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Research on the lignin in heartwood of *Q. robur* L. and *Q. petraea* Liebl. Identification of a fraction of lignin solubilised in wines during ageing in barrels

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Summary : For this work, we brought together the studies carried out on the characterisation of oak wood lignin and on the identification of soluble fractions in wines after ageing in new barrels. The two types of lignin extracted from the heartwood of *Q. robur* and *Q. petraea* present significant differences. They are LE, a lignin extracted in an acid hydroalcoholic medium (PM 23 kDa) and LD, heat-extracted with acidified dioxan (PM 45 kDa). The analysis of the 2D spectra of type TOCYS and HMQC confirms that the two lignins contain mainly monomethoxylated (Guaiacyl)and dimethoxylated (Syringyl) phenolic unit, bonded by dominant ß-O-4 aryl-ether bonds. LD is distinguished from LE by its larger proportion of G unit. During a prolonged period in new barrels made with these two species of oak, a soluble fraction of lignin, whose S/G ratio is approximately equal to 0.5, is demonstrated.

Key words : oak, heartwood, lignins, structure, wines, barrels

INTRODUCTION

Oak heartwood is rich in lignins. Depending on the authors and the extraction methods used, they represent between 25 and 30 % of the dry weight of wood (BROWNING and ISENBERGE, 1952 ; LAI and SARKANEN, 1971 ; FENGEL and WEGENER, 1984 ; JOUIN *et al.*, 1988). Lignin impregnates the cell wall (ROWELL, 1984) and is mainly located in the primary wall (CATESSON, 1980). Since work by KLAUDITZ (1952 and 1957), we have attributed their contribution to the mechanical properties of the wood and to their hydrophobicity. The heterogeneous character of lignin is an important factor (MONTIES, 1980) and is at the origin of the great variability of composition and structure between tree species (FAIX, 1991 ; ROLANDO *et al.*, 1992), within the same species (LAPIERRE, 1986 ; LAPIERRE *et al.*, 1986a and b) and even according to their repartition in the tissue (HARDELL *et al.*, 1980). The works carried out on the structure of oak lignin (MONTIES, 1992) are therefore difficult to generalise and remain dependent on the sampling and the often specific objectives of the study.

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Q. robur and *Q. petraea* are today very widely used for ageing wines and spirits (TARANSAUD, 1976). Their composition and anatomical and mechanical properties have led them to be preferred over other species (STEVENS and TUERNER, 1970 ; SACHSSE, 1984 ; SINGLETON, 1995). The lignins in oak wood are, as in all angiosperms, three-dimensional polymers formed by the co-polymerisation of two phenylpropenoic alcohols : hydroxy-4-methoxy-3-cinamic alcohol (guaiacyl structure ; coniferyl alcohol) and hydroxy-4-dimethoxy-3.5-cinamic alcohol (syringyl structure ; sinapyl alcohol). Throughout the ageing of wines (SINGLETON, 1974 ; FEUILLAT, 1982 ; NAUDIN, 1986) and the maturing of spirits (BRICOUT, 1971 ; PUECH, 1984 ; PUECH, 1992) in barrels, many phenolic aldehydes, resulting from the degradation of the lignins, are diffused. They are mainly vanillin and syringaldehyde and, to a lesser degree, coniferaldehyde and sinapaldehyde (GUYMON and CROWELL, 1968 ; PUECH *et al.*, 1982 ; CAUMIEL, 1983). The lignins may also be partially solubilised ; their presence has been reported essentially in eaux-de-vie (PUECH, 1978 ; VIRIOT *et al.*, 1993). During the work on and the processing of wood in cooperages, many factors are likely to alter the structure of the lignins and to favour their degradation (VIVAS *et al.*, 1991a ; MONTIES, 1992 ; PUECH, 1992). Among them can be mentioned the natural or artificial drying and the bending of staves followed by toasting. Biochemical, chemical and physical mechanisms can be envisaged.

The wooden barrel is a very ancient container, made for the vinification, ageing, transport and storage of wines (TARANSAUD, 1976). But wood is not an inert matter : it favours firstly gaseous exchanges between the wine and the surrounding atmosphere, and then the dissolution of many sapid, odorous constituents which contribute to the organoleptic character of wines (VIVAS *et al.*, 1991b ; VIVAS, 1995). The intensity of these various reactions depends essentially on the period of time spent in the barrels. Over long periods, the wine is altered to such an extent that its composition and quality are radically different from those it started with.

For this work we have brought together our knowledge on the structure of the lignins in two French species widely used in cooperage, *Q. robur* and *Q. petraea*, and have demonstrated a soluble lignin fraction originating from new barrels.

MATERIALS AND METHOD

I - ORIGIN OF THE WOOD SAMPLES

The samples are made up of heartwood from the Allier forests (France) for *Q. petraea* and from the Limousin forests for *Q. robur*. Their botanical characteristics have been verified (JACQUIOT *et al.*, 1973 ; WALKER, 1978). Two series of samples from the same regions but from two different supply sources are made up. The average ages of the trees are 150 and 175 years for the two series of samples of *Q. petraea* and 80 and 110 years for *Q. robur*. The samples are planed down to chips, then reduced to sawdust by being crushed in liquid nitrogen. The different areas of the heartwood – near the sapwood, in the centre of the heartwood and near the centre of the tree – are not distinguished. They are mixed together and constitute an average sample that is representative of the whole heartwood.

II - ORIGIN OF THE WINE SAMPLES

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For our study, we used wines aged in new barrels or in vats, so as to confirm the specificity of response of the method of characterisation of the lignins. The analytical studies covered :

- 7 dry white wines from the Graves (Sauvignon, 1996),
- 11 dry white wines from the Entre-deux-Mers (Sauvignon, 1996),
- 8 red wines from Saint-Emilion (Merlot, 1995),
- 6 red wines from the Médoc (Cabernet Sauvignon, 1995),
- 3 sweet wines from Sauternes (Semillon, 1993),
- 1 red wine from the Médoc (varieties unknown, 1868).

III - Extraction and purification of the wood lignins

Two lignin fractions were selected. They were chosen for their different characteristics. They were the Brauns native lignin, or lignin ethanol « LE », and lignin dioxan « LD ».

1) Extraction and purification of LE

100 g of sawdust is cleaned of its extractive by maceration in 3 x 1 l of water (40°C, 12 hrs), then with 1 x 1 l of chloroform (20°C, 12 hrs). Next, the sawdust is dried in an oven (30°C, 24 hrs) and left to macerate in 5 l of EtOH 95 % vol. adjusted to pH = 5 with acetic acid (24 months at room temperature and in the dark). The extract is filtered, evaporated with a rotary evaporator (40°C) and purified by 5 successive precipitations with water (precipitation : 5 x 1 l of water at 5°C, centrifugation : 3,500 rpm, 15 min.). The resulting powder is washed in 2 x 50 ml of diethyl ether, freeze-dried and kept in a desiccator. Its aspect is pulverulent and light beige in colour.

2) Extraction and purification of LD

100 g of sawdust is cleaned of its extractive by maceration in 3 x 1 l of water (40°C, 12 hrs), then with 1 x 1 l of chloroform (20°C, 12 hrs) and finally by 2 x 1 l of $EtOH/H_2O$ (9 :1) (20°C, 12 hrs). The sawdust is dried in an oven (30°, 24 hrs) before being extracted 6 hrs by 1 l of dioxan containing 100 ml of HCl 12N. The mixture is maintained under reflux. The extraction liqueur is filtered, evaporated with a rotary evaporator (40°C), then treated in the same way as LE. The resulting powder is pale fawn in colour.

IV - Thioacidolysis and GC-MS analysis of the products formed in the form of trimethylsylile derivatives

The method applied is described in detail by Rolando *et al.* (1992). It was developed by Lapierre (1986). It consists of a thioacidolysis of the lignin (BF3 in dioxan/e ; 8.75 :1). The degradation products, presented in the form of thioethylated derivatives, are analysed by GC-MS after silylation (BSTFA/TMS). Only the measures of reagents stipulated by the authors were doubled. GC-MS analysis of the products of thioacidolysis is carried out under the following conditions : CPSil 5 column (25 m, di 0.25 mm), temperature of injector 240°C, temperature programming 120°C to 260°C at a rate of 4°C/min. The Mass Spectrometer is a Saturn 4D™ made by Varian (Ion trap).

V - NMR analysis of the extractable LE and LD lignins

The NMR spectra are obtained after acetylation. The peracetylation of each fraction (LE and LD) is conducted in acetic pyridine/anhydride (1/1, v/v), followed by a precipitation in water. The peracetylated product is collected with chloroform, then carefully dried by nitrogen flow (N2, 50 ml/min.).

The NMR spectra are recorded in deuterated chloroform on a Bruker DPX 400. The TOCYS spectra are recorded for a mixing time of 100 ms. The HMQC spectra are achieved with a B0 field gradient. 64 scans are accumulated with spectral windows of 17 600 Hz and 3 600 Hz respectively for ^{13}C and 1H. The resolution is 68.8 Hz/dot for ^{13}C and 3.6 Hz/dot for ^{1}H.

VI - MEASUREMENT OF THE LIGNINS IN YOUNG WINES (VIVAS *et al.*, 1997)

As long as the wine does not present abundant deposits of phenolic compounds, the lignins can be measured directly in a test sample of wine.

A 20 ml test sample of wine is fully evaporated on a rotary evaporator (< 30°C). Next, the thioacidolysis solution (BF3 in dioxan/ethanethiol, 8.75 :1) is added in proportion to the nature of the product to be analysed. For our samples we used : for the white wines (0.5 vol. of thioacidolysis solution / 1 vol. of wine), for the red wines (1 vol. of solution/ 1 vol. of wine) and for the sweet wines (2.5 vol. of solution/ 1 vol. of wine). We then used the thioacidolysis method described elsewhere (cf. IV).

VII - MEASUREMENT OF THE LIGNINS IN VERY OLD WINES (VIVAS *et al.*, 1998b)

When the wine presents a large amount of deposit, the lignins must be collected from the deposit after centrifugation and dialysis.

Over time, a slow precipitation of the majority of the phenolic compounds takes place. We collect the insoluble phenolic fraction at the bottom of the bottle by centrifugation (10,000 rpm, 10 min.). The elimination of the substances with a low molecular weight that may be present in the precipitate is then carried out by dialysis against 40 l of deionized distilled water. The precipitate is freeze-dried and 50 mg is used to determine the presence of lignins. We use the thioacidolysis method described elsewhere (cf. IV).

RESULTS AND DISCUSSION

I - CHARACTERISATION OF THE EXTRACTABLE LIGNINS IN WOOD : LE AND LD

1) Study by thioacidolysis and GC/MS

Table 1 shows the percentage of identified thioacidolysis products (figure 1). We note that the results of identification and the general look of the chromatograms are identical for both species and both series of samples. Peaks 1 and 2 were attributed to products of thioacidolysis of the xylanes. They essentially come from the xylose units of these polysaccharides. These units are considered as impurities. In the different fractions we find exclusively guaiacyl and syringyl units in variable proportions, depending on the fraction under consideration. The lignins in the heartwood of *Q. robur* and *Q. petraea* belong to the guaiacyl-syringyl lignin group (GS lignins).

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Table I
Principal thioacidolysis products of the lignin fractions of oak heartwood
(Results expressed as % of total identified thioacidolysis products)

Chromato-graphic peak†	Identified products (GC/MS)‡	*Q. robur*		*Q. petraea*	
		LE	LD	LE	LD
	Products of thioacidolysis of xylanes	**22,7**	**22,6**	**22**	**23,6**
1	CHR2-CH2R	19,3	18,4	19,5	19,7
2	CHR2-CHR-CH2R	3,4	4,2	2,5	3,9
	Products of thioacidolysis of lignins	**77,3**	**77,4**	**78**	**76,4**
	Syringyl structures	*37,8*	*48,5*	*40,6*	*44,8*
3	SCHR2	0,8	0,5	0,3	0,7
4	SCH2-CHR2	1	2,8	1,7	2,5
5 et 6	SCHR-CHR-CH2R (erythro/threo)	29,5	33,1	30,7	26,9
7	SCH2-CHR-CHR2	6,5	12,1	7,9	14,7
	Guaiacyl structures	*39,5*	*28,9*	*37,4*	*31,6*
8	GCH2-CHR2	0	1,4	0	2,1
9	GCHR-CHR-CH2R (erythro/threo)	32,1	6	29,2	5,8
10 et 11	GCH2-CHR-CHR2	7,4	21,5	8,2	23,7
	S/G	**0,95**	**1,67**	**1,08**	**1,41**

† cf. VIVAS *et al.*, 1998 ; ‡ cf. figure 1

On the chromatograms of the different fractions, peaks 10, 11 and 5, 6 are the highest and correspond to the principal thioacidolysis products ; they are attributed respectively to the erythro-threo forms of the guaiacyl and syringyl monomers of the lignins. Peak 3 (S form) comes from the end benzaldehyde groups, and disappears by reduction with NaBH4. Peak 4 (S form), present in low proportions in LE and LD, originates from a minor pathway of the reaction which provokes an elimination of the γ-hydroxymethyl groups (ROLANDO *et al.*, 1992) ; this also occurs in a secondary manner during acidolyses (LUNDQUIST, 1976). LD is distinguished from the two other fractions by an increase in the S/G ratio, linked to a reduction in the guaiacyl alkyl aryl ethers (peaks 10, 11) and an increase in their syringyl counterpart (peaks 5, 6 and especially 7). The increase in the G/S ratio is, however, limited by the increase of one of the guaiacyl forms (peak 9). Peak 8 (G form) is absent or not intense enough to be identified in LE. The S/G ratio is close to 1 for LE.

Quantitatively, the comparison of the areas of the different peaks on chromatograms of the same scale, using hexacosane (C26) as the internal calibre, shows that LD is the richer fraction in degradation products. It can be estimated at between 15 and 20 % more than LE. As we had no pure monomer forms we could not quantify the G and S forms in the different fractions. Study by NMR will enable more detailed structural knowledge of the fractions analysed.

2- Study by one- and two-dimensional NMR

Structure of the side chains. On figures 2 and 3, two types of bonds can be observed : β-O-4 and β-β (figure 4). For the β-O-4 bonds, we distinguish the 2 spots which are due, respectively, to the HCα, HCβ and the 2 protons, noted H and H', of the Cγ. The β-O-4 bonds exist in two forms, erythro and threo, demonstrated on the

1D ^1H spectrum ; the HCα has a δ which is close to 6.0 ppm for the erythro form and close to 6.1 ppm for the threo form. The β-β structures present 4 spots attributed to the HCγ and H' Cγ, indicating an axial and equatorial configuration of the bond, tallying with the data from the literature (EDE *et al.*, 1990). The resonance of the axial and equatorial forms is situated in a crowded zone of the spectrum, situated at around 4.5 ppm.

Substitution of the aromatic unit. We attributed, using data taken from the literature (LAPIERRE *et al.*, 1986a ; EDE *et al.*, 1990), the signals on the 1D spectrum of the ^1H of the syringyl unit (δ 6.6 ppm) and guaiacyl unit (δ 6.9 – 7.05 ppm) ; as well as the ^{13}C, the chemical displacements of which are distinct (δ 104 ppm for C2 and C6 and 153 ppm for C3 and C5 of the syringyl units and 112 ppm for C2, 119 ppm for C5 and 123 ppm for C6 of the guaiacyl units).

Quantitative structural analysis. With the attribution of all the protons and carbons of the two lignin fractions carried out (table 2), it is possible to quantify certain struc-

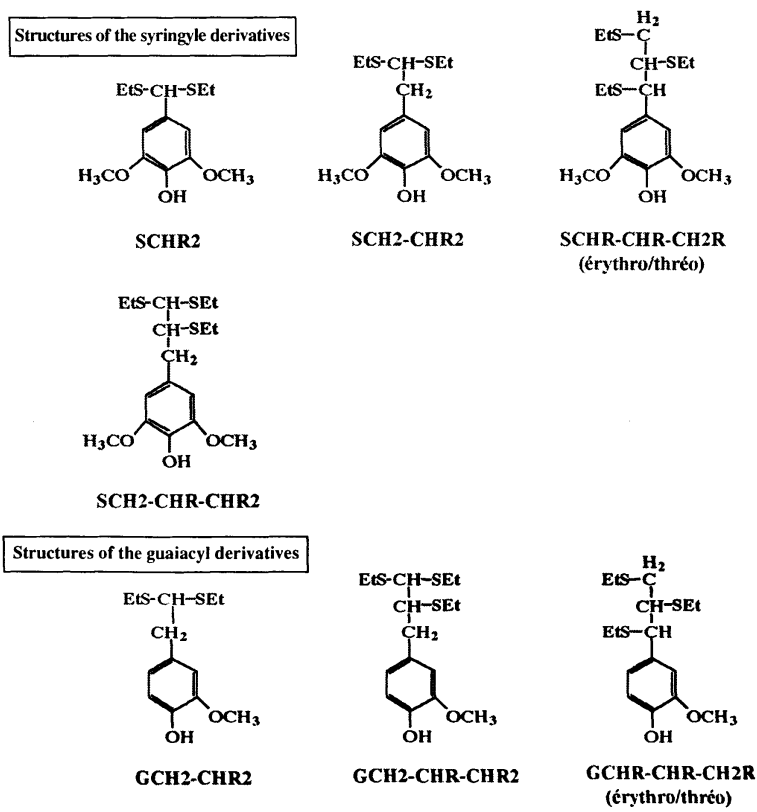

Figure 1 – Structure of the products of thioacidolysis identified in the lignin fractions analysed

(EtS : ethanethiol ; S : syringyl units ; G : guaiacyl units ; R : Ets)

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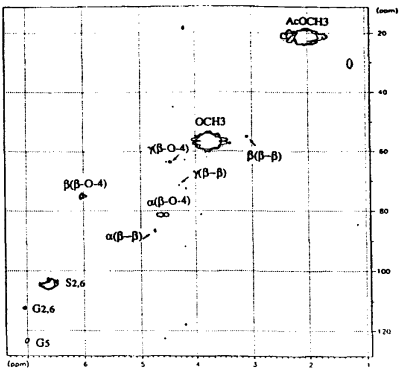

Figure 2 – Detail of the 2D HMQC map for LD

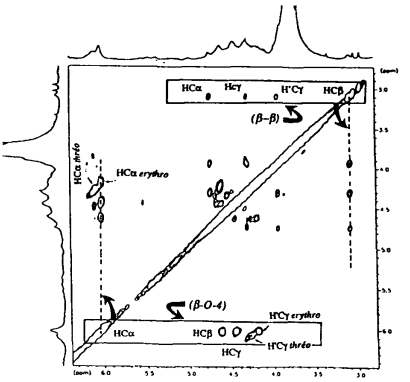

Figure 3 – Detail of a TOCSY experiment on LE

tural aspects of the molecule. LE has a lower S/G ratio than LD (1.1 and 2.7 respectively for LE and LD). This indicates that LE is a lignin with a balanced proportion of S and G units ; LD, on the other hand, presents a preponderance of S units. The β-O-4 erythro/threo ratio is equal to 1 for LE and LD. The β-O-4/G and β-β/G ratios are respectively 1.3 and 0.05 for LE and 1.5 and 0.2 for LD.

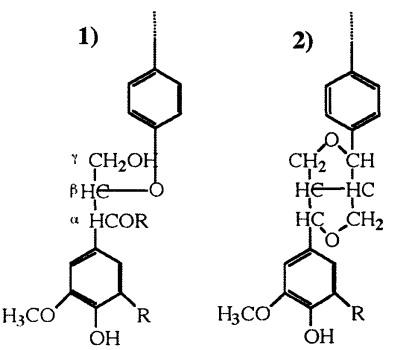

Figure 4 – Principal phenolic units and intermonometric bonds of the lignins in oak wood

(1 : β-O-4 aryl-ether bonds ; 2 : β-β ether « pinoresinol » bond ; R = H guaiacyl unit ; R : OCH_3 syringyl unit)

With knowledge on the one hand of the molar mass of LE and LD, determined by mass spectrometry in positive LSIMS mode (respectively 23 kDa and 45 kDa for LE and LD ; EDE *et al.*, 1990), and on the other of the different ratios obtained from the NMR data cited above, it is possible to calculate the approximate number of each structural element identified. Using this approach, we propose the structural characteristics for the two lignin fractions LE and LD shown in table 3. The ratio (G + 2.S)/OCH3 is close to 0.8 for LE and LD. Theoretically, it should be 1 (1 OCH3 per G unit and 2 per S unit). The estimation of the structure of the molecule is therefore carried out with a 20 % error, which is an acceptable deviation for a macromolecule.

II - DEMONSTRATION AND CHARACTERISATON OF THE SOLUBLE LIGNINS IN WINES

1 - Demonstration of lignins in wine samples

Table II
NMR attribution of the ^{1}H and ^{13}C of the lignin fractions LE and LD
(Chemical shefts d are given in ppm with TMS as reference.)

N°	δ (ppm)		Attributions†
	^{1}H	^{13}C	
1	—	171	COOH of the acetyles
2	—	153	S3,5
3	7.0	123	G6
4	6.9-7.0	119	G5
5	7.05	112	G2
6	6.6	104	S2,6
7	6.0	74	α(β-O-4)*
8	4.75	86	α(β-β)
9	4.6	81	β(β-O-4)*
10	3.9-4.3	72	γ(β-β)#
11	4.1-4.4	63	γ, γ(β-O-4)*
12	3.7	56	OCH3
13	3.1	54	ß(ß-ß)
14	2.3	21	CH3 of the phenolic acetyles
15	2.0	21	CH3 of the acetyles of the aliphatic chains

* with two erythro/threo ; # axial and equatorial ; † cf. figures 2 et 3

Table III
Determination of the proportion of the different elements composing LE and LD lignins
(S : syringyl unit ; G : guaiacyl unit ;
β-O-4 : aryl ether β-O-4 bonds of the side chains ; β-β : C-C bonds of the side chains)

		LE	LD
Side chains	nß-0-4*	10	9
	ß-0-4 erythro/threo	1,0	1,0
	nß-ß*	0,3	1
Aromatic units	nS*	8	16
	nG*	7	6
	S/G†	1,1	2,7
	ß-0-4/G	1,3	1,5
	ß-ß/G	0,05	0,2

* number of β-O-4, β-β bonds and aromatic G and S units calculated from the Mw determined by LSIMS (Mw : LE = 2900, LD = 3900)
† ratio of syringyl/guaiacyl units

It should be noted that for wines that have not been in prolonged contact with wood, the chromatograms do not present the typical lignin products.

Analyses of the thioacidolysates after derivation (trimethylsyllilation) give relatively few peaks identified by GC-MS (figure 5). Using mass spectra recorded with electronic impact, we found two structures originating from the monomehtoxylated units (guaiacyls G) and two from the dimethoxylated units (syringyls S) of the lignins. The spectra recorded for these molecules conform to those recorded for pure lignins

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and the data taken from the literature (ROLANDO *et al.*, 1992). Products 1 and 2 are each presented in the form of two peaks corresponding to their respective erytro/threo forms. The only molecules formally identified as products of thioacidolysis of the lignins are typical of the β-O-4 bonds in the lignins.

The presence of type SG lignins is characteristic of the wood of angiosperms. The identification of these different products of mono- and dimethoxylated thioacidolysis indicates that the wines have stayed in contact with wood for several months. Indeed, although the ellagitannins are very rapidly solubilised, the dissolution of the lignins requires a long stay in the barrels and therefore constitutes an indication of an ageing phase of a few months.

2 – Comparison of the soluble lignins in different wines

We applied the thioacidolysis method to the samples of the collected wines. The wines aged in new barrels all present significant lignin contents. The ratio between the S and G derivatives was calculated (table 4) ; it indicates that all the wines

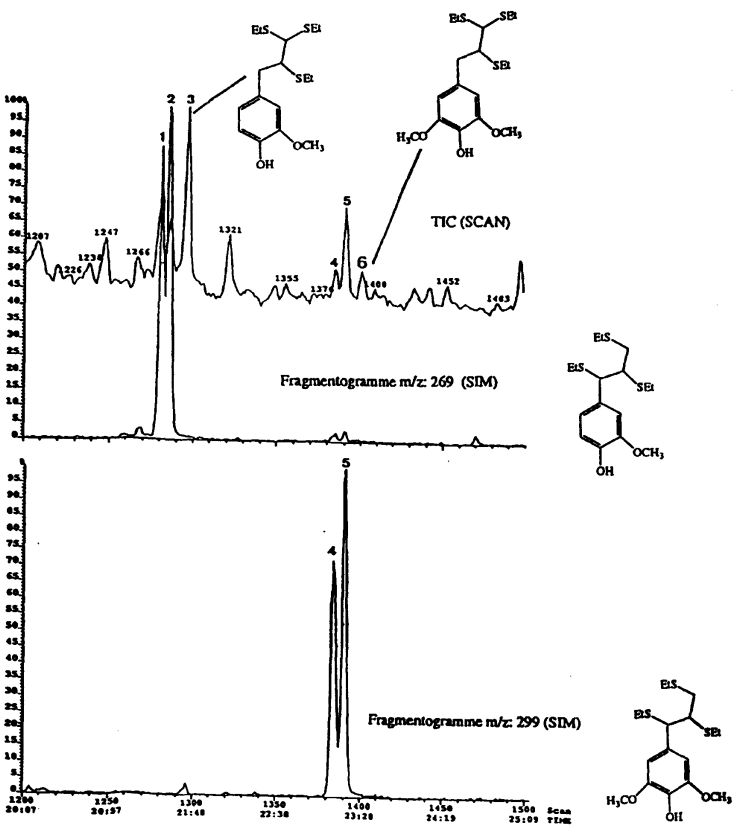

Fig. 5 – Chromatograms of trimethylsyllilated forms originating from the thioacidolysis of soluble lignin fractions in a sample of red wine

Table IV
Application of the thioacidolysis method to different wine samples
Calculation of the S/G ratio

Samples	Dry white wines (n = 18)	Young red wines (n = 14)	Old red wine (1868)	Sweet white wines (n = 3)
S/G	0,5 ±0,12	0,45 ±0,17	0,42	0,47 ±0,08

present quite a similar composition in S and G derivatives of around 0.5. The lignin fraction, therefore, extracted in a hydroalcoholic medium, is characterised by a high proportion of monomethoxylated G units. In contrast to previous work, VIRIOT *et al.* (1993) showed that for eaux-de-vie, a mainly alcoholic medium, the S/G ratio is close to 1, sometimes slightly higher (max S/G : 1.2). These results suggest differences in the extraction conditions, depending on the proportion of water and ethanol.

CONCLUSION

The lignins of high molecular weight, originating from oak heartwood (LD in our study), are rich in syringyl units. This tallies with the results obtained by JOUIN *et al.* (1988) for *Q. robur*, those by LAPIERRE (1986) for *Populus sp.* and the more general ones by ROLANDO *et al.* (1992) for *Populus euramerica*, *Pinus pinaster* and *Picea abies*. The Klason lignin corresponding to the most reticulate form of lignins is richer in syringyl units than LD (S/G : 2.10 according to JOUIN *et al.* (1988), against S/G : 1.4 for LD). Conversely, the lignins of lower mass, which are potentially extractable during the ageing of wines and eaux-de-vie in barrels, are characterised by a balance between syringyl and guaiacyl units. It appears that LD is the purer lignin fraction, while LE, in that its extraction protocol is close to the conditions of wines and eaux-de-vie, is probably the lignin fraction which will be extracted and be found in wines and eaux-de-vie.

1D and 2D homo and heteronuclear ^{1}H and ^{13}C NMR study confirms previous interpretations. It also enables us to further the structural study of lignin fractions. High molecular mass prevents us from leading to an exact structure ; but the application of quantitative NMR (ROBERT and GAGNAIRE, 1980 ; GAGNAIRE and ROBERT, 1982) gives access to the monomeric composition and to the principal types of bonds : LE is a GS lignin and LD is an S lignin with a majority of β-O-4 bonds between the elementary phenolic units. In future, the synthesis of ^{13}C-enriched oak lignins should be envisaged in order to provide more detailed structural information. The interest of such an approach is strengthened by the fruitful results obtained on poplar lignin (MONTIES *et al.*, 1982 ; LAPIERRE *et al.*, 1984).

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BIBLIOGRAPHY

BRICOUT J., 1971. Analyse de quelques constituants dérivés du chêne dans les vieilles eaux-de-vie d'Armagnac. *Ann. Technol. Agric.*, **20**, (3), 217-223.

BROWNING B.L. and ISENBERG I.H., 1952. *In : Wood chemistry*. L.E. Wise and E.C. Jahn (Eds.), Academic press, New York, 1259.

CATESSON A.M., 1980. Les tissus végétaux. Ultrastructure, biogenèse. *In : Les polymères végétaux : polymères pariétaux et alimentaire non azotés*. B. Monties (Ed.). Bordas, Paris, 1-29.

CAUMIEL M., 1983. *Le Cognac*. Pour la science. 48-56.

EDE R.M., BRUNOW G., SOMOLA L.K. et LEMMETYINEN J., 1990. Two-dimensional ^{1}H-^{1}H chemical shift correlation and J resolved NMR studies on isolated and synthetic lignins. *Holzforschung*, **44**, 95-101.

FAIX O., 1991. Classification of lignins from different botanical origins by FT-IR spectroscopy. *Holzforschung*, **45**, Suppl., 21-27.

FENGEL D. et G. WEGENER, 1984. *Wood : Chemistry, ultrastructure, reaction*. De Gruyter, Berlin, 58 p.

GUYMON J.F. et E.A. CROWELL, 1968. Separation of vanillin, syringaldehyde and other aromatic compounds in the extracts of French and American woods by brandy and aqueous solution. *Qual. Plant. Mat. Veget.*, **16**, 320-333.

HARDELL W.E., G.J. LEARY, M. STOLL et U. WESTERMARK, 1980. Variation in lignin structure in defined morphological part of service. Variation in lignin structure in defined morphological parts of birch. *Svensk. papperstid.*, **83**, 44-49 and 71-74.

JACQUIOT C., Y. TENARD et D. DIROL, 1973. *Atlas d'anatomie des bois des angiospermes. Essences feuillues*. Tome I et II. CTB-CNRS, Paris.

JOUIN D., M.-T. TOLLIER and B. MONTIES, 1988. Lignification of oak wood. I- Lignin determination in sapwood and heartwood. *Cellulose Chem. Technol.*, **22**, 231-243.

KLAUDITZ W., 1952. Zur biologisch-mecanischen wirkung des lignins in stammholz der nadel und laubhör. *Holzforschung*, **6**, 70-82.

KLAUDITZ W., 1957. Zur biologisch-mechanischen wirkung des lignins in stammholz der nadel und laubhözer. *Holzforschung*, **11**, 110-116.

LAI Y.Z. et K.V. SARAKANEN, 1971. *In :Lignins : Occurence, formation, structure, reactions*, Chap. 5. Sarakanen K.V. and Ludwig C.H. (Eds.), p 150.

LAPIERRE C., 1986. Hétérogénéité des lignines de peuplier : Mise en évidence systématique. *Thèse doctorat d'état*. Université Paris-sud, 305 p.

LAPIERRE C., B. MONTIES et C. ROLANDO, 1986a. Thioacidolysis of poplar lignins : Identification of monomeric syringyl products and characterization of guaïacyl-syringyl lignin fraction. *Holzforschung*, **40**, 113-118.

LAPIERRE C., B. MONTIES et C. ROLANDO, 1986b. Preparative thioacidolysis of spruce lignin : Isolation ans identification of main monomeric product. *Holzforschung*, **40**, 47-51.

LUNDQUIST K., 1976. Low-molecular weight lignin hydrolysis products. *Appl. Polymer. Symp.*, **28**, 1393-1407.

MONTIES B., 1980. Les lignines. *In : Les polymères végétaux. Polymères pariétaux et alimentaires non azotés*. Monties B. (Ed.), Bordas, Paris, 122-155.

MONTIES B., 1992. Composition chimique des bois de chêne : Composés phénoliques, relations avec quelques propriétés physiques et chimiques susceptibles d'influencer la qualité des vins et des eaux-de-vie. *In : Le bois et la qualité des vins et des eaux-de-vie*. Guimberteau G., ed. Vigne et Vin Publications Internationales, Bordeaux, 59-72.

PUECH J.-L., 1978. Vieillissement des eaux-de-vie en fûts de chêne. Extraction de la lignine et de ses produits de dégradation. *Thèse*, Université Paul Sabatier, Toulouse.

PUECH J.-L., 1984. Characteristics of oak wood and biochemical aspects of armagnac ageing. *Am. J. Enol. Vitic.*, **35**, 77-81.

PUECH J.-L., 1992. Influence du bois de chêne sur les caractéristiques analytiques des eaux-de-vie. *In : Le bois et la qualité des vins et des eaux-de-vie*. Guimberteau G. (Ed.), Vigne et Vin Publications Internationales, Bordeaux, 123-134.

PUECH J.-L., R. LEAUTE, G. GLOT, L. NONDEDEU et H. MONDIES, 1982. Étude de la lignine et de ses produits de dégradation dans les eaux-de-vie de Cognac. *Bull. Groupes Polyphénols*, **11**, 605-611.

ROLANDO C., B. MONTIES et C. LAPIERRE, 1992. Thioacidolysis. *In : Methods in lignins chemistry*. Lin S.Y. and Dence

C.W. (Eds.), Springer-Verlag, Berlin, 334-349.

ROWELL R., 1984. The chemistry of solid wood. *Adv. Chem. Ser. ACS 207*, 1-614.

SACHSSE H., 1984. *Einheimische nutzhölzer und ihre bestimmung nach makroskopischen merkmalen*. Pareys Studientexte 44, Verlag Paul Parey, Berlin, 160 p.

SINGLETON V.L., 1974. Some aspects of wooden container as a factor in wine maturation. *In : Chemistry of wine making*. ACS serie 137, Webb A.D. (Ed.), 311 p.

SINGLETON V.L., 1995. Maturation of wines and spirits : Comparisons, facts, and hypotheses. *Am. J. Enol. Vitic.*, **46**, 98-115.

STEVENS W.C. et N. TURNER, 1970. *Wood bending handbook*. Her majesty's stationery office, London, 109 p.

TARANSAUD J., 1976. *Le livre de la tonnellerie*. La roue à livres diffusion, Paris.

VIRIOT C., A. SCALBERT, C. LAPIERRE et M. MOUTOUNET, 1993. Ellagitannins and lignins in ageing of spirits in oak barrels. *J. Agric. Food Chem.*, **41**, 1872-1879.

VIVAS N., Y. GLORIES, B. DONECHE et E. GUEHO, 1991a. Observations sur la microflore du bois de chêne au cours de son séchage naturel. *Ann. Sci. Nat. Bot.* (Paris), **11**, 149-153.

VIVAS N., GLORIES Y. et FRANÇOIS J., 1991b. Mise au point sur l'élevage des vins rouges en fût de chêne. *Rev. Œnol.*, **17**, 62, 17-21.

VIVAS N., 1995. La qualité du bois de chêne et son utilisation pour la vinification et l'élevage des vins. *J. Sci. Tech. Tonnellerie*, **1**, 1-16.

VIVAS N., BOURGEOIS G., VITRY C. et GLORIES Y. 1997. Characterisation by thioacidolysis of lignins from red wines aged in oak barrels. *In « In Vino Analytica Scientia »*, 208-211.

VIVAS N., PIANET I., BOURGEOIS G., VITRY C., SERVENS C. et GLORIES Y., 1998a. Lignin fractions from *Quercus robur* L. and *Quercus petraea* (Matt) Liebl., the main oak species used for barrel making. *Am. J. Enol. Vitic.*, **49**, 49-55.

VIVAS N., BOURGEOIS G. et GLORIES Y., AUGUSTIN M. et VITRY C., 1998b. Détection CPG/SM des biomarqueurs spécifiques de l'élevage des vins en barriques. *Analusis*, **26**, 88-92.

WALKER F.S., 1978. Pedunculate and sessile oaks : Species determination from differences in their wood. *In : Dendrochronology in Europe*. Fletcher J. (Ed.), British archeological reports, series 51, 329-338.