Evolution of Castalagin and Vescalagin in Ethanol Solutions. Identification of New Derivatives

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Brandies, cognac, armagnac, whiskeys, and rums are aged in oak barrels to improve their organoleptic properties. During this period, numerous compounds such as ellagitannins are extracted from the wood and can subsequently be transformed into new derivatives by chemical reactions. Model solutions of castalagin and vescalagin have been studied to determine the behavior of polyphenols in ethanol−water. Upon prolonged exposure to 40 and 70% (v/v) ethanol at room temperature, hemiketal derivatives containing ethoxy groups have been characterized by LC/MS and NMR. These compounds further evolve to afford the corresponding ketals. They have also been detected in the extracts of oak wood stored under similar conditions.

Keywords: *Oak wood; ellagitannins; ethanol; derivatives; HPLC; LC/MS; NMR*

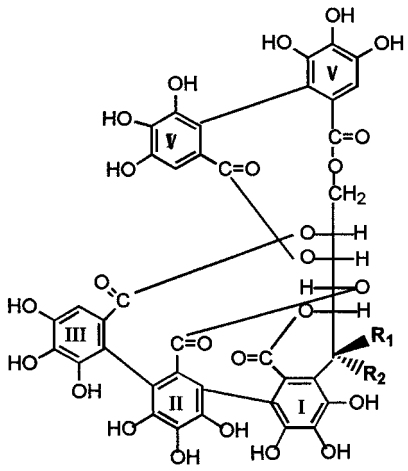
INTRODUCTION

Heartwood of pedunculate oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* Liebl.) is very much in demand as wood for cooperage due to its physicochemical properties. It is hard and flexible and is easily shaped for barrel staves. Moreover, oak wood provides both good heat insulation and remarkable impermeability, and its extracts contribute to improving the organoleptic qualities of wines and brandies.

The extracts contain aliphatic compounds (hydrocarbons, fatty acids), lactones, carotenoids, norisoprenoids, phenolics (lignans, coumarins, and phenols), and ellagitannins. The latter compounds can accumulate in oak heartwood and form up to 2−3% of the dry matter (Masson et al., 1995). Eight ellagitannins from oak wood have been identified to date: castalagin and vescalagin (Mayer et al., 1969, 1971), forming ∼50% of the ellagitannins, grandinin (Nonaka et al., 1989), and roburins A−E (Hervé du Penhoat et al., 1991).

The maximum alcohol content by volume of *appellation contrôlée* spirits and distilled spirits from wine (Armagnac and Cognac) is 72%, but it is sometimes higher in brandies, rums, and whiskeys. Commercial brands of these spirits generally have an alcohol content of 40% (v/v). Neither young nor old spirits aged in oak barrels contain ellagitannins (Puech and Moutounet, 1992; Viriot et al., 1993).

Ellagitannins, and particularly castalagin and vescalagin, may be hydrolyzed during the aging of spirits (Viriot et al., 1993) or may be oxidized because spirits always contain oxygen (Mourgues et al., 1973), thus leading to polymerization reactions (Rabier and Moutounet, 1991). However, none of these hypotheses has been verified. In 1978, Puech showed that tannins exposed to alcohols or spirits such as Armagnac or Cognac incorporated ethoxy moieties into their structures.



Vescalagin R_1 = OH ; R_2 = H

Castalagin R_1 = H ; R_2 = OH

The results of a study of the behavior of castalagin and vescalagin in 40 and 70% (v/v) model aqueous ethanol solutions are described here.

EXPERIMENTAL PROCEDURES

Materials and Reagents. The wood used for the extraction of ellagitannins was a sample of pedunculate oak identified from morphology of leaves (Dupouey and Badeau, 1993) (*Q.*

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robur L.) from the Limousin region. The heartwood was dried for 3 years at room temperature (~20−25 °C) and then ground until the particle size was <0.5 mm. The authentic samples of castalagin, vescalagin, castalin, and vescalin used as reference substances for chromatographic characterization were supplied by Mayer (Mayer et al., 1969, 1971). The water used for chromatography was of Milli-Q (Millipore) quality. All other chromatographic eluents were of HPLC quality.

Ellagitannin Extraction. Tannins were extracted according to Peng's (1991) procedure. Acetone was evaporated under reduced pressure at 40 °C, and the water was removed by freeze-drying. Crude extraction yield was 7%.

Analytical HPLC. A Millipore−Waters chromatograph equipped with an oven, a temperature control unit, a 490 E variable-wavelength detector, two model 510 pumps, and a 717 automatic injector with a SIM (system interface module) was used for HPLC. The Maxima 820 program was run on a PowerMate 386/25 (NEC).

The ellagitannins were separated on a Lichrospher RP 18 end-capped (250 × 4 mm, 5 μm) (Merck, Darmstadt, Germany) column equipped with a precolumn from the same supplier. The oven temperature was maintained at 25 °C. All solvents were filtered and degassed prior to use, and samples were filtered on a 0.45-μm HV Millipore membrane. Solvent A was a mixture of water and phosphoric acid (999:1) (v/v), and solvent B was methanol. Isocratic elution was used to separate castalagin, vescalagin, and their corresponding derivatives. The flow rate was 1 mL/min, and detection was conducted at three wavelengths, 240, 254, and 280 nm.

Preparative HPLC. Castalagin and vescalagin were purified by submitting the wood extract to preliminary separation using Puech's (1988) method. The fraction containing the ellagitannins was subsequently fractionated on a reverse-phase silica gel column. The experimental setup consisted of two Gilson 306 pumps, a Jasco 875 UV detector, an axially compressible Microsorb FAST PCLC C18 (21.4 × 50 mm) (Rainen Instruments) column filled with grafted silica gel (particle size = 3 μm), and a Hewlett-Packard recorder. Isocratic elution conditions were as follows: 96% solvent A (a 999:1 v/v mixture of water and phosphoric acid) and 4% solvent B (methanol) at 25 °C with a 15 mL/min constant flow rate. Ten milligrams of the sample was injected, and detection was conducted at 280 nm.

A similar procedure was used to purify the ellagitannin derivatives, except that the samples were subjected to a 0−20% linear gradient of solvent B for 10-min period followed by washing of the column with methanol. The flow rate was 15 mL/min, and 10 mg of the sample was injected.

The resulting fractions were concentrated under reduced pressure and chromatographed on a Sephadex LH-20 (6 cm × 1 cm, Pharmacia) column to remove phosphoric acid. The column was washed with water until neutrality, and the ellagitannins were eluted with methanol. After evaporation of methanol, the fractions were redissolved in water and lyophilized.

Thin-Layer Chromatography. Silica gel layers on aluminum plates (F254, 5 cm × 10 cm) were used. The eluent was a 1:2:7 (v/v/v) mixture of water/formic acid/ethyl acetate. To reduce trailing, the silica gel was pre-eluted with the solvent and then dried.

Liquid Chromatography/Mass Spectrometry (LC/MS). An API I-plus spectrometer was used (Sciex, Thornhill, ON, Canada). Ionization was performed with an electrospray source, and the ions were collected with a simple quadrupole covering a mass range (m/z) of 0−2400. Detection was performed in the positive-ion mode with an ionization potential of 5000 V at the injector and an opening potential of 60 V. The spectrometer was calibrated with a polypropylene glycol sample with a range of 59−2012 amu. Detection was carried out with a 0.3 step from 200 and 1500 amu and a dwell time of 0.8 ms. The chromatograph consisted of an Applied Biosystem 140B pump, a 785A UV detector, and a Supersher 100 RP 18 (12.5 cm × 2 mm; particle size = 5 mm) column supplied by Merck. A 20 μL aliquot was injected, and detection was conducted at 280 nm. The eluent was a binary mixture of solvent A (H_2O/HCOOH 98:2) and solvent B (CH_3CN/H_2O/HCOOH 80:18:2). The elution scheme included a first step with 5% B, a second step with a 5−30% gradient of solvent B, and finally a step of a 30−50% gradient of B. The flow rate was 200 μL/min.

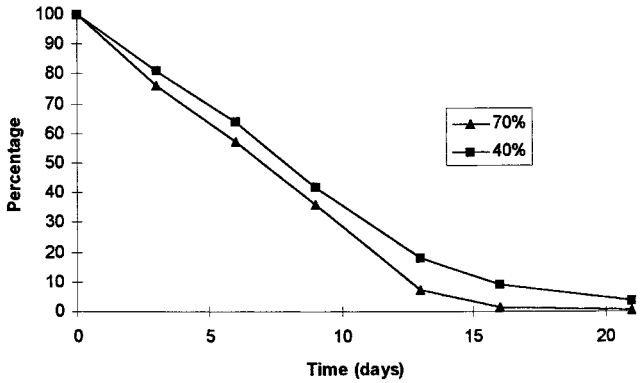


Figure 1. Vescalagin content in water−ethanol.

NMR Spectroscopy. Spectra (400.13 ^{1}H and 100.6 MHz ^{13}C) were recorded at 25 °C in acetone-d_6, MeOH-d_4, DMSO-d_6, and a mixture of acetone-d_6/D_2O (0.5 mL/2 drops). The digital resolution of the proton spectra was 0.5 Hz/point, and the acquisition time was 2.01 s. ^{13}C spectra were recorded with complete proton decoupling, an acquisition time of 1.11 s, digital resolution of 0.9 Hz/point, and a recycle time of 4.1 s. INAPT (Bax et al., 1985) spectra were acquired under similar conditions by using a 5 Hz filter for polarization transfer. Steady-state NOE experiments were performed by applying low-power irradiation at the offset frequency of the saturated spin for 5 s. The recycle time was 12 s for the latter experiments.

Two-dimensional correlation spectra were recorded on a Bruker AM400 in the aforementioned solvents (COSY and HETCOR) and on a Varian UNITY INOVA 500 equipped with a 3-mm reverse probe in a mixture of ethanol-d_6/D_2O (70:30) (DQCOSY, HSQC, and HMBC).

RESULTS AND DISCUSSION

Purification of Ellagitannins. Ellagitannins were purified twice by preparative chromatography on a reverse-phase silica gel column. This was followed by preparative HPLC to isolate vescalagin and castalagin. The purity of these compounds was determined by analytical HPLC and thin-layer chromatography. This approach has the advantage of not requiring preliminary chromatography on a Sephadex LH-20 column, thus avoiding oxidation of the hexahydroxydiphenoyl esters (A. Scalbert, private comunication).

These aliquots were directly placed in the injector of the HPLC apparatus. The presence of ethanol in the solvent mixture causes broadening of the peaks as it is a stronger eluent than the mobile phase (Scalbert et al., 1990). The validity of the measurements was controlled after evaporation of the ethanol and dissolution in water.

Transformation of Ellagitannins in Water−Ethanol. This study was conducted on aqueous solutions of vescalagin and castalagin in 40 and 70% ethanol−water at room temperature. The pH of this solution was similar to that of spirits (pH ~4.5). These solutions were placed in tightly sealed containers to prevent the evaporation of ethanol and periodically exposed to air when samples were taken to monitor the reaction.

The changes the vescalagin and castalagin contents determined by analytical HPLC are given in Figures 1

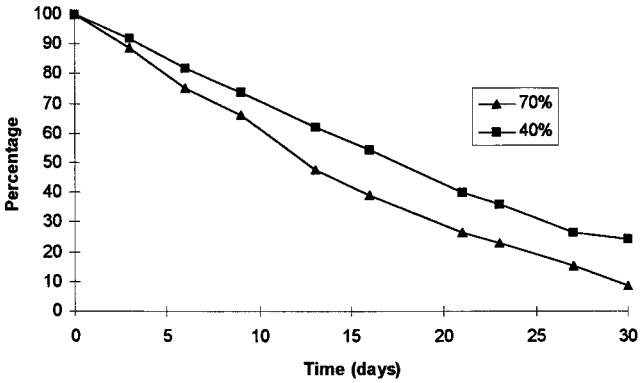


Figure 2. Castalagin content in water−ethanol.

and 2. The progressive disappearance of the tannins seems to be influenced both by the structure of the ellagitannin and the amount of ethanol in the solution. This decrease is more rapid in the case of vescalagin regardless of the alcohol content of the solutions. This difference in stability and therefore reactivity between the stereoisomers has already been mentioned in the literature (Haslam, 1985; Vivas, 1995) and is considered to be the result of two factors: on the one hand, the greater flexibility of the glucose C1 and OH1 in vescalagin and, on the other hand, the greater hydrophilic character of the C1 region in vescalagin. It should be noted that the rate of evolution increases with the amount of alcohol, suggesting that ethanol participates in this transformation. Thus, the ellagitannins appear to be unstable in water−alcohol, and transformation is complete within 30 days in the case of the 70% solutions.

These results suggested that new compounds were formed from the ellagitannin precursors, and it was interesting to try to detect such derivatives. Figure 3 shows the chromatograms obtained after prolonged exposure of the ellagitannins to water−alcohol. For each tannin this treatment leads to the formation of a new compound. The retention times of these derivatives are 27.4 and 30.2 min for the vescalagin and the castalagin derivatives, respectively. The rate of formation of these products is directly related to the rate of transformation of the corresponding ellagitannins, and it increases with the percentage of ethanol in the solutions.

Various hypotheses have been put forward to explain the disappearance of ellagitannins under similar conditions such as hydrolysis, oxidation, and polymerization as well as reaction with ethanol. To shed light on the transformation of castalagin and vescalagin into new compounds in water−ethanol, we undertook the characterization of the resulting derivatives.

Purification of the Derivatives. Purification was conducted on the water−ethanol solutions (70% v/v) of castalagin (**1**) and vescalagin (**3**) after a period of 1 month. The purity of the castalagin (**2**) and vescalagin (**4**) derivatives was verified by analytical HPLC and by LC/MS. The UV spectra of these compounds, which were recorded with a diode array detector (Waters), were identical to those of the ellagitannin precursors.

Mass Spectrometry. The molecular ions (*m*/*z*) of compounds **2** and **4** were determined to be 977 ± 0.3 amu (M − H). The corresponding difference in molecular weight with respect to the ellagitannin precursors (+44) corroborates a reaction mechanism requiring the par-

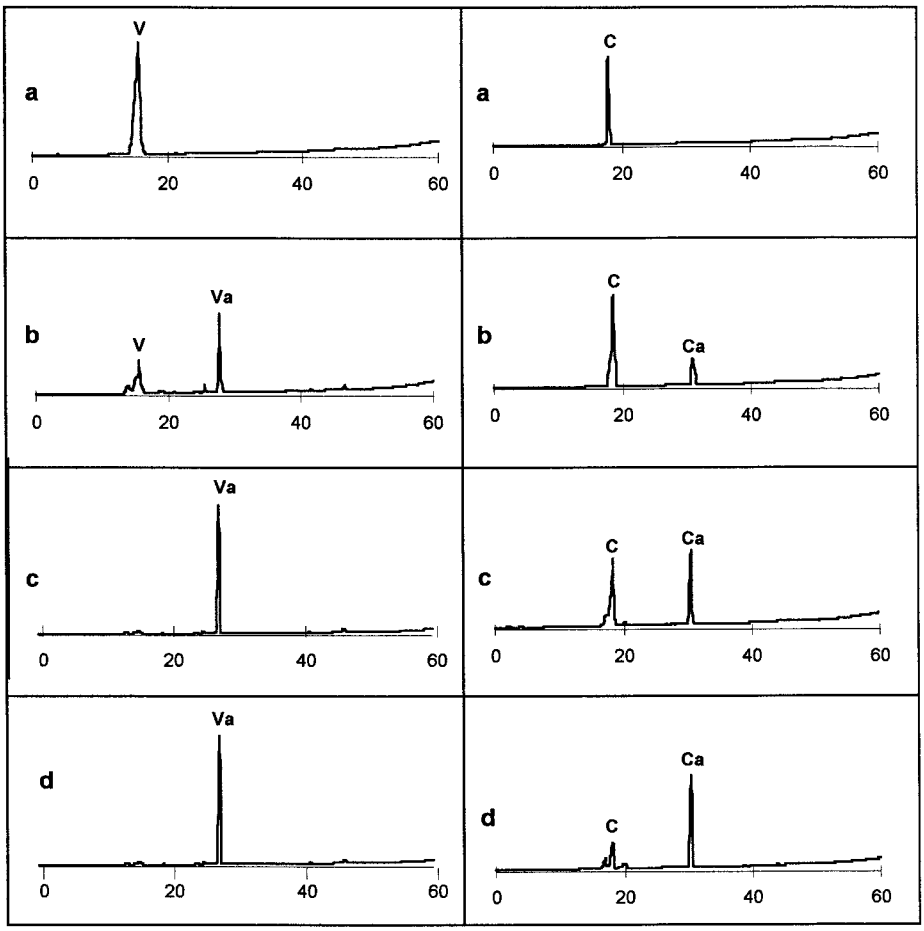


Figure 3. Chromatograms of water−ethanol (70% v/v) solutions of vescalagin (V) and castalagin (C): (a) freshly prepared solution; (b) $t = 6$ days; (c) $t = 13$ days; (d) $t = 21$ days; Va, vescalagin derivative; Ca, castalagin derivative.

Table 1. 100.6 MHz ^{13}C Chemical Shifts and Multiplicity[a] of Castalagin (1), Vescalagin (3), Their Derivatives (2 and 4), and 3-Cyclohexene-1,2-dione (5) in Acetone-d_6 (Referenced to the Solvent Signal δ_{Me} 29.8)

	1, castalagin	**2**, castalagin derivative	**3**, vescalagin	**4**, vescalagin derivative	**5**, 3-cyclohexenyl-1,2-dione
glucose					
C1	66.10 (d)	61.54 (d)	63.71 (d)	59.52 (d)	
C2	72.38 (d)	74.73 (d)	76.01 (d)	80.95 (d)	
C3	64.79 (d)	64.73 (d)	66.71 (d)	68.44 (d)	
C4	67.71 (d)	67.99 (d)	67.55 (d)	68.44 (d)	
C5	69.48 (d)	69.23 (d)	69.41 (d)	68.60 (d)	
C6	63.83 (t)	63.29 (t)	63.83 (t)	62.86 (t)	
aromatic rings					
C1′	121.14, 123.95	123.59, 123.92	123.06, 123.13	123.34, 123.72	
	123.96, 125.41	124.07, 125.06	123.45, 124.98	123.85, 124.77	
	126.26		126.07		
C2′, C6′	106.09 (d)	106.15 (d)	105.73 (d)	106.36 (d)	
	106.83 (d)	106.70 (d)	106.54 (d)	106.52 (d)	
	107.39 (d)	106.86 (d)	106.95 (d)	106.84 (d)	
	110.79, 112.50	108.61, 111.66	111.45, 112.69	109.39, 111.68	
	112.76, 113.12	112.23, 113.40	112.94, 113.38	112.16, 113.72	
	113.95, 114.02	114.05	114.03, 114.26	113.73	
	114.19		115.28		
C3′, C5′	141.88, 142.47	142.58, 142.92	142.15, 142.65	141.93, 141.93	
	142.60, 142.75	142.92, 143.42	142.74, 142.79	142.52, 142.79	
	142.84, 143.14	143.64, 143.64	142.83, 143.15	142.79, 143.46	
	143.70, 144.24	144.11, 144.11	143.51, 143.54	143.46, 143.94	
	144.24, 145.21		143.67, 145.74	144.15	
C4′	133.09, 134.34	133.42, 134.49	133.30, 134.21	133.42, 134.54	
	134.84, 134.94	135.18, 135.18	134.67, 134.97	134.96, 135.08	
	135.78		135.57		
carbonyls					
COOR	161.75, 163.73	160.46,[b] 164.34[c]	163.20, 163.77	160.69, 165.22	
	164.77, 164.79	165.48, 165.71	164.72, 165.34	165.56, 166.04	
	167.04	166.79	167.40	166.57	
dione ring					
C1″		198.16		199.18	197.9
C2″		169.24		168.63	185.5
C3″		140.89[b]		d	137.5
C4″		152.52		155.15	153.1
C5″		43.16		43.93	36.3
C6″		80.89		82.20	46.9
CH2		61.10		61.32	
CH3		12.57		12.51	

[a] Multiplicity was determined from a *J*-modulated spectrum. [b] Correlated to H2 in the corresponding INAPT spectrum. [c] Correlated to H3 in the corresponding INAPT spectrum. [d] Undetermined.

ticipation of a molecule of ethanol. Incorporation of a molecule of ethanol in the place of a hydrogen atom seemed highly probable. Moreover, the fact that identical molecular weights were detected for **2** and **4** suggested that the tannin derivatives were stereoisomers and that the integrity of the chiral center at C1 was not affected by the reaction. Fragmentation achieved with −140 V source contained two ions at 301 and 675 amu. Therefore, the part of the molecule containing the ellagic acid fragment was not modified by the transformation of **1** and **3** into **2** and **4**. The ions at 301 and 675 amu may correspond, respectively, to ellagic acid and castalin incorporating an ethanol molecule.

NMR Spectroscopy. Compounds **2** and **4** were studied by ^{1}H and ^{13}C spectroscopy in acetone-d_6. The chemical shifts of the C2 signals of the carbohydrate moieties of vescalagin and castalagin, 72.38 and 76.01 ppm, respectively, are characteristic, and by analogy the resonances at 74.73 and 80.95 ppm were assigned to C2 of derivatives **4** and **2**. From a heteronuclear correlation (HETCOR) spectrum it was possible to identify the corresponding H2 signals, and in turn the complete proton coupling graph (H1 to H6a,b) of the glucose residue was obtained from the homonuclear correlation (COSY) spectrum. The assignment of the remaining carbon signals was then extracted from the HETCOR spectrum. The resonances of an ethanol moiety were also detected in the aliphatic region of the proton (1.37 and 4.36 ppm) and carbon (12.5 and 61 ppm) spectra. These data are assembled in Tables 1 and 2.

The chemical shift range of the aromatic carbons of the nonahydroxytriphenoyl (NHTP) and hexahydroxydiphenoyl (HHDP) groups are also characteristic, and these signals have been grouped according to the atom positions (carbon substituted by an ester group, C1′; ortho carbons, C2′ and C6′; meta carbons, C3′ and C5′; and para carbons, C4′) for compounds **1**−**4** in Table 1. Perusal of the carbon data indicates that derivatives **2** and **4** contain one fewer aromatic ring than the parent compounds **1** and **3**. However, the ellagitannin derivatives contain 43 carbons instead of 41 carbons, as is the case for castalagin and vescalagin. Once the two signals of the ethanolic moiety are accounted for, six carbon signals remain unassigned (for example, 43.16, 80.89, 140.89, 152.52, 169.24, and 198.16 ppm in the case of **2**). In the case of compound **2**, the disappearance of an NHTP or a HHDP aromatic ring was confirmed by the low-field region of the proton spectrum, which contains the signals of 12 phenolic protons instead of the 15 phenolic protons expected for **1** or **3**. Chemical exchange is much faster in the case of the sample of compound **4**, undoubtedly because of a higher concentration of H_2O.

Comparison of the six unassigned carbon signals with those of 3-cyclohexenyl-1,2-dione (**5**) (5,5-dimethyl-3,4-

Table 2. 400.13 MHz ^{1}H Chemical Shifts and Multiplicity (*J* in Hertz) of Castalagin (1), Vescalagin (3), Their Derivatives (2 and 4), and 3-Cyclohexene 1,2-dione (5) in Acetone-d_6 (Referenced to the Solvent Signal δ_{Me} 2.2)

	1, castalagin	**2**, castalagin derivative	**3**, vescalagin	**4**, vescalagin derivative
glucose				
H1	5.85	5.64 (m)	5.03	4.70 (d, 1)
H2	5.18	5.18 (d, 6.3)	5.40	5.17 (d, 1)
H3	5.18	6.15 (d, 7.7)	4.5	5.62
H4	5.4	5.57 (t, 8.3)	5.35	5.62
H5	5.75	5.62 (m)	5.77	5.62
H6a	5.25	5.09 (dd, 12.7, 3.9)	5.51	5.10 (dd, 12.2, 4.9)
H6b	4.25	4.12 (dd, 12.7, 2.4)	4.18	4.09 (dd, 12.2, 3.9)
OH1		5.31 (d, 5.9)		4.87[a]
aromatic rings				
H2′	6.92, 6.94, 6.8	6.88, 7.0, 6.80	6.94, 6.95, 6.78	6.80, 6.82, 7.08
OH		7.6−8.8 (12H)		
dione ring				
H5″		5.58 (d,3.4)[b]		5.62[c]
OH6″		4.7 (br s)		a
CH2		4.36 (m)		4.36
CH3		1.37 (t, 7)		1.36 (t, 7)

[a] Signals of exchangeable protons are very broad and could not be detected unambiguously. [b] See insert in Figure 4, MeOH-d_4. Coupling constant data in Hz: H1 (dd; 6.5, 3.4), H2 (d; 6.5), H3 (d; 8.5), H4 (t; 8.5), H5 (∼ddd; 8.5, 3.5, 2), H5″ (d; 3.4). [c] Masked by overlapping signals in the region near 5.62 ppm; integral indicates 4H: H3, H4, H5, and H5″.

Table 3. 400.13 MHz ^{1}H Steady-State Nuclear Overhauser Effects[a] of Tannin Derivatives (2 and 4) in Acetone-d_6

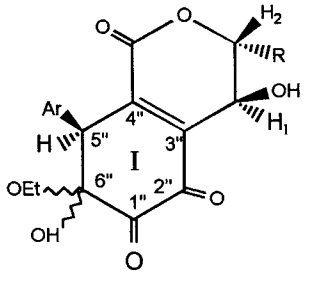
compound	saturated spin	detected spins, % NOE			
2, castalagin derivative	H3	H2	H4		
		4	5		
	CH2	H5″	CH3		
		1	7		
	OH1	H1	H3	aromatic protons (−)	phenolic OHs and OH6″ (+)
		10	2		
	OH6″	H5″		aromatic protons (−)	phenolic OHs and OH1 (+)
		4			
4, vescalagin derivative	H1	H2	5.62	OH1[b]	
		4	6	4	
	H2	H1	OH1[b]	aromatic protons (−)	
		4	5		
	CH2	OH6″[b]	CH3		
		3	5		

[a] Experimental conditions: preirradiation delay, 5 s; recycle time, 12 s. [b] A unique signal was observed for these two exchangeable protons.

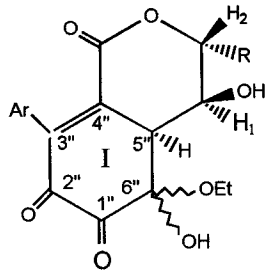
dioxo-1-cyclohexene-1-carbaldehyde; Martin et al., 1987) revealed a related structure. This model compound contains two methyl groups at C6″ (46.9 ppm) instead of both an −OR and an −OH moiety. This explains the lower chemical shift when compared to that of **2** or **4** (∼80 ppm). Similarly, the C2″ signal resonates at lower field in the case of **5** (185.5 ppm) when compared to the signals (169.24 and 168.63 ppm) of **1** and **3**, which may be due to the two α-methyl groups of the former compound and/or the more extended π-electron system of the latter ellegitannin derivatives.

Two regioisomers A and B could be proposed for compound **2** on the basis of the chemical shift data. Optimum spectral dispersion for the region containing the H1 and H5″ resonances was obtained for a spectrum acquired in MeOH-d_4 with decoupling of H3, and an expansion of this spectrum is given in the inset in Figure 4; the coupling constants have been indicated in a footnote to Table 2. The 3.4 Hz coupling constant detected for H1 was compatible with either the $^5J_{H1,H5''}$ coupling of regioisomer A or the $^3J_{H1,H5''}$ coupling of regioisomer B as the former value is enhanced in the presence of unsaturated bonds.

To validate regioisomer A or B through selective long-range heteronuclear coupling (typically between protons and carbons separated by three bonds), INAPT spectra



A



B

of **2** were recorded. In the spectrum acquired with selective excitation of H2, a correlation with C3″ resonating at 140.89 ppm argued in favor of regioisomer A.

Steady-state NOEs were recorded to identify the position of the ethyl group (Table 3). In the case of **2**, strong effects were observed between H5″ and OH6″ as expected and, for both **2** and **4**, a weaker effect was detected between OH6″ and the ethylene protons of the ethyl moiety. This strongly suggested that the −OEt substituent was attached to C6″ as indicated in the structure of the regioisomer A. The NOE difference spectra also contained correlations due to chemical exchange between the residual water and the OH

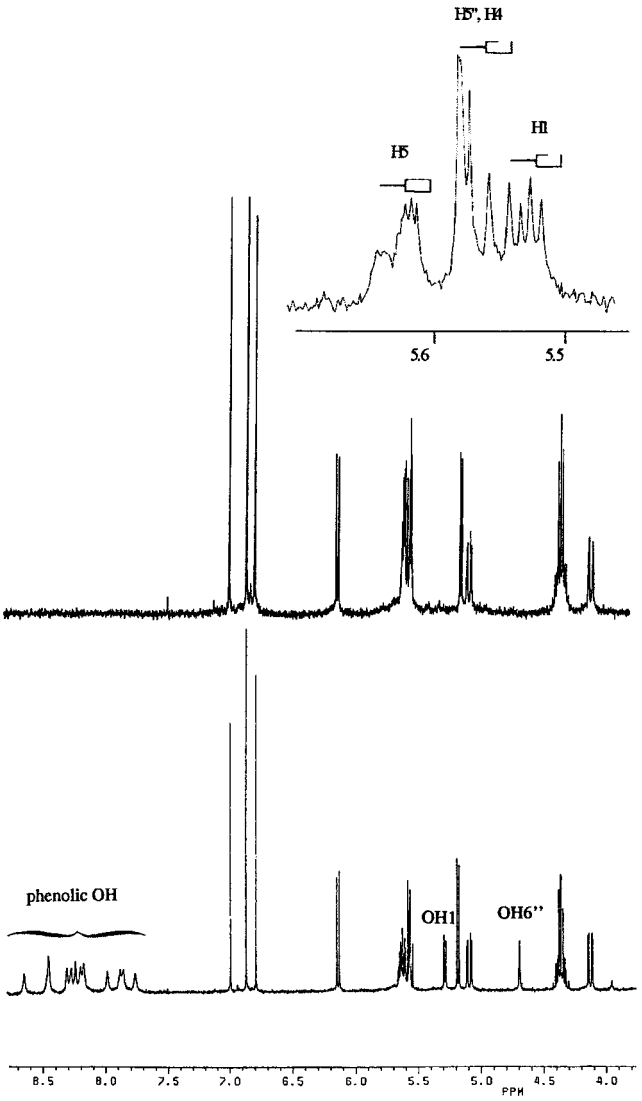


Figure 4. 400.13 MHz ^{1}H spectra of the castalagin derivative (**2**) in acetone-d_6 (bottom) and in a mixture of acetone-d_6/water (top). The signals of OH1 and OH6″ are labeled. (Inset) Expansion of the corresponding spectrum in MeOH-d_4, which was acquired with decoupling of H3. The H5 and H1 multiplets and the overlapping doublets for H5″ and H4 are indicated.

protons. Water was added to the sample of **2** in acetone-d_6 to accelerate the exchange of the hydroxyl groups and afforded proof that the OH6″ proton corresponded to a hydroxyl moiety. The resonances of interest are labeled in the spectra of **2** (Figure 4), recorded in acetone-d_6 (bottom) and in a mixture of acetone-d_6/D_2O (top). The intensity of the signals of OH1, OH6, and the phenolic protons decreased notably upon addition of water, confirming the structure proposed for the ellagitannin derivatives. A possible mechanism for the formation of the cyclohexenyl dione ring has been given in Figure 5. Oxidation of castalagin (Feldman et al., 1996) followed by Michael addition would be expected to afford the castalagin derivative (**2**).

It is noted that the chemical shifts of the C5″ and C6″ were shifted to high field with respect to related compounds (Quideau et al., 1996), although that of C5″ is analogous to the chemical shift reported for a benzylic carbon in related mongolicain A (Tanaka et al., 1996). To obtain further proof for the structure of **2** indicated in Figure 6, a 70:30 ethanol-d_6/D_2O solution of castalagin was prepared in an NMR tube. According to the mechanism in Figure 5, these conditions should afford

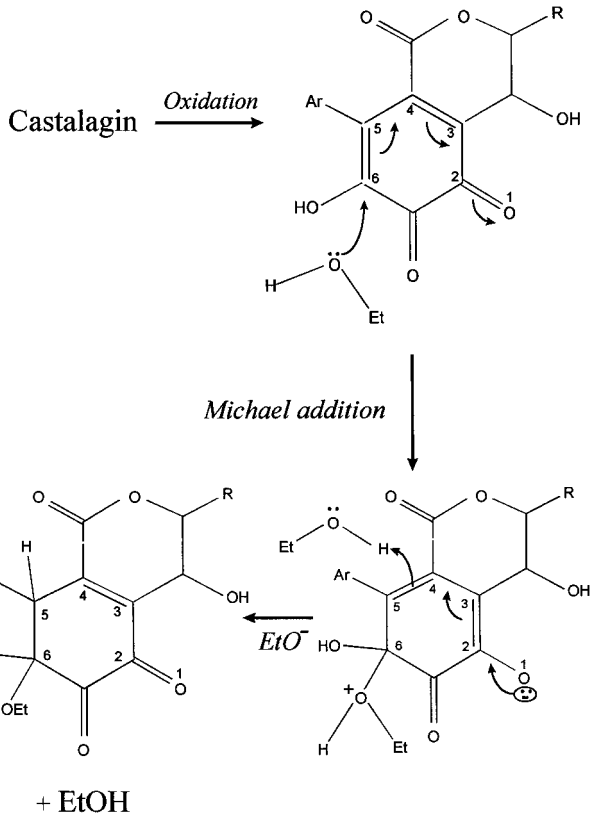


Figure 5. Oxidation−ethoxylation mechanism of castalagin. The π system is numbered from 1 to 6; it should be noted that this numbering does not correspond to the carbon positions of 3-cyclohexenyl-1,2-dione that have been given in the scheme of the regioisomers A and B.

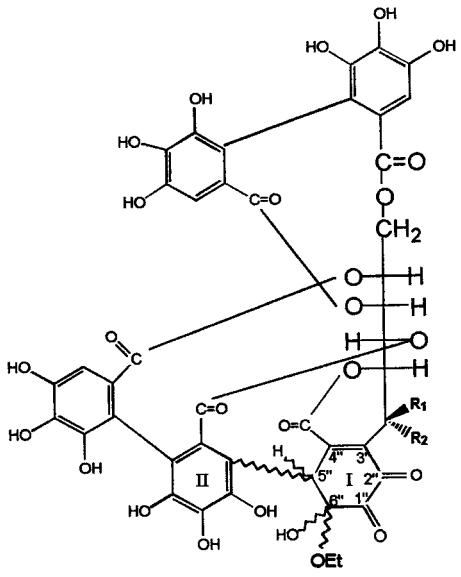


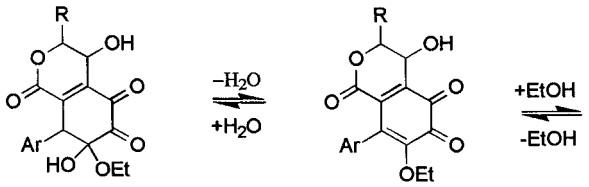
Figure 6. Castalagin and vescalagin derivative. Castalagin, R_1 = H; R_2 = OH. Vescalagin, R_1 = OH; R_2 = H.

compound **2** with a deuterium in the place of H5″. Spectra recorded after 3 months confirmed this hypothesis, as the signals of **2**, which represented 10% of the resulting mixture of **1** and **2**, were very similar to those in Tables 1 and 2. The only notable difference was the absence of the resonances of H5″ and C5″ due to deuterium exchange with the solvent.

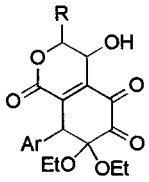
Two chiral centers are created during the transformation of **1** into **2**, and theoretically four diastereoisomers could be formed. The stereoselective formation of a

unique product is unexpected and may reflect a fairly concerted process governed by steric factors or a reversible transformation leading to the thermodynamic product. It is also possible that chemical exchange is occurring at C6″, effectively masking the existence of both configurations at this center.

HPLC analysis of the 70% ethanol solutions of castalagin and vescalagin 6 months later indicated the presence of new compounds with retention times of 48.7 and 46.7 min, respectively. LC/MS spectra of these compounds showed that the molecular weight was 1006 amu for both derivatives (+28 amu with respect to **2** and **4**). These results suggest that the hemiketal structures change to the ketal forms by means of a mechanism which can be schematized as follows:



Hemiketal



Ketal

Conclusions. Castalagin and vescalagin represent >50% of the ellagitannins in oak wood. These molecules are soluble in water−alcohol solutions containing a high percentage of ethanol. Ellagitannins have never been detected in spirits such as armagnac, cognac, whiskey, rum, and brandies. Numerous hypotheses have been put forward to explain this phenomenon, but none of the explanations has been verified. Storage of castalagin and vescalagin in 40 and 70% ethanol−water (v/v) has shown that the ethoxy moieties are added to the ellagitannins, leading to hemiketal and ketal structures. These molecules should be present in the spirits. However, one cannot exclude the possibility that such molecules undergo further rearrangements during aging in oak barrels, which can last for several decades. It is likely that the six other ellagitannins present in oak wood are modified in spirits by means of reaction mechanisms that are identical to those observed for castalagin and vescalagin.

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