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Analysis of oak tannins by liquid chromatography-electrospray ionisation mass spectrometry

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Abstract

Extractable tannins were analysed by liquid chromatography-electrospray ionisation mass spectrometry in two oak species, North American white oak (*Quercus alba*) and European red oak (*Quercus robur*). They mainly included various glucose gallic and ellagic acid esters. The structures were partially determined, and they included grandinin/roburin E, castalagin/vescalagin, gallic acid, valoneic acid bilactone, monogalloyl glucose, digalloyl glucose, trigalloyl glucose, ellagic acid rhamnose, quercitrin and ellagic acid.

Keywords: Tannins; Gallic acid; Ellagic acid; Glucose ester

1. Introduction

Tannins are complex polyphenols present in most plant species. They are proposed to play key roles in the chemical defences of the plant species. They are conventionally divided into condensed and hydrolysable tannin molecules. Condensed tannins have a flavonoid core as a basic structure, and the hydrolysable tannins are glucose esters of gallic and ellagic acids. Hydrolysable tannins are considered to present greater risks to animal health [1,2]. Wood species differ in their tannin content both qualitatively and quantitatively. Generally, hardwood, e.g. oak, contains more tannins than softwood [3].

Occupational sinonasal cancer risk is associated with exposure to hardwood dust [4] although the proximate carcinogens in hardwood dust particles are not known [5]. A chemical marker for the wood dust would greatly facilitate exposure analysis and the estimation of the absorbed internal doses. Tannin in wood are promising candidates in this respect. Plant tannins have been studied previously [6–10] and also commercial tannin extracts have been analysed by liquid secondary ion mass spectrometry (LSIMS) and fast ion bombardment mass spectrometry (FAB-MS) [11,12].

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We used mass spectrometer fitted with an electrospray ionisation interface with prior seperation by high-performance liquid chromatography (HPLC) to analyse tannins in two oak species. The mass spectrometer was operated in the negative ion mode, while further structural confirmation was accomplished by tandem mass spectrometry, utilising the parent and daughter ion scans of the fragment ions characteristic of tannins. Some phenols were also identified with the data from the literature.

2. Experimental

2.1. Chemicals and materials

Gallic (GA), ellagic acids (EA) and quercitrin were purchased from Sigma (St. Louis, MO, USA). North American white oak *(Quercus alba)* and European red oak (*Quercus robur)* blocks were ground to dust with a hand-operated milling cutter. Water was purified in a Milli-Q Water purification system (Millipore, MA, USA). Methanol (HPLC-grade) was purchased from Rathburn Chemicals (Walkerburn, Scotland). Diethyl ether (p.A.) and formic acid (98–100%) were purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

All chromatographic analysis was performed using a Finnigan MAT LCQ ion trap mass spectrometer fitted with an electrospray ionisation source (San Jose, CA, USA). The liquid chromatographic system was a Rheos 4000 HPLC (Flux Instruments, Danderyd, Sweden) equipped with a Lachrom autosampler L-7200 Merck Hitachi (Hitachi, Tokyo, Japan) and Symmetry C_{18} column (5 μm; 150×2.1 mm I.D.) with a guard column (Waters, Milford, MA, USA).

2.3. Extraction

Finely ground dust (300 mg) was extracted with 100 ml of methanol–water solution (80:20, vol/vol) at room temperature for 24 h. After filtration, the methanol was removed by vacuum distillation, and the aqueous residue was extracted three times with

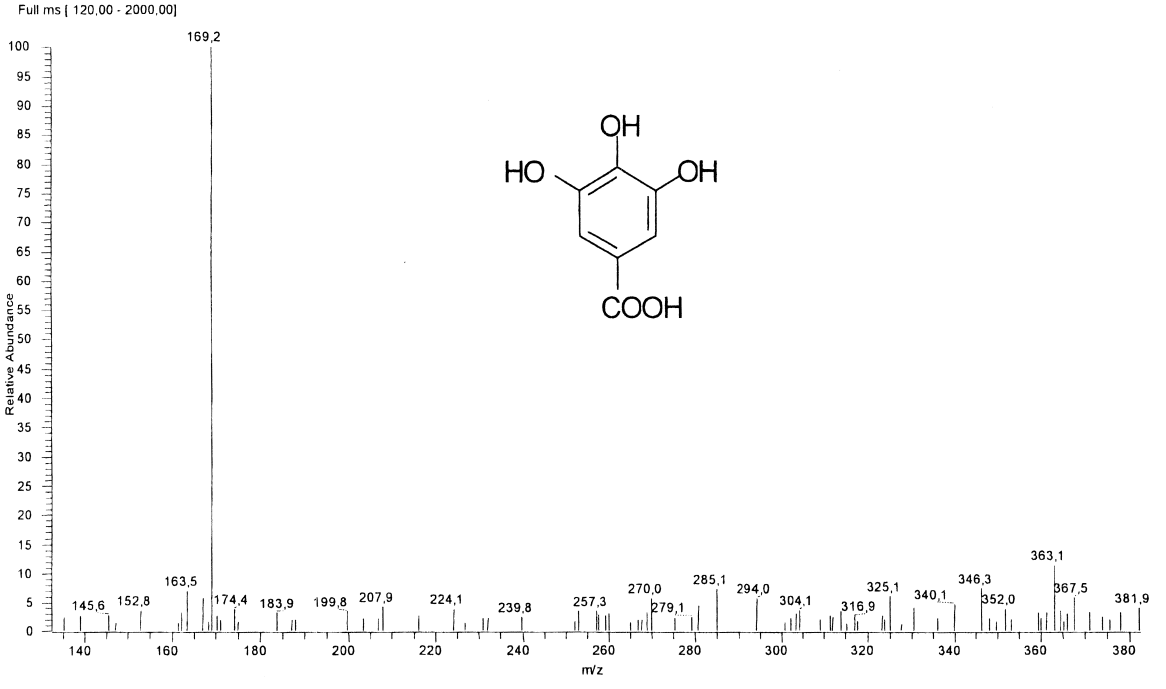


Fig. 1. (a) Chemical structure and molecular ion peak $(M-H)^-$ 169 of gallic acid standard in negative ion mode.

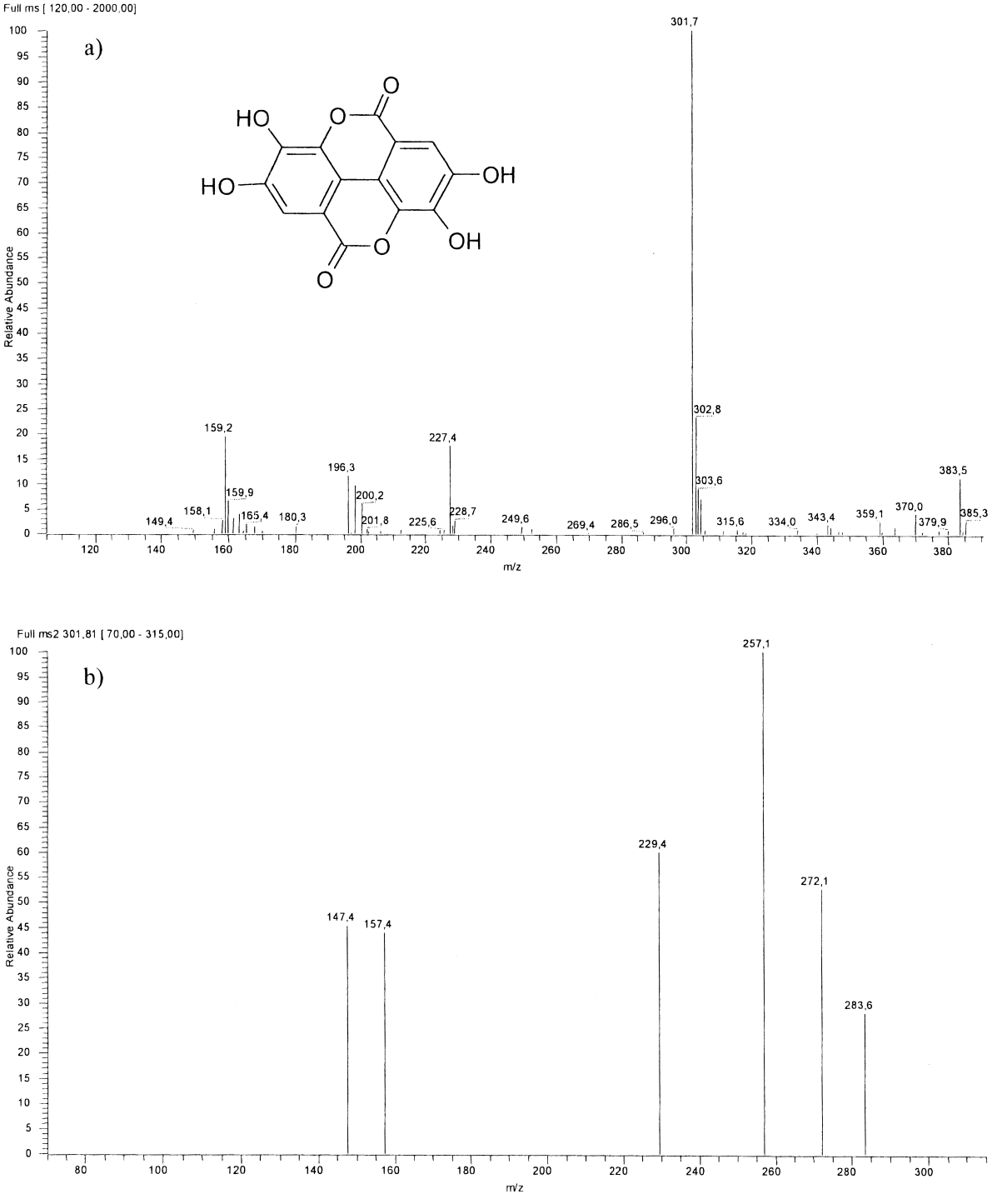


Fig. 2. (a) Chemical structure and molecular ion peak $(M-H)^-$ 301 of ellagic acid standard and (b) fragment ion peaks in negative ion mode.

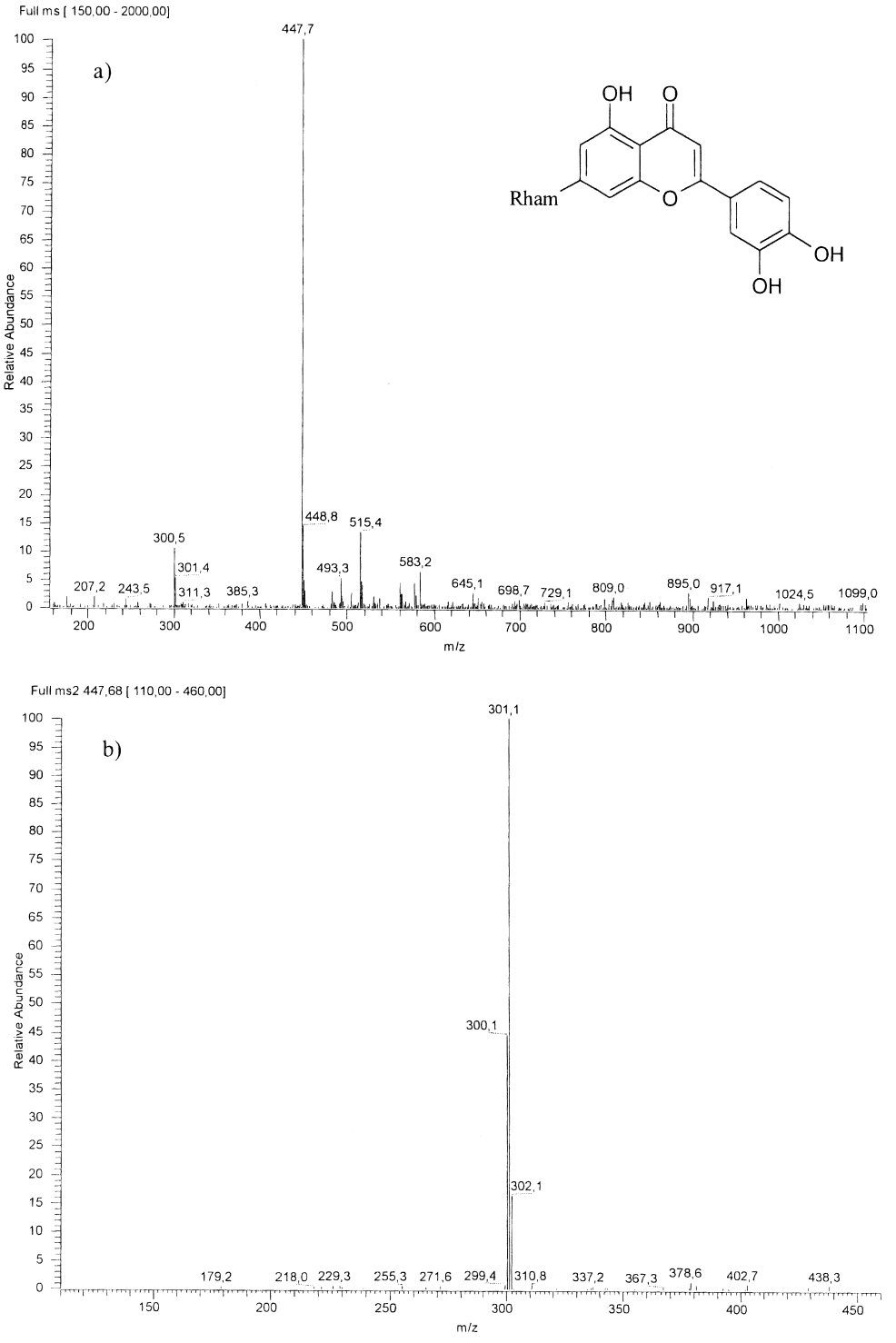


Fig. 3. (a) Chemical structure (Rham=rhamnose) and molecular ion peak $(M-H)^-$ 447 of quercitrin standard and (b) fragment ion peaks in negative ion mode.

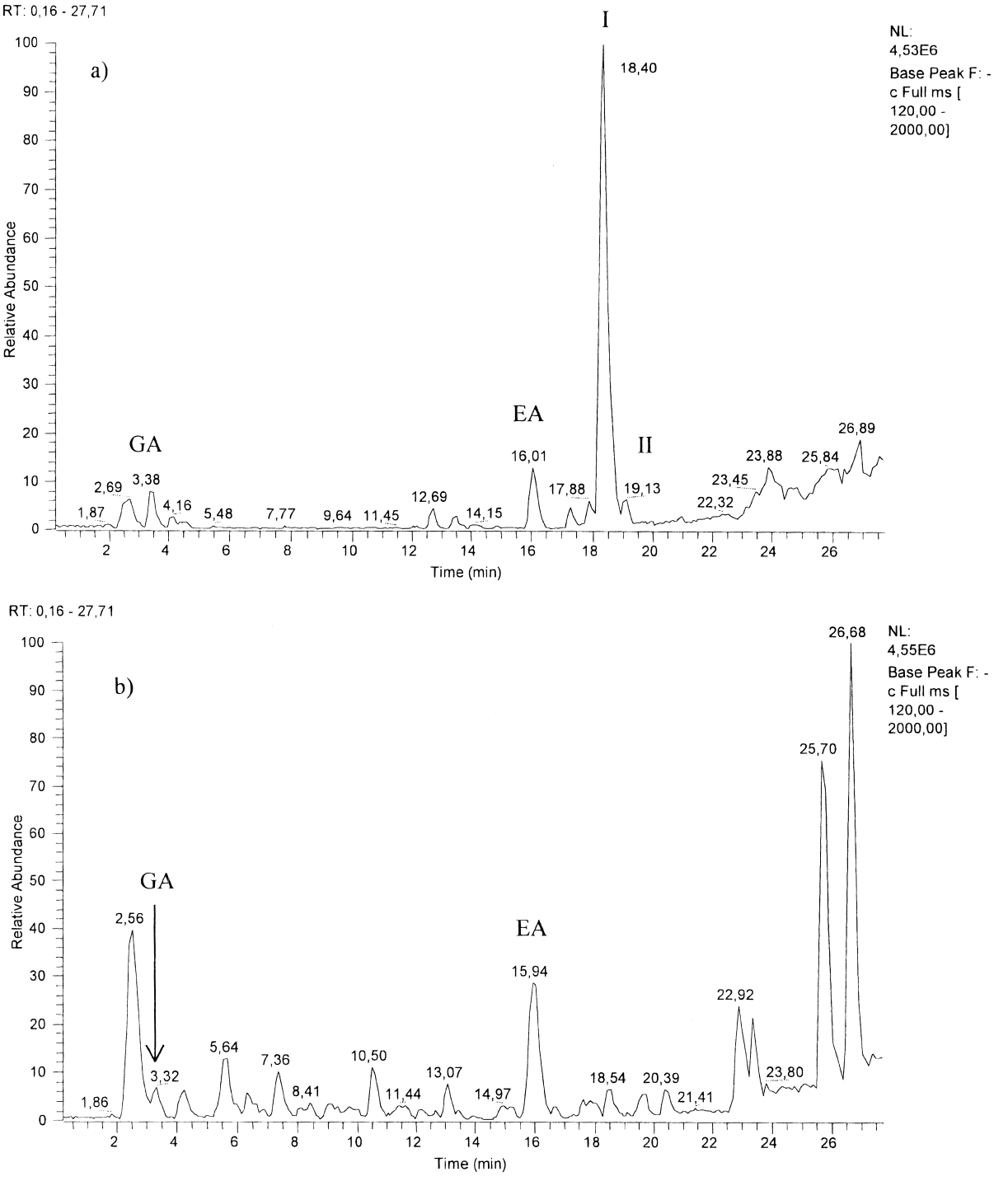


Fig. 4. Base peak ion chromatogram of (a) white oak dust and (b) red oak dust obtained by negative ion HPLC–ESI-MS. Gallic acid (GA), ellagic acid (EA) and unknown compounds I and II (Table 1) are marked in chromatograms.

25 ml of diethyl ether. The diethyl ether was then evaporated to dryness and the residue was redissolved in 1 ml of water–methanol–formic acid (80:20:1).

2.4. Liquid chromatography

Methanol containing 1% formic acid (A) and water with 1% formic acid (B) were used as eluents. The gradient of A from 20 to 100% was run in 40 min. An automatic injection volume of 20 μl with a flow-rate of 200 μl/min was used.

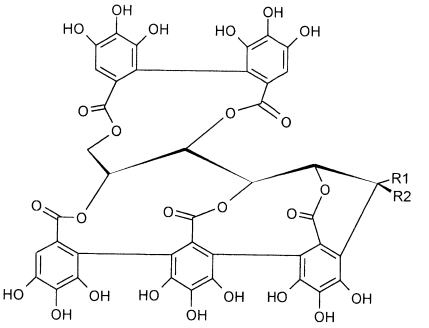
2.5. Electrospray mass spectrometry

All extracts were analysed in the negative ion mode. The electrospray conditions were optimised by direct injection of the standards. The capillary temperature was 225°C, source voltage 3 kV, sheath gas flow 92 (arbitrary units, scale 0–100 units), capillary voltage −25 V and tube lencs offset −30 V. The ion trap was used in automatic gain control mode and the maximum ion time was 200 ms.

3. Results and discussion

Tannins present in the two oak species were identified based on deprotonated molecule $(M-H)^-$ and basic fragments. Furthermore, gallic acid, ellagic acid and quercitrin determination were based on their retention times, and comparison of the MS–MS fragmentation was done using authentic standards (Figs. 1–4). The negative ion mass spectra of gallic acid show an ion at m/z 169 $(M-H)^-$. The single fragment ion peak of GA at m/z 125 $(170\text{-H-}CO_2)^-$ was an indicator of trihydroxy phenol moiety.

The glucose GA esters were mainly identified by looking for GA moieties. For example, the loss of one galloyl ester in case of trigalloyl glucose (Fig. 5) gives a fragment ion peak at m/z 465 $(636\text{-H-GA})^-$ and at m/z 483 $(636\text{-H-}152)^-$ [11]. They were attributed, respectively, to dehydrated digalloyl glucose $(484\text{-H-}H_2O)^-$, and digalloyl glucose $(484\text{-H})^-$. Both oak dusts also contained an identified peak (Table 1) with the fragment ion peak at m/z 287 $(332\text{-H-}CO_2)^-$ proposed to be monogalloyl glucose. Digalloyl glucose had peaks at two different



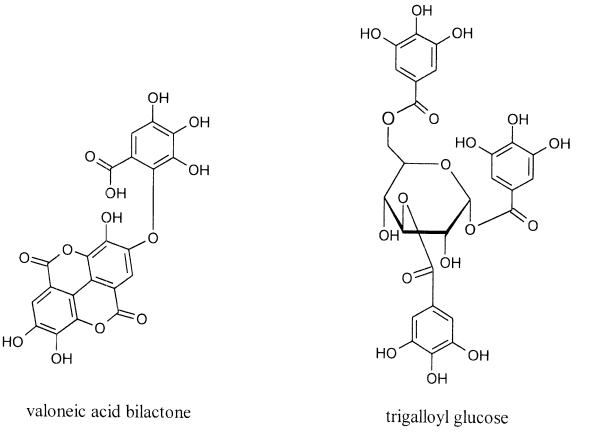


Fig. 5. The structures of castalagin, vescalagin, valoneic acid bilactone and trigalloyl glucose.

retention times (Fig. 6). At retention time 5.6 min, the fragment ion peaks were at m/z 271 $(484\text{-H-}212)^-$, m/z 313 $(484\text{-H-GA})^-$ and m/z 211 $(484\text{-H-}272)^-$, the middle one was attributed to a loss of GA (Table 1). Grandinin/roburin E and castalagin/vescalagin (Fig. 5) were identified in the oak extract by their molecular weight [13]. These have also been found in commercial tannin extracts made from chestnut and oak [11,12]. The fragment ion peak of castalagin/vescalagin at m/z 631 $(934\text{-H-EA})^-$ indicated castalin/vescalin and loss of ellagic acid.

We found two compounds, not reported in oak extract earlier. They contained valoneic acid bilactone (Fig. 5) and ellagic acid rhamnoside. Valoneic acid bilactone had a fragment ion peak at m/z 425 $(470\text{-H-}CO_2)^-$ [14]. The deprotonated molecule m/z

Table 1
Retention times, molecular ions, daughter ions and neutral loss fragments of the HPLC-ESI-MS analysis of tannins present in white oak (*Quercus alba)* and red oak (*Q. robur*)[a]

Compound	Retention time (min)	[M-H]-ion	Daughter ions	Neutral loss fragments
Both oak species				
Grandinin/roburin E	2.4	1065	975, 987, 1028	90, 78, 37
Vescalagin/castalagin	2.6	933	631	302
Gallic acid	3.3	169	125	44
Not identified	3.3	347	303	44
Valoneic acid bilactone	4.3	469	425	44
Digalloylglucose	5.6	483	271, 313, 211	212, 170,272
Trigalloyl glucose	7.4	635	465, 483	170, 152
Valoneic acid bilactone	9.1	469	425	44
Not identified	12.2	293	249	44
Not identified	13.1	401	342, 327, 207, 59, 74, 194	
Monogalloyl glucose	13.2	331	287	44
Ellagic acid rhamnoside	15.0	447	301, 300	146, 147
Digalloyl glucose	15.2	483	453	30
Ellagic acid	15.8	301	257, 229,272	44, 72, 29
Not identified	15.8	572	556, 183, 373	16, 389, 199
Not identified	17.5	585	537, 359	48, 226
Not identified	17.9	461	315	146
Not identified	17.9	287	227, 269, 209	60, 18, 78
Not identified	22.9	725	679, 517,643	46, 208, 82
Not identified	26.6	831	669, 679, 517	162, 152, 314
Not identified	25.7	533	485	48
Not identified	26.7	533	485	48
Only in white oak				
Quercitrin	17.2	447	301, 300	146, 147
Compound I	18.4	487	271, 211	216, 276
Compound II	19.3	639	487	152

[a] The most important compounds are in bold print

447 at retention time 15.0 min had a fragment ion peak at m/z 301 (448-H-146)$^-$ and 300 (448-H-147)$^-$, ion m/z 301 further fragmentating to m/z 284, 258 and 185, and was thus tentatively identified as ellagic acid rhamnoside (Table 1). Quercitrin standard also had molecular peak m/z 447, which fragments were at m/z 301 and 300 (Table 1 and Fig. 3), but it had longer retention time (17.2 min) and the ion peak m/z 301 was further fragmentating to m/z 179 and 271. Quercitrin was present only in white oak extract.

The white oak extract also showed a compound with a pseudomolecular ion at m/z 487 with a retention time of 18.4 min (compound I) (Fig. 4). It fragmented into ions at m/z 271 and 211, which were found also in digalloyl glucose (Table 1). Ion m/z 639 (compound II) (Fig. 4), which presented at retention time 19.3 min, fragmented to ion m/z 487. Further fragmentation of 487 gave ions at m/z 271 (487-216)$^-$, 335 (487-152)$^-$, 211 (487-276)$^-$, 469 (487-H_2O)$^-$ and 169 (487-318)$^-$, ion m/z 469 indicating deprotonated valoneic acid bilactone and m/z 169 deprotonated gallic acid. Compounds I and II (proposed structures in Fig. 7) were not present in the red oak extract.

A summary of the identified and unidentified compounds in both oak extracts is presented in Table 1. White oak and red oak differed in both content and amounts of tannin. It is noted that Vivas et al. [11] found also vescalin/castalin (M=632) in commercial tannin extracts made from oak. They were not present in detectable amounts in our patterns.

According this study tannins in oak species are mainly hydrolysable ones. Chemical composition of

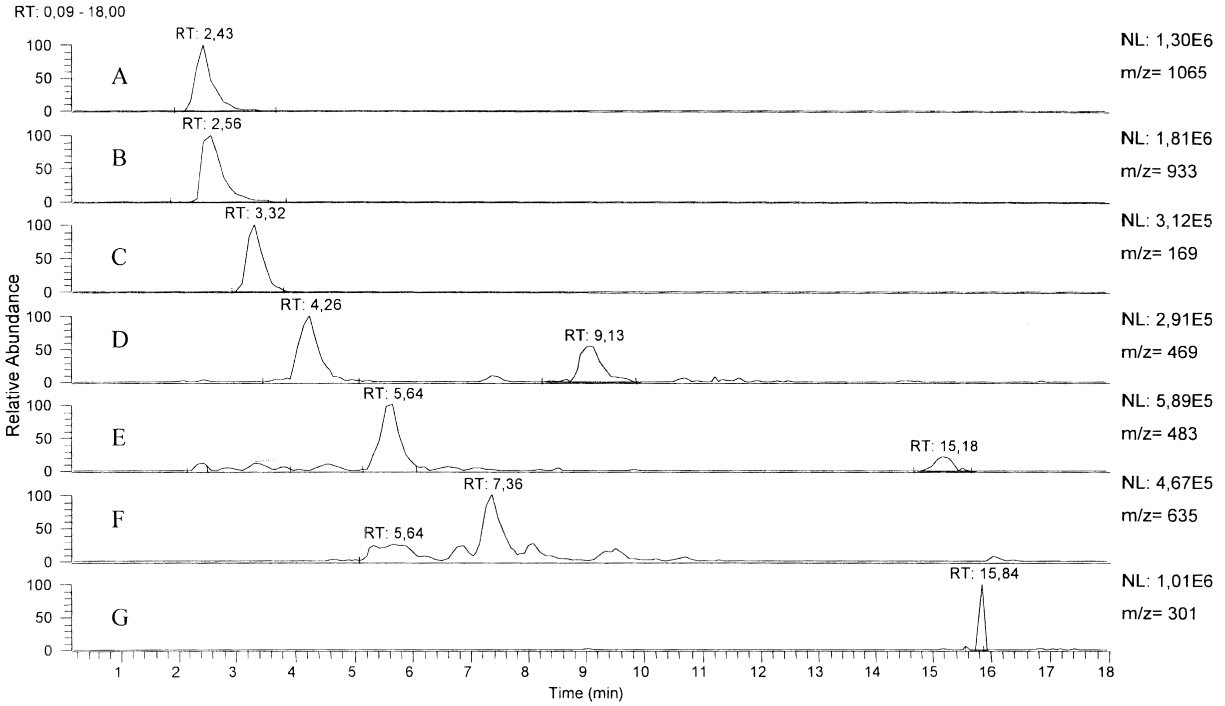


Fig. 6. Selected ion chromatograms (A) for m/z 1065, grandinin/roburin E; (B) for m/z 933, castalagin/vescalagin); (C) for m/z 169, gallic acid; (D) for m/z 469, valoneic acid bilactone; (E) for m/z 483 digalloyl glucose, (F) for m/z 635, trigalloyl glucose; (G) for m/z 301, ellagic acid) obtained by HPLC–ESI-MS analysis of red oak.

other hard and soft wood species still remain to be studied. It is hoped that an investigation of these species will lead to a better understanding of the content of tannins in a variety of hard and soft woods, leading to a series of specific compounds that can be correlated to the carcinogenity of a variety of wood dusts.

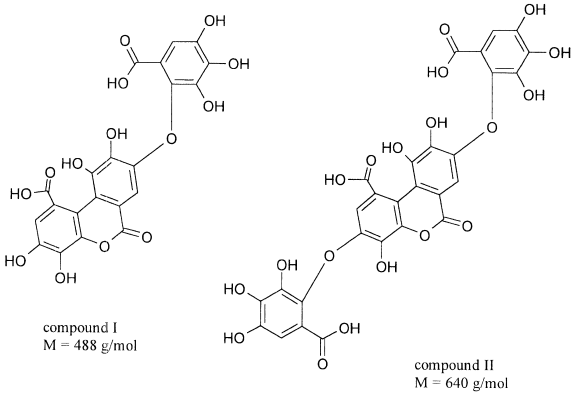


Fig. 7. Proposed structures for two compounds present in white oak.

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