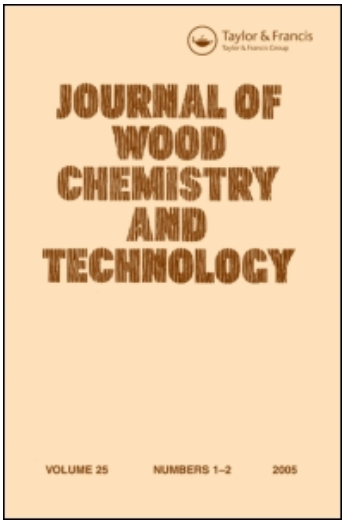

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Feruloyl Esters of ω-Hydroxyacids in Cork Suberin

José Graça[a]; Helena Pereira[a]
[a] Departamento de Engenharia Florestal, Institute Superior de Agronomia, Lisboa Codex, Portugal

To cite this Article Graça, José and Pereira, Helena(1998) 'Feruloyl Esters of ω-Hydroxyacids in Cork Suberin', Journal of Wood Chemistry and Technology, 18: 2, 207 — 217
To link to this Article: DOI: 10.1080/02773819809349577
URL: http://dx.doi.org/10.1080/02773819809349577

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 18(2), 207-217 (1998)

FERULOYL ESTERS OF ω-HYDROXYACIDS IN CORK SUBERIN

José Graça and Helena Pereira
Instituto Superior de Agronomia
Departamento de Engenharia Florestal
P - 1399 Lisboa Codex, Portugal

ABSTRACT

Feruloyl esters of ω-hydroxyacids were found in the alcoholysis products of suberin from *Quercus suber* cork. Their identification was made from mass spectra and by comparison with synthetic model compounds. The ω-hydroxyls of suberinic hydroxyacids are thought to be the ester-bonding points between suberin aliphatics and associated aromatics.

INTRODUCTION

Suberin is the polymer responsible for the insulating and hydrophobic properties of the outer tissues of plants with secondary growth, especially of tree periderms. The proportion of suberized cells and their suberin content in the outer bark of different species varies widely. The periderm of the West Mediterranean-based cork-oak (*Quercus suber* L.) is probably the best example of a totally

suberized and suberin-rich plant tissue. This is the result of a very active and long-living phellogen, producing 80-200 phellem cells per year,[1] with an overall suberin content of *ca.* 40%.[2] The cork layer attains several centimetres of thickness in *ca.* 10 years, allowing its commercial exploitation and utilization for the production of cork stoppers. The production and industrial transformation of cork is a major economic sector in Portugal.

Analytically suberin is usually considered as the materials solubilized by ester-cleavage techniques, normally base- or acid-catalysed hydrolysis, alcoholysis and hydrogenolysis, which give a mixture of monomers, mostly long-chain ω-hydroxylated acids and α,ω-diacids, with small quantities of 1-alkanoic acids and 1-alkanols.[3,4] In *Q. suber* cork, the more abundant chain length for the saturated monomers is 22 carbons (22C), but 18C is the dominant overall carbon aliphatic chain. Moreover, almost all 18C acidic monomers have mid-chain modifications: unsaturation, epoxide or *vic*-diol substitution.[3,5,6] An important amount of glycerol is also solubilized, which has been shown recently to be esterified to suberinic acids, thus indicating that glyceryl-acyl linkages are part of the suberin structure.[7] Together with these fatty-like aliphatic monomers, depolymerization of suberin solubilizes varying amounts of phenolic compounds, usually hydroxycinnamic acids, of which ferulic acid is the most commonly found.[8-10] After suberin extraction, most of the remaining materials are polyphenols with a lignin-like character.[2,11-13] Ester linkage of aliphatics to aromatics in the suberin structure has long been hypothesized.[14,15] Isolated fractions from *Pseudotsuga menziesii*[16] and *Q. suber*[17] cork suberins have been characterized as esters between phenolic acids and ω-hydroxyacids. Also, alkyl ferulates[18-20] and *p*-coumaryl alkanoates[21] were found in the extractives of suberin-rich tissues and have been regarded as possible suberin polymer constituents. The occurrence of ferulates of ω-hydroxylated fatty acids in *Q. Suber* suberin is presented here and its consequences for the polymer structure discussed.

RESULTS AND DISCUSSION

Feruloyl esters of ω-hydroxyacids in the methanolysis products

Methanolysis of extractive-free suberized *Q. suber* cork tissue gave a mixture of organic-soluble suberinic monomers (Table 1), in relative quantities close to published results.[3,5] In addition, several peaks in the high temperature region of the ion chromatogram (0.4%) were identified as methyl esters of feruloyl esters of ω–hydroxyacids, by their EI mass spectra on GC-MS of the TMSi-derivatized mixture. They showed a fragmentation pattern which was consistent with an aromatic compound (high intensity $[M]^+$), but with ions common to an alkyl chain (*m/z* 55) and aliphatic methyl ester groups (*m/z* 74 and 87). Also, the intense ion at *m/z* 249 was consistent with a feruloyl ion, resulting from the expected carbonyl-initiated α-cleavage in the ester, and its abundance was concordant with the stability imparted by the totally conjugated feruloyl moiety. Confirmation of identification was obtained by synthesis of the feruloyl ester of 16-hydroxyhexadecanoic acid methyl ester.

The feruloyl ester-linked ω-hydroxyacids included all the even saturated members from 16C to 24C, a 18C monounsaturated and probably the 18C 9,10 *vic*-diol substituted one. The major peak (75% of ferulates) was the feruloyl ester of 22-hydroxydocosanoic acid. The second most abundant (15%) was identified as the feruloyl ester of a 18C monounsaturated ω-hydroxyacid ($[M-CH_3OH]^+$ present, with *m/z* 74 and 87 comparatively less abundant), probably the 9-unsaturated, which is the one always found in the suberinic mixtures.[3,4] A peak (1%) was tentatively identified as the methyl ester *bis*TMSi derivative of the 18-*O*-feruloyl ester of the 9,10,18-trihydroxyoctadecanoic acid. The $[M]^+$ was absent but the complementary ions associated with the facile cleavage between the trimethylsiloxy carrying carbons 9 and 10 were present, *viz.*, *m/z* 479 for the fragment including the feruloyl moiety, *m/z* 259 for the remaining fragment ion. The ω-hydroxyalkanoic acids and the ω-hydroxy-mid chain-substituted acids found esterified as ferulates are the same and in comparable relative proportions

TABLE 1

Aliphatic Suberin Monomers, Ferulic Acid and Feruloyl Dimers in the $CHCl_3$-Soluble Methanolysis Products of *Quercus suber* Cork* (hydroxyls as TMSi-derivatives, carboxyls as methylesters).

	%	
1-alkanols		1.5
1-alkanoic acids		2.5
ω–hydroxylated acids		52.4
saturated chain	21.1	
22C saturated	13.6	
18C 9,10-substituted	25.8	
9,10-epoxide	8.1	
9,10-*vic*-diol	4.1	
9,10-unsaturated	13.6	
others		5.5
α,ω–diacids		41.1
saturated chain	8.4	
22C saturated	5.6	
18C 9,10-substituted	32.7	
9,10-epoxide	18.2	
9,10-*vic*-diol	7.4	
9,10 -unsaturated	7.1	
Ferulic acid		1.0
Feruloyl esters		0.4
Others unidentified		1.1
Total		100

* Estimated by the integrated areas in a GC-MS chromatogram.

of thoses found as free monomers in the methanolysis products. The notable exceptions are the absence of the feruloyl esterified 18-hydroxy-9-epoxyoctadecanoic acid and the low proportion of the presumed ferulate of the 9,10,18-trihydroxyoctadecanoic acid. Minor peaks with similar mass spectrum of the more abundant feruloyl esters (16C, 18:1C, 22C), appear earlier in the ion chromatogram. This suggests the presence of *cis* and *trans* isomers, arising from

the prop-2-ene part of the feruloyl moiety. The main peaks with the longer retention times are presumably the *trans* isomers.

Check for artifacts during the methanolysis

The presence of these ester-linked dimers after an ester-cleaving procedure such as the alcoholysis used raises the question of possible artifacts, an eventual source being the acidification of the methanolysate extract, where acid-catalysed esterification can occur. The possibility of artifacts was checked in three ways: could the ferulates be found without the acidification step; could the ferulates be formed during the methanolysis or the consequent acidification; what is the stability of these esters under the methanolysis conditions used. The analysis of the depolymerized suberin done directly in the methanolysate before acidification showed the same mixture of compounds, including the ferulates in the same approximate proportions (data not shown). A methanolysis in the conditions used for suberin depolymerization (same proportion of sodium methoxide/ mass of substrate and same concentration of methoxide in solution) was applied to a mixture of ferulic acid and methyl 16-hydroxyhexadecanoate. Analysis of the solution content before and after acidification showed no formation of ferulates. The stability of the ferulates under equivalent methanolysis conditions was studied on synthetic methyl 16-*O*-feruloyloxyhexadecanoate. About half the ferulate was transesterified in 5 min, but after 2 h 4% was still present. No ferulate was detectable only after 24 h (see Experimental for details). The stability of these esters may be due to the large conjugation system within the feruloyl moiety up to the carbonyl group, thereby decreasing the reactivity of the carbonyl towards the nucleophilic attack by the methoxide base.

An earlier identification of the ferulic acid ester of 22-hydroxydocosanoic acid in the methanolysate products of *Q. suber* cork suberin was made,[14] but the authors claimed that caffeic acid was the aromatic moiety originally present. In fact, by carrying out an ethanolysis instead of the methanolysis, they found an ethoxyl group in the aryl 3- position and concluded that a hydroxyl was in this position of the ring, being methylated or ethylated depending on the alcoholysis

reagent involved. To see if this could be the case here, we ran an ethanolysis, under the same conditions as used for methanolysis, on cork, caffeic acid and ferulic acid. Ethylation of the 3-*O* position on caffeic acid was not observed, neither was substitution of the methoxyl by an ethoxyl group in the equivalent position on ferulic acid. In the cork ethanolysis products the depolymerized ferulic acid was present as its ethyl ester. Also, in the ethanolysate products of cork, compounds with mass spectra assignable to feruloyl esters of ω-hydroxyacids were present. They showed fragmentation of the feruloyl part (*m/z* 266, 249, 236, 219 and 209) similar to the one found after methanolysis, showing no change on the hydroxycinnamoyl moiety. Molecular ions were 14 Amu higher, corresponding with the formation of ethyl esters instead of methyl esters at the aliphatic carboxylic end. Thus, we concluded that ferulic acid was the hydroxycinnamic acid present in the cork suberin methanolysate, both in the free and dimeric forms. Also, the transesterification as ethyl esters at the carboxilic end of the ω-hydroxyacid moiety of the ferulates, shows them to be originally esterified.

Ferulic acid linkages to suberin aliphatics

In *Q. suber* cork suberin ω–hydroxyl and carboxylic groups of aliphatic acids are known to be all esterified.[22,23] It was recently found that in this suberin glycerol is esterified to all families of suberinic acids, and existing in quantities enough to esterify most of the carboxilic groups present.[7] In the methanolysis extracts we have now found small quantities of ferulic acid esterified to the same ω-hydroxylated acids found in bigger proportions as solubilized monomers, after the ester-hydrolysing procedure. Together this suggests that primary hydroxyls in ω-hydroxylated suberinic acids may act as the preferential ester-linking groups to aromatics in *Q. suber* suberized cell walls. A structure linking a glyceryl esterified ω-hydroxyacid (22-hydroxydocosanoic) and further esterified to an hydroxycinnamic acid (caffeic acid) has already been reported in the extractable wax of suberin-rich tissue of potato wound periderm.[24] Also, an enzyme was isolated from the suberized cell layers of wound-healing potato, able to catalyse *in vitro* the transfer of ferulic acid from feruloyil-CoA to ω-hydroxyhexadecanoic

acid, producing the corresponding ferulate.[25]

Ferulic acid (or other hydroxycinnamic acids) is found after suberin depolymerization only in small quantities, which is far from enough to esterify all the primary hydroxyls in suberinic acids. In our case, the ω–hydroxylated suberinic acids accounted for *ca.* 52% of the total suberinic acid monomers, but even higher values have been found for *Q. suber* cork (64%,[5] 71%[3]). Additional ferulic carboxylic groups for esterification to the ω–hydroxyls may be supplied by the polymeric phenolics that accompany suberin aliphatics in suberized cell walls. In potato periderm, it was found that the insoluble aromatics associated with suberin are almost exclusively a ferulic acid-derived polymeric structure.[13] In *Q. suber* cork, these aromatics were shown to be, at least in part, a typical guaiacyl lignin,[12] but ferulic acid is also known to act as a link between polymers of different structural nature, namely lignin and polysaccharides.[26,27] Eventually, also in suberized cell walls, ferulic acid acts as the ester-linking bridge between polymeric aliphatics and aromatics.

EXPERIMENTAL

Plant material

Nine year-old reproduction cork (outer-bark) from *Q. suber* was cut in small pieces and all lenticular tissue or phloem remains removed, to obtain a "pure" suberized tissue sample. This material was ground in a Wiley mill and in an ultracentrifuge mill, and the 40-60 mesh fraction recovered. The isolated cork was Soxhlet-extracted successively with $CHCl_3$ (6 h), EtOH (12 h), H_2O (18 h) and MeOH (12 h), dried at 50° and further under vacuum at room temperature.

Cork methanolysis

Dry extracted cork (2.5 g) was refluxed (2 h) in 150 mL of dry MeOH in which 25 mg Na per g of cork were dissolved (18 mM CH_3ONa/MeOH). The

methanolysate was filtered trough a G3 crucible, its pH adjusted to 6 with 0.35 M H_2SO_4 in MeOH and the filtrate evap. almost to dryness under vacuum. The residue was transferred to a separatory funnel with 100 mL $CHCl_3$ and 50 mL H_2O, the organic phase being further extracted with 50 mL H_2O (x2). For GC-MS analysis aliquots were taken in the methanolysate before acidification and from the $CHCl_3$ phase. The remainder of the latter was dried and weighted for suberin quantification (51% based on extractive-free cork).

Methanolysis for artifact control

Ca. 0.11 mmol of ferulic acid (22 mg) and methyl 16-hydroxyhexadecanoate (31 mg) were stirred in a solution of 1.3 mg Na in 3.15 mL of dry MeOH (18 mM CH_3ONa/MeOH), kept in an oil bath at 68°C. 100 μL portions were taken at 1, 2 and 3 h and analysed by GC-MS. No ferulate synthesis was observed. The methanolysate was filtered, acidified to pH 5.8, evaporated, and the residue partitioned between 1mL $CHCl_3$ and 1 mL H_2O. GC-MS analysis of the $CHCl_3$ phase showed no ferulate synthesis.

Ca. 21 mg of methyl 16-*O*-feruloyloxyhexadecanoate was stirred in a solution of 0.53 mg Na in 1.25 mL of dry MeOH (18 mM CH_3ONa/MeOH) kept in an oil bath at 68°C. 50 μL portions were taken each time for GC-MS analysis and the % of methyl 16-*O*-feruloyloxyhexadecanoate calculated as its peak area/total peak area (x 100), in the total ion chromatograms: 0 min (95.3%), 5 min (47.1%), 10 min (33.9%), 15 min (22.7%), 30 min (19.9%), 45 min (18.8%), 60 min (16.5%), 90 min (9.9%), 120 min (4.0%), 180 min (3.5%), 16 h (1.6%), 24 h (0%).

Ethanolysis

The same procedure as described above for methanolysis was carried out with dry EtOH as the alcoholysis reagent. Ethanolysis of 2.5 g of cork, 0.5 g of ferulic and 0.5 g of caffeic acid were examined. GC-MS analysis were carried out in the $CHCl_3$ phase.

Synthesis of methyl 16-*O*-feruloyloxyhexadecanoate

16-Hydroxyhexadecanoic acid (Tokyo Kasei) was quantitatively methylated with CH_2N_2-Et_2O. 90 mg (0.32 mmol) of methyl 16-hydroxyhexadecanoate, 62 mg (0.32 mmol) of ferulic acid and 2.8 mg of *p*-toluenesulfonic acid were dissolved in 1 mL of dry pyridine, 72 mg (0.35 mmol) of DCC (1,3-dicyclohexylcarbodiimide) added and the mixture stirred at 60°C during 18 h.[28] The yield of the synthesis, as determined by GC-MS analysis from the pyridine solution, was 39% (area of feruloyl ester/area of feruloyl ester + area of ferulic acid, in the total ion chromatogram). The pyridine solution was filtered, evaporated and the residue dissolved in $CHCl_3$ (2 mL) and H_2O (1 mL) added to wash the organic phase. The $CHCl_3$ solution was applied to TLC plates (0.25 mm) and eluted with $CHCl_3$-EtOAc (85:15). 21 mg of methyl 16-*O*-feruloyloxyhexadecanoate were recovered in the band of Rf 0.55-0.63.

Ethyl ferulate was synthesised with the same DCC technique [ferulic acid 195mg (1 mmol), EtOH 1 mL (17 mmol), *p*-toluenesulfonic acid 13 mg, DCC 257 mg (1.1 mmol)] in quantitative yield.

GC-MS analysis

Portions (50-250 μL) from the solutions for analysis were dried under N_2 and further under vacuum, and then trimethylsilylated with pyridine-BSTFA (1:1). GC-MS were run on a HT-5 capillary column (25 m, 0.22 mm, 0.1 μm), at 70 kPa of helium column head pressure. Split injections were made with the following conditions: Injector 325°C; oven temperatures for alcoholysis mixtures from cork, 150°C (3 min), 10°Cmin^{-1} up to 220°C, 1°Cmin^{-1} up to 250°C, 5°Cmin^{-1} up to 325°C (15 min); oven temperatures for synthesis mixtures and alcoholysates of standard compounds, 150°C (3 min), 10°Cmin^{-1} up to 325°C (30 min). MS conditions, transfer line 325°C, source temp 220°C, EI at 70eV. Quantifications were made based on peak areas observed on total ion chromatograms.

MS data

EIMS, 70 eV. Synthetic methyl 16-*O*-(4-*O*-trimethylsilylferuloyl)-oxyhexadecanoate, *m/z* (rel. int): 534 $[M]^+$ (51), 266 $[TMSiOC_6H_3OCH_3C_2H_2CO_2H]^+$ (12), 249 $[TMSiOC_6H_3OCH_3C_2H_2CO]^+$ (89), 87 (22), 74 (41), 73 (100). Synthetic ethyl 4-*O*-trimethylsilylferulate: 294 $[M]^+$ (68), 264 (99), 249 (21), 236 (13), 219 (33), 192 (48), 73 (100). From *Q. suber* cork methanolysate: methyl 16-*O*-(4-*O*-trimethylsilylferuloyl)-oxyhexadecanoate, 534 (53), 266 (20), 249 (92), 87 (25), 74 (57), 73 (100); methyl 22-*O*-(4-*O*-trimethylsilylferuloyl)-oxydocosanoate: 618 $[M]^+$ (63), 266 (28), 249 (100), 87 (25), 74 (47), 73 (69); methyl 18-*O*-(4-*O*-trimethylsilylferuloyl)-oxyoctadec-9-enoate: 560 $[M]^+$ (9), 528 $[M\text{-}CH_3OH]^+$ (0.3), 266 (5), 249 (100), 87 (5), 74 (10), 73 (43); methyl 18-*O*-(4-*O*-trimethylsilylferuloyl)-9,10-*bis*(trimethylsiloxy)oxyoctadecanoate (tentative): 479 $[(C_6H_3CH_3OTMSiOC_2H_2CO)OC_8H_{16}CHOTMSi]^+$ (21), 340 (6), 323 (2), 259 $[CH_3CO_2C_7H_{14}CHOTMSi]^+$ (21), 249 (80), 155 (31), 73 (100).

ACKNOWLEDGEMENTS

We thank A. Velez Marques for discussion on synthesis and stability of feruloyl esters.

REFERENCES

1. H. Pereira, J. Graça and C. Baptista, IAWA Bull. n.s., 13, 389 (1992).

2. H. Pereira, Wood Sci. Technol., 22, 211 (1988).

3. P. Holloway, Phytochemistry, 22, 495 (1983).

4. P. Kolattukudy and K. Espelie, In Natural Products of Woody Plants, Vol. I, Chap. 6.4, J. Rowe (ed.) Springer Verlag, Berlin, 1989.

5. M. Arno, M. Serra and E. Seoane, E., An. Quim., 77C, 82 (1981).

6. M. Bento, H. Pereira, M. Cunha, A. Moutinho, K. van den Berg and J. Boon, Phyto. Anal.,in press.

7. J. Graça and H. Pereira, Holzforschung, 51:225 (1997).

8. R. Riley and P. Kolattukudy, Plant Physiol, 56, 650 (1975).

9. R. Ekman, Holzforschung, 37, 205 (1983).

10. O. Borg-Olivier and B. Monties, Phytochemistry, 32, 601 (1993).

11. W. Cottle and P. Kolattukudy, Plant. Physiol., 69, 393 (1982).

12. A. Marques, H. Pereira, D. Meier and O. Faix, Holzforschung, 50, 393 (1996).

13. M. Bernards, M. Lopez, J. Zajicek and N. Lewis, J.Biol. Chem., 270, 7382 (1995).

14. A. Guillemonat and J. Traynard, Bull. Soc. Chim. France, 142 (1963).

15. P. Kolattukudy, Rec. Adv. Phytochem., 77, 185 (1977).

16. H. Hergert and E. Kurth, TAPPI, 35, 59 (1952).

17. H. Hergert, For. Prod. J., 8, 335 (1958).

18. J. Adamovics, G. Johnson and F. Stermitz, Phytochemistry, 16, 1089 (1977).

19. A. Baldé, M. Clayes, L. Pieters, V. Wray and A. Vlietinck, Phytochemistry, 30, 1024 (1991).

20. M. Bernards and N. Lewis, Phytochemistry, 31, 3409 (1992).

21. P. Houghton, Phytochemistry, 28, 2693 (1989).

22. C. Agullo and E. Seoane, Chem. Ind., 608 (1981).

23. C. Agullo and E. Seoane, An. Quim., 78C, 389 (1982).

24. A. Schmutz, T. Jenny and U. Ryser, Phytochemistry, 36, 1343 (1994).

25. S. Lofty, J. Negrel and F. Javelle, Phytochemistry, 35, 1419 (1994).

26. S. Fry, Ann. Rev. Plant. Physiol., 37, 165 (1986).

27. T. Lam, K. Iiyama and B. Stone, Phytochemistry, 31, 1179 (1992).

28. K. Holmberg. and B. Hansen, Acta Chem Scand., 33B, 410 (1979).