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# Comparison of direct and indirect methods of measuring the precursors of β-methyl-γ-octalactone and their application to the analysis of Sessile oak wood [*Quercus petraea* (Matt.) Liebl.]

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**Abstract**

A new indirect method for measuring the level of β-methyl-γ-octalactone precursors in oak wood by GC–MS is described. This level is calculated from the difference between the amount of free β-methyl-γ-octalactone and the amount formed after hydrolysis and lactonization. It is compared to the level of a precursor of *cis*-β-methyl-γ-octalactone, a 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid, determined directly by HPLC. These two methods are applied to 12 powdered samples of Sessile oak wood and the results show that the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid is by far the most abundant precursor of β-methyl-γ-octalactone in this wood. 

*Keywords:* Wood; *Quercus petraea*; Methyloctalactone; Lactone; Glucopyranosyloxymethyloctanoic acid

## 1. Introduction

Identified for the first time in whisky by Suomalainen and Nykänen [1] and then in oak wood by Masuda and Nishimura [2], β-methyl-γ-octalactone (also called whisky or oak lactone) is one of the main volatile compounds of oak wood extracted by barrel-aged wines and brandies [3–5]. Of the four β-methyl-γ-octalactone isomers, only the forms 3*S*,4*S* (*cis*) and 3*S*,4*R* (*trans*) are found in oak wood [6–9]. These two isomers, possessing a characteristic aroma of coconut, celery and fresh wood, have different flavour perception thresholds. The values obtained from a racemic mixture in white wine were 92 μg/l for the *cis* isomer and 460 μg/l for the *trans* isomer [10]. Detection thresholds in air determined by gas chromatography (GC)–sniffing of a racemic mixture were 1 μg/l for the *cis* form and 20 μg/l for the *trans* form [11].

The level of β-methyl-γ-octalactone increases in wood extracts that are heated in a strong acidic medium and this indicates the existence of a precursor in oak wood [5].

During the air-drying of oak staves, Chatonnet et al. [12] observed an increase in the levels of *cis*- and *trans*-β-methyl-γ-octalactone and a change in the relative abundance of the two isomers in favour of the more odorous *cis* form. Sefton et al. [13] showed

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that the level of β-methyl-γ-octalactone increases or decreases as a function of the origin of the oak, the drying site and season. These different authors explain the formation of β-methyl-γ-octalactone by the presence of one or several precursors and the loss of β-methyl-γ-octalactone by evaporation or leaching.

The structure of a precursor to β-methyl-γ-octalactone isolated from oak wood, 3-methyl-4-(3′,4′-dihydroxy-5′-methoxybenzoyloxy)octanoic acid, was proposed by Otsuka and co-workers [14,15]. However, neither the structure nor the presence of this compound in oak wood has been confirmed.

Based on the work of Otsuka and co-workers [5,14,15], Chatonnet [10] developed an indirect method of measuring the precursor(s) of β-methyl-γ-octalactone. The level of precursors was determined from the difference between the level of free β-methyl-γ-octalactone and total β-methyl-γ-octalactone (after hydrolysis and lactonization in a hot acid medium). In this article we will show that the proposed experimental protocol gives inaccurate results and we present an alternative method based on the same principle but carried out under more appropriate conditions.

We recently showed that a precursor of *cis*-β-methyl-γ-octalactone, the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid (Fig. 1) identified from the wood of *Platycarya strobilacea* Sieb. et Zucc. by Tanaka and Kouno [16], is also found in Sessile oak wood [*Quercus petraea* (Matt.) Liebl.], a European white oak species used for cooperage [17]. In this article we will describe a simple and rapid method of measuring this precursor by high-performance liquid chromatography (HPLC).

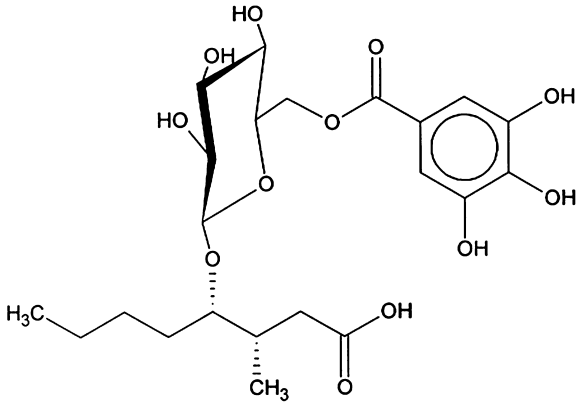


Fig. 1. A precursor of β-methyl-γ-octalactone: the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid.

These direct and indirect methods will subsequently be compared using 12 powdered samples of Sessile oak wood.

## 2. Material and methods

### 2.1. Chemical products and solvents

A mixture of *cis* and *trans* racemic β-methyl-γ-octalactone, racemic γ-decalactone, racemic nonan-4-ol and racemic octan-3-ol (all from Aldrich), methanol, chloroform, ethanol, diethyl ether, hydrochloric acid and sulfuric acid (all from Merck), pentane, dichloromethane and anhydrous sodium sulfate (all from Carlo Erba), potassium hydroxide and orthophosphoric acid (both from Prolabo) were used.

The 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid was extracted from the wood of Sessile oak A (see below) using methanol and then isolated by semi-preparatory HPLC as previously described [17].

### 2.2. Plant material

Twelve Sessile oaks [*Quercus petraea* (Matt.) Liebl.] about 200 years old were selected in November 1995 and November 1996 from the forest of Bercé (Sarthe, France). The species was determined from the morphological characteristics of twigs, leaves and acorns collected from each of the 12 trees [18].

### 2.3. Sampling

The 12 trees, identified by the letters A to L, were cut into staves having dimensions of 105×8 cm to 13×3 cm, destined for the manufacture of barrels. From each oak, a stave about 2.5 m up the tree bole was placed in a freezer at −18°C immediately after being cut. Care was taken to ensure that each of the

staves studied came from the same wood zone of the cut logs.

A sample about 15 cm long was taken from the middle of each of the 12 staves and then ground into a powder with a particle size of less than 0.5 mm.

### 2.4. Measuring wood moisture content

The percentage moisture content of wood powders was determined by the difference in the mass of fresh wood and wood dried in an over at 103°C for 24 h.

### 2.5. Indirect quantitative determination of the precursors of β-methyl-γ-octalactone

#### 2.5.1. Extraction of total β-methyl-γ-octalactone

In an Erlenmeyer flask, 20 ml of methanol and 4 ml of potassium hydroxide in methanol (742.6 mg/l) are added to 2 g of wood powder. After being agitated for 1 h, 48 ml of 12 *M* sulfuric acid is cautiously added to the mixture, and the extraction continues under agitation for another 2 h. The internal standard, γ-decalactone, is then added (106.8 μg). After filtration through a glass fibre filter, three successive extractions using 40, 20 and 20 ml of a pentane–dichloromethane (2:1) mixture are carried out in a separatory funnel. The organic phases are combined, dried with anhydrous sodium sulfate and then the extract is concentrated down to about 1 ml using a Vigreux column (water bath temperature of 36°C). A second standard (114.2 μg of nonan-4-ol) is then added in order to determine the proportion of the internal standard that is recuperated.

#### 2.5.2. Extraction of free β-methyl-γ-octalactone

In an Erlenmeyer flask, 24 ml of methanol is added to 2 g of wood powder. After 1 h under agitation, 48 ml of water is added to the mixture and extraction continued under agitation for another 2 h. The extraction protocol for free β-methyl-γ-octalactone is then carried out as described above.

#### 2.5.3. Identification and quantification of the cis and trans isomers of β-methyl-γ-octalactone by gas chromatography–mass spectrometry

A Hewlett-Packard 6890 Series chromatograph was equipped with a DB WAX capillary column (30 m×0.25 mm, 0.5 μm; J&W Scientific, USA) and an ''on column'' injector (30 to 250°C at 180°C/min). The helium gas vector flow was maintained at 1 ml/min throughout the analysis. The oven temperature program was as follows: 30 to 100°C at a ramp rate of 70°C/min, constant for 2 min, 100°C to 200°C at a rate of 3°C/min, 200°C to 245°C at 45°C/min and then constant for 30 min.

A Hewlett-Packard 5973 mass spectrometer was used. Ionisation was achieved under the electron impact mode (ionisation energy of 70 eV), the source, transfer line and quadrupole temperatures were 250°C, 230°C and 106°C, respectively.

Detection was carried out in scan mode (*m*/*z* 35 to *m*/*z* 350 a.m.u.) for the identification of compounds and in the selected ion monitoring (SIM) mode for their quantification.

The *cis* and *trans* isomers of β-methyl-γ-octalactone are identified by co-injection of the synthetic products. The ions chosen in the SIM mode for the calculation of response factors and quantification are: *m*/*z* 99 (*cis*- and *trans*-β-methyl-γ-octalactone), *m*/*z* 85 (γ-decalactone) and *m*/*z* 55 (nonan-4-ol) due to their relatively high abundance. Two other ions per compound are used as qualifiers: *m*/*z* 71 and 87 (*cis*- and *trans*-β-methyl-γ-octalactone), *m*/*z* 55 and 128 (γ-decalactone) and *m*/*z* 73 and 83 (nonan-4-ol).

The chromatography response factors, of β-methyl-γ-octalactone with respect to γ-decalactone and γ-decalactone with respect to nonan-4-ol, are calculated from a range of standard concentrations and verified before each measurement using a standard solution. After calculating the extraction yield of γ-decalactone, the levels of free and total β-methyl-γ-octalactone are determined and the level of the precursors expressed as μg of β-methyl-γ-octalactone released per g of dry wood.

### 2.6. Quantitative determination of a precursor of cis-β-methyl-γ-octalactone: 6′-O-gallate derivative of (3S,4S)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid

#### 2.6.1. Extraction of the precursor

The 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid is extracted from 1 g of wood powder by 10 ml of methanol during 12 h in a stoppered Erlenmeyer

flask placed on a table of agitation. The solution is filtered and then analysed by HPLC.

*2.6.2. Quantification of the precursor by HPLC*

The HPLC system consisted of Millipore–Waters (Milford, MA, USA) components: two Waters 510 pumps, a Waters 717 automatic injector, an oven and a Waters 490E multiple-wavelength detector. A LiChrospher RP18 endcapped (Merck, Darmstadt, Germany) column (250 mm×4 mm, 5 μm) and pre-column (4 mm×4 mm, 5 μm) were maintained at 28°C. Solvent A: water–$H_3PO_4$ (999:1) and solvent B: $CH_3OH$–water (50:50) were used for the following elution gradient: 50% of solvent B for 5 min, 50 to 80% over 5 min, 80% for 5 min, 80 to 100% over 10 min then 100% during 10 min. The flow was 0.8 ml/min and detection was carried out at 272 nm.

The chromatographic response factor of the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid is established from three standard concentration ranges of the pure product isolated as previously described [17].

## 3. Results and discussion

### *3.1. Indirect quantitative determination of the precursors of β-methyl-γ-octalactone*

The level of β-methyl-γ-octalactone precursors is determined from the difference between the levels of free β-methyl-γ-octalactone and total β-methyl-γ-octalactone (after hydrolysis and lactonization).

The method described by Chatonnet [10] (method 1) (Fig. 2) was applied to a synthetic solution of β-methyl-γ-octalactone. The results are shown in Table 1. The amount of free β-methyl-γ-octalactone is around 26% less than the initial level of the solution. After hot acid hydrolysis of the solution, this undere-

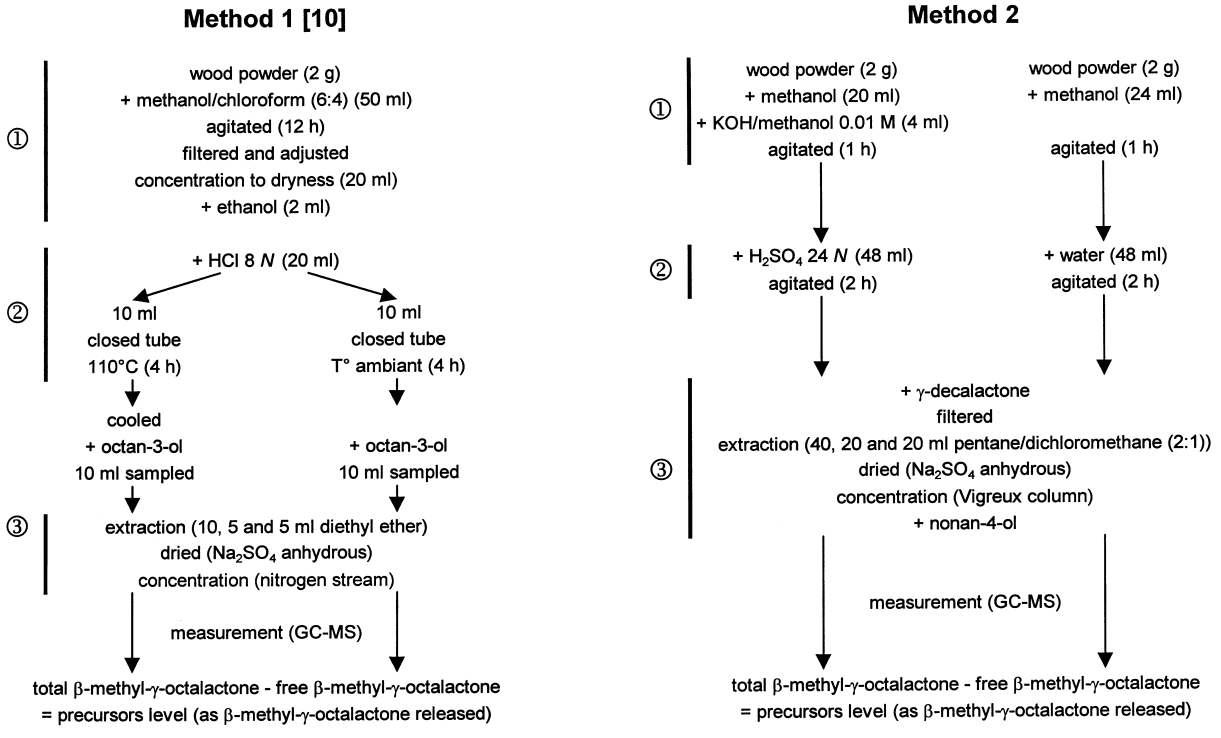


Fig. 2. Parallel presentation of two indirect methods of measuring the precursors of β-methyl-γ-octalactone. Method 1 is the protocol proposed by Chatonnet [10] and method 2 is a new protocol that we propose in this study.

Table 1
Recovery (%) and variation of the *cis*/*trans* ratio of a synthetic β-methyl-γ-octalactone solution[a] using the published indirect method [10] (method 1) and the new one (method 2) for measuring free and bound β-methyl-γ-octalactone presented in Fig. 1

| | Analysis conditions of free β-methyl-γ-octalactone | | | | Analysis conditions of total β-methyl-γ-octalactone | | | |
|---|---|---|---|---|---|---|---|---|
| | Method 1 | | Method 2 | | Method 1 | | Method 2 | |
| | Recovery (%) | *cis*/*trans* ratio | Recovery (%) | *cis*/*trans* ratio | Recovery (%) | *cis*/*trans* ratio | Recovery (%) | *cis*/*trans* ratio |
| Analysis 1 | 71 | 1.2 | 97 | 1.3 | 67 | 0.5 | 94 | 1.2 |
| Analysis 2 | 71 | 1.2 | 98 | 1.3 | 66 | 0.6 | 94 | 1.2 |
| Analysis 3 | 79 | 1.3 | 99 | 1.2 | 71 | 0.6 | 95 | 1.2 |

[a] The *cis*/*trans* ratio of the synthetic β-methyl-γ-octalactone used was 1.3.

stimation is of the order of 32% and a change in the isomeric ratio is also observed in favour of the more thermally stable *trans* isomer (the percentage of the *cis* isomer decreases from 55 to 37%).

The experimental protocol proposed by Chatonnet [10] leads therefore to inaccurate results. The alternative method (method 2) that we propose (Fig. 2) is based on the same principles but under more appropriate conditions. This new method was applied to the same synthetic β-methyl-γ-octalactone solution, and the results are summarised in Table 1. Unlike the results obtained from method 1 (Table 1), any loss of β-methyl-γ-octalactone or decline in the relative abundance of the *cis* isomer are minimal.

A comparison of the two methods, each divided into three consecutive steps, is presented in Fig. 2:

Step 1: Extraction of free β-methyl-γ-octalactone and precursors from oak wood. When the methanol–chloroform extract is concentrated down to dryness in method 1, there can be a loss of β-methyl-γ-octalactone by evaporation (up to 25% in our tests). This concentration is not carried out in method 2.

Step 2: Hydrolysis and lactonization of precursors. The new method separates the step of saponification (using potassium hydroxide in methanol) from those of hydrolysis and/or lactonization (in 12 *M* sulfuric acid). These reactions are carried out at room temperature. This limits the loss of β-methyl-γ-octalactone to about 6% and any change in the isomeric ratio (the decline in the percentage of the *cis* isomer is very small) (Table 1).

Step 3: Extraction of β-methyl-γ-octalactone and subsequent concentration and analysis by GC–MS. Concentration under a nitrogen gas flow increases the likelihood of β-methyl-γ-octalactone volatilisation (method 1). In the new experimental protocol (method 2), concentration using a Vigreux type column limits this loss. In addition, the use of two internal standards in this new method allows the estimation of extraction yields of β-methyl-γ-octalactone from that of the internal standard γ-decalactone which possesses a similar structure.

In order to confirm the results obtained from synthetic β-methyl-γ-octalactone solutions, the two methods were applied to the same oak wood powder (tree L). The results are shown in Tables 2 and 3. Method 1 underestimates the level of precursors by about 40% compared to the values obtained from method 2. Both parts of the protocol proposed by Chatonnet [10] give rise to significant errors in the final results: the levels of free and total β-methyl-γ-octalactone are underestimated by about 32 and 36%, respectively and there is a change in the isomeric ratio in favour of the *trans* isomer during acid hydrolysis (the percentage of the *cis* isomer changes from 92 to 70%).

The new method gives more reliable results. Despite several sources of possible error through the extraction and concentration procedures, the relative standard deviation (RSD) after repeating the analysis three times on the same wood powder was only 5% (Table 3).

Having isolated and identified a precursor of *cis*-β-methyl-γ-octalactone in Sessile oak wood, namely the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid [17], we developed a simple and rapid method of measuring this compound by HPLC so as to compare its levels with

Table 2
Application of the indirect method of measuring β-methyl-γ-octalactone precursors proposed by Chatonnet [10] (method 1) to an oak wood powder (tree L)

| Method 1 | Free β-methyl-γ-octalactone[a] | | | | Total β-methyl-γ-octalactone[a] | | | | Precursors[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *trans* | *cis* | % *cis* | *trans*+*cis* | *trans* | *cis* | % *cis* | *trans*+*cis* | *trans* | *cis* | % *cis* | *trans*+*cis* |
| Analysis 1 | 6.3 | 61.6 | 91 | 67.9 | 39.1 | 89.1 | 69 | 128.3 | 32.8 | 27.6 | 46 | 60.4 |
| Analysis 2 | 6.0 | 68.9 | 92 | 74.9 | 39.7 | 91.5 | 70 | 131.2 | 33.6 | 22.6 | 40 | 56.3 |
| Analysis 3 | 6.1 | 68.9 | 92 | 75.0 | 39.1 | 93.2 | 70 | 132.3 | 33.0 | 24.3 | 42 | 57.2 |
| Mean | 6.1 | 66.5 | | 72.6 | 39.3 | 91.3 | | 130.6 | 33.1 | 24.8 | | 58.0 |
| Standard error | 0.2 | 4.2 | | 4.1 | 0.3 | 2.0 | | 2.1 | 0.4 | 2.5 | | 2.1 |
| RSD (%) | 3 | 6 | | 6 | 1 | 2 | | 2 | 1 | 10 | | 4 |

[a] As μg per g of dry wood.
[b] As μg of β-methyl-γ-octalactone released per g of dry wood.

Table 3
Application of the new method of indirectly measuring the precursors of β-methyl-γ-octalactone (method 2) to an oak wood powder (tree L)

| Method 2 | Free β-methyl-γ-octalactone[a] | | | | Total β-methyl-γ-octalactone[a] | | | | Precursors[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *trans* | *cis* | % *cis* | *trans*+*cis* | *trans* | *cis* | % *cis* | *trans*+*cis* | *trans* | *cis* | % *cis* | *trans*+*cis* |
| Analysis 1 | 9.2 | 99.2 | 91 | 108.4 | 11.6 | 191.7 | 94 | 203.3 | 2.3 | 92.5 | 98 | 94.8 |
| Analysis 2 | 9.3 | 95.6 | 91 | 104.9 | 11.7 | 194.5 | 94 | 206.2 | 2.3 | 99.0 | 98 | 101.3 |
| Analysis 3 | 8.8 | 98.5 | 92 | 107.3 | 11.2 | 188.7 | 94 | 199.8 | 2.3 | 90.2 | 97 | 92.5 |
| Mean | 9.1 | 97.8 | | 106.9 | 11.5 | 191.6 | | 203.1 | 2.3 | 93.9 | | 96.2 |
| Standard error | 0.3 | 1.9 | | 1.8 | 0.3 | 2.9 | | 3.2 | 0.0 | 4.6 | | 4.6 |
| RSD (%) | 3 | 2 | | 2 | 2 | 2 | | 2 | 1 | 5 | | 5 |

[a] As μg per g of dry wood.
[b] As μg of β-methyl-γ-octalactone released per g of dry wood.

those of β-methyl-γ-octalactone precursors measured by our new indirect method 2.

### *3.2. Quantitative determination of a precursor of cis-β-methyl-γ-octalactone: the 6′-O-gallate derivative of (3S,4S)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid*

The precursor is measured by reversed-phase HPLC on a $C_{18}$ column. Detection occurs at the maximum absorbance wavelength for the compound: 272 nm (Fig. 3). The product isolated from the wood of tree A appears as two peaks representing 95% (*erythro* form) and 5% (presumed *threo* form) of the total area [17]. The response factor is determined from three standard concentration ranges of the isolated compound assuming a 95% purity.

Four extraction solvents were tested: the methanol

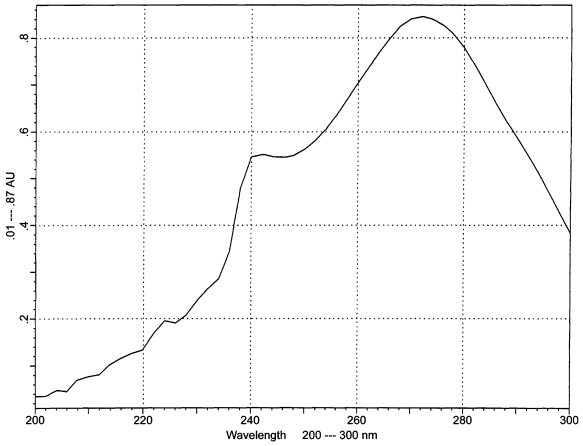


Fig. 3. UV spectra of the 6′-*O*-gallate derivative of 4-β-D-glucopyranosyloxy-3-methyloctanoic acid.

Table 4
Test of the repeatability of the method of measuring the 6′-*O*-gallate derivative (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid by HPLC (the levels[a,b] of this precursor were determined in fresh wood of Sessile oak A)

| | Level of precursor[a] | Level of β-methyl-γ-octalactone released[b] |
|---|---|---|
| Analysis 1 | 510.5 | 163.2 |
| Analysis 2 | 485.9 | 155.3 |
| Analysis 3 | 502.7 | 160.7 |
| Analysis 4 | 491.9 | 157.3 |
| Analysis 5 | 485.1 | 155.1 |
| Mean | 495.2 | 158.3 |
| Standard error | 11.1 | 3.5 |
| RSD (%) | 2 | 2 |

[a] As μg per g of dry wood.
[b] As μg of β-methyl-γ-octalactone that could be released from the precursor per g of dry wood.

used for the extraction and identification of the precursor from the wood of tree A [17], the methanol–chloroform (6:4) mixture used in the methodology proposed by Chatonnet [10], an acetone–water (7:3) mixture and ethanol–water (88:12) mixtures. These last two mixtures are often used for the extraction of wood phenolic compounds as the water causes the swelling of cell walls and thereby facilitates extraction [19]. After 6 h of extraction, the extraction yields (expressed as a percentage of the most efficient solution) of the solvents were: acetone–water: 100%, methanol: 95%, methanol–chloroform: 93% and ethanol–water: 71%. We chose methanol as the extraction solvent as it is also used in the new method of indirect quantification described previously (method 2) and carried out extractions over 12 h in order to obtain an extraction yield close to that obtained with acetone–water.

Using the chosen chromatographic conditions, it is not possible to quantify the *threo* form of the precursor as the retention time is very close to that of ellagic acid. In this study therefore only the *erythro* form is measured, which clearly appears to be the dominant form in the wood of the 12 Sessile oaks studied.

To test the repeatability of the method, the wood powder of tree A (Table 4) was analysed five times and the RSD obtained was 2%.

### *3.3. Application of the methods of quantification to 12 powders of Sessile oak wood*

The indirect method (method 2) of quantifying the precursors of β-methyl-γ-octalactone was applied to 12 powders of fresh wood taken from staves of trees A to L, the results being shown in Table 5.

The levels of β-methyl-γ-octalactone vary greatly among the 12 Sessile oaks studied. Trees G, H and K

Table 5
Level[b] of β-methyl-γ-octalactone precursors in fresh wood of 12 oaks determined by the new method of indirect measurement (method 2)

| Tree | Free β-methyl-γ-octalactone[a] | | | | Total β-methyl-γ-octalactone[a] | | | | Precursors[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *trans* | *cis* | % *cis* | *trans*+*cis* | *trans* | *cis* | % *cis* | *trans*+*cis* | *trans* | *cis* | % *cis* | *trans*+*cis* |
| A | 14.6 | 96.0 | 87 | 110.6 | 28.9 | 232.1 | 89 | 261.0 | 14.3 | 136.1 | 90 | 150.4 |
| B | 6.0 | 62.1 | 91 | 68.1 | 10.2 | 130.9 | 93 | 141.0 | 4.2 | 68.8 | 94 | 73.0 |
| C | 9.4 | 51.0 | 85 | 60.4 | 13.3 | 118.3 | 90 | 131.6 | 4.0 | 67.3 | 94 | 71.3 |
| D | 14.8 | 77.3 | 84 | 92.1 | 28.7 | 192.4 | 87 | 221.1 | 13.9 | 115.1 | 89 | 129.0 |
| E | 4.0 | 62.7 | 94 | 66.7 | 7.3 | 160.9 | 96 | 168.2 | 3.3 | 98.2 | 97 | 101.5 |
| F | 5.4 | 22.2 | 80 | 27.6 | 10.1 | 112.7 | 92 | 122.8 | 4.7 | 90.6 | 95 | 95.3 |
| G | 0.0 | 0.0 | – | 0.0 | 0.0 | 0.0 | – | 0.0 | 0.0 | 0.0 | – | 0.0 |
| H | 0.0 | 0.0 | – | 0.0 | 0.0 | 0.0 | – | 0.0 | 0.0 | 0.0 | – | 0.0 |
| I | 37.3 | 15.2 | 29 | 52.5 | 48.7 | 34.9 | 42 | 83.6 | 11.4 | 19.7 | 63 | 31.1 |
| J | 20.2 | 18.7 | 48 | 38.9 | 24.6 | 35.7 | 59 | 60.3 | 4.4 | 17.0 | 79 | 21.4 |
| K | 0.0 | 0.0 | – | 0.0 | 0.0 | 0.0 | – | 0.0 | 0.0 | 0.0 | – | 0.0 |
| L | 9.1 | 97.8 | 91 | 106.9 | 11.5 | 191.6 | 94 | 203.1 | 2.3 | 93.9 | 98 | 96.2 |

[a] As μg per g of dry wood.
[b] As μg of β-methyl-γ-octalactone released per g of dry wood.

contain neither any free β-methyl-γ-octalactone nor any β-methyl-γ-octalactone precursors. The levels of precursors in oaks A–F are high and greater than the level of free β-methyl-γ-octalactone possessed by these trees particularly in tree F, although in trees B and L these levels are similar. Contrary to all these trees, oaks I and J contain higher amounts of the *trans* than the *cis* isomer of free β-methyl-γ-octalactone and possess much lower levels of precursors.

However in all the trees analysed containing β-methyl-γ-octalactone, the isomer formed from the hydrolysis and lactonization of precursors is mainly the *cis* isomer. For those trees containing high levels of this isomer in the free form (oaks A–F and L), the proportion of *cis*-β-methyl-γ-octalactone formed from precursors is equally high, varying between 89 and 98% (Table 5). For trees possessing more of the *trans* than the *cis* isomer in free form (oaks I and J), the proportion of *cis*-β-methyl-γ-octalactone formed from precursors was lower although nonetheless dominant at 63% for tree I and 79% for tree J. A major change in the isomeric ratio of total β-methyl-γ-octalactone, in favour of the *cis* isomer, is therefore observed for these two trees and to a lesser extent for the other seven trees. This change in the isomeric ratio cannot be due to the hydrolysis and lactonization conditions because as we have shown above these lead to an opposing modification in favour of the more thermally stable *trans* isomer. We are able to assume therefore that β-methyl-γ-octalactone precursors possess primarily an *erythro* configuration, as was the case for the precursor that we isolated and identified.

Table 6 shows the results for the measurement of the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid for the same 12 wood samples. The level of the precursor varied from 0 to 495 μg per g of dry wood. In order to compare the levels of precursors measured by the two different methods, we have expressed the quantity of the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid as μg of β-methyl-γ-octalactone that could be liberated from this precursor per g of dry wood (Table 6).

The results of the two methods are very similar (Tables 5 and 6) and a very high correlation is found between the two series of values ($r=0.993$). The ratio between the level measured by direct quantification and that measured by the indirect method varied from 0.8 for tree C to 1.1 for tree E. The 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid is therefore the principal and perhaps the only precursor to *cis*-β-methyl-γ-octalactone in the wood of the 12 Sessile oaks studied, as the small amount of *trans* isomer observed could derive from epimerization of the *erythro* form under the conditions of hydrolysis and lactonization chosen. However, the presence of minor precursors having a *threo* configuration giving rise to *trans*-β-methyl-γ-octalactone cannot be excluded. Indeed, we have already shown in the wood of tree A the existence of a minor isomer of the 6′-*O*-gallate derivative of 4-β-D-glucopyranosyloxy-3-methyloctanoic acid which was tentatively identified as a *threo* form [17].

A reason for the deficit observed in the level of *cis*-β-methyl-γ-octalactone precursors, when measured by the indirect method compared to direct quantification, is likely to be due to the fact that β-methyl-γ-octalactone is not entirely liberated in the hydrolysis and lactonization conditions used, even if the yields are superior to those obtained using the method proposed by Chatonnet [10]. These

Table 6
Levels[a,b] of the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid in the fresh wood of 12 Sessile oaks

| Tree | Level of precursor[a] | Level of β-methyl-γ-octalactone released[b] |
|---|---|---|
| A | 495.2 | 158.3 |
| B | 242.4 | 77.5 |
| C | 185.5 | 59.3 |
| D | 383.2 | 122.5 |
| E | 351.7 | 112.4 |
| F | 332.0 | 106.1 |
| G | 0.0 | 0.0 |
| H | 0.0 | 0.0 |
| I | 104.0 | 33.3 |
| J | 71.6 | 22.9 |
| K | 0.0 | 0.0 |
| L | 301.1 | 96.3 |

[a] As μg per g of dry wood.
[b] As μg of β-methyl-γ-octalactone that could be released from the precursor per g of dry wood.

results agree with the values shown in Table 1 concerning the epimerization and incomplete recuperation of synthetic β-methyl-γ-octalactone under the conditions of indirect measurement.

## 4. Conclusion

This study describes a new method of indirectly measuring the precursors of β-methyl-γ-octalactone which is more reliable and precise than the method previously proposed, as well as a simple and rapid method of measuring a precursor found in Sessile oak wood, the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid.

The level of precursors was found to be highly variable among the 12 Sessile oaks studied. Trees possessing high levels of free *cis*-β-methyl-γ-octalactone have equally high levels of precursors and therefore levels of this isomer and the flavour potential of the wood are liable to increase. In contrast, trees having a high level of free *trans*-β-methyl-γ-octalactone possess much lower levels of precursors and the proportion of *cis*-β-methyl-γ-octalactone formed from these precursors was lower than in the former trees. Therefore any change in their flavour potential is very limited.

The results obtained from comparing the methods of direct and indirect measurement, suggest that the 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid is by far the most abundant precursor to β-methyl-γ-octalactone in Sessile oak wood.

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