Ellagitannins and Lignins in Aging of Spirits in Oak Barrels†

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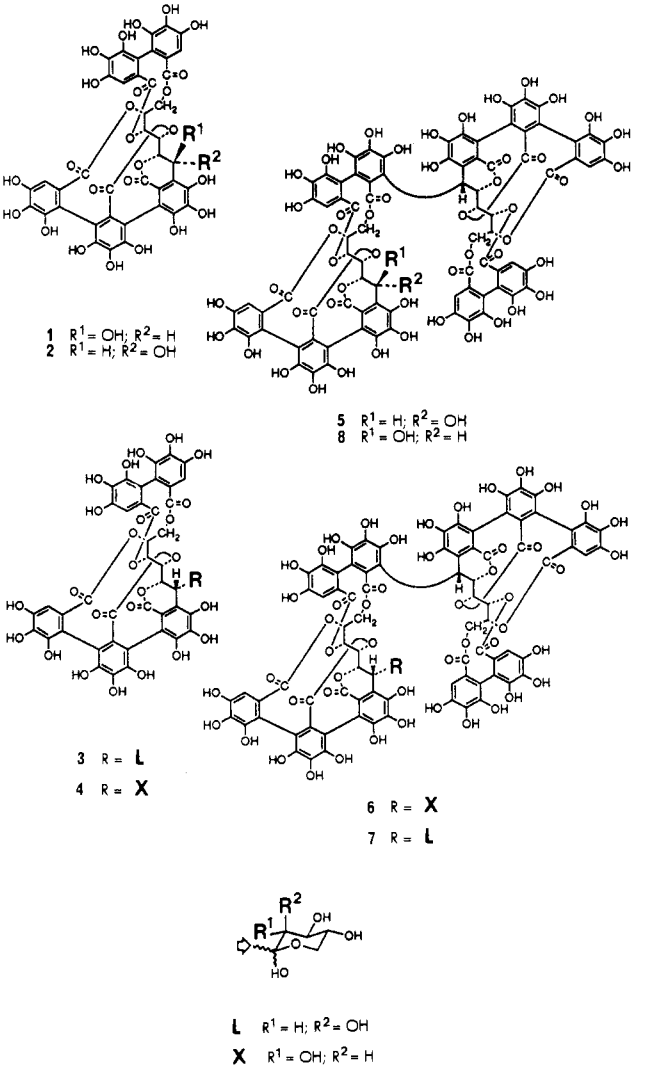
The solubilization and evolution of high molecular weight polyphenols, ellagitannins and lignins, have been studied by selective degradation methods and by gel-permeation chromatography in cognacs and brandies of different ages. Both ellagitannin and lignin contents largely exceed those of low molecular weight phenols such as vanillin or syringaldehyde. Ellagitannins are quickly solubilized during the first years of aging and are simultaneously degraded into ellagic acid. Lignin oligomers are more slowly released and are the main polyphenols in old spirits. The content of free phenolic groups in these lignin oligomers is much higher than that of native oak wood lignins. This content remains constant during the entire aging period, which suggests that lignin oligomers are not significantly degraded during spirit aging.

INTRODUCTION

Wood barrels in a form nearly identical to those used today were invented by the Celts. They superseded amphoras for wine transportation to Italy in the third century B.C. (Johnson, 1989). Since then, aging in wood casks has become an integral part of the production process of high quality wines or spirits, such as cognac, armagnac, whisky, bourbon whiskey, rum, and various brandies (Johnson, 1985). Oak wood is most largely used for this purpose, due to its unique mechanical, physical, and chemical properties. Oak species are either European oaks, *Quercus robur* L. and *Q. petraea* Liebl., or American oaks, *Q. alba* L. and related white oaks (Singleton, 1974a). Oak wood contributes to the formation and the development of color and flavor in wines and brandies.

Wood is made of various polymers, cellulose, hemicelluloses, and lignins, which form an insoluble network (Fengel and Wegener, 1984). In addition to these insoluble polymers, oak wood also contains about 10% (dry weight) of phenolic extractives, which are essentially ellagitannins (Scalbert et al., 1988, 1989). These ellagitannins are easily extracted from wood powder by water–alcohol or water–acetone mixtures (Peng et al., 1991) and are fully soluble in water and less soluble in pure alcohols.

The structures of the main ellagitannins have been established. Those of the isomers vescalagin (**1**) and castalagin (**2**) have been known for more than 20 years (Mayer et al., 1967) although a reverse configuration for the asymetric carbon was proposed more recently (Nonaka et al., 1990). Several compounds related to vescalagin and castalagin have also been purified (Scalbert et al., 1989) and characterized (Hervé du Penhoat et al., 1991a,b). They differ by the presence of an additional substituent on the C-1 of the glucose-chain backbone. This substituent can be a pentose, either lyxose **L** or xylose **X**, as in grandinin (**3**) or roburin E (**4**); it can also be vescalagin or castalagin itself as in the dimers roburin A, B, C, and D, (**5**), (**6**), (**7**), and (**8**), respectively.



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Not all ellagitannins in oak heartwood are extractible. Some resist extraction by water–alcohol mixtures or any other organic solvents (Peng et al., 1991). These insoluble ellagitannins can be estimated by acid treatment of the residue of extraction and determination of the resulting ellagic acid. They may represent up to 50% of the total ellagitannins in oak heartwood. Their content increases

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Table I. Maximum Concentrations (Milligrams per Liter) of Various Polyphenols in Different Spirits Aged in Oak Barrels

phenolic compd	max concn in aged spirit	refs	phenolic compd	max concn in aged spirit	refs
vanillin	2.4	a	scopoletin	0.5	b
syringaldehyde	3.1	a	gallic acid	17	c
vanillic acid	2.0	a	ellagic acid	60	d
syringic acid	2.4	a	ellagitannins	127	d
coniferaldehyde	2.2	a	lignins	840	e
sinapaldehyde	1.1	a			

[a] Delgado and Gomez-Cordoves, 1987; Nabeta et al., 1987; Salagoity et al., 1987; Puech, 1988; Puech et al., 1991. [b] As in a and Tricard et al., 1987. [c] Delgado and Gomez-Cordoves, 1987; Puech et al., 1991. [d] Puech et al., 1990; Puech et al., 1991. [e] Puech et al., 1985; Puech et al., 1991.

with the age of the wood, from the outer part to the inner part of heartwood.

All wood polymers and extractives are liable to be degraded and (or) solubilized during the aging of wines or spirits. Spirits offer a good model to study the solubilization of wood components as, contrary to wines where large amounts of polyphenols and polysaccharides originate from grape, all nonvolatile solutes originate from wood.

Lignin-derived phenols have been the most largely studied compounds in aged spirits. Most authors have focused on low molecular weight phenolic compounds such as vanillin or syringaldehyde, which are present in low concentrations (Table I). Much less effort has been paid to the study of larger molecular weight phenolic compounds due to the difficulties in their analysis. Puech et al. (1990, 1991) have, however, shown that the concentrations of ellagitannins and lignins exceed those of all low molecular weight phenols (Table I). We report here more detailed analyses of these high molecular weight polyphenols in brandies and cognacs of various ages. Selective methods based on the chemical degradation of ellagitannins and lignins are applied to their quantitative determination. The molecular weight distribution of polyphenols is studied by gel-permeation chromatography.

EXPERIMENTAL PROCEDURES

Materials. Ellagic acid, gallic acid monohydrate, vanillin, syringaldehyde, vanillic acid, and syringic acid (Fluka), *n*-hexadocosane (Janssen), *n*-docosane (Sigma), and polystyrenes (Supelco) were used as standards without further purification.

Oak sapwood and heartwood samples were obtained from a 90-year-old *Q. petraea* tree and collected 2 months after the tree was felled, 6 m above the base of the trunk (Peng et al., 1991). The sapwood sample includes growth rings 1–7 and the heartwood sample growth rings 44–52. Samples were air-dried and ground in a Retsch mill SM1 (particle size less than 60 mesh).

Brandies were aged in a new or used barrel (volume, 400 L). No toasting of the new barrels was done after they were bent. No addition of spirit or brandy was made during aging.

Cognacs were aged in used barrels (volume, 350 L). These used barrels were 6 years old, in average, when they received the spirit. In order to limit the effect of barrel variability, all samples were a mixture of three (two for the 30-year-old cognac) cognacs of identical age kept in separate barrels. To compensate for the volume loss due to evaporation (2–3% per year), addition of a cognac of identical age, aged in an extra barrel, was regularly carried out. The alcohol grades of the various samples were 69.4, 69.0, 64.6, 61.1, and 56.4 for 1-, 2-, 10-, 20-, and 30-year-old cognacs, respectively.

Barrels used to age both spirits were made from European oaks (*Q. robur* or *Q. petraea*), grown in Limousin (France), as is the rule for cognacs and armagnacs. No extraneous compounds were added to the analyzed brandies or cognacs.

Fractionation of Brandy Solutes. Brandy (500 mL) was concentrated under reduced pressure (all evaporations at a temperature below 40 °C) to about 100 mL and fractionated by extraction with diethyl ether and ethyl acetate. The organic extracts were dried on sodium sulfate. The various extracts were concentrated in a rotating evaporator and freeze-dried.

Determination of Total Phenols. They were determined by the Folin–Ciocalteu assay with gallic acid as a standard (Scalbert et al., 1989).

Analysis of Low Molecular Weight Phenolic Compounds by Gas Chromatography. Extracts (about 15 μg) were silylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (50 μL) and GC grade pyridine (5 μL) at room temperature for 1 h. Silylated samples (2 μL) were introduced with a moving needle-type injector (temperature of the injector, 250 °C) onto a fused silica capillary column coated with poly(dimethylsiloxane) (30 m × 0.32 mm i.d., film thickness 0.25 μm, SE 30 Supelco). Elution was carried out with helium as the carrier gas (inlet pressure, 0.6 bar) and with a temperature program from 110 to 250 °C at 3 °C/min. Detection was carried out either with a flame ionization detector or with a Nermag R10–10B quadrupole spectrometer operating in the electron impact mode (70 eV).

Estimation of Low Molecular Weight Phenolic Compounds by High-Performance Liquid Chromatography. Low molecular weight phenols were chromatographed by HPLC on a Merck LiChrospher RP18e (5–μm) column (25 cm × 4 mm i.d.). Flow speed was 1 mL/min. Cognac volume injected (autosampler) was 20 μL. Various elution gradients were used according to the nature of the phenols analyzed.

Ellagic acid: solvent A, H_2O/H_3PO_4 1000:1; solvent B, MeOH; linear gradient from 0% to 90% B in 30 min; detection, 370 nm.

Gallic acid: solvent A, H_2O/H_3PO_4 1000:1; solvent B: MeOH; linear gradient from 0% to 10% B, in 40 min; detection, 280 nm. For determination of gallic acid, the ethanol in the cognac was removed under reduced pressure prior to injection.

Vanillin, syringaldehyde, vanillic acid, and syringic acid: solvent A, H_2O/H_3PO_4 1000:1; solvent B, acetonitrile; linear gradient from 0% to 60% B in 36 min, detection, 280 nm for the acids and 313 nm for the aldehydes.

Linearity was observed for concentrations lower than 0.30 mg/mL (ellagic acid and the aldehydes), 0.22 mg/mL (vanillic and syringic acids), or 1 mg/mL (gallic acid).

Determination of Ellagitannins (Peng et al., 1991). Aged spirits (0.5 mL) were added in a Teflon-lined screw-cap glass tube to 4 mL of MeOH and 0.5 mL of aqueous HCl, 6 M. The reaction was run at 120 °C for 160 min. Ellagic acid produced by hydrolysis of the hexahydroxydiphenoyl units of the ellagitannins was determined, together with the preexisting free ellagic acid, by HPLC as described above. Bound hexahydroxydiphenoyl units (ellagitannins) were determined by the difference between total ellagic acid and preexisting free ellagic acid. Results are expressed in vescalagin (the major ellagitannin of oak wood) equivalents, provided that 1 mol of ellagic acid corresponds to 1 mol of vescalagin.

All attempts to estimate the content of the various ellagitannin monomers and dimers by reverse-phase HPLC failed, even after removal by vacuum evaporation of ethanol, which is known to interfere with the separation (Scalbert et al., 1990). The characteristic peaks of the various ellagitannins found in wood extract were not observed. It is possibly due to complexation with unknown compounds in the cognac (Viriot et al., 1993a). Attempts to estimate ellagitannins with nitrous acid according to the Bate–Smith method (Scalbert et al., 1989) also failed, due to the lack of sensitivity of the method.

Determination of Lignins and of Their Monomeric Composition by Thioacidolysis (Lapierre et al., 1991a). Dried solutes of aged spirits (10 mg, 5–20 mL) or wood (10 mg) were added to dioxane/ethanethiol 8.75:1 (v/v) and 0.2 M BF_3 (10 mL) in a tube fitted with a Teflon-lined screwcap, under an atmosphere of nitrogen. The reaction was allowed to proceed at 100 °C (oil bath) for 4 h, with stirring. The cooled reaction mixture, together with a few milliliters of water used to rinse the tube, was added to a sodium bicarbonate solution (0.4 M, 5 mL), dichloromethane (10 mL), and an internal standard solution (*n*-hexadocosane in dichloromethane, 100 μg/mL, 1 or 2 mL). pH was set at 2–3 with a 0.4 M sodium bicarbonate aqueous solution. The whole mixture

Table II. Total Solutes and Polyphenol Contents (Milligrams per Liter) in Brandies Aged in New and Used Barrels

armagnacs	dry extract	total phenols	ellagitannins	lignins	ellagic acid
7 years old, new barrel	2174	742	29	114	34
Et_2O	306	117	0	0	23
EtOAc	390	186	5	30	9
H_2O	1478	367	26	33	0
10 years old, used barrel	868	109	0	55	10

was extracted with dichloromethane (2 × 10 mL). The organic layer was dried over sodium sulfate and the solvent removed under reduced pressure. The solids were redissolved in 1 mL of dichloromethane. An aliquot (5–10 μL) was silylated and analyzed by gas chromatography as above. Lignin concentrations were calculated from the yields of thioacidolysis monomers. Calculation was based on the assumption that both cognac and wood lignins give the same yields of thioacidolysis monomers. This yield (G + S = 2000 μmol/g of lignin) was established from an oak sapwood sample of known lignin content (Klason method lignin = 22%).

Thioacidolysis of Permethylated Cognac Extracts (Lapierre and Rolando, 1988). Diazomethane was generated by reaction of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Aldrich, 2 g in 20 mL of diethyl ether) with potassium hydroxide (5 g in 18 mL of aqueous methanol, 55% v/v). The recovered diazomethane solution was added (7 mL) to freeze-dried solutes of cognac or to wood powder (10 mg). The reaction mixture was stirred for 5–15 h at room temperature. The solvent was then removed and the methylation repeated three times. The permethylated extract was freeze-dried and submitted to thioacidolysis as described above except that here the internal standard was *n*-docosane (50 μg/mL for cognacs and 200 μg/mL for wood, 2 mL).

Gel-Permeation Chromatography (Viriot et al., 1993b). Freeze-dried solutes (1 mg) of cognac were acetylated in Ac_2O/Pyr 1:1 (500 μL) at room temperature. Reagents were removed under reduced pressure after the addition of toluene. THF (1 mL) was added to solubilize the acetylated polyphenols which were analyzed on two Ultrastyragel columns, 500 and 1000 Å, 300 × 7.8 mm i.d. (Waters), used in series. THF (Chromasol SDS) was delivered at a 1-mL/min flow rate. Detection was carried out at 280 nm. Various polystyrenes and acetylated ellagitannin monomers and dimers (2 mg/mL; injection, 20 μL) were used as standards. Retention times for the standards were the following: polystyrene (PS) 2000, 15.5 min; PS 4000, 14.3 min; PS 9000, 13.3 min; PS 23 000, 11.7 min; PS 35 000, 11.5 min; vescalagin and castalagin, 16.5 min; roburin E and grandinin, 16.3 min; roburins A, B, C, and D, 15.6 min; ellagic acid, 18.3 min; methyl ester of gallic acid, 18.8 min; vanillin, 20.4 min; and syringaldehyde, 20.2 min. The identity of the different peaks obtained with wood extracts was confirmed by their spectra (230–380 nm) obtained with an on-line UV 117 detector (Gilson). Spectra were similar to those of the corresponding nonacetylated compounds. Ellagitannins showed no maxima in the wavelength region studied, whereas ellagic acid showed a maximum at 370 nm.

RESULTS

Total solutes (dry extract) and polyphenols were estimated in two brandies, one aged 7 years in a new oak barrel and the other one aged 10 years in an old barrel (Table II). The old barrel released in the brandy about seven times less materials than the new barrel.

In the brandy aged in the new barrel, the concentration of polyphenols is about 0.7 g/L; they represent one-third of the dry extract. The other two thirds are essentially polysaccharides as shown by the presence of major signals in the 60–100 ppm region of the ^{13}C-NMR spectrum of the water-soluble fraction (not shown).

Solutes were fractionated into three pools according to their solubility in diethyl ether, ethyl acetate, and water, and the phenols were analyzed by HPLC, gas chromatography, and mass spectroscopy. About one-fifth of the total phenols are soluble in diethyl ether and correspond essentially to low molecular weight phenols such as ellagic acid, gallic acid, and its ethyl ester and to a lesser extent vanillin, syringaldehyde, vanillic acid, and syringic acid (Figure 1a and Table II). The ethyl acetate fraction contains lesser amounts of the same low molecular weight phenols incompletely extracted by diethyl ether together with ellagitannins and lignin oligomers. Minor compounds such as coniferyl alcohol, methoxyhydroquinone, 2,5-dimethoxyhydroquinone, homovanillic acid, and protocatechuic acid were also identified (Figure 1b). Traces of a polyphenol of molecular weight 708 were tentatively assigned to the lignan lyoniresinol, previously identified in oak heartwood and brandy (Nabeta et al., 1987; Dada et al., 1989).

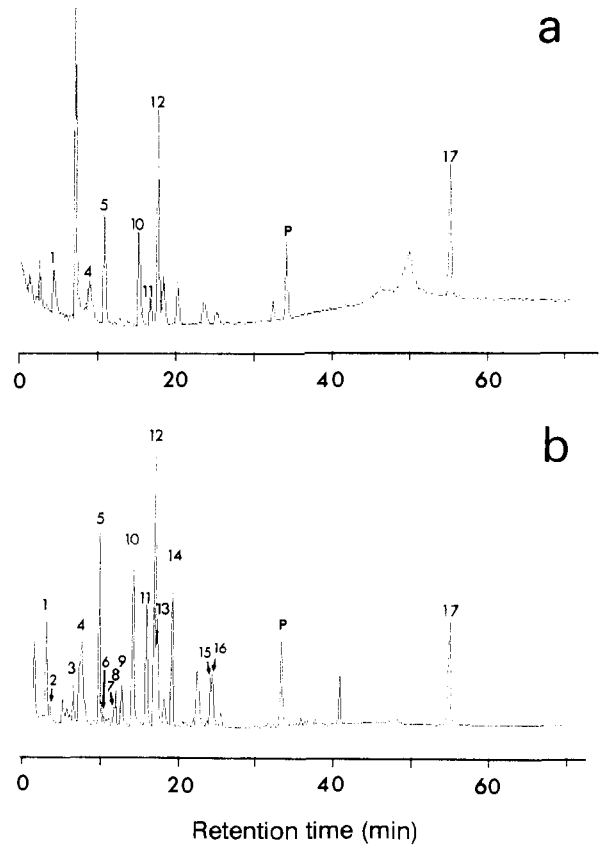

Figure 1. Gas chromatograms of silylated brandy solutes (monitored as total ion current detected in the mass spectrometer: (a) diethyl ether extract and (b) ethyl acetate extract; 1, vanillin; 2, methoxyhydroquinone; 3, 2,5-dimethoxyhydroquinone; 4, syringaldehyde; 5, vanillic acid; 6, homovanillic acid; 7, coniferyl alcohol; 8, 3,4-dihydroxybenzoic acid; 9, myristic acid; 10, syringic acid; 11, ethyl gallate; 12, gallic acid; 13, ethyl palmitate; 14, palmitic acid; 15, linoleic acid; 16, oleic acid; 17, ellagic acid; and P, phthalate (contaminant from solvents).

Most of the polyphenols present in the brandy are water-soluble, insoluble in the two previous organic solvents, and include the major part of ellagitannins and about half of the lignin oligomers. In contrast to the phenols soluble in diethyl ether, water-soluble polyphenols do not give well-individualized peaks or spots but bumps or streaks, by HPLC and paper chromatography, respectively. These polyphenols are probably heterogeneous in structure and composition and may correspond largely to oligomers and polymers.

Ellagitannins, lignins, and ellagic acid, as well as vanillin, syringaldehyde, vanillic acid, and syringic acid, were estimated in cognacs aged in oak barrels during increasing periods of time (Table III). Concentrations of these various

Table III. Total Solutes and Polyphenol Contents (Milligrams per Liter) in Cognacs of Various Ages

age (years)	dry extract	total phenols	ellagitannins	lignins	ellagic acid	gallic acid	vanillin	syringaldehyde	vanillic acid	syringic acid
1	297	92	10	12	7	3	0.6	1.1	0.9	0.8
2	597	190	25	29	12	3	1.8	3.2	1.5	1.0
10	1882	553	31	127	32	22	5.8	10.9	3.1	4.0
20	2705	684	17	201	44	23	6.8	13.3	4.0	5.6
30	3615	833	4	219	55	26	7.2	14.2	5.4	6.4

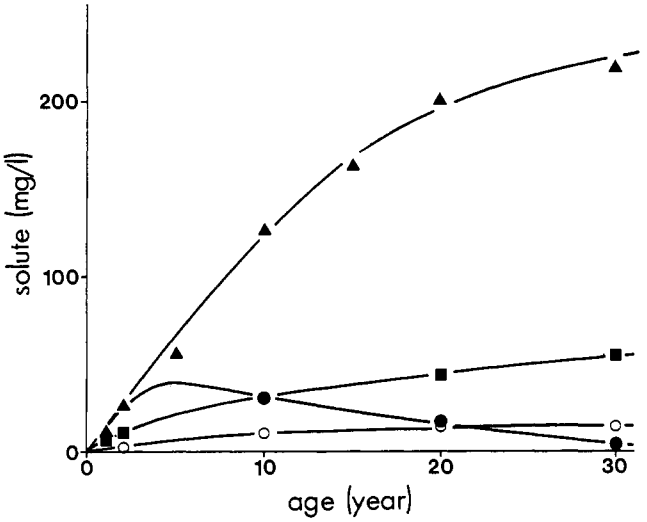

Figure 2. Polyphenol concentrations in cognacs of different ages: (●) ellagitannins, (■) ellagic acid, (▲) lignin oligomers, and (○) syringaldehyde.

phenolics are very similar in both the 7-year-old brandy aged in a new barrel and in the 10-year-old cognac aged in a used barrel (6 years old in average). High molecular weight polyphenols (ellagitannins and lignins) are the main phenolic compounds. Low molecular weight phenols (ellagic acid, gallic acid, vanillin, syringaldehyde, vanillic acid, and syringic acid) account for only a minor proportion of the total phenols (about 14%).

The concentration of dry extract and most phenols increases with the duration of aging. The rate of accumulation of the various solutes is not constant during all of the aging period. It is significantly reduced after 30 years of aging (Table II; Figure 2). This decrease in the rate of accumulation should still be more evident when the concentration of the spirit in the barrel, due to evaporation, is taken into account.

Some differences in the kinetics of accumulation are observed between the various phenols. Leveling-off of vanillin and syringaldehyde concentrations after 20 years of aging is more pronounced than that of dry extract, total phenols, lignin, vanillic acid, or syringic acid. More significant, the evolution of ellagitannin concentration shows a very distinct pattern when compared to all other solutes: the concentration increases quickly during the initial years to reach a maximum value after about 5 years of aging and decreases to a near-zero value after 30 years (Figure 2). As a consequence, the younger cognacs contain about as much ellagitannins as lignin oligomers whereas the older ones contain mainly lignin oligomers.

The possible occurrence of polyphenolic polymerization during aging was examined by determination of the proportion of free phenolic and etherified units in noncondensed lignin oligomers and by gel-permeation chromatography. The proportion of phenylpropane units with free and etherified phenolic groups remains constant during the 30 years of aging (Table IV). Large differences are, however, observed when cognac lignins are compared to those of oak wood. The proportion of etherified guaiacyl and syringyl units in cognac lignin oligomers is respectively 42% and 33% lower than in *in situ* lignins of oak wood.

Table IV. Monomer Composition and Proportion of Free Phenolic Units of Lignins in Cognacs of Various Ages and in Oak Wood

	S/G[a]	$G_{OH}/(G_{OH}+G_{OR})$[b]	$S_{OH}/(S_{OH}+S_{OR})$[b]
cognac			
1 year	1.30	0.65	0.39
2 years	1.26	0.63	0.37
10 years	1.18	0.64	0.37
20 years	1.11	0.61	0.34
30 years	1.05	0.63	0.33
wood			
sapwood	1.88	0.34	0.04
heartwood	2.04	0.35	0.04

[a] S = syringyl, G = guaiacyl, molar ratio. [b] G_{OH}, S_{OH} = free phenolic noncondensed units; G_{OR}, S_{OR} = etherified noncondensed units.

Some differences were observed between the gel-permeation chromatograms of the various cognacs (Figure 3). In younger cognacs (1–10 years old), the absorption maximum (16.5 min) has an identical retention time to that of pure vescalagin or castalagin. A shoulder at 15.7 min corresponds to ellagitannin dimers. The peak at 18.3 min is ellagic acid. All low molecular weight phenols (gallic acid and vanillin, etc.) are well separated from the other phenols, ellagitannins or lignins. Older cognacs (20 and 30 years old) differ from younger cognacs as the peak and the shoulder characteristic of ellagitannin monomers and dimers are no longer recognized. The curve profile is smoother. The absorption maximum at 16.9 min has a retention time identical to a polystyrene of molecular weight 780. The chromatograms of 20- and 30-year-old cognacs are very similar; no difference in the retention time at the absorption maximum could be observed.

DISCUSSION

Ellagitannins in Spirit Aging. During the aging of spirits in oak barrels, ellagitannins may take part in the following reactions. (1) They are solubilized by the spirit and diffuse through the wood. (2) Soluble ellagitannins may be hydrolyzed with formation of ellagic acid. (3) They may be degraded without formation of ellagic acid; this would occur if the hexahydroxydiphenoyl residue in ellagitannins is involved in an oxidative reaction. (4) Insoluble ellagitannins may also be hydrolyzed with formation of ellagic acid which could diffuse through the wood into the spirit. (5) Ellagic acid may eventually be degraded; the extent of ellagic acid degradation is probably low, as it was observed that it is remarkably stable in an acidic methanol–water solution, even at 120 °C (Peng et al., 1991).

The measurements made here on cognacs of different ages show that ellagitannins are quickly solubilized and degraded (Figure 2). Ellagic acid is more slowly released and should arise either from the solubilization of free ellagic acid present in the wood or from the degradation of soluble and insoluble ellagitannins. The relative concentrations of free ellagic acid and ellagitannins in oak wood show that the second hypothesis is more likely.

Indeed, if we suppose that free ellagic acid results exclusively from the solubilization of the free ellagic acid

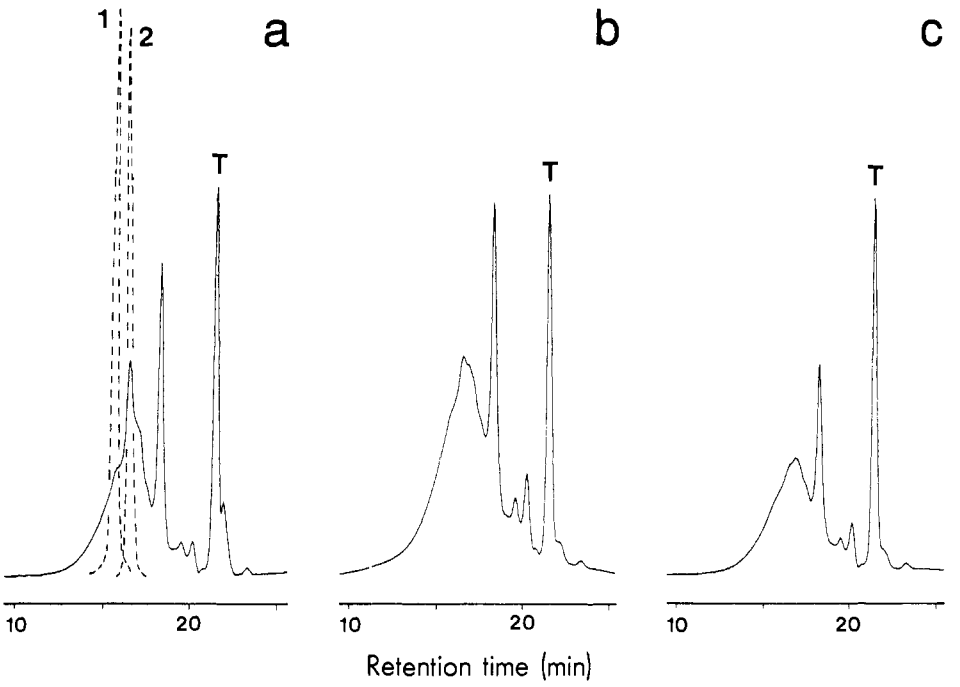

Figure 3. Gel-permeation chromatograms of cognacs of different ages: (a) 2 years old, (b) 20 years old, and (c) 30 years old. Dotted-line: standards; 1, roburin A, and 2, vescalagin. T, toluene.

contained in the wood (2.3–3.8 mg/g of heartwood; Viriot et al., 1993a), we can calculate (barrel volume, 350 L; wood density, 720 kg/m^3) that a 55 mg/L concentration of free ellagic acid in cognac (Table III) would require exhaustive extraction of the wood in contact with the spirit on a minimal depth of about 3 mm. If this is so, there would be no reason why soluble ellagitannins (75 mg/g of heartwood; Peng et al., 1991) should not be extracted on a similar depth. The ellagitannin concentration in cognacs would then be 1265 mg/L, a value which far exceeds the maximum found for ellagitannins or even total phenols in any sample analyzed (Table III).

On the other hand, if we suppose that all free ellagic acid in cognacs results from the hydrolysis of soluble and insoluble ellagitannins in wood (75 and 25 mg/g, respectively; Peng et al., 1991), the depth of wood extraction required to account for the maximum concentration of tannins found in cognac would be about 0.3 mm. This value should, however, be taken as a minimum as we cannot exclude that some ellagitannins are oxidatively degraded without formation of ellagic acid, either in the processing of barrels or during aging.

The present results show that, among the possible implications of ellagitannins in spirit aging, solubilization and hydrolysis with formation of ellagic acid are more likely to occur. The extent of oxidative degradation is so far unknown. Further experiments are required to determine its implication in spirit aging.

Gallic acid concentration in cognacs is of the same magnitude as that of ellagic acid (Table III). Oak heartwood contains low amounts of gallic acid (0.9–2.0 mg/g; Viriot et al., 1993a) which are again insufficient to account for the amount of gallic acid in cognacs. Gallic acid in cognacs probably results from the hydrolysis of galloyl esters. The identification of ethyl gallate in brandy (Figure 1) can only be explained by transesterification of galloyl esters by ethanol. Galloyl esters are major constituents of galls grown on oak (Haddock et al., 1982) and have been identified in barks (Nishimura et al., 1986) and leaves (Sheu et al., 1990) of some oak species. Their occurrence in wood of European oak has, however, never been reported.

Lignins in Spirit Aging. Lignins in European oak, determined by the Klason method, represent 22–25% of the dry weight of wood (Fengel and Wegener, 1984). It is likely that the lower values are closer to the actual content as insoluble ellagitannins (the content of which varies between samples) will behave as lignins in the determinations.

Oak wood lignins are made of both syringylpropane and guaiacylpropane units. The main intermonomeric linkages are alkyl aryl ether linkages between the propane side chain of one unit and the phenolic group of another unit. Other intermonomeric linkages are carbon–carbon linkages in various bonding patterns. The phenylpropane units taking part in these last linkages are often called condensed units, in contrast to noncondensed units, as they resist depolymerization with acids or alkalis.

Lignins are insoluble unless the alkyl aryl ether linkages are broken by mechanical or chemical treatments (e.g., solvolysis in an ethanol/HCl mixture at 120 °C). This is also what probably happens under milder conditions during the aging of spirits in an oak barrel. Lignin monomers or oligomers are thus solubilized.

The occurrence of several phenols deriving from lignins, such as vanillin, syringaldehyde, vanillic acid, syringic acid, coniferaldehyde, or sinapaldehyde, has been commonly reported in various spirits aged in oak barrels (see references in Table I). Coniferyl alcohol, bound to the lignin polymer through etherification of its phenolic group, is a common constituent of lignins. Vanillin and syringaldehyde are usually present in woods in trace amounts: (1.2–3.6) × 10^{-3} mg/g (Nabeta et al., 1987). These values are too low to account for the concentrations of vanillin and syringaldehyde in cognac. In spirits, vanillin and syringaldehyde, as well as vanillic acid and syringic acid, more likely result from the oxidative degradation of guaiacylpropane and syringylpropane units, respectively, either during the seasoning and heat treatment of oak staves or later during the aging of spirits in the barrel.

Vanillic acid and syringic acid could originate from the direct degradation of lignins or from the oxidation of vanillin and syringaldehyde as suggested by the leveling-off of the concentrations of these two last compounds after

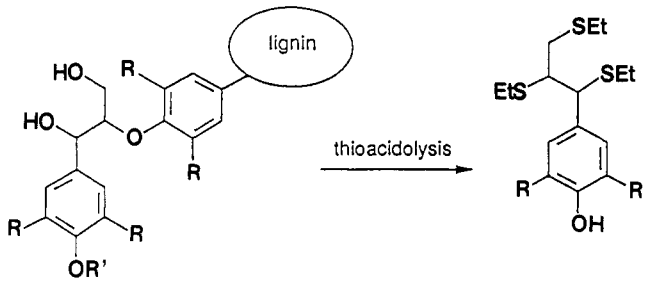

Figure 4. Cleavage of alkyl aryl ether linkages between noncondensed units in lignins by thioacidolysis. R = H or OCH_3; R' = H or alkyl group of lignin.

20 years of aging. Other low molecular weight phenols, such as the presently identified methoxyhydroquinone, 2,5-dimethoxyhydroquinone, homovanillic acid, or protocatechuic acid, possibly derive from lignins through so far unknown oxidative reactions. All these low molecular weight phenols, in contrast to lignin oligomers, represent only a minor fraction of the total phenols present in cognac or brandies (Table III).

Lignin oligomers were assayed by thioacidolysis which cleaves specifically the alkyl aryl ether linkages; lignin noncondensed units give soluble phenylpropane monomeric thioethers which can be analyzed and estimated by gas chromatography (Figure 4; Rolando et al., 1992). Thioacidolysis allows estimation of lignins (from dimers to polymers) without any interference from low molecular weight phenols such as vanillin or syringaldehyde.

This method, applied here for the first time to the analysis of aged spirits, shows that the content of lignin oligomers far exceeds that of vanillin or syringaldehyde (Table III). The maximum lignin concentration estimated (160 mg/L) would require exhaustive extraction of the wood in contact with the spirit on a minimal depth of 0.13 mm. This value can be compared to the 0.27 mm required to account for the presence of ellagic acid which would result mainly from the hydrolysis of ellagitannins. This difference may possibly be due to the slower release of lignins in cognacs over the 30 years of aging, as compared to ellagitannins, or to a possible underestimation of lignin content in cognacs (see below).

Lignins in the solubilization process are chemically altered, and some differences in their structures in both aged cognac and oak wood were observed. The relative proportions of syringylpropane to guaiacylpropane of lignin oligomers in cognacs differ from those of oak sapwood and heartwood (Table IV). Lignins in wood are heterogeneous and vary in monomer composition (syringyl to guaiacyl ratio; Monties, 1989) and in the proportion of the various types of intermonomeric bonds (Lapierre et al., 1991b). The present results show that guaiacyl-rich lignins are preferentially solubilized in cognacs. An easier solubilization of guaiacyl-rich lignins was also reported in kraft (Gellerstedt et al., 1988) or neutral sulfite cook (Kolar et al., 1979) of birch wood. A decrease of the syringylpropane to guaiacylpropane ratio in cognacs with the duration of aging was observed. It could possibly be explained by the higher sensitivity of syringyl units toward oxidation (Sultanov and Wallis, 1991).

More differences between cognac and oak wood lignins relate to the proportion of free phenolic units. It is about 35% higher in cognacs for both guaiacyl and syringyl units. This increase in the number of free phenolic units results from the cleavage of the alkyl aryl ether linkages at the origin of lignin solubilization. The constant proportion of free phenolic units over the 30 years of aging (Table IV) may correspond to the minimum number of broken alkyl aryl ether linkages required for lignin solubilization.

The constant proportion of free phenolic units in cognac lignins indicates that chemical alteration of lignin oligomers during aging is limited. Indeed, further depolymerization during aging would increase the proportion of free phenolic units, and repolymerization through oxidation would involve the reactive free phenolic groups and decrease their content.

Relative Concentrations of Polyphenols in Spirits. The sums of the concentration values of the different polyphenols estimated individually in cognacs (ellagic acid, gallic acid, vanillin, syringaldehyde, vanillic acid, syringic acid, ellagitannins, and lignins) account for only 38–46% of the total phenol concentration (Table III). This may be due to the choice of methods and standards used in the assays or to the existence in the spirits of unknown compounds which may interfere with these assays.

The Folin–Ciocalteu method is superior to most other colorimetric methods for the total phenol determination as most phenols, whatever their substitution pattern is, show close molar absorption coefficients (Singleton, 1974b; Scalbert, 1992). However, total phenols in cognacs may be overestimated if they give a lower absorption per mass unit than the standard (gallic acid). Their ability to reduce the Folin–Ciocalteu reagent may be diminished if they are partially oxidized during aging. Total phenols may also be overestimated if other constituents, present in cognacs and not considered in the present study, are also able to reduce the Folin–Ciocalteu reagent. Sugars are present in large amounts in spirits aged in oak barrels (about 500 mg/L in a 30-year-old cognac; Viriot et al., 1992), but their contribution to color formation in the Folin assay is negligible (Moutounet, 1981). Hydroxymethylfurfural, identified in cognacs, does not respond to the Folin assay.

The discrepancy mentioned above may also be due to the underestimation of the different individual classes of polyphenols. This is unlikely for ellagic acid, vanillin, syringaldehyde, vanillic acid, and syringic acid as pure standards were available and used for the HPLC determinations. On the other hand, ellagitannin concentrations determined by acid degradation and estimation of the resulting ellagic acid are underestimated. These values are calculated as castalagin–vescalagin equivalents. Castalagin and vescalagin, the two major ellagitannins in oak wood, give 1 mol of ellagic acid per mole, just as the dimers which have, however, a twice higher molecular weight. The presence of dimers in oak wood (they represent 25% of the total tannins; Viriot et al., 1993a) will result in an underestimation of ellagitannin concentrations by as much as 25%.

Lignin oligomers in cognacs may also be underestimated. In the present study, the amount of cognac lignins is expressed as oak wood lignin equivalents. It is assumed that both types of lignins give similar yields in thioacidolysis monomers. This yield depends however on the condensation degree of the lignins. Nothing is known so far on the relative condensation degree of cognac lignins. However, they are likely more condensed than the native oak lignins, as they are formed through cleavage of alkyl aryl ether linkages, characteristic of noncondensed units. A relatively high condensation degree will lead to their underestimation. The concentration of lignins in cognacs should thus be taken as indicative and probably as a minimum value.

The lignin concentrations determined here can be compared to those previously published. Puech (1988) determined the lignin concentration in armagnac by estimation of their methoxyl groups. He reported a

concentration of "lignocomplex" of 814 and 363 mg/L for two armagnacs aged in a new and old barrel, respectively. These values are much higher than the ones determined here although the values of total phenols (670 and 216 mg/L, same assay and same standard as in this paper) are very close to those measured here (Table II). It is likely that the lignin contents determined by Puech are overestimated; the factor used to convert methoxyl values into lignocomplex values (×9.7) is determined on a lignin fraction prepared by mild ethanolysis of oak wood (pH 5, 7 months; Puech, 1978). This fraction is probably not a pure lignin as a pure guaiacyl–syringyl lignin (methoxyl content, 22%) would give a theoretical conversion factor of 4.5. If this theoretical conversion factor is used, the lignin contents reported by Puech would thus become 378 and 168 mg/L. These values are closer to the ones reported here, although still significantly higher, and do not exceed the total phenol concentrations.

The underestimation of lignin contents in the present study would thus be confirmed. In all cases, it is beyond doubt that lignins are the major polyphenols in aged cognacs and brandies.

Molecular Weight Distribution of Polyphenols in Spirits. The curve profiles obtained by gel permeation chromatography differ between young and old cognacs. The young cognac chromatrograms can be compared to those obtained from oak wood extracts (Viriot et al., 1993a). The maxima are the same and correspond to ellagitannin monomers (vescalagin, castalagin, grandinin, and roburin E) and dimers (roburins A, B, C, and D). The curve profiles of young cognacs are, however, smoother, and dimers do not appear as a peak but as a shoulder. This may be explained by a partial chemical alteration of these ellagitannins or by a contribution of overlapping lignin oligomers.

In older cognacs, the curve profile is even smoother, and ellagitannins peaks are no longer recognized. This is in agreement with the quantitative data presented above, which show that in older cognacs, lignin oligomers predominate over ellagitannins which are largely degraded. Lignin oligomers have a wider polymolecularity. A molecular weight at the maximum of the curve profile can be estimated by comparison with polystyrene standards. The obtained value, 780, would represent oligomers of two to three units (an average molecular weight per monomeric unit of 300 for an acetylated guaiacyl–syringyl lignin is assumed). This degree of polymerization is likely to be underestimated as the use of linear polystyrene polymers as standards will lead to an underestimation of the actual molecular weight of lignins by a factor as high as 2.5, as lignins are branched polymers (Himmel et al., 1990).

A comparison of the chromatograms of the 20- and 30-year-old cognacs shows no difference in molecular weight distributions. Polyphenol polymerization does not appear to be a prominent phenomenon in cognac aging.

Variations in Polyphenol Nature and Content with Oak Wood Quality. Three factors may influence the nature and content of polyphenols which accumulate in spirits: the nature of the oak wood (species and geographical origin), the wood transformation (seasoning and heating) during cooperage manufacture, and the history of the cask. Few data exist on the influence of these factors on the accumulation of ellagitannins and lignins in spirits. Ellagitannins, because of their solubility and higher reactivity, are more likely to be affected by these factors than lignins. Furthermore, ellagitannin contents vary largely according to the wood sample considered.

Ellagitannin structures and contents are very similar in the two European oaks used for cooperage (*Q. petraea* and *Q. robur*; Scalbert et al., 1986). The structures of the main ellagitannins in American oak have not yet been described. The ellagitannin content was found to be lower in American oak than in European oak by several authors (Singleton, 1974a; Puech, 1984; Puech et al., 1991). However, it should be confirmed by the analysis of a larger number of samples.

It is well known in the cooperage and spirit industry that the tannin content varies with the geographical origin of the wood. This has not been confirmed yet by chemical analysis due to the large variations between samples (Puech, 1984). Again, such a statement still awaits confirmation by analysis of a larger number of samples.

Seasoning and heating of oak wood may affect the ellagitannin and lignin contents in spirits. Such treatments may induce degradation (Sarni et al., 1990) or leaching of part of the ellagitannins. They might also break some of the intermonomeric alkyl aryl ether linkages in the lignin polymer and facilitate their solubilization during aging.

Lastly, the spirit industry uses both new and used barrels for aging. The proportion and content of the different polyphenols will be largely determined by the history of the barrel. The total amount of extracted phenols is much lower when an old barrel is used (Puech et al., 1985; this paper). It is particularly obvious for ellagitannins which are totally absent after 10 years of aging in a used barrel (Table II). The lignins are comparatively less affected.

CONCLUSIONS

Polyphenols originating from oak wood are major solutes of spirits aged in oak casks. They represent about a third of the dry extract, and their concentration increases regularly with the duration of aging. The main polyphenols are high molecular weight compounds, either ellagitannins or lignin oligomers. The proportions of ellagitannins and lignins vary with the duration of aging. Ellagitannins are wood extractives which do not require cleavage of covalent bonds to be solubilized; their maximum concentration is reached in the first years of aging. Solubilization of ellagitannins competes with their chemical degradation, most likely through hydrolysis with formation of ellagic acid.

Lignins, in contrast to ellagitannins, are insoluble cell wall polymers. Their solubilization requires the cleavage of covalent linkages. It is relatively slow and proceeds during the entire aging period. A young spirit will thus contain a high proportion of ellagitannins, whereas in an old spirit, lignin oligomers will largely predominate.

Oak wood lignins are solubilized most likely through the cleavage of alkyl aryl ether bonds. These bonds are the most labile and major intermonomeric linkages in lignins. Lignin oligomers then diffuse through the wood into the spirit and seem to be not much altered chemically over 30 years of aging. Guaiacyl-rich lignins are preferentially solubilized.

Solubilization of lignins and degradation of ellagitannins are thus largely explained by hydrolytic reactions. Oxidative reactions also contribute to the transformation of polyphenols during spirit aging, as exemplified by the formation of vanillin, syringaldehyde, and quinones. Their contribution to lignin solubilization or ellagitannin degradation remains, however, unexplored.

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