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Varietal Thiols in Wine: Discovery, Analysis and Applications

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1. INTRODUCTION

Aroma compounds are produced in grapes (varietal aroma) and/or throughout the wine-making process (from oak barrels, for example). For a better understanding, they are classified according to their formation period.

- Varietal aroma compounds: Grapes are nonaromatic fruits, except for a few varieties such as Muscat, which is rich in monoterpenols. However, grapes allow the production of quality wines, in which the aromatic sensations are important. That specificity is due to the presence of odorless compounds in grapes, called varietal precursors that could generate, during wine making, odoriferous compounds typical of the used grape variety.

  Most of the yeast substrates during fermentation, such as sugars, lipids, and nitrogen- or sulfur-containing compounds, are also aroma precursors but are not considered as specific precursors because they lead to the formation of aroma compounds through complex biochemical reactions and the original structure of the precursors is not yet recognizable in the formed aroma compounds. On the contrary, varietal aroma compounds are already present in grapes either as a free form, which means volatile and directly perceptible by the olfactory receptors, or as a bound form, meaning linked by a covalent bond to a nonvolatile moiety (amino acid, sugar, etc.). The cleavage of that chemical bond could occur during the technical operation of wine making and lead to a so-called varietal aroma compound in which the original skeleton of the volatile moiety, biosynthesized in the plant, is preserved,[1] even if the cleavage mechanism is due to the yeast in some cases, as for the varietal thiols.
- Prefermentation aromas: These substances, including C_6 compounds, appear between harvest and alcoholic fermentation through enzymatic reactions occurring when berries are crushed.
- Fermentation aromas: These compounds, such as ethyl esters and fusel alcohols, are secondary products of microorganism metabolism (yeast or lactic acid bacteria) and are responsible for the vinous and fruity olfactive characteristics of the product.
- Postfermentation aromas: Such compounds are formed during wine aging and involve chemical or biochemical conversion of volatile compounds. They are responsible for the complexity of the bouquet of old wines.

Among these compounds, the varietal thiols, especially 4-mercapto-4-methylpentan-2-one (4MMP, **1**), 3-mercaptohexyl acetate (3MHA, **2**), and 3-mercaptohexan-1-ol (3MH, **3**), have been identified as key molecules of young wines elaborated with many varieties. These compounds belong to the class of varietal aromas because they result from the cleavage of odorless precursors present in grapes or musts by yeast during alcoholic fermentation. The positive contribution of varietal thiols was first pointed

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out by Du Plessis and Augustyn,[2] who demonstrated that the guava-like aroma found in South African Sauvignon Blanc wines is mainly due to the presence of 4MMP. In contrast with light sulfur compounds, such as carbon sulfide, ethanethiol, methanethiol, and hydrogen sulfide (bp < 90 °C), which are mainly produced at high levels during alcoholic fermentation and are responsible for olfactory defects, varietal thiols occur at very low concentrations in some *Vitis vinifera* wines, exhibiting pleasant odors such as blackcurrant bud, passion fruit, and grapefruit (Table 1). Other nonvarietal sulfur compounds can positively contribute to the wine aroma through coffee and meaty notes (Table 1).

Over the past two decades, the interest in the contribution of varietal thiols in young wines has grown considerably, especially for the wine industry. Most literature reports dealing with varietal thiols in wine focus on these compounds and demonstrate the central role played by the different branches of chemistry in the understanding and control of biochemical processes responsible for the release of these powerful odoriferous compounds. This review is intended to describe the synthetic routes and their contribution to studies of the biochemical transformations occurring during wine making. The analytical procedures developed in several works are also discussed, as they contribute to the general knowledge of the enological and viticultural aspects impacting the levels of thiols in wines.

In accordance with new rules for the international nomenclature of chemical compounds, the prefix "sulfanyl" must normally replace the prefix "mercapto"; however, this change was not made in this review to preserve the more familiar nomenclature of these varietal thiols.

2. DISCOVERY: OCCURRENCE, BIOGENESIS, AND SENSORY ROLES PLAYED BY VARIETAL THIOLS

Volatile varietal thiols such as 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and its acetate (3MHA) contribute positively to the fruity notes of young wines at very low concentrations close to part-per-trillion levels. Their biogenesis during wine making has involved many investigations during the past 20 years with the aim of better understanding and managing the quality of wine by developing several analytical tools and chemical synthesis strategies.

2.1. Most Relevant Varietal Thiols

The most relevant varietal thiols, compounds **1**−**7**, are gathered in Table 1, and this review focuses on only **1**−**3**. The importance of sulfur compounds in wine aroma was first highlighted by Du Plessis and Augustyn in 1981.[2] Indeed, they demonstrated the similarity between a neutral wine fortified with synthetic 4MMP and Chenin Blanc or Colombard wines exhibiting a strong guava-like aroma.[2] In addition, wine treatment with copper sulfate was found to decrease this guava-like aroma in aromatic wines, which enhanced the presumption of the contribution of this thiol compound to the specific aroma of such wines.

4MMP was formally identified in Sauvignon Blanc[3] and later in Scheurebe,[4] Maccabeo,[5] Gewürztraminer, Riesling, Muscat, Colombard, Petit Manseng, and Tokay wines.[6]

3MH and 3MHA are more ubiquitous than 4MMP, as they have been identified in a wide range of varietal wines such as Sauvignon Blanc,[7] Petite Arvine,[8] Petit and Gros Manseng,[6b,9] Melon B. and Bacchus,[10] Semillon,[6b] Verdejo,[11] and Koshu,[12] as well as in red grapes such as Grenache,[13] Merlot, and Cabernet Sauvignon,[14] and Rosé wines from Provence.[15]

More recently, 3MH was detected for the first time in Sauvignon Blanc grape juices from Adelaide hills vineyard that underwent an exogenous enzymatic treatment, at levels of around 100 ng/L.[16]

2.2. Other Qualitative Sulfur Compounds

Others sulfur compounds (Table 1, compounds **8**−**18**) contribute qualitatively to the aroma of wine. Some of them (**14**−**18**) are not very important and are rarely reported in literature. In contrast, 2-mercaptoethyl acetate (**8**) and 3-mercaptopropyl acetate (**9**), reminiscent of toasted and roasted meatlike nuances, have been identified in white wines such as Semillon and Sauvignon Blanc.[17] At the same time, 3-mercapto-2-methylpropanol (**10**), identified in red Bordeaux wines made from Cabernet Sauvignon and Merlot, give broth and sweat odors for concentrations ranging from 25 to 10000 ng/L in wine.[14b] Furanthiol derivatives, such as 2-furanmethanethiol (**11**), bring a strong roast edcoffee aroma to Petit Manseng and red Bordeaux wines made from Merlot, Cabernet Franc, and Cabernet Sauvignon[18] when fermented or aged in oak barrels. This is because furfural extracted from oak is required for the biogenesis of furanthiol derivatives.[19] Other mercaptans such as ethyl-3-mercaptopropionate (**12**) and benzenemethanethiol (**13**) have been identified in aged Champagne wines and seem to be responsible for empyreumatic notes with very low perception thresholds.[20] The biogenesis formation of this latter compound has never been formally established, but the addition of hydrogen sulfide to benzaldehyde could be hypothesized[20b], similarly to the mechanism of furanmethanethiol formation from furfural.[19]

2.3. Biogenesis

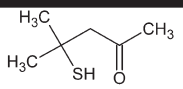
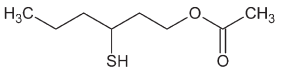
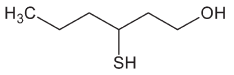
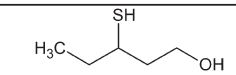
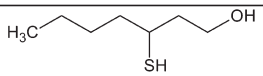
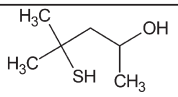
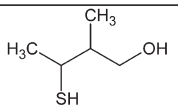
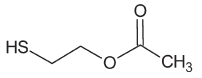
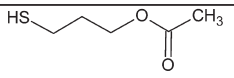
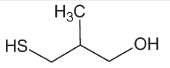
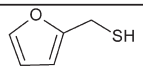
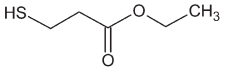
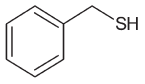
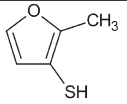
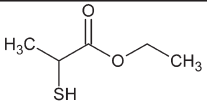
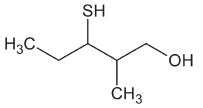
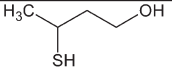
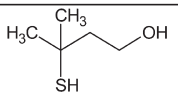
4MMP, 3MH, and 3MHA are powerful odoriferous thiols and constitute varietal aromas because they are released during alcoholic fermentation from odorless precursors occurring in grapes and musts[21] (Figure 1).

Three biogenesis pathways are commonly accepted to explain the release of 4MMP and 3MH in wine. The biogenesis of 3MHA is quite particular, because 3MHA is produced from 3MH during fermentation, by the action of the yeast ester-forming alcohol acetyltransferase, encoded by the ATF1 gene.[22]

The first pathway involves cysteinylated precursors, which were initially identified in Sauvignon Blanc grapes and then in Merlot and Cabernet Sauvignon,[14a] Semillon,[24] Petit and Gros Manseng, Riesling, Melon B. and Gewürztraminer,[25] and Koshu,[12] especially for Cys3MH. These *S*-cysteine conjugates are cleaved by the yeast, through its beta-lyase activity,[23b] during alcoholic fermentation. *S*-3-(hexan-1-ol)-cysteine (Cys3MH) is more ubiquitous and abundant in grapes than *S*-3-(4-mercapto-4-methylpentan-2-one)-cysteine (Cys4MMP).[14a,26] These *S*-cysteinylated precursors occur widely in plants, as reported by Starkenmann et al.[27] and could constitute a powerful source of aroma in industry.

Despite the fact that the distribution of Cys3MH diastereomers varies from one grape variety to another, (*S*)-Cys3MH is always the most abundant compound.[28] The diastereomeric distribution of Cys3MH ranges from 44%/56% to 48%/52% and from 29%/71% to 35%/65% for (*R*,*R*)-Cys3MH and (*R*,*S*)-Cys3MH diastereomers, respectively, in healthy and botrytized Semillon and Sauvignon Blanc musts.[29] The differences in the enantiomeric distributions for 3MH and 3MHA observed in

Table 1. Volatile Thiols Identified in *Vitis vinifera* Wines[a]

Origins	Numbers	Molecules	Names	Odors	Perception thresholds in model solution (ng/L)	References	Concentration range reported in literature (ng/L)
VARIETAL THIOLS	1		4-methyl-4-mercaptopentan-2-one **(4MMP)**	Box-tree, blackcurrant bud	0.8	2,3c,6b	until 400
	2		3-mercaptohexyl acetate **(3MHA)**	Box-tree	4.2 in racemic mixture	6b,7a	until 2500
	3		3-mercaptohexan-1-ol **(3MH)**	Grape fruit, passion fruit	60 in racemic mixture	6b,7b	until 19000
	4		3-mercaptopentan-1-ol	Grape fruit	950	93	90-300
	5		3-mercaptoheptan-1-ol	Grape fruit	35	93	25-75
	6		4-methyl-4-mercaptopentan-2-ol **(4MMPOH)**	Citrus zest	55	6b,7b	until 90
	7		2-methyl-3-mercaptobutan-1-ol	Raw onion	nr	93	80-150
NON-VARIETAL THIOLS	8		2-mercaptoethyl acetate	Meaty	nr	17	na
	9		3-mercaptopropyl acetate	Meaty	nr	17	na
	10		3-mercapto-2-methylpropan-1-ol	Broth, sweat	3000 (in water)	14b	25-10000
	11		2-furanmethanethiol	Coffee	0.4	18	0.4-62
	12		Ethyl-3-mercaptopropionate	Meaty	200	20a,94	40-12000 (in sparkling wines)
	13		Benzenemethanethiol	Smoky	0.3	20b	10-40
	14		2-methyl-3-furanthiol	Meaty	0.4-1.0 (in water)	95	> 100
	15		Ethyl-2-mercaptopropionate	Fruity	500	20a	na
	16		2-methyl-3-mercaptopentan-1-ol	Raw onion	nr	6a	na
	17		3-mercaptobutan-1-ol	Onion, leek	nr	6a	na
	18		3-methyl-3-mercaptobutan-1-ol	Cooked leek	1500	6b,7b	na

[a] na, not available.

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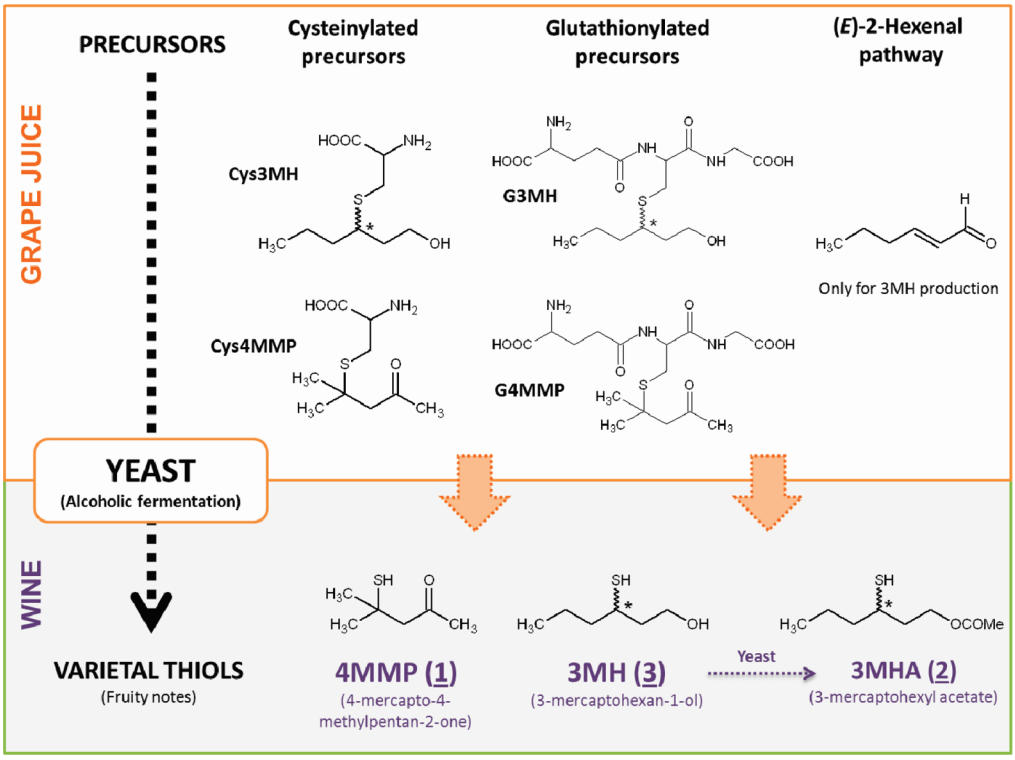

Figure 1. Different biogenesis pathways for 4MMP (**1**), 3MH (**3**), and 3MHA (**2**) during alcoholic fermentation.

wines from healthy or botrytized grapes could have a significance in a sensory aspect, especially for 3MHA (see section 2.4).

The second pathway concerns the glutathionylated precursors: *S*-3-(hexan-1-ol)-glutathione (G3MH) identified in grapes of Sauvignon Blanc,[30] Melon B.,[26a] Riesling,[25] Gewürztraminer,[25] Chardonnay,[28] Pinot Grigio,[28] and Koshu[12] and *S*-3-(4-mercapto-4-methylpentan-2-one)-glutathione (G4MMP) occurring in Sauvignon Blanc,[31] Riesling,[26a] and Gewürztraminer.[26a] The mechanism of thiol release from glutathionylated precursors has been investigated only for G3MH. Indeed, the percolation of a Sauvignon Blanc or Gros Manseng must through an immobilized γ-glutamyltranspeptidase column results in an increase in Cys3MH, suggesting that G3MH could be a pro-precursor.[30] Recently, it was demonstrated that a *Vitis vinifera* cell culture was able to produce Cys3MH from G3MH, deciphering part of Cys3MH biogenesis in grapes.[32] In addition, that work pointed out that *Botrytis cinerea* is a powerful activator of Cys3MH biogenesis.[32] However, *Botrytis cinerea* in grapes is known to produce laccase, a powerful and nonselective oxidase. Thus, the oxidation mechanism induced by its presence could be detrimental to the final quality of wine and particularly to thiol levels.

In contrast, model[12,33] or Sauvignon Blanc[25] musts were spiked with synthetic G3MH and then fermented with VIN13 or VL3 as yeast strains. The release of 3MH in the resulting wine demonstrated that G3MH constitutes another precursor of 3MH. A similar outcome was observed for G4MMP in experiments on Sauvignon Blanc must.[34] Consequently, G3MH could play two different roles according to enological conditions: pro-precursor of Cys3MH[30,32] and precursor of 3MH.[12,25,33]

Glutathionylated precursors occur at lower levels in grapes than cysteinylated precursors,[26a] except in Australian grape juices.[28] G3MH is more abundant than G4MMP in grapes, which is consistent with the equal abundances of the corresponding thiols in wines. The G3MH concentration was found to range from 0.2 to 7.3 μg/L in several grape varieties from France such as Sauvignon Blanc, Melon B., Riesling, and Gewürztraminer,[26a] whereas the concentration reached 1250−2770 μg/L for Sauvignon Blanc, Chardonnay, Riesling, and Pinot Grigio from Australia.[28] This difference in precursor concentration between Australian and French grapes is surprising because the grapes belong to the same *Vitis vinifera* variety.

G4MMP levels were found to be considerably lower in Sauvignon Blanc, Riesling, and Gewürztraminer grapes ranging from 0.03 to 4.3 μg/L.

The distribution of G3MH diastereomers differs according to the grape variety, but the most abundant compound is always (*S*)-G3MH, where the (*S*) refers to the absolute configuration of the asymmetric carbon bound to the cysteine residue.[28]

The enzymatic cleavage of Cys3MH does not seem stereoselective because the enantiomeric distribution of 3MH, which is close to a racemic mixture in wine made from healthy grapes, is in coherence with the diastereomer proportions of the precursor in berries. Nevertheless, this observation has to take into account the fact that the diastereomer distribution of G3MH is highly in favor of (*S*)-G3MH, which can modulate the enantiomeric proportions of thiols in wine.

Finally, the third biogenesis pathway involves C_6 unsaturated compounds, such as (*E*)-2-hexenal, which undergo a sulfur addition during alcoholic fermentation.[35] To date, the sulfur donor has not yet been identified, but it could be H_2S, cysteine, glutathione, or other molecules in must having an available free thiol function.

2.4. Sensory Contribution

Varietal thiols, such as 4MMP, 3MH, and 3MHA, contribute to the typing of young white wines as Sauvignon Blanc. Their

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perception thresholds are often very low, in the range of nanograms per liter.[6b] Although they occur at trace levels, their concentrations are always above their perception thresholds, bringing much appreciated fruity notes in wines.

4MMP, reminiscent of box tree and blackcurrant bud,[3] often exhibits concentrations less than 70 ng/L, except in Scheurebe wines with concentrations close to 400 μg/L,[4] for a perception threshold equal to 0.8 ng/L in hydroalcoholic solution.[6b] The level of 400 μg/L for 4MMP in Scheurebe seems particularly high. The levels reported for the other compounds in the same wine lead us to think that the real concentration of 4MMP is 400 ng/L, as mentioned in several books dealing with enology.[6a,21] 3MH and 3MHA, which are more abundant than 4MMP, give the heavily studied fruity notes, such as passion fruit and grapefruit, to white or rosé wines,[6b,13,36] with perception thresholds close to 60 and 4.2 ng/L, respectively.[6b] In addition, 3MH is responsible for blackcurrant odors in red wines.[37]

The enantiomeric forms of both 3MH and 3MHA exhibit different abundances, perception thresholds, and olfactory perceptions in wines. For healthy Sauvignon Blanc and Semillon grapes, 3MH is present as a racemic mixture, whereas the *R*/*S* ratio is close to 30%/70% for 3MHA.[38] In the case of botrytized grapes, the enantiomeric ratio for 3MH varies from 50%/50% to 30%/70% for the *R*/*S* forms.[6a,38] During alcoholic fermentation, only the enantiomeric ratio of 3MH varies from 40%/60% for *R*/*S* forms at the beginning of the process to 50%/50% in the finished wines. The enantiomers of 3MH and 3MHA also exhibit different perception thresholds and smells. Indeed, (*R*)- and (*S*)-3MH are reminiscent of grapefruit and passion fruit for very similar perception thresholds: 50 and 60 ng/L, respectively, in hydroalcoholic solution. (*R*)-3MHA smells of passion fruit, whereas (*S*)-3MHA is more herbaceous with a characteristic odor of boxwood, for perception thresholds ranging from 9 to 2.5 ng/L in hydroalcoholic medium.[38]

Gas chromatography−olfactometry (GC−O) constitutes the best way to screen the odor-active molecules in wine aromas. This technique uses the human nose as a detector for compounds eluting from the GC column. The first experiments conducted on Sauvignon Blanc extracts analyzed by GC−O revealed a specific odor zone, reminiscent of blackcurrant bud, on the chromatogram[3a] that was later identified as the 4MMP contribution. However, olfactometry does not take into account the antagonist and synergic effects between volatiles in a complex matrix such as wine (aroma enhancers or depressors).

In parallel with olfactometry measurements, comparisons between the concentration of a specific volatile and its perception threshold can help in determining the most active odorants in wine. Using aroma extract dilution analysis (AEDA), 4MMP was identified as the major olfactory contributor in Scheurebe wines.[39] Based on the same approach, Sauterne wines were found to exhibit a very strong bacon−petroleum odor due to 3-methyl-3-mercaptobutanal and 2-methylfuran-3-thiol, with others polyfunctional thiols such as 3-methylbut-2-ene-1-thiol, 3-mercaptopropyl acetate, 3-mercaptohexan-1-ol, and 3-mercaptoheptanal contributing to the global aroma.[40] A similar study conducted on botrytized Sauternes wines revealed the importance of 3MH for the grapefruit aroma of such wines.[41] In support of qualitative determination, quantitative aspects such as the calculation of odor-active values (OAVs, defined as the concentration-to-perception threshold ratio) were applied for the identification of impact odorants in Spanish wines.[5,42] All three of the compounds 4MMP, 3MH, and 3MHA were found to influence the global aroma of aged red wines,[42a] whereas 3MHA constituted the most active odorant in Marmajuelo and Verdello wines.[42b]

Finally, odor reconstitution experiments represent the best approach to measure quantitatively the contribution of the matrix to the wine aroma. A global strategy consisting of qualitative and quantitative determinations of impact odorants followed by omission tests with synthetic aroma models have demonstrated that 3MH is the most important odorant in Grenache rosé wines.[13] Sensory and quantitative chemical analyses are often carried out in parallel to characterize odoriferous molecules in wines. Recently, sensory analysis of Sauvignon Blanc from New Zealand demonstrated that 3MH and 3MHA can be used to predict the tropical character of wines and show good correlation with their respective sensory attributes.[43] Ferreira et al.[13] and Masson and Schneider[15] used reconstitution of dearomatized wines to estimate the contributions of 15 volatiles to perception and, in particular, the implication of varietal thiols such as 3MH and its acetate as the key aromas in Rosé wines.[15]

3. ANALYSIS: SYNTHESIS INVESTIGATIONS AND APPLICATIONS

Varietal thiols and their precursors are present at trace levels in complex matrixes such as wine or grape must, and to be quantified, they require the use of very sensitive and accurate analytical methods such as the stable isotope dilution assay (SIDA). In addition, relationship studies to elucidate the biogenesis of these odoriferous molecules involve the use of labeled molecules as powerful tracers during alcoholic fermentation. Consequently, several chemical syntheses of thiols and their precursors in either natural or deuterated form have been developed in the past 20 years.

3.1. Varietal Thiols

Retrosynthetic routes for varietal thiols can be easily summarized as Michael additions of a sulfur donor to α,β-unsaturated ketones, aldehydes, or esters. 3MH and 4MMP can be synthesized by the addition of a sulfur nucleophile to (*E*)-2-hexenal or mesityl oxide (Figure 2). Deuteration of such molecules requires the introduction of deuterium atoms in a nonenolizable position to obtain stable compounds under acidic or basic conditions. This condition warrants the reliability of quantification by mass spectrometry using these molecules as internal standards. Labeling can be performed either by using deuterated starting material such as commercially available [$^2H_{10}$]-mesityl oxide or by introducing deuterium atoms through a chemical reaction such as reduction.

3.1.1. Chemical Strategies. Syntheses of natural and deuterated analogues of 4MMP, 3MH, and 3MHA were initially reported in the literature[44] to develop a quantification method by SIDA−GC−ITMS/MS.[10] To be efficiently separated by mass spectrometry, such labeled molecules require a shift of a mass equal to at least 2 Da in their molecular weights; otherwise, the contribution of ^{13}C could interfere with the results. Kotseridis and co-workers[44]obtained [$^2H_{10}$]-4MMP (Figure 3), [$^2H_2$]-3MH (Figure 4), and [$^2H_5$]-3MHA (Figure 4) in good yields and purities using Michael addition of triphenylsilanethiol to either [$^2H_{10}$]-mesityl oxide or (*E*)-hex-2-enoate. The use of a solid hydrogen sulfide equivalent renders the preparation of these thiols a not-so-unpleasant operation.

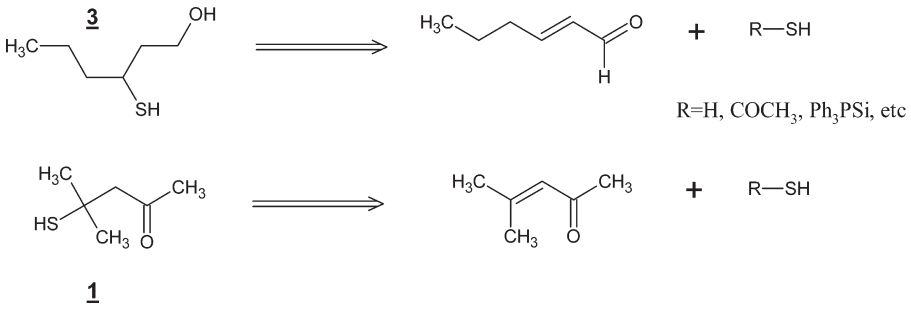

Figure 2. Retrosynthetic routes for 3MH (**3**) and 4MMP (**1**). [Note that the order of compounds is correct for a "retrosynthesis", which displays the targeted compound first, followed by a double arrow (characteristic of retrosynthesis) pointing toward the starting material on the right-hand side.]

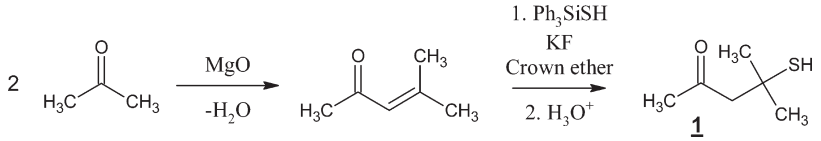

Figure 3. Synthesis procedure of 4MMP (**1**) according to the method of Kotseridis et al.[42] [$^2H_{10}$]-4MMP (**1**-d_{10}) was obtained under the same conditions using pure [$^2H_6$]-acetone as the starting material.

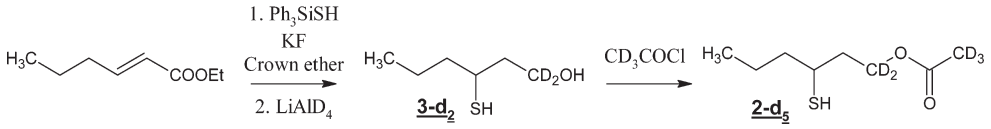

Figure 4. Synthesis of [$^2H_2$]-3MH (**3**-d_2) and [$^2H_5$]-3MHA (**2**-d_5) according to the method of Kotseridis et al.[42]

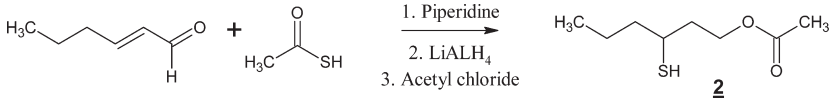

Figure 5. Synthesis procedure of natural 3MHA (**2**) according to the method of Vermeulen et al.[45]

Subsequently, improved procedures for the synthesis of these thiols have been developed. Vermeulen and Collin[45] reported combinatorial synthesis to obtain polyfunctional thiols including 3MHA easily (Figure 5). Based on an initial Michael addition of thioacetic acid to α,β-unsaturated ketones or aldehydes followed by reduction using aluminum hydride and acetylation using acetyl chloride, they synthesized 21 different mercapto esters.[45] This strategy seems to be powerful for assessing a significant number of mercaptans in a single experiment. Nevertheless, the separation of each molecule from the global mixture and their use as internal standards or biosynthetic markers remain problematical. Similarly, Vermeulen et al. reported the synthesis of 4MMP using piperidine and hydrogen sulfide.[46] The use of hydrogen sulfide as a sulfur donor makes this strategy less attractive than the strategy of Kotseridis et al.,[44] which employs Ph_3SiSH as a sulfide equivalent.

More recently, deuterated versions of 3MH and 3MHA have been synthesized using a Wittig olefination of butyraldehyde, followed by a Michael addition of thioacetic acid to the resulting α,β-unsaturated ester, which, in turn, is followed by a reduction step with aluminum deuteride to introduce two deuterium atoms.[47] Methyl esterification with acetyl chloride of [$^2H_2$]-3MH produced the corresponding [$^2H_2$]-3MHA. This strategy was as efficient as that of Kotseridis et al.[44] in terms of reported yields and purities. Hebditch and co-workers also synthesized [$^2H_1$]-4MMPOH. Such labeling did not provide a sufficient molecular weight shift in mass spectrometry, however, so this molecule cannot be used as an internal standard for the quantification of the natural compound in wine.

The first enantioselective synthesis of 3MH was performed by an asymmetric epoxydation of (*E*)-2-hexenol under Sharpless conditions using diethyl L-(+)- and D-(−)-tartrate and following the procedure of Pickenhagen and Brönner-Schindler.[48] Treatment of the epoxides with thiourea gave the corresponding thiiranes with inversion of the absolute configuration. Reduction with Vitride [sodium bis(2-methoxyethoxy)aluminum hydride] in tetrahydrofuran (THF) produced both enantiomers of 3MH (Figure 6). This strategy was employed to synthesize both enantiomers of 3MH in order to develop a method for determining the absolute configuration and conformation of organic molecules containing two or more chromophores.[49] An alternative synthetic approach was developed to obtain (*R*)-3MH (**3b**)[50] in better yield than by Pickenhagen and Brönner-Schindler's synthesis (8.5% for the total enantioselective synthesis). For this purpose, (*E*)-2-hexenal was subjected to an asymmetric conjugated addition of benzylthiol in the presence of substituted

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pyrrolidine as an organocatalyst; subsequent cleavage of the sulfide moiety produced the expected pure product with a high enantiomeric excess (84%) and better yield (32%) (Figure 7). Despite their good yields, purities, and enantiomeric excesses, these strategies have not been established as routine applications in the field of wine aroma research.

Each enantiomer of 3MH and 3MHA has been synthesized in its natural form following an enantiopure strategy[38] inspired by the enantioselective synthesis of the 1-methoxyhexane-3-thiol.[51] Indeed, ethyl-3-oxohexanoate was selectively reduced by Baker's yeast to afford the corresponding alcohol in its (*R*) absolute configuration, which underwent Mitsonobu reactions to give both enantiomers of 3MH and 3MHA (Figure 8). These enantiomerically pure molecules have been used as powerful standards for an analytical method focused on the determination of 3MH enantiomeric distribution in wines of Semillon and Sauvignon Blanc.

According to their individual needs, the chemical strategies for synthesizing varietal thiols reported in the literature present some advantages and drawbacks and are summarized in Table 2.

3.1.2. Quantification. Quantification of varietal thiols at trace levels in wine requires optimized sample preparation and sensitive analytical methods. Several analytical approaches combining either selective isolation or preconcentration steps allow measurements at sub-part-per-billion levels. In addition, such molecules presenting a free thiol function are highly reactive and can react with polyphenols or be oxidized into their corresponding disulfides at the pH of wine.[52] Consequently, analytical methods should overcome these difficulties by using labeled internal standards.

Tominaga and co-workers reported the first sample preparation allowing for the selective extraction of thiols from a dichloromethane extract of wine using a reversible interaction between the SH function and sodium *p*-hydroxymercuribenzoate.[53] Analysis of these extracts by GC−MS enabled the quantification of 3MH, 3MHA, 4MMP, 4MMPOH (**4**), and 3-mercapto-3-methylbutan-1-ol in Sauvignon Blanc wines from Bordeaux and Sancerre with good repeatability (CV < 10% for all compounds). However, this methodology presents two major drawbacks: The detection and quantification of 4MMP is based on only one fragment, which might not provide satisfactory specificity, and the basic conditions used for the extraction of sodium *p*-HMB complexes on the cationic resin could degrade 3MHA into 3MH. In addition, varietal thiols are highly reactive compounds that could be oxidized into the corresponding disulfides during sample preparation. Consequently, using this method to quantify such compounds in wine could give unreliable results. Recently, the removal of fatty acids

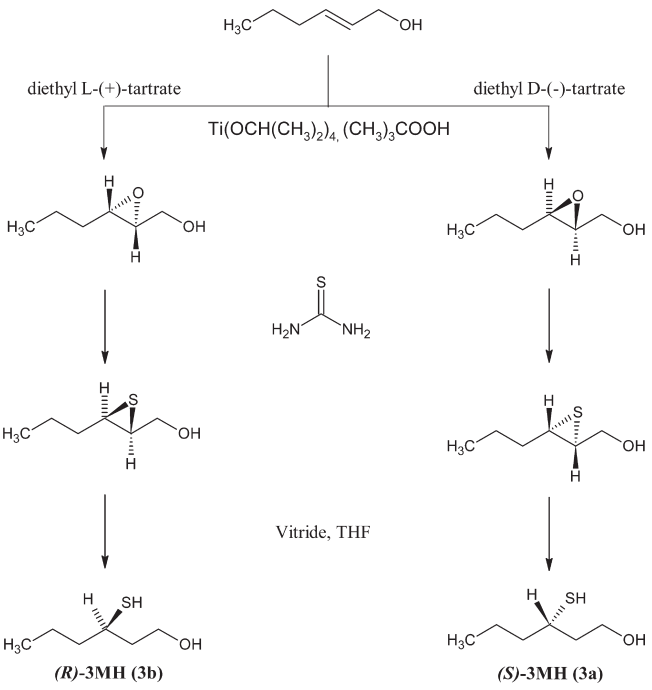

Figure 6. Synthesis procedure of 3MH (**3**) according to the method of Pickenhagen and Brönner-Schindler.[48]

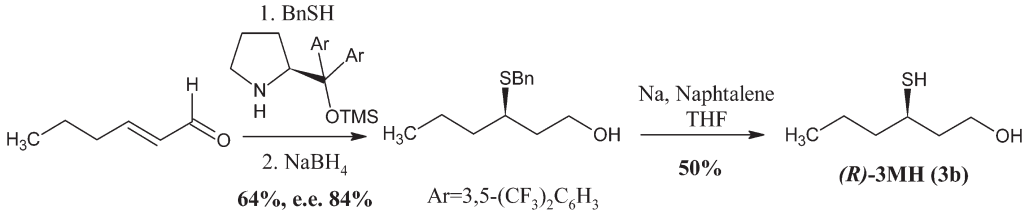

Figure 7. Synthesis procedure of (*R*)-3MH (**3b**) according to the method of Scafato et al.[50]

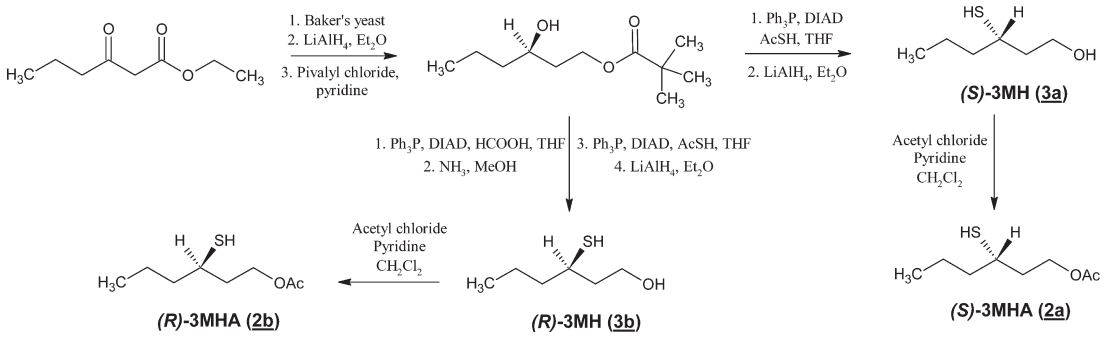

Figure 8. Enantioselective synthesis procedure of 3MH (**3a** and **3b**) and 3MHA (**2a** and **2b**) according to the method of Tominaga et al.[38]

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Table 2. Chemical Strategies to Synthesize Varietal Thiols in Natural and Deuterated Forms

target compound(s)	chemical strategy	yield (%)	purity[a] (%)	feasibility	advantages (+)/ drawbacks (−)	ref
		Analysis: SIDA Quantification (Racemic Mixture)				
[$^2H_2$]-3MH [$^2H_5$]-3MHA 4MMP/[$^2H_{10}$]-4MMP	addition of Ph_3SiSH to α,β-unsaturated ester or ketone	[$^2H_2$]-3MH: 31 [$^2H_5$]-3MHA: 15 [$^2H_{10}$]-4MMP: 18	[$^2H_2$]-3MH: 95 [$^2H_5$]-3MHA: 95 [$^2H_{10}$]-4MMP: 85	easy	(+) Ph_3SiSH as a solid hydrogen sulfide equivalent	44
[$^2H_2$]-3MH [$^2H_2$]-3MHA	Wittig olefination of butyraldehyde followed by Michael addition of thioacetic	[$^2H_2$]-3MH: 37 [$^2H_2$]-3MHA: 26	[$^2H_2$]-3MH:100 [$^2H_2$]-3MHA:100	easy	(+) nonexchangeable position for deuterium (+) thioacetic as a sulfide equivalent (−) difficulties in isolating the first intermediate product	47
[$^2H_{10}$]-3MH	Swern oxidation of butanol-d_{10}, followed by Wittig−Horner reaction, followed by Michael addition of thioacetic acid	17	na	easy	(+) nonexchangeable position for deuterium (+) thioacetic as a sulfide equivalent	65
		Analysis: Quantification (Enantiomeric Distribution)				
(*R*)-3MHA (*S*)-3MHA (*R*)-3MH (*S*)-3MH	reduction of ethyl-3-oxo-hexenoate by baker yeast, followed by Mitsunobu reactions	na	ee: 98 (for all compounds)	difficult	(−) five to seven steps to isolate expected compounds	38
(*R*)-3MH	asymmetric addition of BnSH to (*E*)-2-hexenal	32	ee: 84	easy	(+) only two steps to afford the product (+) no metal used as organocatalyst	50
		Sensory Characterization				
3MHA	addition of thioacetic acid to (*E*)-2-hexenal	na	na	easy	(−) compound obtained in a mixture with 20 others compounds	45
4MMP	addition of H_2S to mesityl oxide	na	na	difficult	(−) difficult handling of H_2S (−) compound obtained in a mixture without purification	46
(*R*)-3MH (*S*)-3MH	asymmetric epoxydation of (*E*)-2-hexenol under Sharpless conditions	8.5	ee: 90	difficult	(+) high ee (−) four steps (−) low yield	48

[a] na, not available; ee, enantiomeric excess.

and interfering compounds by means of a bed packed with LichrolutEN resin was found to improve the selectivity and sensitivity of mercaptan (2-furanmethanthiol, 4MMP, 3MH, and 3MHA) analysis based on a *p*-hydroxymercuribenzoate extraction.[54] In addition, the possibility of fixing some organomercury salt using SPE material has considerably simplified the extraction of thiols from wine.[55]

A similar method based on a liquid−liquid extraction followed by a specific trapping of thiols on Affigel 501 (a cross-linked agarose gel with phenylmercuric gel) enables the quantification of 4MMP by GC−AED and that of 3MH and 3MHA by GC−MS with detection limits below their respective perception thresholds.[10] For the first time, the use of labeled 3MH, 3MHA, and 4MMP as internal standards was able to overcome oxidation problems due to the similar reactivities of thiol functions from natural and labeled molecules in wine.[10]

Other sample preparation approaches have focused on preconcentration steps to limit the use of huge quantities of organic solvents and to minimize the volume of wine (now close to 500 mL). A direct quantification method by the purge-and-trap method followed by GC−EIMS allows the quantification of 3MH and 3MHA in wines with detection limits in the vicinity of their perception thresholds.[56] This technique constitutes an improvement of previously reported methods by eradicating the liquid−liquid extraction step.

Table 3. Analytical Methods Allowing the Quantification of the Three Varietal Thiols of Interest (3MH, 3MHA, and 4MMP) in Wine

target compound	sample preparation	analysis	quantification[a]	LOD[b] (ng/L)	repeatability[b] (RSD %)	advantages (+)/ drawbacks (−)	ref
3MH 3MHA 4MMP	CH_2Cl_2 extraction, followed by purification using *p*-hydroxymercuribenzoate	GC−MS (SIM)	internal calibration (ISTD: 4-methoxy-2-methyl-2-mercaptobutane)	na	<10	(−) only one ion per compound (−) sample preparation could induce thiol oxidation (not taken into account in the quantification) (−) basic conditions of the purification step could degrade 3MHA into 3MH (−) use of mercury derivatives	53
3MH 3MHA 4MMP	CH_2Cl_2/pentane extraction, followed by purification on Affi Gel 501	GC−MS/MS, GC−AED for 4MMP	SIDA (ISTDs: $[^2H2]$-3MH, $[^2H_5]$-3MHA, and $[^2H_{10}]$-4MMP)	3MH: 1 3MHA: 0.7 4MMP: 5	3MH: 9 3MHA: 3 4MMP: 12	(+) SIDA overcomes errors due to oxidation problems during sample preparation (−) LOD of 4MMP above its perception threshold (−) use of mercury derivatives	10
3MH 3MHA 4MMP	SPE extraction, followed by re-extraction using *p*-hydroxymercuribenzoate	GC−MS (SIM)	internal calibration (ISTD: 2-octanol)	3MH: 15 3MHA: 5 4MMP: 0.8	3MH: 14 3MHA: 11 4MMP: 15	(+) LODs below perception thresholds (−) complex sample preparation (−) injector equipped with a large-volume injection device required (−) use of mercury derivatives	54
3MH 3MHA 4MMP	extraction on an SPE cartridge containing *p*-hydroxymercuribenzoate	GC−MS (SIM)	internal calibration (two ISTDs: 4-methoxy-2-methylbutan-2-thiol and 3-mercapto-4-methylbutylformate)	3MH: 13 3MHA: 5 4MMP: 1.5	3MH: 8 3MHA: 9 4MMP: 12	(+) extraction step easier (−) use of mercury derivatives	55
3MH 3MHA	purge-and-trap extraction	GC−MS (SIM)	internal calibration (ISTD: 6-mercaptohexanol)	3MH: 48 3MHA: 36	na	(+) no solvent extraction (+) no matrix effect (−) LODs above perception thresholds	96
3MH	CH_2Cl_2 extraction and back-extraction in 1 M NaOH solution, followed by derivatization with PFBBr in aqueous extract	GC−MS (SIM)	SIDA (ISTD: $[^2H_{10}]$-3MH)	30	<2.5	(−) only one compound analyzed among the three compounds of interest (+) SIDA overcomes matrix effects (+) up to 25 samples analyzed per day	16
3MHA	on SPME fiber derivatization with 2,3,4,5, 6-pentafluorobenzylbromide (PFBBr)	GC−MS (SIM)	internal calibration (two ISTDs: hexanthiol and benzylthiol)	3	15	(+) automated method (+) only 10 mL of wine (−) robustness of the derivatization procedure (−) limited linear ranges	55

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Table 3. Continued

target compound	sample preparation	analysis	quantification[a]	LOD[b] (ng/L)	repeatability[b] (RSD %)	advantages (+)/ drawbacks (−)	ref
3MH 3MHA 4MMP	benzene extraction, followed by derivatization with PFBBr in organic extract	GC−MS (SIM)	internal calibration (two ISTDs: 4-methoxy-α-toluenethiol and 3-mercapto-3-methylbutylformate)	3MH: 7 3MHA: 0.6 4MMP: 0.1	na	(+) only 6 mL of wine (−) use of benzene (−) injector equipped with a large-volume injection device required	58
3MH 3MHA 4MMP	4MMP oximation in wine, followed by in situ SPE derivatization with PFBBr	GC−MS (SIM)	SIDA (ISTDs: $[^2H_2]$-3MH, $[^2H_5]$-3MHA, and $[^2H_{10}]$-4MMP)	3MH: 2 3MHA: 0.3 4MMP: 0.1	3MH: 19 3MHA: 5 4MMP: 11	(+) 12 samples analyzed simultaneously (+) SIDA overcomes matrix effects	59
3MH	4MMP oximation in wine, followed by in situ SPE derivatization with PFBBr and SPME injection	GC−MS (SIM)	SIDA (ISTDs: $[^2H2]$-3MH, $[^2H_5]$-3MHA, and $[^2H_{10}]$-4MMP)	3MH: 1.3	3MH: 6	(+) SPME prevents sensitivity losses due to the rapid damage of the analytical system encountered for liquid injection	60
3MHA 4MMP				3MHA: 0.25 4MMP: 0.03	3MHA: 4 4MMP: 9	(+) SIDA overcomes matrix effects	
(*R*)-3MH (*S*)-3MH (*R*)-3MHA (*S*)-3MHA	CH_2Cl_2 extraction, followed by purification using *p*-hydroxymercuribenzoate	GC−MS (SIM)	internal calibration (ISTD: 4-methoxy-2-methyl-2-mercaptobutane)	na	na	(−) no validation data	38

[a] ISTD, internal standard. [b] na, not available.

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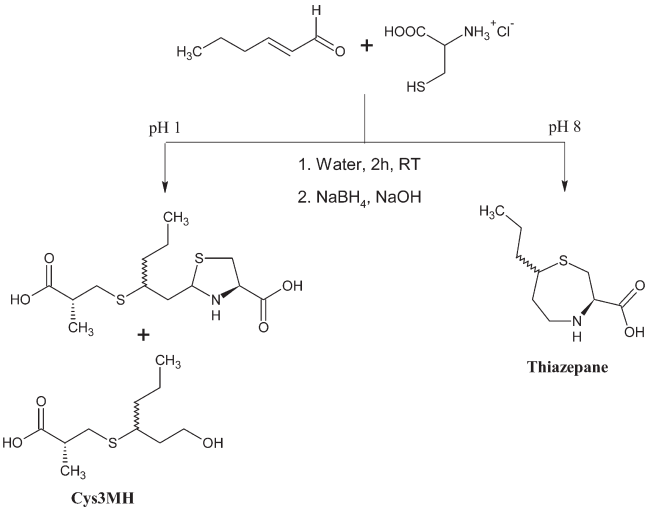

Figure 9. Influence of pH on Cys3MH synthesis.[61]

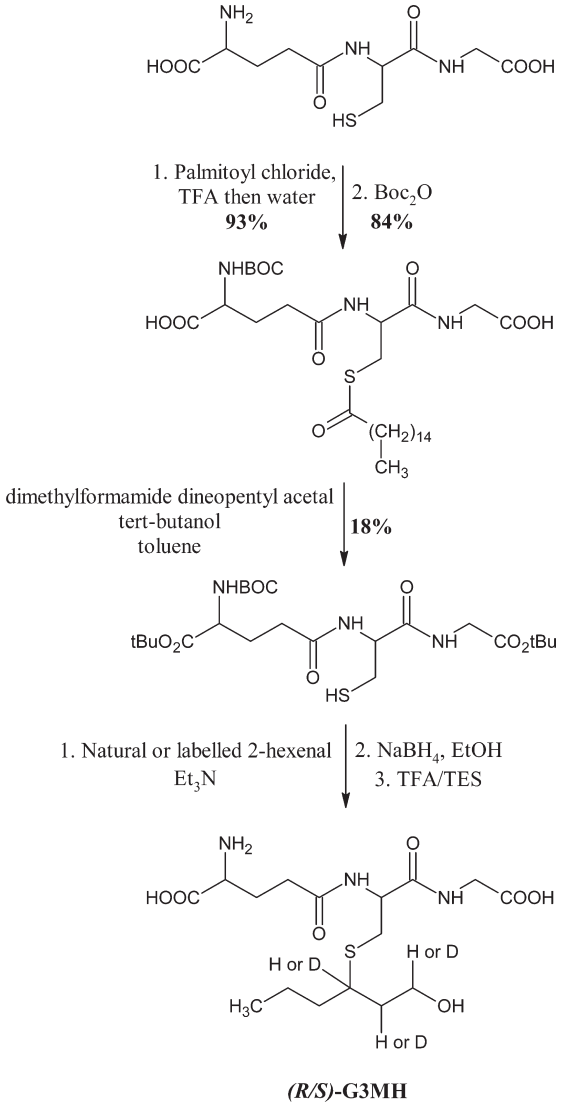

Figure 10. Synthesis procedure of natural and deuterated (*R*/*S*)-G3MH according to Roland et al.[25]

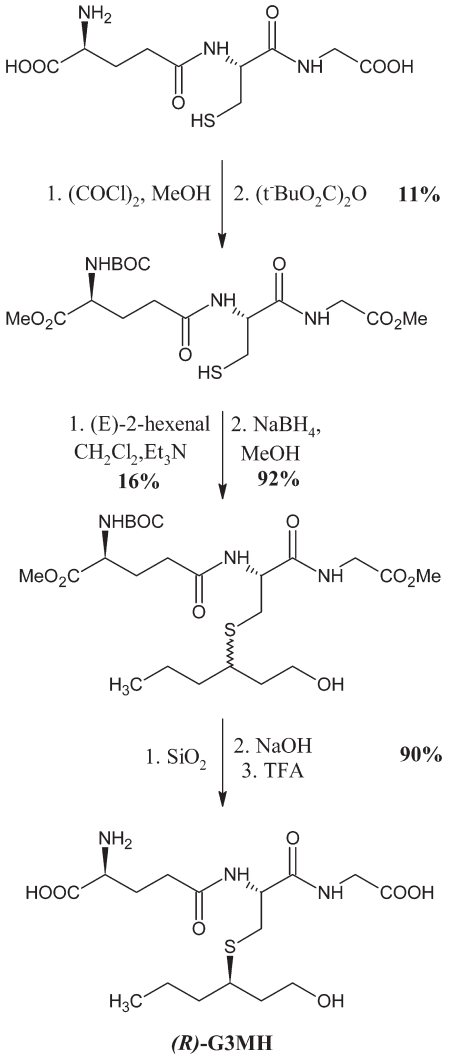

Figure 11. Synthesis procedure of (*R*)-G3MH according to Grant-Preece et al.[33]

To improve the detection and enhance the stability of thiols, derivatization procedures can be considered as powerful methods. Several derivatization reagents, such as the pentafluorobenzylbromide (PFBBr), can provide the total conversion of 3MHA and 2-methylfuranthiol into the corresponding derivatives,[57] warranting their quantification with satisfactory repeatability by SPME−GC−NCI−MS. The "on-fiber derivatization" procedure is highly innovative and allows the total automation of the method. Several improvements of the original method[57] have been performed to apply this derivatization procedure to other polyfunctional thiols occurring in wine with limits of detection far below the perception thresholds: optimization of derivatization reaction conditions,[58] development of an in situ SPE derivatization procedure,[59] decrease of matrix effects by using SIDA,[16,60] and adaptation of the derivatization procedure to an aqueous wine extract.[16]

Using established chromatographic conditions,[53] the enantiomeric ratio of 3MH and 3MHA has been measured in wines of Semillon and Sauvignon Blanc.[38] For this purpose, the enantiomers were separated on a Lipodex chiral column composed of heptakis-(2,3,6-tri-*O*-pentyl)-α-cyclodextrines.

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Table 4. Chemical Strategies to Synthesize Thiol Precursors in Natural and Deuterated Forms

target compound(s)	chemical strategy	yield[a] (%)	purity[a] (%)	feasibility	advantages (+)/ drawbacks (−)	ref
		Relationship Studies: Synthetic Medium				
Cys3MH Cys4MMP	Michael addition of L-cysteine to either (*E*)-2-hexenal or mesityl oxide	na	na	easy	(−) no characterization, yield, or purity data available	23b
(*R*)-Cys3MH (*S*)-Cys3MH	multistep synthesis	(*R*)-Cys3MH: 38 (*S*)-Cys3MH: 39	isomeric purity: 98	difficult	(+) high isomeric purity	65
G3MH	Michael addition of glutathione to (*E*)-2-hexenal	na	98.5	easy	(−) no characterization data available (−) G3MH in mixture with glutathione	30
G4MMP	Michael addition of glutathione to mesityl oxide	79	na	easy	(+) good yield and easy reproducible	31
		Relationship Studies: Natural Medium				
$[^2H_8]$-2-hexenal	oxidation of $[^2H_{10}]$- butanol, followed by Wittig reaction	7	na	easy	(−) low yield due to the difficult recovery of butanal-d_{10}	35
G3MH $[^2H_2]$-G3MH	multistep synthesis	<14 (both compounds)	na	difficult	(−) low yield (−) G3MH in mixture with glutathione	25
G3MH $[^2H_9]$-G3MH	Michael addition of glutathione or cysteine to (*E*)-2-hexenal or mesityl oxide	G3MH: 34 G3MH-d_9: 44	na	G3MH: easy G3MH-d_9: easy	(+) good yields (+)easy reproducible synthesis except for (*R*)-G3MH	33
(*R*)-G3MH $[^2H_6]$-Cys4MMP	(*R*)-G3MH: multistep synthesis	(*R*)-G3MH: na Cys4MMP-d_6: 83		(*R*)-G3MH: difficult	(−)isolation of (*R*)-G3MH difficult to achieve	
$[^2H_{10}]$-G4MMP	Michael addition of glutathione to $[^2H_{10}]$- mesityl oxide	84	na	easy	(+) good yield, easy reproducible synthesis	34
Cys3MH $[^2H_2]$-Cys3MH $[^2H_8]$-Cys3MH	Michael addition of L-cysteine to natural or labeled (*E*)-2-hexenal ($[^2H_2]$ or $[^2H_8]$)	na	na	easy	(−) no characterization data	62
		Analysis: SIDA Quantification				
$[^2H_6]$-Cys4MMP	Michael addition of L-cysteine to $[^2H_{10}]$- mesityl oxide	na	70	easy	(−) mixture of expected product and cysteine	47
$[^2H_2]$-2-hexenal	deuteration of hexynol, followed by mild oxidation with MnO_2	93	93	easy	(+) no purification step (−) huge excess of MnO_2	25
$[^2H_2]$-Cys3MH (labeling on cysteine moiety)	Michael addition of $[^2H_2]$-cysteine to (*E*)-2-hexenal	25	na	easy	(+) usable as analytical standard (−) unusable as tracers	64
		Chemical Mechanism Study				
Cys4MMP	Michael addition of cysteine to mesityl oxide	66	90	easy	(−) Cys4MMP obtained in mixture with cysteine	97

[a] na, not available.

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Table 5. Influence of Yeast Strain on Varietal Thiol Release under Enological Conditions[a]

	conversion yields under enological conditions (%)			
strain	Cys4MMP → 4MMP	Cys3MH → 3MH	G3MH → 3MH	G4MMP → 4MMP
		S. cerevisiae		
VL3c	0.06[81b]–0.8[98]	0.31[98]		
EG8	0.5[81b]–0.7[98]	0.41[98]		
VL1	0.2[81b]			
522d	0.06[81b]			
VIN13	0.6[98]	0.39[98]	**4.5**[25]	**0.3**[34]
VIN7	1.3[98]	0.30[98]		
QA23	1.3[98]	0.23[98]		
NT116	0.5[98]	0.29[98]		
ES1		**0.48–0.81**[62]		
		Interspecific Hybrids (*S. cerevisiae* × *S. bayanus*)		
H1–H9	3.5–10.9[84]			

[a] Model conditions or **natural** conditions.

To advise the analytical chemist who would be interested in developing a quantification method for these three varietal thiols in wine, Table 3 provides an overview of all published strategies with sample preparation details, types of analysis, and major advantages and drawbacks.

3.2. Precursors

As for the synthesis of thiols , cysteinylated and glutathionylated precursors have been obtained in their natural or deuterated forms by means of Michael additions of cysteine or free glutathione to α,β-unsaturated ketones, aldehydes, or esters such as mesityl oxide or (*E*)-2-hexenal derivatives. Synthesis of such labeled molecules provides very useful analytical standards for the development of quantitative method based on SIDA.

In addition, for these molecules to be used as powerful tracers for relationship studies, labeling should be introduced in the aroma moiety of the precursor. Indeed, the yeast is able to cleave these aroma precursors to release the corresponding labeled thiols in wine. The formal identification of these labeled thiols in wine unequivocally confirmed the relationships with their precursors.

3.2.1. Chemical Strategies. The first reported syntheses of thiol precursors concerned Cys3MH, Cys4MMP, and Cys4MMPOH in their natural forms. They were based on the Michael addition of L-cysteine hydrochloride to either (*E*)-2-hexenal or mesityl oxide to give the pure precursors after reduction with sodium borohydride for Cys3MH and Cys4MMPOH.[23b] Cys3MH formation by Michael addition of cysteine chloride to (*E*)-2-hexenal was found to be pH-dependent.[61] Indeed, major differences between acidic and basic conditions were observed after the reduction step with sodium borohydride: At pH 1, two compounds were isolated (single and double adducts), whereas at pH 8, only the thiazepane was formed (Figure 9). The influence of pH was not measured in Tominaga et al.'s synthesis for cysteinylated precursors,[23b] which could involve the production of byproducts.

Concerning the deuterated analogues, [1,1′-$^2H_6$]-Cys4MMP was synthesized using a strategy similar to that described previously with [$^2H_{10}$]-mesityl oxide as a pure starting material.[47] Different labeling strategies were reported for the synthesis of Cys3MH. Indeed, the number of deuterium atoms introduced into (*E*)-2-hexenal can be easily modified from two to eight, as already reported in the literature.[25,35,62] The deuteration of hexyn-1-ol provides [2,3-$^2H_2$]-(*Z*)-2-hexenol selectively, which affords [2,3-$^2H_2$]-(*Z*/*E*)-2-hexenal after oxidation with manganese dioxide.[25] Another approach allows the synthesis of [$^2H_8$]-(*E*)-2-hexenal from [$^2H_{10}$]-butanol, which is first oxidized through a Dess–Martin reaction and then elongated through a Wittig reaction.[35] These molecules, [2,3-$^2H_2$]-(*Z*/*E*)-2-hexenal or [$^2H_8$]-(*E*)-2-hexenal, are useful for the synthesis of deuterated Cys3MH and G3MH.

One strategy adapted from the synthesis of Luisier et al.[63] referred to the labeling of the cysteine residue instead of the aroma moiety in the Cys3MH molecule using [3,3-$^2H_2$]-DL-cysteine as a starting material.[64] Even if this compound can be used as an internal standard for Cys3MH quantification, its use in biogenesis experiments is not convenient, because there is no doubt that yeast will use the cysteine moiety as a nitrogen source.

Most products of Cys3MH syntheses are reported as racemic mixtures because both diastereomers of this precursor occur in grapes and musts. However, the separation of the different Cys3MH diastereomers on silica gel from a racemic mixture was reported in the literature, in an effort to investigate the diastereoselectivity of yeast cleavage responsible for the release of the thiols.[65]

Syntheses of glutathionylated precursors have been reported more recently in the literature. Indeed, G3MH was first synthesized in natural form by adding reduced glutathione to (*E*)-2-hexenal to obtain the expected racemic product in a mixture with glutathione.[30] Several improvements of this original synthesis have contributed to the decrease of the amount of free glutathione in the final mixture with G3MH.[25,33] These approaches are based on the strategy proposed by Falck et al., giving synthetic building blocks of glutathione.[66] Natural and labeled G3MH have been synthesized using a strategy involving acid-labile protecting groups and deuterated [$^2H_2$]-(*Z*/*E*)-2-hexenal[25] (Figure 10). (*R*)-G3MH was separated from the racemic mixture of the same synthesis (Figure 11), providing pure analytical standards used for relationship studies[33] and analytical development.[28]

The G4MMP synthesis requires the Michael addition of glutathione to mesityl oxide to give the natural compound.[31] A similar synthesis with deuterated mesityl oxide as the starting material affords the deuterated analogue.[33] To the nonspecialist chemist, the synthesis of G4MMP is a simpler procedure than that of G3MH.

All of the chemical strategies for thiol precursors are summarized in the Table 4, which provides key aspects of each published synthesis (yield, purity, feasibility, and advantages and drawbacks).

3.2.2. Relationship Studies Using Labeled Precursors. To date, only 10–15% of the total 3MH in wine has been confirmed to result from both the cysteinylated precursor and the hexenal pathway. Depending on the must composition and the wine-making process, this range can vary dramatically.

Because varietal thiols are the results of different biogenetic pathways, the measurement of conversion yields from each precursor can be based only on deuterated markers. This technique presents another advantage because experiments can be performed in real grape must and can thus take into account the impact of must composition on the ability of yeast to convert the precursors into thiols (see section 4.2.2).

The first pathway elucidated with this technique was the hexenal pathway, contributing to the production of 3MH, formally

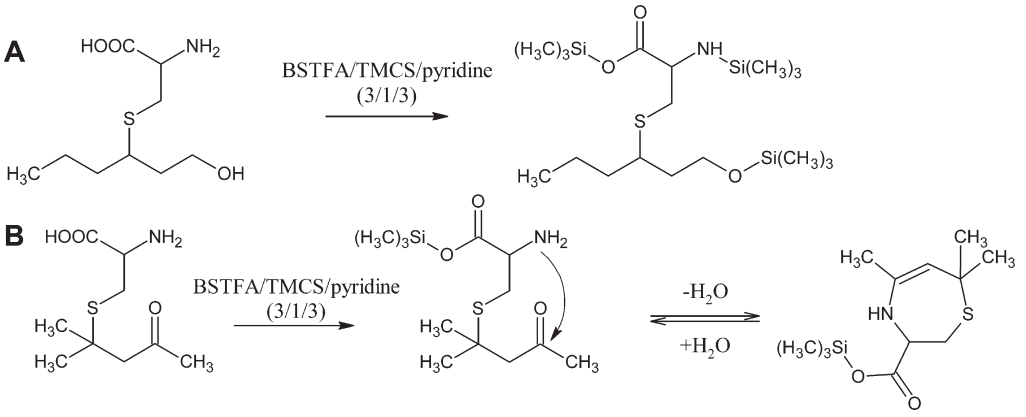

Figure 12. Tominaga et al.'s derivatization procedure to afford trimethylsilylated derivatives of (A) Cys3MH and (B) Cys4MMP.[23b]

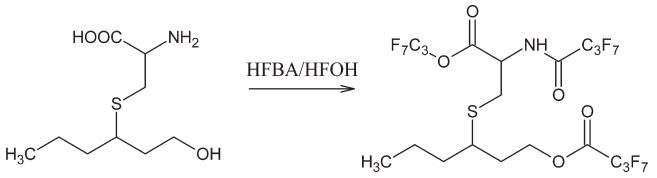

Figure 13. Thibon et al.'s derivatization procedure for the separation of both diastereomers of Cys3MH.[29]

confirmed by adding [$^2H_8$]-hexenal to a Melon B. must.[35] The release of [$^2H_8$]-3MH in the corresponding wine demonstrated that (*E*)-2-hexenal constitutes an additional pathway for 3MH production. This pathway contributes up to 10% of the total 3MH released in the Melon B. wine. The same technique was also used to establish 4MMP formation from mesityl oxide (using [$^2H_{10}$]-mesityl oxide), but as mentioned in the original study, the eventual precursors have not be yet identified in grapes or musts.

Subileau and co-workers[62] measured the yield of [$^2H_8$]-Cys3MH converted into [$^2H_8$]-3MH in a Sauvignon Blanc must from two different origins (Gers and Languedoc) using two different yeast strains. Regardless of the must origin and yeast type, the molar conversion yield was always below 1% (Table 5), explaining only between 3% and 7% of the total 3MH in the resulting wines.

Using the same strategy, [$^2H_{2,3}$]-G3MH was added to a Sauvignon Blanc must to investigate other biogenesis origins that could explain the total production of 3MH in wine.[25] The identification of [$^2H_{2,3}$]-3MH in the resulting Sauvignon Blanc wine showed the direct connection between G3MH and 3MH under enological conditions. The conversion rate of G3MH into 3MH was estimated as being close to 4.5%, irrespective of the initial amount of [$^2H_{2,3}$]-G3MH spiked in the must (Table 5). Similar experiments demonstrated a direct relationship between G4MMP and 4MMP using a Sauvignon Blanc must initially spiked with [$^2H_{10}$]-G4MMP.[34] The conversion yield of 0.3% explained 20% of the total 4MMP release.

The levels of the three different precursors reported in the literature and the mean conversion yields determined experimentally cannot explain the total amount of thiols present in wines. This observation points out the eventual presence in must of other precursors, especially derivatives of the already identified precursors (aldehyde or cyclic forms). However, modulation of the conversion yield by the nitrogen composition cannot be excluded (see section 4.2).

3.2.3. Quantification. The very low quantities (part-per-billion levels) of cysteinylated and glutathionylated precursors in grapes requires not only appropriate sample preparation (extraction and purification) but also sensitive analytical methods. The development of quantification methods was initially based on indirect procedures such as the cleavage of the precursors into their corresponding thiols or the derivatization of these molecules.

The first quantification method for cysteinylated precursors was based on the specific cleavage of *S*-conjugate molecules into the corresponding thiols by percolating grape musts through a column containing an immobilized tryptophanase enzyme (EC 4.1.99.1).[26b] The released volatile thiols were then quantified by isotopic dilution using the separation and detection methods previously described by Tominaga and co-workers.[53] Satisfactory sensitivity and reproducibility were reported despite the use of internal standards containing only one deuterium atom on an exchangeable position.

Several derivatization procedures have been developed for the enhancement of the volatility and reduction of the thermal degradation of cysteinylated precursors, so that they can be analyzed by gas chromatography. Trimethylsilation of Cys3MH, Cys4MMP, and Cys4MMPOH using a mixture of bis(trimethylsilyl)-trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS), and pyridine (3/1/3) gave stable derivatives (Figure 12) that were easily analyzable by GC−MS.[23b] This method was dedicated to the formal identification of only Cys3MH and Cys4MMP as precursors of the corresponding thiols by comparing the mass spectra of the trimethylsilylated compounds obtained from synthetic solutions and from crude extracts containing sulfur flavor.[23b] For Cys3MH, an improved version of this procedure was used to measure the influence of this compound on the aromatic potential of Merlot and Cabernet-Sauvignon.[14a] Presently, Cys3MH is purified, from a very small volume of must (500 μL) compared to the 45 L in the original method,[23b] by affinity chromatography (Chelating Sepharose 4B column) and subsequent derivatization using a mixture of BSTFA/TMCS/pyridine.[14a] The use of [^{15}N]-Cys3MH as an internal standard warrants a more reliable quantification.[14a] More recently, the derivatization of Cys3MH through a perfluoro-acylation reaction allowed the separation of the two diastereomers on a BPX35 capillary column and their quantitative analysis by GC−ITMS/MS.[29] The derivatization used a mixture of heptafluorobutyric anhydride (HFBA) and heptafluorobutanol (HFOH) (Figure 13).

Direct analytical methods (i.e., without a derivatization step) were later developed for the quantification of Cys3MH[64] and then for

Table 6. Analytical Methods Allowing the Quantification of Thiol Precursors in Musts and Wines

target compound(s)	sample preparation[a]	analysis	quantification[b]	LOD (μg/L)	repeatability (RSD %)	advantages (+)/ drawbacks (−)	ref
CysMH Cys4MMP	enzymatic cleavage of cysteinylated precursors, followed by analysis of released thiols according to the method of Tominaga et al.[23b]	GC−MS (SIM)	SIDA (ISTDs: [$^2H_1$]-Cys3MH and [$^2H_1$]-Cys4MMP)	na	Cys3MH: 5 Cys4MMP: 6	(−) 500 mL of must (−) only one quantifier ion per compound (−) labeled standards with only one deuterium atom introduced on a exchangeable position	26b
Cys3MH	purification of Cys3MH from must by affinity chromatography (Chelating Sepharose 4B columns), followed by derivatization with a BSTFA/TMCS/pyridine mixture	GC−MS (SIM)	SIDA (ISTD: [^{15}N]-Cys3MH)	na	5	(+) 500 μL of clarified must (−) only 1 Da of mass shift between natural and labeled Cys3MH	14a
(*R*)-Cys3MH[c] (*S*)-Cys3MH[c]	purification of Cys3MH from must by affinity chromatography (Chelating Sepharose 4B columns), followed by derivatization with a HFBA/HFOH mixture	GC−MS/MS	internal calibration (ISTD: *S*-benzyl-L-cysteine)	0.3	5	(+) separation of Cys3MH diastereomers	29
Cys3MH	SPE (Supelclean Envi18)	HPLC−MS (SIM)	SIDA (ISTD: [$^2H_2$]-Cys3MH)	3	3	(+) one-step sample preparation	64
Cys3MH Cys4MMP G3MH G4MMP	extraction on a cation-exchange resin, followed by purification by SPE (Sep-Pak)	nanoLC−MS/MS (MRM)	SIDA (ISTDs: [$^2H_2$]-Cys3MH, [$^2H_6$]-Cys4MMP, and [$^2H_2$]-G3MH) and external calibration for G4MMP	Cys3MH: 0.3 Cys4MMP: 0.1 G3MH: 0.06 G4MMP: 0.008	Cys3MH: 7 Cys4MMP: 12 G3MH: 7 G4MMP: 27	(+) simultaneous quantification of four precursors in must (+) 1.2 mL of must	26a
(*R*)-Cys3MH[c] (*S*)-Cys3MH[c] (*R*)-G3MH[c] (*S*)-G3MH[c]	SPE (Strata SDB-L cartridge)	HPLC−MS/MS (MRM)	SIDA (ISTDs: [$^2H_8$]-(*R*)-Cys3MH, [$^2H_8$]-(*S*)-Cys3MH, [$^2H_9$]-(*R*)-G3MH, and [$^2H_9$]-(*S*)-G3MH)	(*R*)-Cys3MH: 0.04 (*S*)-Cys3MH: 0.04 (*R*)-G3MH: 0.08 (*S*)-G3MH: 0.13	(*R*)-Cys3MH: 10 (*S*)-Cys3MH: 10 (*R*)-G3MH: 7 (*S*)-G3MH: 8	(+) separation of Cys3MH and G3MH diastereomers (+) quantification in must and wine samples	28

[a] SPE, solid-phase extraction. [b] ISTD, internal standard; MRM, multiple reaction monitoring. [c] For (*R*)-Cys3MH, (*S*)-Cys3MH, (*R*)-G3MH, and (*S*)-G3MH, the letter in parentheses describes only the absolute configuration of carbon involved in the thioether bond.

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both cysteinylated and glutathionylated precursors.[26a,28] Presently, these methods use liquid chromatography instead of gas chromatography, which is more suitable for these nonvolatile molecules.

Cys3MH was quantified in Petite Arvine must and wine by SIDA−HLPC−MS.[64] The cysteinylated precursor was extracted from must or wine using SPE (Supelclean Envi-18), separated on a reverse-phase Nucleosil column, and then detected in negative ionization mode (APCI) by mass spectrometry. With limits of detection close to 3 μg/L, this analytical method is very convenient for quantifying even trace amounts of Cys3MH in must and wine.

Both cysteinylated and glutathionylated precursors of 3MH and 4MMP were first quantified in Sauvignon Blanc, Melon B., Gewürztraminer, and Riesling musts by SIDA−LC−MS/MS.[26a] Using a minimal volume of must, precursors were extracted using cation-exchange resin (Dowex) and then purified by SPE (Sep-Pak) on a reverse phase C18. The detection was performed in multiple reaction monitoring (MRM) mode, providing high sensitivity and specificity. Using a similar method, diastereomers of Cys3MH and G3MH were quantified for the first time in several musts by SIDA−HPLC−MS/MS with detection in MRM mode.[28] This method used a shorter sample preparation, as the must or wine samples were purified only by SPE using a reverse-phase C18 column (Strata SDB-L).

Table 6 provides an overview of all reported methods allowing the quantification of thiol precursors in must and/or wine, presenting the key parameters such as limit-of-detection (LOD) and repeatability data.

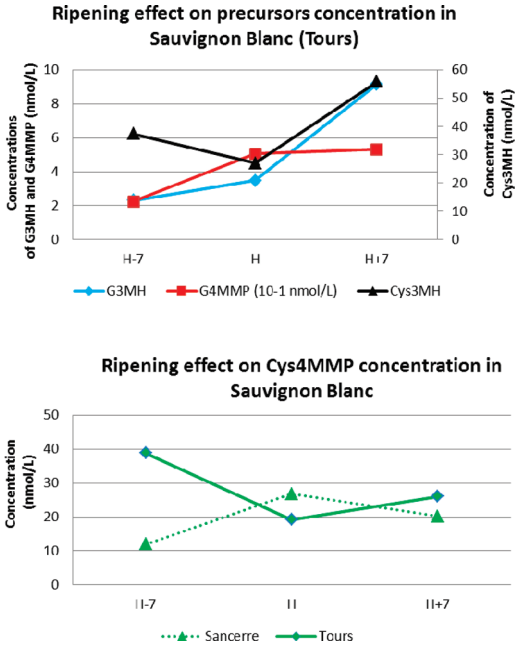

Figure 14. Influence of ripening on cysteinylated and glutathionylated precursors of 3MH and 4MMP for Sauvignon Blanc grapes from Sancerre and Tours. (H − 7, H, and H + 7 indicate the harvest date plus or minus 7 days.) Reprinted with permission from ref 67. Copyright 2010 American Chemical Society.

4. APPLICATIONS: VITICULTURAL AND ENOLOGICAL ASPECTS OF THIOL FORMATION IN WINES

4.1. Viticulture

The evolution of aromatic potential responsible for the release of varietal thiols was carefully studied to better understand the biogenesis of such molecules in the berries during ripening.

In 2000, Peyrot des Gachons and co-workers measured the evolution of cysteinylated precursors (Cys3MH, Cys4MMP, and Cys4MMPOH) in Sauvignon Blanc grapes from Bordeaux, from one month before harvest and for two consecutive vintages.[26b] The comparison between vintages demonstrated a huge difference in precursor contents probably depending on the must composition (assimilable nitrogen, for example). Ripening was found to directly affect the concentration of precursors by increasing their total initial amount in the berries. A similar study was conducted on Sauvignon Blanc and Melon B. musts for both glutathionylated and cysteinylated precursors of 3MH and 4MMP.[67] For Sauvignon Blanc grapes from two French vineyards (Sancerre and Tours), the concentrations of Cys3MH, G3MH, and G4MMP increased considerably during ripening (Figure 14). The Cys4MMP evolution was different according to the considered location (Sancerre versus Tours). For Melon B. grapes, no variation in precursor contents was measured during ripening.

The concentrations of cysteinylated precursors in Sauvignon Blanc grapes are modulated by water deficit.[68] The amount of Cys3MH is directly proportional to the water deficit, whereas the amount of Cys4MMP is inversely proportional to it. These observations have to be correlated with the production of polyphenol and hydroxycinnamic acids in Sauvignon Blanc grapes under mild water deficit, which are involved in negative aspects. For the cysteinylated and glutathionlated precursor of 3MH, it has recently been reported that elevation and soil composition can influence the amount of such molecules in Koshu grapes.[12]

The concentration of Cys3MH is also influenced by the *Botrytis cinerea* infestation of grapes.[24,29,64] Indeed, Cys3MH amounts were found to be considerably higher in botrytized Sauvignon Blanc and Semillon musts than in unaffected grapes.[29] In Sauvignon Blanc grapes affected by *Botrytis cinerea*, the increase of Cys3MH content, which is more important than the concentration effect in berries, occurs during the beginning of the botrytization process, that is, between the stages of healthy grapes and "pourri plein" (entirely botrytized but not desiccated).[24] The influence of over-ripening on the Cys3MH concentration is lower than that observed for botrytization: 10- and 100-fold increases, respectively. Similar observations were made in Petite Arvine must affected by rot, which exhibited a higher level of Cys3MH than healthy grapes.[64]

The influence of *Botrytis cinerea* on finished wines has also been noted.[38] Indeed, Sauvignon Blanc and Semillon wines affected by the Noble rot were found to contain higher concentrations of 3MH than wines obtained from healthy grapes.

The concentration of thiol precursors appears to be linked to the must composition such as the assimilable nitrogen depending on the vineyard management. Indeed, a moderate water deficit results in a higher Cys3MH concentration in Sauvignon Blanc grapes.[68] In addition, the vine nitrogen status influences the concentrations of cysteinylated precursors (Cys3MH, Cys4MMP, and Cys4MMPOH), glutathione, and polyphenol compounds in Sauvignon Blanc grapes.[69] The higher the nitrogen supply, the greater the increase in the cysteinylated precursors and glutathione contents. Similarly, the combination of nitrogen and sulfur foliar supply in Sauvignon Blanc vines enhances the concentrations of varietal thiols (4MMP, 3MH, and 3MHA) in the resulting wines.[70]

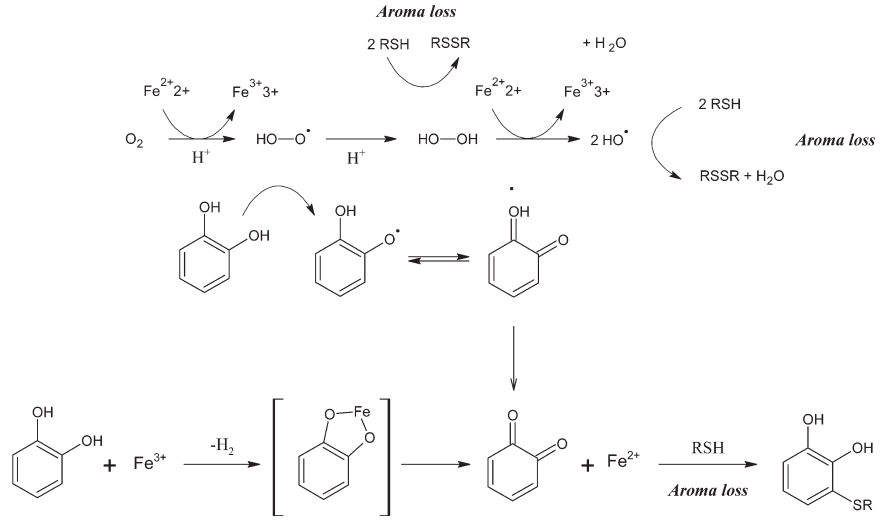

Figure 15. Possible mechanisms involved in thiol trapping in the presence of oxygen according to Nikolantonaki et al.[52b]

Foliar nitrogen and sulfur addition to Sauvignon Blanc vines before veraison improves the aromatic expression of the wines without increasing the vigor and *Botrytis cinerea*. The increase in the concentrations of varietal thiols in wines from foliar pulverization experiments is probably due to the increase of assimilable nitrogen, which modulates the conversion yield of the yeast.

4.2. Enology

4.2.1. Elaboration of Must. The elaboration of musts constitutes a key step in the wine-making process (white wines) because of oxidation reactions. Indeed, the crushing of berries involves the release of *trans*-caftaric acid, which is oxidized to *o*-quinones by polyphenoloxidase in the presence of oxygen. Until glutathione is present in the medium, quinones undergo a Michael addition of glutathione to form the so-called grape reaction product (GRP)[71] and then condense with other polyphenolic substrates such as flavonoids. GRP is not subject to further oxidation by polyphenoloxidase and does not contribute to the browning of the must. Nevertheless, in the case of infestation by *Botrytis cinerea*, the laccase can oxidize GRP in the corresponding quinone of GRP, which, in the presence of glutathione, can produce the double adduct (GRP2) or transform into brown polymers. As long as glutathione is available, the quinones are trapped and cannot participate in coupled reactions, leading to an irreversible browning of the must.

At this step of wine making, cysteinylated and glutathionylated precursors of 3MH and 4MMP are not oxidizable because of the chemical stability of the thioether bond under oxidative conditions. Consistent with this observation, Roland and co-workers[67] reported that, during the controlled oxidation of musts of Melon B. and Sauvignon Blanc, cysteinylated precursors and G4MMP exhibit no degradation, whereas G3MH levels increase. They hypothesized a reaction between glutathione and (*E*)-2-hexenal, where the latter is formed by the action of lipoxygenase on linolenic acid during pressing.[72] This reaction could explain the production of G3MH during prefermentation operations.[67]

Some wine-making processes such as skin contact and pressing influence the extraction of aroma precursors that are compounds susceptible to induce modifications in the finished wines. The localization of these molecules in the berry (skin and/or pulp) modulates their extraction during wine making. Interestingly, Cys3MH is mostly localized in the skin, whereas Cys4MMP is present in both the skin and the pulp of Sauvignon Blanc grapes.[73] Because of its preferential localization in skin (60%), Cys3MH was found to increase in concentration in Merlot and Cabernet Sauvignon grape juices with prolonged skin contact and a higher maceration temperatures (25 °C).[14a] Moreover, Cys3MH is also influenced by winery pressing because its concentration in must was found to increase significantly after 32 h of skin contact at 2 atm of pressure.[74] More recently, the distributions of Cys3MH, G3MH, and G4MMP in both Sauvignon Blanc and Melon B. grapes were measured.[75] For both grape varieties, precursors were in major part detected in the skin, except for G3MH in the Melon B. berries. As for Cys3MH, [74] G3MH was more extracted at the end of pressing for the elaboration of Sauvignon Blanc grape juices. [75] Nevertheless, the use of such a practice must be moderate because it also involves a better extraction of polyphenolic compounds, which are prejudicial for overall white wine quality. In addition, such compounds are substrates of oxidation reactions in bottled wines susceptible to affect the thiols' stability during wine storage (see section 4.2.3). Finally, additional investigations performed on Koshu grapes showed that Cys3MH and G3MH are preferentially located in the leaves, skin, and juice of this grape variety. [12]

4.2.2. Yeast and Fermentative Conditions Influencing Thiol Release. Varietal thiols are released during alcoholic fermentation by *Saccharomyces cerevisiae* yeast through its beta-lyase activity. Indeed, a cell-free enzyme extract of *Eubacterium limosum* (containing carbon−sulfur lyase enzymes) or purified tryptophanase from *Escherichia coli* was found to release 4MMP and 3MH, respectively, from their odorless *S*-cysteine conjugate precursors.[23b,76] The mechanism of release of these molecule was first investigated for 4MMP by deleting genes encoding putative *S. cerevisiae* yeast carbon−sulfur lyases.[77] According to Howell and co-workers,[77] four genes, namely, BNA3, CYS3, GLO1, and IRC7, seemed to be implicated in 4MMP release whereas Thibon et al.[78] demonstrated that only the putative cystathionine beta-lyase Irc7p was able to achieve the conversion of Cys-4MMP into 4MMP.[78] IRC7 is regulated by Ure2p/Gln3p proteins.[78]

The conversion of Cys-3MH appears to be more complex, and IRC7 is not the only gene involved. Because of a probable additive effect, the identification of the others enzymes will be

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very difficult. In addition, the enantiomeric distribution of 3MH might be influenced by the stereoselectivity of Irc7p.[78] This result is not consistent with the observation of Thibon and co-workers,[29] who hypothesized the nonstereoselectivity of the enzyme responsible for the cleavage because the enantiomeric distributions of 3MH and its cysteinylated precursor are similar in must as well as in wine. It should be noted that these studies concerned only cysteinylated precursors and no data are available on the genetic determination of the conversion of glutathionylated precursors. In addition, 3MH is also released through the hexenal pathway, but as the sulfur donor has not yet been identified, no investigation of possible genetic determination could be achieved.

3MHA results from acetylation of this unidentified sulfur donor by yeast ester-forming alcohol acetyltransferase, as encoded by the ATF1 gene.[79] The overexpression of ATF1 gene in the VIN13 yeast strain implicated a significant increase of 3MHA, whereas the overexpression of the IAH1 gene, encoding for ester-degrading enzyme, resulted in lower 3MHA contents.[79a] The selection of the yeast strain, as a modulator of varietal thiols release and aromatic quality of wines, represents a crucial step in wine making.

Few studies have been performed on the transportation of yeast cell precursors. In synthetic media, Gap1p (general amino-acid permease) constitutes at least one transporter of Cys3MH, whose activity regulates thiol production.[80] Thus, the production of varietal thiols by yeast, in such a medium, is modulated by the nitrogen catabolite repression mechanism, such as the uptake of nitrogen-poor sources. Indeed, the substitution of diammonium phosphate (DAP) by urea as the sole source of nitrogen was found to involve an increase of 3MH in synthetic medium.[80] On grape must, even if Gap1p has not be confirmed as a transporter precursor, addition of DAP, which eventually prolongs nitrogen catabolic repression (NCR), has been shown to decrease thiol release.

According to Thibon and co-workers,[78] NCR through Ure2p influences not precursor uptake, but rather only the kinetics of their absorption: derepressed strains exhibited a higher intake of precursors at the beginning of fermentation. This observation could be interesting from a technological point of view if the enzymes responsible for the cleavage are active only at this time of the process.

Some commercial yeast strains such as VL3c, EG8, VIN13, and VIN7[81] have demonstrated their ability to release varietal thiols under enological conditions. 4MMP and 3MH formation in wine can be modulated by yeast strains. Indeed, VIN7 and VIN13 yeast strains exhibit better conversion yield in cleaving *S*-cysteine conjugates for 4MMP and 3MH, respectively.[81c] When compared to parent yeast alone, a combination of *S. cerevisiae* strains, such as VIN7 and QA23, resulted in the overproduction of both 3MH, up to 200 ng/L, and 3MHA, up to 20 ng/L, in Sauvignon Blanc.[82] Recent investigations have demonstrated that cofermentation with *Pichia kluyveri* (non *S. cerevisiae* yeast) generates more 3MH and 3MHA in Sauvignon Blanc wines.[83] In addition, interspecific hybrid *S. cerevisiae* × *S. bayanus* var. uvarum was found to enhance the production of 4MMP from its *S*-cysteine precursor compared to its parent *S. cerevisiae*.[81d,84] Even if yeast strains can modulate the release of varietal thiols during alcoholic fermentation, the conversion yields are always below 10% for both classes of precursors (Table 5), providing no explanation for the total biogenesis of such molecules in wine.

Fermentation temperature influences the release of varietal thiols, but the reported data appear to be quite variable. More specifically, fermentation conducted at 20 °C instead of 13 °C was found to result in more 4MMP, 3MH, and its acetate in model medium and wines, despite the yeast strain used.[85] On the contrary, warmer conditions (28 °C instead of 18 °C) result in larger amounts of 4MMP being released. However, this observation seemed to be highly strain-dependent.[81a]

The diastereoselective cleavage of Cys3MH by yeast was investigated to explain the enantiomeric ratios of 3MH released in wine.[65] For this purpose, (*R*)-Cys3MH and (*S*)-Cys3MH were treated with apotryptophanase enzyme, where the absolute configuration is given with respect to the asymmetric carbon bonded to cysteine. (*R*)-Cys3MH gave only the corresponding (*R*)-3MH with a yield close to 82%. A similar outcome was observed for (*S*)-Cys3MH cleavage, which resulted in (*S*)-3MH (43%) and traces of (*R*)-3MH. Equivalent results were observed for *Saccharomyces cerevisiae* AWRI 1655 instead of the apotryptophanase enzyme. Similar investigations have been performed for the cleavage of G3MH during fermentation, under synthetic conditions.[33] The fermentation of the single (*R*)-G3MH diastereomer with the VIN13 yeast strain releases both (*R*)-3MH and (*R*)-Cys3MH, with a conversion yield close to 3% for the thiol production.

4.2.3. Aging. Thiols are chemically unstable because they are easily oxidizable in disulfide under mild oxidative conditions.[86]

At bottling, oxygen enters the headspace of a wine bottle in lower quantities (1−2 mg/L) than during storage over a period of 24 months (1−10 mg/L)[87] and can induce some oxidation reactions responsible for aroma loss. The oxygen transfer rate (OTR) during storage is stopper-dependent because some synthetic stoppers allow the entrance of oxygen into bottles at a relatively high rate, whereas screw caps and technical corks are well-known for their oxygen barrier properties.[9,88] Nevertheless, in several cases, the use of cork can cause the specific absorption of volatile compounds into the stopper. This phenomenon, called scalping, could be responsible for the loss of fruity aroma in Sauvignon Blanc wines by trapping 3MH and 3MHA in the cork.[89]

Depending on the type of wine, oxygen can be beneficial or detrimental for the aroma. Interestingly, oxygen participates in red wine maturation by enhancing the color and decreasing the global astringency of the wine.[90] In white wines, however, oxygen is highly prejudicial because it involves the loss of fruity aromas and the development of oxidized notes and induces a browning of the color.[91] Indeed, varietal thiols can react with electrophilic molecules in wine to form some specific adducts with polyphenolic compounds.[52b] The presence of (+)-catechin and (−)-epicatechin, together with Fe(III) catalyzing their oxidation into quinones, favors the disappearance of such thiols (Figure 15).

Nevertheless, the absence of oxygen in white wines at bottling and during the storage is characterized by the production of reduced dominant odors in such products. Consequently, an acceptable compromise has to be achieved during storage to protect white wine's aroma against oxidation reactions. One solution constitutes the aging on lees (before bottling) and the presence of sulfur dioxide, natural glutathione, and anthocyanins (in red and rosé wines) such as the malvidin-3-glucoside, which have a protecting effect against the loss of fruity notes in wine.[52a,89,92] Other + +techniques, such as the addition of glutathione as an antioxydant (trapping of quinones), have been discussed by the International Organization of Vine and Wine (OIV) to preserve the aroma of white wine during storage and to counterbalance the oxidation reactions.

5. CONCLUSIONS

Wine aroma is strongly influenced by viticultural and enological practices. Although fruity notes in young wines have been

heavily studied, varietal thiols are not readily manipulated, as many compounds influence wine quality in a positive or negative way. The presence of varietal thiols in wine results from many factors affecting the precursor concentrations. This occurs at all levels: (i) in grapes, (ii) in their extracts, (iii) in their release during fermentation, and (iv) in their conservation at a convenient level until the wine is consumed.

The presence of precursors in grapes depends on several viticultural factors, such as nitrogen and water nutrition, vine management, and maturity. These aspects are currently well-known, even if the mechanisms implicated are not totally elucidated.

Extraction of these precursors from grapes requires attention even if, in this form, they are not directly affected by the oxidation reactions occurring during must elaboration.

During fermentation, the conversion of these precursors into thiols remains the key step, and it is the topic of the majority of research studies in the field. Studies performed by research groups all around the world have allowed the identification of numerous precursors and the determination of their conversion, which depends on the grape composition (assimilable nitrogen, concentration and location of precursors in berries), as well as on the yeast genetic and fermentation conditions. In most of these studies, organic chemistry is necessary for the identification and quantification of thiols and their precursors and for the better comprehension of yeast contributions and physiology. As the discovery of some precursors is very recent, many aspects in this field have not been investigated.

After alcoholic fermentation, when thiols have been released, all of the technology employed must be focused against oxidation. Consequently, chemistry and biochemistry again remain helpful, as they offer the only tool to understand the various mechanisms (oxidation or nucleophilic addition) and, thus, propose means to avoid the aroma loss due to the disappearance of those compounds.

Many research studies should be performed in the future to better understand the interactions between thiols and other volatile or nonvolatile compounds, which are the keys to explain olfactive sensations and, thus, the wine aroma quality. This is a new interdisciplinary perspective that will require numerous disciplines, from chemistry to human neurophysiology.

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Aurélie Roland was born in 1984 and was graduated from the National School of Chemistry of Montpellier in 2007. She obtained her Ph.D. degree in Food Sciences in 2010 with work focused on the influence of oxidation mechanisms occurring during must elaboration on the aroma quality of Sauvignon Blanc and Melon B. wines from the Loire valley. Her Ph.D. work was published in six papers in peer-reviewed journals and presented at three international conferences. Now, she is working at the National Institute for Agronomic Research (Montpellier, France) on the development of analytical methods based on stable isotope dilution assays for the quantification of aroma compounds in wines.

Agricultural engineer and enologist Rémi Schneider received his Ph.D. in Food Science from the University of Montpellier (France) in 2001. He is now working at the French Wine Technical Institute (IFV) in Montpellier and coordinates a Joint Technical Unit between INRA (National Institute for Agricultural Research) and IFV, dedicated to new methods for the characterization of grape and wine quality. His research is focused on the analysis of aromas and aroma precursors. An expert at the OIV (International Organization for Vine and Wine), he is also member of the American Chemical Society.

Alain Razungles received his diploma in Agronomy in 1977, his Ph.D in Food Sciences in 1985, and his Habilitation in 2001 from the University of Montpellier. He is a professor of enology at Montpellier SupAgro and performs his research at the Sciences for Enology research unit at the National Institute for Agronomic Research (INRA) in Montpellier. His research interests concern three topics: wine aroma compounds and precursors in grapes, sensory analysis, and wine technology.

Florine Cavelier received her Ph.D. degree in organic chemistry from Montpellier University (France) in 1989. She spent two years as a Royal Society Fellow at the Dyson Perrins Laboratory under the supervision of Professor Jack Baldwin in Oxford, U.K. In 1991, she obtained an academic position at the Centre National de la Recherche Scientifique (CNRS). She is currently Research Director at the Institute of Biomolecules Max Mousseron (IBMM) in Montpellier. She is office member of the "Groupe Français des Peptides et Protéines" and member of the European Peptide Society. Her research interests focus on non-natural amino acids and biologically active peptides.

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ABBREVIATIONS

3MH	3-mercaptohexan-1-ol
3MHA	3-mercaptohexyl acetate
4MMP	4-mercapto-4-methylpentan-2-one
4MMPOH	4-mercapto-4-methylpentan-2-ol
AEDA	aroma extract dilution analysis
APCI	atmospheric-pressure chemical ionization
bp	boiling point
BSTFA	bis(trimethylsilyl)trifluoroacetamide
Cys3MH	*S*-3-(hexan-1-ol)-cysteine
Cys4MMP	*S*-3-(4-mercapto-4-methylpentan-2-one)-cysteine
Cys4MMPOH	*S*-3-(4-mercapto-4-methylpentan-2-ol)-cysteine
DAP	diammonium phosphate
DIAD	diisopropyl azodicarboxylate
G3MH	*S*-3-(hexan-1-ol)-glutathione
G4MMP	*S*-3-(4-mercapto-4-methylpentan-2-one)-glutathione
GAP1p	general amino-acid permease
GC−AED	gas chromatography−atomic emission detection
GC−EIMS	gas chromatography−electron impact mass spectrometry
GC−MS	gas chromatography−mass spectrometry
GC−O	gas chromatography−olfactometry
GRP	grape reaction product
HFBA	heptafluorobutyric anhydride
HFOH	heptafluorobutanol
HPLC−MS	high-performance liquid chromatography−mass spectrometry
ITMS/MS	ion trap tandem mass spectrometry
LOD	limit of detection
MRM	multiple reaction monitoring
NCI	negative chemical ionization
NCR	nitrogen catabolic repression
OAV	odor active values
OTR	oxygen transfer rate
PFBBr	pentafluorobenzylbromide
SIDA	stable isotope dilution assay
SIM	selecting ion monitoring
SPE	solid-phase extraction
SPME	solid-phase microextraction
SPME−GC−NCI−MS	solid-phase microextraction−gas chromatography−negative chemical ionization−mass spectrometry
THF	tetrahydrofuran
TMCS	trimethylsilylchlorosilane

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