5**, **6** and **7** than the *in situ* cork lignin. This indicates a higher degree of condensation of the isolated lignin as compared to *in situ* lignin. This condensation is probably associated to acid-catalysed condensation reactions (products **5** and **6**) and oxidative coupling reactions (product **7**) during the organosolv treatment in air atmosphere.

The aromatic fraction of suberin extracts is composed essentially of G units (96–98 %). This is attributed par-

Table 2. Molar proportions of products **1–9** (see Fig. 1) as obtained by the permanganate oxidation of cork, desuberized cork, cork methanolysis extracts (extracted suberin) and organosolv cork lignin.

Sample	Molar Proportions (%)									H:G:S
	1	2	3	4	5	6	7	8	9	
Cork (extractive free)	3	66	4	7	7	–	8	5	–	3:93:4
Desuberized Cork										
0.01 % $NaOCH_3$	2	59	3	8	5	–	8	7	8	6:91:3
0.02 % $NaOCH_3$	2	57	3	9	6	tr	9	6	8	6:91:3
0.03 % $NaOCH_3$	3	63	4	11	9	tr	7	3	–	3:93:3
3.00 % $NaOCH_3$	3	51	3	19	11	tr	8	5	tr	3:94:3
Extracted Suberin										
0.01 % $NaOCH_3$	–	78	tr	6	2	–	4	5	5	2:98:0
0.02 % $NaOCH_3$	–	66	tr	8	2	–	11	7	6	3:97:0
0.03 % $NaOCH_3$	–	56	tr	10	3	–	14	12	5	2:98:0
3.00 % $NaOCH_3$	–	58	2	17	2	–	7	9	5	2:96:2
Isolated Lignin	2	60	3	6	9	2	12	6	–	2:93:5

tially, at least, to ferulic acid structures originally esterified to aliphatic hydroxyacids chains, which was identified by GC-MS in the suberin extracts obtained (unpublished results). Minor proportions of S units (2 %) were only found in suberin extracts obtained upon strong alkaline extraction conditions (3 % $NaOCH_3$). Other aromatic structures found in suberin extracts, even with very mild extraction conditions, include biphenyl structures (yielding products **7** and **9**), diarylether structures (yielding product **8**) and, probably, β–5 and α–6 condensed structures (yielding products **4** and **5**, respectively), involving essentially G type units. Condensed H type structures are present in suberin extracts. This can be attributed to complete condensation of phenolic H type structures through radical coupling reactions of phenoxyl mesomeric forms in solution during cork reflux in the alkaline methanolic solution in air atmosphere, leading to condensed structures which upon permanganate oxidation, give oxidation product **9**. The occurrence of these radical coupling reactions is evidenced from the absence of product **9** in the permanganate oxidation products of intact cork.

When cork is progressively desuberized, the proportion of condensed structures involving the 5 and 6 positions of the aromatic ring (products **4** and **5**), increases in desuberized cork and extracted suberin (Table 2). This is mainly a consequence of *in situ* condensation reactions occurring in the increasingly stronger alkaline medium used for desuberization through the quinone methyde mechanism.

The study of cork dioxane lignin and suberin extracts using ^{13}C NMR, GC and MS is in progress with the aim to deepen the knowledge of the nature of the aromatic fraction of cork and its interaction with the aliphatic part of suberin.

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