IDENTIFICATION OF THREE DIFFERENT TOCOPHERYL ESTER SERIES IN WOOD EXTRACTIVES FROM SEVERAL SPECIES OF EUCALYPTUS

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ABSTRACT
Three different series of tocopheryl esters, which have rarely been reported in plants, have been identified in the wood from several species of eucalypt, including *Eucalyptus globulus*, *E. nitens*, *E. maidenii*, *E. grandis* and *E. dunnii*. These series of compounds were characterized intact by gas chromatography/mass spectrometry. Based on their mass spectra, the compounds were identified as three series of α-tocopherol, β-tocopherol and δ-tocopherol esterified to long chain fatty acids. The different series of tocopherol esters were esterified to fatty acids in the range from C₁₂ to C₂₀ including the unsaturated oleic (C₁₈:₁) and linoleic (C₁₈:₂) acids, with the presence of exclusively the even carbon atom number homologues and the C₁₈:₁ and C₁₈:₂ being the most prominent.

I. INTRODUCTION
Lipophilic extractives from wood consist of complex mixtures of many different compounds, from the low-molecular-weight resin and fatty acids to high molecular weight compounds, such as waxes, sterol esters and triglycerides. The chemical composition of the lipophilic fraction of *Eucalyptus globulus* wood, a raw material for manufacturing of kraft pulp, has already been reported (Gutiérrez et al. 1999; Freire et al., 2002). However, a more detailed analysis of the high molecular weight compounds, performed in the present work, indicated the presence of tocopheryl esters, which have not been reported before in eucalypt wood. Therefore, the aim of this study is to characterize the α-, β- and δ-tocopheryl ester series in the wood from different eucalypt species (*E. globulus*, *E. nitens*, *E. maidenii*, *E. grandis* and *E. dunnii*). These compounds have rarely been reported in plants (Pereira et al., 2002) and this is the first time that they are reported among eucalypt wood extractives. The intact tocopheryl esters from eucalypt woods were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) using short- and medium-length high-temperature capillary columns, respectively, with thin films, according to the method previously described (Gutiérrez et al., 1998). The total extracts were also fractionated using a solid-phase extraction (SPE) protocol to concentrate and separate the tocopheryl esters from the rest of the lipophilic compounds. Characterization of intact individual compounds was achieved based on their mass spectra.

II. EXPERIMENTAL
Samples
The wood samples (*E. globulus*, *E. nitens*, *E. maidenii*, *E. grandis* and *E. dunnii*) were provided by ENCE pulp mill in Pontevedra (Spain). For the isolation of lipids, the samples, previously debarked and ground to sawdust, were extracted with acetone during 8 h in a Soxhlet apparatus. The acetone extracts were evaporated to dryness, and redissolved in chloroform for chromatographic analysis of the lipophilic fraction.

Solid-phase extraction (SPE) fractionation
The lipid extracts from eucalypt woods were fractionated by a SPE procedure (Gutiérrez et al., 1998) in aminopropyl-phase cartridges (500 mg). The dried chloroform extract was taken up in a minimal volume (< 0.5 ml) of hexane-chloroform (4:1) and loaded into a cartridge column previously conditioned with hexane (4 ml). The column was eluted with 8 ml of hexane and the isolated fraction further dried under nitrogen producing a fraction enriched in wax esters.

GC and GC/MS analyses of lipophilic extracts
The GC analyses of the extracts were performed in an Agilent 6890N GC system using a short-fused silica capillary column (DB-5HT, 5 m × 0.25 mm I.D., 0.1 μm film thickness). The temperature program was started at 100°C with a 1 min hold and then raised to a final temperature of 350°C at 15°C/min, and held for 3 min. The injector and flame-ionization detector temperatures were set at 300 and 350°C, respectively. The carrier gas was helium at a rate of 5 ml/min, and the injection was performed in splitless mode. Peaks were quantified by area in the GC chromatograms. The GC/MS analyses were performed with a Varian model Star 3400 GC equipped with an ion trap detector (Varian Saturn 2000) using a medium-length (12 m) capillary column of the same characteristics described above. The oven was heated from 120 (1 min) to 380°C at 10°C/min and held for 5 min. The transfer line was kept at 300°C. The injector was temperature programmed from 120 (0.1 min) to
380°C at a rate of 200°C/min and held until the end of the analysis. Helium was used as carrier gas at a rate of 2 ml/min.

III. RESULTS AND DISCUSSION

Most studies concerning the analysis and characterization of high molecular weight fatty acid esters, proceeds by alkaline hydrolysis of the intact molecules, followed by separate analyses of the respective neutral constituents and the acyl moieties. This methodology, however, loses potentially useful information on the nature of the intact esters. In this work, we have analysed the wax ester fraction by GC/MS using a medium-length (12 m) high-temperature capillary column with thin film that allows the analysis of intact high molecular weight ester waxes without prior saponification. This GC/MS method was used to analyze the SPE hexane fraction that contained the tocopheryl esters. The mass spectra of selected tocopheryl esters, namely α- and β-tocopheryl tetradecanoate, as well as their structures, are shown in Figure 1. The mass spectrometric fragmentation patterns of these series are very simple, and show a base peak corresponding to the tocopheryl fragment ion due to the loss of the fatty acid, resulting in the ions at $m/z$ 430, $m/z$ 416 and $m/z$ 402 for the α-, β- and δ-tocopheryl esters, respectively. These fragments, as also occurs with the corresponding free tocopherols, undergo a retro-Diels-Alder reaction with hydrogen transfer to give an intense ion at $m/z$ 165, 151 and 137 respectively, and also and α-cleavage to give a weaker ion at $m/z$ 205, 191 and 177 (for the α-, β- and δ-tocopheryl esters, respectively).

Figure 1. Structures and mass spectra of α- and β-tocopheryl tetradecanoate.
Three series of tocopheryl esters, namely α-, β- and δ-tocopheryl esters, were found in all the eucalypt woods analyzed here. The distribution of the three homologous series of tocopheryl esters found in the SPE hexane fraction of *E. globulus* is shown in Figure 2. The abundance of the α-, β- and δ-tocopheryl esters series in the different eucalypt species selected for this study is shown in Table 1. The α- and β-tocopheryl ester series were the most abundant whereas the δ-tocopheryl ester series was present only in trace amounts.

![Figure 2. Representative GC/MS fragmentograms: m/z 402 showing the homologous series of δ-tocopheryl alkanoates; m/z 416 showing the homologous series of β-tocopheryl alkanoates; and m/z 430 showing the homologous series of α-tocopheryl alkanoates. Cₙ refers to carbon chain length of the esterified fatty acids.](image)

Table 1. Abundance of α-, β- and δ-tocopheryl esters series in the woods from the different eucalypt species.
Three different series of tocopheryl esters have been identified for the first time in the wood of different eucalypt species (E. globulus, E. nitens, E. maidenii, E. grandis and E. dunnii). The different series of α-, β- and δ-tocopheryl ester were esterified to fatty acids in the range from C12 to C20, including the unsaturated oleic (C18:1) and linoleic (C18:2) acids with the presence of exclusively the even carbon atom number homologues and the C18:1 and C18:2 being the most prominent.

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VI. REFERENCES