SUBERIN COMPOSITION FROM DIFFERENT BARK LAYERS OF *QUERCUS SUBER* L. BY PY-GC/MS IN THE PRESENCE OF TETRAMETHYLAMMONIUM HYDROXIDE (TMAH)

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In previous studies we found significant differences in the suberin content with respect to the bark layer of *Quercus suber* samples. In this study the monomer composition of suberin from the two bark layers (i.e., back and cork) of three provenances (Extremadura, Castile-la Mancha, and Portugal) was investigated using pyrolysis in the presence of tetramethylammonium hydroxide (TMAH) with gas chromatography-mass spectrometry (GC/MS). The major compounds released were octadec-9-enedioic acid, docosanedioic acid, and 9,10-epoxyocta-decanedioic acid with mean values of 17%, 14.5%, and 11%, respectively. The former is more abundant in back than in cork, and the latter in cork than in back with mean differences, in terms of percentages, between the back and cork of 4.3 ± 0.81 and 2.2 ± 0.52, respectively.

Keywords: Quercus suber; Suberin; Pyrolysis GC-MS; Tetramethylammonium hydroxide (TMAH)

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INTRODUCTION

Cork, the outer bark of *Quercus suber* L., is a natural raw material composed mainly of suberin and lignin (hydrophobic biopolymers), which contribute about 40% and 20%, respectively, to its dry weight. The remainder comprises polysaccharides (ca. 20%) of a hydrophilic character, and extractables (ca. 15%) (Pereira 2007). This heterogeneous chemical composition of cork provides numerous bonding possibilities for a wide range of pollutants, and confers on cork unique characteristics that make it a very interesting natural material to investigate.

The distribution of these biopolymers is quite variable within the cork bark due to the different characteristics of its layers (back, cork, and belly). In previous studies we reported significant differences for suberin and holocellulose contents with respect to the bark layer, while no significant differences were found for lignin (Jové et al. 2011).

The monomer composition of suberin has been extensively studied by gas chromatography mass spectrometry (GC-MS) prior to suberin depolymerization (Gandini et al. 2006). Other techniques such as infrared (FT-IR) spectroscopy, nuclear magnetic resonance (^{13}C NMR and ^{1}H NMR) (Gil et al. 1997; Neto et al. 1995) and thermochemolysis in the presence of a methylating agent (tetramethylammonium hydroxide, TMAH) (del Río et al. 1998) have also been applied to solid cork to

characterize the suberin *in situ*. In particular, the latter technique can also be performed directly in a pyrolyzer unit, which has proven to be very efficient, less laborious, and easy to perform with very low amounts of sample, because the products are methylated *in situ* prior to gas chromatographic analysis (del Río et al. 1996).

In this study, as supplementary material related to a previous publication (Jové et al. 2011), we investigate the monomer composition of suberin from different bark layers (i.e., cork and back) by Py-GC/MS in the presence of TMAH.

EXPERIMENTAL

Reagents and Samples

The *Quercus suber* cork sample was taken from cork strips supplied by a cork factory and originating from boards of reproduction cork intended for the production of stoppers. The cork strips were cut into three layers with a hand saw at three radial positions: the outermost layer (the back), 6-10 mm thick; the cork middle part used for cork stoppers, 26-32 mm thick; and the innermost layer of cork (the belly), 3-5 mm thick. In this study, only the belly part of the sample was selected (Jové et al. 2011).

Each sample was cut into small pieces (< 10 mm) and milled using a ZM-200 ultra centrifugal mill (Retsch). The granulated samples were sieved, and the 40 to 60 mesh granulometric fraction (0.25 to 0.42 mm grain size) was used for the subsequent analyses. The separated back and cork layers from Extremadura (EXT-06), Castile-la Mancha (CMANCH), and Portugal (POR) were analyzed.

Methods

Pyrolysis in the presence of TMAH was performed with approximately 1.0 mg of sample in finely powdered form, mixed with approximately 5 μL TMAH (25% w/v in methanol), in a 2020 micro-furnace pyrolyzer (Frontier Laboratories Ltd.) connected to an Agilent 6890 GC/MS system equipped with a DB-5MS (Agilent J&W) fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 5973 mass selective detector (EI at 70 eV). The pyrolysis was performed at 500ºC. The oven temperature was programmed from 40ºC (1 min) to 300ºC at 6ºC min^{-1} (10 min). Helium was used as carrier gas (1 ml min^{-1}). The injector and detector were set at 300ºC. The compounds were identified by fragmentography and by comparing their mass spectra with those of the Wiley and NIST libraries and values reported in the literature. The summed peak areas were normalized to 100 and the data for two analyses were averaged.

RESULTS AND DISCUSSION

The chromatograms of the products released after pyrolysis in the presence of TMAH of the cork samples are shown in Fig. 1. Table 1 shows the list of the identified compounds, as well as their relative distribution in the different samples.

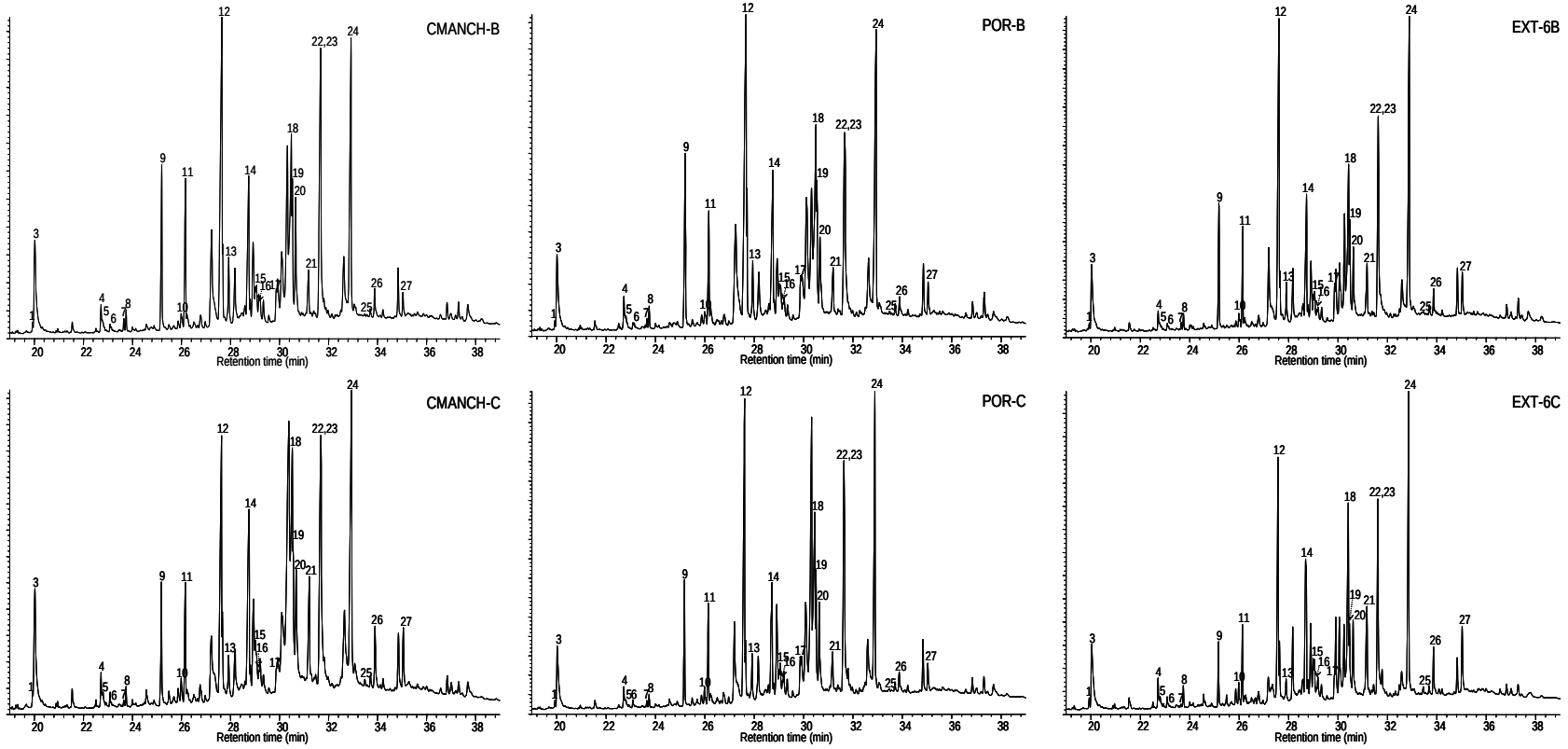

Figure 1. Gas chromatograms of the products released after pyrolysis in the presence of TMAH from the samples of Extremadura (EXT-06), Castile-La Mancha (CMANCH), and Portugal (POR). The bark layers are designed by "B" (the back) and "C" (the cork). The peak numbers correspond to the compounds identified in Tables 1 and 2.

Table 1. Composition (%) of the Products (as their methyl derivatives) Released after Pyrolysis in the Presence of TMAH from the Cork Samples of Extremadura (EXT-06), Castile-La Mancha (CMANCH) and Portugal (POR). The bark layers are designed by "B" (the back) and "C" (the cork).

Label	Compound	EXT6-B	EXT6-C	CMANCH-B	CMANCH-C	POR-B	POR-C
1	hexadecenoic acid	0.3	0.5	0.3	0.4	0.2	0.3
2	ferulic acid	5.9	7.0	6.8	7.5	5.2	5.4
3	hexadecanoic acid	0.4	0.5	0.5	0.4	0.5	0.3
4	octadec-9,12-dienoic acid	0.9	1.5	1.0	0.9	1.0	0.9
5	octadec-9-enoic acid	0.2	0.3	0.4	0.3	0.3	0.3
6	octadecanoic acid	0.1	0.2	0.2	0.2	0.2	0.1
7	16-hydroxyhexadecanoic acid	0.3	0.2	0.4	0.2	0.3	0.2
8	1-eicosanol	0.6	1.0	0.6	0.5	0.7	0.5
9	hexadecanedioic acid	5.4	2.8	5.5	3.7	6.6	4.9
10	eicosanoic acid	0.3	0.2	0.4	0.5	0.4	0.3
11	18-hydroxyoctadec-9-enoic acid	4.1	3.2	5.0	3.4	3.8	3.8
12	octadec-9-enedioic acid	19.2	15.1	17.8	12.6	21.3	17.7
13	octadecanedioic acid	2.0	1.4	2.6	1.6	2.3	2.1
14	docosanoic acid	5.3	6.5	6.0	8.5	6.5	6.1
15	18-hydroxy-9,10-epoxyoctadecanoic acid	1.1	1.4	1.0	0.9	0.7	1.2
16	20-hydroxyeicosanoic acid	0.4	0.3	0.9	0.4	0.2	0.5
17	10-hydroxyoctadec-9-enedioic acid	1.7	1.2	1.2	0.9	1.7	1.8
18	9,10-epoxyoctadecanedioic acid	9.6	11.6	9.4	12.2	10.2	12.0
19	eicosanedioic acid	3.6	3.2	3.2	2.6	3.2	3.8
20	9,10-dihydroxyoctadecanedioic acid	3.3	3.1	4.5	4.7	3.6	4.7
21	tetracosanoic acid	2.9	5.3	1.9	4.2	2.1	2.4
22	22-hydroxydocosanoic acid	4.2	5.0	5.1	5.3	3.6	4.3
23	9,10,18-trihydroxyoctadecanoic acid	9.0	6.3	10.4	10.2	8.6	9.8
24	docosanedioic acid	15.9	16.1	12.4	13.7	14.6	14.4
25	hexacosanoic acid	0.1	0.5	0.2	0.4	0.2	0.2
26	24-hydroxytetracosanoic acid	1.2	2.3	1.2	2.1	0.7	1.0
27	tetracosanedioic acid	2.0	3.4	1.1	1.9	1.2	1.3

A predominance of the C_{18} and C_{22} monomers, particularly the dicarboxylic acids, was observed in all samples. The most abundant members of the C_{18} family were octadec-9-enedioic acid (**12**) (mean value in percentage and standard deviation of 17±3), 9,10-epoxyoctadecanedioic acid (**18**) (11±1.2), and 9,10,18-trihydroxyoctadecanoic acid (**23**) (9.1±1.5). It is interesting to note that octadec-9-enedioic acid is more abundant in the back than in the cork, while 9,10-epoxyoctadecanedioic acid is more abundant in the cork than in back. The higher amount of suberin in cork with respect to the back could be attributed to the presence of this compound.

Among the trihydroxyacids, 9,10,18-trihydroxyoctadecanoic acid was also found to be the most abundant compound in cuticles from apple fruit and *Agave Americana* (del Río et al. 1998). Its presence was also shown in the early stages of fruit development (Baker et al. 1982). With respect to the C_{22} family, the most abundant compounds are docosanedioic acid (**24**) (mean value 14.5±1.4), docosanoic acid (**14**) (6.5±1.1), and 22-hydroxydocosanoic acid (**22**) (mean value 4.6±0.7). Except for 22-hydroxydocosanoic acid (**22**), which was slightly more abundant in the cork than in the bark, no general trends in the abundance between bark layers were observed. Among the C_{16} monomers, hexadecanedioic acid (**9**) (mean value in percentage and standard deviation of 4.8±1.4) was detected and its abundance was found higher in the back than in the cork in all samples. Significant amounts of ferulic acid (**2**) were also detected (6.3±0.9) in all samples.

Although the distribution of the compounds identified is similar to that previously reported for the monomer constituents of these samples using different depolymerization methods (Gandini et al. 2006), the relative abundance of each monomer is somehow different. Graça and Pereira (2000) reported a lower presence of octadec-9-enedioic acid (0.5%) and docosanedioic acid (4.5%) and a higher presence of 9,10-epoxyoctadecanedioic (23%) in cork samples from the Alentejo region of Portugal.

It is interesting to point out that while the cork suberins analyzed in this study resulted in mainly C_{18} and C_{22} monomers, the major monomers present in the suberin isolated from other plants (i.e. the chalazal region of the inner seed coat of grapefruit) are the C_{22} and C_{16} monomers (del Río et al. 1998). As occurs in the suberin from corks, a significant contribution of docasanoic and docosanedioic acids were also found in those suberins (del Río et al. 1998).

Figure 2 schematically depicts the monomeric composition (%) of cork suberins by major chemical families. It is clear that the monomeric units in cork suberin are predominantly constituted by diacids and with an important contribution of hydroxyacids. It is also shown that diacids are slightly higher in back than in cork.

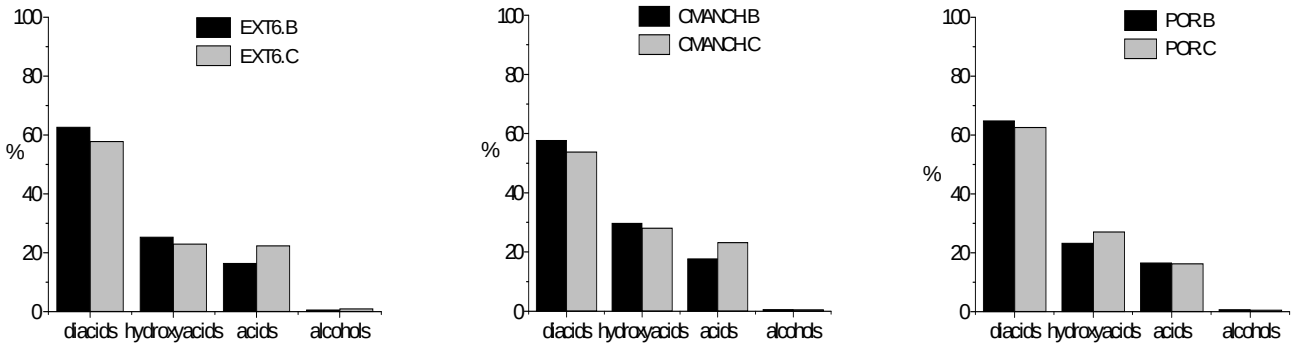

Figure 2. Schematic monomeric composition of cork suberins from Extremadura (EXT), Castile-La Mancha (CMANCH), and Portugal (POR) by family class in percentage of composition

CONCLUSIONS

1. Suberin composition from different bark layers was analyzed using pyrolysis in the presence of tetramethyl-ammonium hydroxide (TMAH).
2. The results indicated a predominance of the C_{18} and C_{22} monomers.
3. The major monomers found were octadec-9-enedioic acid (mean value 17%) and 9,10-epoxyoctadecanedioic (mean value 11.2%) . The former was more abundant in back than in cork, while the later was more abundant in cork than in back.
4. Diacids are the most abundant chemical family (~ 60%), being slightly higher in back than in cork, followed by the hydroxyacids (~ 30%).

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