Identification, Quantitation, and Sensory Evaluation of Thiols in Bordeaux Red Wine with Characteristic Aging Bouquet

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ABSTRACT: Great Bordeaux red wines are known for their distinctive aging bouquet. However, the nature of volatile chemicals underpinning this sensory expression is not fully understood. This work investigated the empyreumatic aging bouquet of a collection of premium Bordeaux red wines using silver-ion (Ag^+) solid-phase extraction, cryogenic heart-cutting multidimensional gas chromatography mass spectrometry/olfactometry, and comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. In doing so, a substantial number of "meaty" odors were revealed. Three detected "meaty" notes were tentatively or unequivocally attributed to furan thiols. Among them, 2-methyltetrahydrofuran-3-thiol (**1**) with a pleasant "meaty" aroma was reported in wine for the first time. Its *trans* isomer (*trans*-**1a**) was resolved from its racemate by chemical modification, which confirmed its presence in wine. The odor detection threshold of *trans*-**1a** in the model wine was determined at 55 ng/L. Moreover, an additive effect between **1** and literature-known 2-methyl-3-furanthiol was observed. By a new ultra high-performance liquid chromatography quadrupole Orbitrap high-resolution MS method, the concentration of *trans*-**1a**, in addition to those of 2-methyl-3-furanthiol and 2-furfuryl thiol, was measured in the wines at ng/L levels.

KEYWORDS: *meaty, thiols, aging bouquet, aroma, wine*

1. INTRODUCTION

Premium red wines produced in Bordeaux are renowned for their symbolic "Bordeaux style" aging bouquet associated with a great diversity of aromatic attributes, previously described as "dominant fruity/floral notes and some roasted and licorice odors, together with hints of empyreumatic nuances."[1] Analytical and sensorial characterization of aging bouquet has been described for premium aged red wines produced in Bordeaux[2,3] and other regions.[4] The aroma nuances of a wine are driven by the volatile molecules in the headspace acting as stimuli for the human olfactory system.[5] The volatile compounds identified in a wine are diverse in chemical families, concentration ranges, and sensory contributions.[6] Those with exceedingly low odor detection thresholds (ODTs) are often aroma-critical compounds because of their greater sensory impacts.[7] Volatile thiols are such aroma-active odorants that have been actively attracting research interest[8] since the identification of 4-methyl-4-sulfanyl-pentan-2-one (4MSP, boxtree odor, ODT 0.8 ng/L in the model wine), the first thiol reported in wines.[9] Other subsequently identified volatile thiols such as 3-sulfanylhexan-1-ol (3SH, grapefruit odor), 3-sulfanylhexyl acetate (3SHA, passion fruit odor), 2-furfurylthiol (FFT, coffee-like odor), and 2-methyl-3-furanthiol (2M3FT, cooked meat odor) have become widely recognized among the most potent volatile aroma compounds in wine.[10] Certain thiols were particularly linked to the empyreumatic notes in aged wines.[11−13] The identification of thiols ignited research focusing on thiol biosynthesis and fermentative biotransformation, demonstrating the significance of discovering new volatile compounds, particularly those having meaningful sensory contributions, for aroma research.[5]

From the perspective of analytical chemistry, qualitative and quantitative analyses of thiols in wine is intrinsically challenging[5] because these highly reactive molecules are mostly present at ultratrace quantities (~ng/L) in a complex wine matrix.[10] Historically, many of the first identifications of thiols in wines were achieved by odorant screening with conventional gas chromatography mass spectrometry/olfactometry (GC−MS/O) after selective extraction of thiols from wine. In short, thiols were selectively extracted by reversible affinity chromatography.[10] Afterward, the prepared organic extracts were analyzed by GC−MS/O for odorous zones (OZs) of interest. If the retention index, odor descriptors, and mass spectra of the selected OZs are obtained and match those of pure reference standards, then full identification can be concluded. The identification of 4MSP, 3SH, 3SHA, FFT, and 2M3FT in wine, along with many other more,[14] was achieved using such an analytical approach due to the extraordinarily sensitive human olfactory system employed in GC−MS/O screening.[10] However, severely toxic organomercuric chemicals are universally involved in the selective thiol extraction step, posing health and environmental concerns. Meanwhile, more

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robust chromatography separation and detection systems facilitated more thiol identifications in wine.[14,15] A recently proposed protocol described the identification of new thiols in red wine through oak-wood accelerated reductive treatment.[16] Another alternative approach to identify thiols in foods and beverages[10] was to conduct thiol-specific chemical derivatization and to screen thiol derivatives by high-performance liquid chromatography (HPLC) combined with high-resolution MS (HRMS) or tandem MS (MS/MS). Thiol-specific derivatization was needed to facilitate HPLC separation and to provide diagnostic ions for MS screening.

Recently, a solid-phase extraction (SPE) method using silver-ion (Ag^+) cartridges for selective thiol extraction was developed in our laboratory, with a particular focus on qualitative thiol screening.[17] In reported demonstrative applications, Ag^+ SPE was carried out to isolate thiol fractions from two Bordeaux red wines. Preserved free thiols were analyzed by Deans switch facilitated heart-cutting (H/C) multidimensional (MD) GC−MS/O.[17] This method offered a new analytical workflow to explore unknown trace volatile thiols in wine.

In this study, we investigated a pool of premium Bordeaux red wines with marked empyreumatic[11,12] aging bouquet in the hope to decipher unknown volatiles involved in their typical aging aroma expression. Preliminary benchtop sensory evaluation after the addition of traces of copper (Cu^{2+}) instantly depleted the empyreumatic aging bouquet of selected wines, indicating the involvement of thiols. Therefore, thiol fractions were isolated by selective Ag^+ SPE protocol.[17] The obtained thiol fractions were screened for the OZs of interest by cryogenic H/C MDGC−MS/O. A large number of OZs reminiscent of pleasant "meaty," "boxtree," and "thiols" was detected, demonstrating the organoleptic importance of thiols to the aging bouquet of the selected wines. A "meaty" smelling thiol, 2-methyltetrahydrofuran-3-thiol (**1**), was identified in wine for the first time. Chemical modification and density functional theory (DFT) calculations confirmed the structural assignment of *trans*-**1a** in wine. Tentatively, structures of two additional "meaty" OZs are proposed based on their mass spectra. In addition to H/C MDGC−MS/O screening, Ag^+ SPE thiol fractions were further explored by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC−TOF/MS), revealing more thiol candidates. The knowledge demonstrated in this study has expanded our understanding of the contribution of thiols to the aging bouquet of wine.

2. MATERIALS AND METHODS

2.1. Chemicals. The following chemicals were purchased from commercial suppliers: D-(−)-ribose (≥99%, Sigma-Aldrich, Saint-Quentin-Fallavier, France), L-cysteine (≥99.5%, Sigma-Aldrich), ethylenediaminetetraacetic acid (EDTA, ≥ 99.995%, Sigma-Aldrich), *cis*/*trans*-2-methyltetrahydrofuran-3-thiol (**1**, food grade, ≥ 97%, Sigma-Aldrich), 2M3FT (≥95%, TCI Europe, Zwijndrecht, Belgium), FFT (≥98%, Sigma-Aldrich), and 2-sulfanylpropanoate (≥95%, Enamine, Kyiv, Ukraine). Solvents (VWR, Paris, France) used for sample preparation were of HPLC grade or higher. Dichloromethane (CH_2Cl_2) was distilled before use. Water was purified by a Milli-Q ultrapure water purification system (Merck Millipore, Guyancourt, France). Solvents used for HRMS analyses were of Optima LC/MS grade (Thermo Fisher Scientific, Illkirch-Graffenstaden, France). The stock solutions of thiol standards were prepared by weighing each pure compound on a balance and making the desired volumes with HPLC-grade ethanol. Working solutions were obtained by volumetrically diluting the respective stock solutions. EDTA (∼1 mg/mL) was added to thiol solutions to minimize oxidations. Solutions of **1** and FFT were prepared every 2 months to ensure their integrity. 2M3FT was always prepared fresh. Thiol solutions were stored in an inert atmosphere, protected from light, and kept at −20 °C.

2.2. Wine Samples. Eight Bordeaux red wines (Table 1) were obtained from local producers for their expression of aging bouquet with empyreumatic nuances and were subjected to thiol screening by GC−MS/O and H/C MDGC−MS/O. Two wines (W9, W10, 1500 mL/bottle) from the same producer as W1, vintage 2011 and 2012, respectively, were used for assessing the GC × GC−TOF/MS performance.

Table 1. Selected Premium Bordeaux Red Wines with Distinctive Empyreumatic Aging Bouquet for GC−MS/O and H/C MDGC−MS/O Screening

wine code	vintage	variety[a]	alcohol (v/v, %)	volume (mL/bottle)	Appellation d'Origine Contrôlée
W1	2009	Merlot 65%, Cabernet Franc 30%, Cabernet Sauvignon 5%	13.5	1500	Saint-Emilion[b]
W2	2007	Cabernet Sauvignon 55%, Merlot 35%, Cabernet Franc 5%	13.0	1500	Saint-Julien[b]
W3	2007	Merlot, Cabernet Sauvignon*	13.5	750	Saint-Julien
W4	2001	Merlot, Cabernet Sauvignon*	13.5	750	Saint-Julien
W5	2015	Merlot, Cabernet Sauvignon*	13.5	750	Saint-Julien
W6	2009	Merlot, Cabernet Sauvignon*	13.0	3000	Listrac-Médoc
W7	2009	Merlot, Cabernet Sauvignon*	13.0	750	Listrac-Médoc
W8	2006	Cabernet Sauvignon 65%, Merlot 35%	12.5	375	Pessac-Léognan[b]

[a]Blending percentage not specified. [b]Classified growth.

2.3. Ag^+ SPE. The extraction of thiols from wine was conducted according to a recently described protocol.[17] A large-volume SPE barrel was fitted onto a Ag^+ SPE cartridge (MetaSep IC-Ag cartridge, GL Sciences, Tokyo, Japan). An aliquot of wine (one or several 750 or 1500 mL bottles) was extracted by CH_2Cl_2 (5% v/v solvent to sample ratio) three times. The resulting organic phases were pooled. Ag^+ SPE cartridge was preconditioned by 10 mL of CH_2Cl_2. Afterward, the collected organic extracts were loaded onto a Ag^+ SPE cartridge, followed by three washes, using CH_2Cl_2 (10 mL), acetonitrile (20 mL), and CH_2Cl_2 (10 mL) in sequence. Final elution was conducted by 5 mL of H_2O, 20 mL of freshly prepared L-cysteine solution (10 g/L in H_2O, sparged with N_2 before use), and 10 mL of CH_2Cl_2. The collected elutes (5 mL of H_2O, 20 mL of L-cysteine solution, and 10 mL of CH_2Cl_2) were combined together and stirred for 15 min, and the organic phases were pooled, dried on anhydrous Na_2SO_4, and concentrated under a gentle stream of N_2. For method blanks[18] (negative control samples), the entire extraction protocol was carried out as described above, whereas the wine sample (0 mL) was omitted, and the same batch solvents, reagents, laboratory ware, and apparatuses were used to gauge any possible artifact inputs originated from the laboratory environment and sample preparation.

2.4. Maillard Reaction Generating "Meaty" Flavor Compounds. A thermal reaction was conducted to generate "meaty" flavor compounds. A mixture was prepared by dissolving L-cysteine (100 mg) and D-(−)-ribose (90 mg) in 20 mL of phosphate buffer (0.2 M, pH 6.0).[19] Five aliquots (5 × 4 mL) of mixture were transferred to five 10 mL thick-walled glass tubes (Pyrex) and sealed with Teflon-coated screw caps. The tubes were protected from light and autoclaved at 120 °C for 1 h. The autoclaved mixture was cooled to room temperature, combined, and extracted by the Ag^+ SPE

protocol described above. The final extract (~50 μL) was analyzed by H/C MDGC−MS/O.

2.5. GC−MS/O and H/C MDGC−MS/O. GC−MS/O and H/C MDGC−MS/O were conducted according to a previously described method[17] using a 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to a 5977A MS detector (Agilent Technologies). The selectable MDGC−MS/O system was fitted with a G4513A autosampler (Agilent Technologies), an olfactometry detection port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) connected to a flow of humified N_2 (Air Liquide, Floirac, France), a low thermal mass series II (LTM-II) module, a Deans switch plate, a three-way splitter, and a cryogenic trapping system (CTS2, Gerstel) using liquid nitrogen as the coolant. Helium (Air Liquide) was used as the carrier gas. The GC conditions were as follows−injector: liquid injection in the splitless mode at 240 °C; host GC oven kept at constant temperature of 240 °C; LTM ^{1}D column (30 m × 0.25 mm i.d., 0.25 μm, DB-5MS or DB-35MS, Agilent J&W) with a flow rate of 1.5 mL/min; LTM ^{2}D column (30 m × 0.25 mm i.d., 0.25 μm, DB-WAX or DB-35MS, Agilent J&W) with a flow rate of 2.5 mL/min. For the conventional GC−MS/O analysis, the Deans switch was deactivated to bypass the ^{2}D column. In the H/C mode, Deans switch was activated, and cryogenic trapping was simultaneously applied. CTS2 was kept at 240 °C prior to the H/C, decreased at a rate of −720 °C/min to −150 °C (the duration of H/C), and increased to 240 °C at a rate of 720 °C/min (after H/C). H/C window was selected based on GC−O data to target particular OZs of interest. MS transfer line temperature, MS source, and MS quadrupole were at 260 °C, 230 °C, and 100 °C, respectively. Mass spectra were recorded in the full scan mode (35−350 m/z) using electron ionization at 70 eV. ODP was kept at 230 °C. A maximum of 2 μL of extracts was injected. Two trained expert panelists were instructed to use a free vocabulary to describe the odors that were perceived at the sniffing port and to rank the intensity of perceived odors on a numerical scale from 1 to 5, with 1 being "weakest" and 5 as "strongest." The panel was encouraged to focus on pleasant thiol-like odors. Linear retention indices (LRIs) were calculated by C_8−C_{20} *n*-alkanes (Sigma-Aldrich) for conventional GC−MS/O and for the ^{1}D of MDGC−MS/O runs. LRIs for the ^{2}D of the MDGC−MS/O were calculated according to a previously described procedure.[20] The collected sensory descriptors and their LRIs were compiled and compared to our in-house database and to NIST 2014. Method blank and instrument blank were analyzed to validate possible new identification of unknown thiols.

2.6. Comprehensive GC × GC−TOF/MS. GC × GC−TOF/MS system consisted of an Agilent 7890A gas chromatograph (Agilent Technologies), a Pegasus BT4D time-of-flight mass spectrometer (Leco Corporation, Saint Joseph, MI), and a L-PAL3 GC autosampler (Leco Corporation). A 0.5 μL organic extract was injected at 230 °C in the splitless mode (1 min). Helium (Air Liquide) was used as the carrier gas at a flow rate of 1.2 mL/min. Capillary columns, DB-5MS 50 m × 0.25 mm i.d., 0.25 μm film thickness (Agilent J&W) and RTXi-17Sil MS 1.3 m × 0.25 mm i.d., 0.25 μm film thickness (Restek, Bellefonte, PA), were used for the ^{1}D and ^{2}D separation, respectively. The oven program for the first column was 55 °C (1 min) and a 4 °C/min ramp to 280 °C (1 min). The secondary oven was kept at 5 °C higher relative to the primary oven temperature. The modulator was held at 15 °C higher relative to the secondary oven temperature. The modulation period was set for 5 s and was performed by using a QuadJet dual-stage thermal modulator with liquid nitrogen. The transfer line temperature was set at 260 °C. MS ion source temperature was 250 °C. MS was operated in the electron ionization mode at 70 eV, and the mass scan range was 40−400 m/z with an acquisition rate of 200 spectra/s. Data acquisition and processing were performed by using LECO ChromaTOF software (version 5.51). *n*-Alkane standards (C_8−C_{20}, Sigma-Aldrich) were analyzed for LRIs.

2.6.1. Data Curation. For each sample, automated baseline correction, deconvolution, and integration were processed using LECO ChromaTOF software for peaks with signal-to-noise (S/N) > 25. Furthermore, peak identification was obtained by comparing to commercial libraries (NIST 2017, FFNSC 3) for ΔLRI ≤ 30 and/or a minimum mass spectrum similarity score (MSSS) of 800 out of 1000.

2.7. Preparation of 1a and 1b. The *trans*- and *cis*- stereoisomers of **1** (*trans*-**1a** and *cis*-**1b**, respectively) were obtained from commercially available racemic **1** through the following procedures (Figure 3a). The initial stereoisomeric ratio of *trans*-**1a** and *cis*-**1b** in racemic **1** was determined by GC−EI−MS.

Step 1: *S*-(2-methyloxolan-3-yl) benzenecarbothioate (**2**): To a suspension of potassium carbonate (10.6 g, 76.3 mmol) in 60 mL of acetone were added racemic **1** (2.6 mL, 3.0 g, 25.4 mmol) and benzoyl chloride (4.4 mL, 5.4 g, 38.2 mmol). The reaction mixture was stirred at room temperature. Thin-layer chromatography (TLC) was conducted on silica gel 60 F_{254} TLC aluminum sheets (Merck) to monitor reaction. Spots were revealed with UV at 254 nm and potassium permanganate stain. The total conversion of **1** was achieved in 3.5 h. The mixture was extracted with EtOAc (3 × 40 mL). The organic phases were pooled and washed by H_2O (3 × 40 mL), neutralized with saturated $NaHCO_3$ solution (120 mL), and washed by H_2O (2 × 120 mL) until pH to 7. The organic phase was separated, dried over anhydrous magnesium sulfate, and filtered. The filtrate was collected, and the solvent was removed under a vacuum to obtain a crude colorless liquid **2** (5.95 g).

Step 2: The crude product **2** (3 g) was purified by silica gel 60 (Merck, 70−230 mesh, 0.040−0.063 mm) flash chromatography (*n*-pentane/Et_2O, 1/9, v/v) to give two colorless liquids: **2a** (330 mg, R_f = 0.28) and **2b** 1.195 g (R_f = 0.20).

For atom numbering used for NMR description, refer to Figure S1 of the Supporting Information.

trans-(±)3-*S*-Benzoyl-2,5-anhydro-1,4-dideoxy-3-thiopentitol (**2a**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (d, J = 6.1 Hz, 3H, CH_3), 1.98 (dddd, J = 13.1 (4a−4b), 7.8 (4b−5a), 6.4 (3−4b), 5.3 (4b−5b) Hz, 1H, H4b), 2.59 (dddd, J = 13.1 (4a−4b), 8.6 (3−4a), 7.6 (4a−5b), 6.9 (4a−5a) Hz, 1H, H4a), 3.75 (app. td, J = 8.5, 6.8, 1H, H3), 3.98−3.81 (m, 2H, H2, H5a), 4.03 (ddd, J = 8.7 (5a−5b), 7.7 (4a−5b), 5.4 (4b−5b) Hz, 1H, H5b), 7.46 (m, 2H, H_{ar}3′,5′), 7.58 (tt, J = 7.4, 1.3 Hz, 1H, H_{ar}4′), 7.95 (dd, J = 8.4, 1.3 Hz, 2H, H_{ar}2′,6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.54 (CH_3), 33.63 (C4), 46.88 (C3), 66.98 (C5), 80.32 (C2), 127.38 (C_{ar}2′,6′), 128.80 (C_{ar}3′,5′), 133.66 (C_{ar}4′), 136.98 (C1′), 191.59 (C═O).

cis-(±)3-*S*-Benzoyl-2,5-anhydro-1,4-dideoxy-3-thiopentitol (**2b**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (d, J = 6.0 Hz, 3H, CH_3), 2.07 (dddd, J = 13.3 (4a-4b), 8.0 (4b−5b), 6.1 (4b−5a), 4.3 (3−4b) Hz, 1H, H4b), 2.57 (dddd, J = 13.3 (4a−4b), 8.6 (4a−5a), 7.5 (3−4a), 6.1 (4a−5b) Hz, 1H, H4a), 3.82 (app. td, J = 8.4 (4a−5a and 5a−5b), 6.5 (4b−5a) Hz, 1H, H5a), 4.01 (app. td, J = 8.0 (5a−5b and 4b−5b), 6.1 (4a−5b) Hz, 1H, H5b), 4.21−4.31 (m, 2H, H2, H3), 7.45 (m, 2H, H_{ar}3′,5′), 7.58 (tt, J = 7.5, 1.3 Hz, 1H, H_{ar}4′), 7.95 (dd, J = 8.4, 1.4 Hz, 2H, H_{ar}2′,6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.13 (CH_3), 33.84 (C4), 46.53 (C3), 66.25 (C5), 76.92 (C2), 127.38 (C_{ar}2′,6′), 128.77 (C_{ar}3′,5′), 133.60 (C_{ar}4′), 137.08 (C-1′), 191.65 (C═O).

Step 3: **2a** (330 mg, 1.5 mmol) was added to a degassed aqueous lithium hydroxide solution (LiOH, 0.1 mM, 45 mL), and the reaction mixture was stirred under an argon atmosphere for 5 h. The reaction mixture was extracted with *n*-pentane (2 × 20 mL). The aqueous phases were separated and acidified with 5 M HCl to bring pH to 3−4. The aqueous phase was extracted with CH_2Cl_2 (20 mL), dried over anhydrous magnesium sulfate, and filtrated. The filtrate was concentrated by gentle rotary evaporation, and a white precipitate was formed. The white precipitate was washed with a small amount of ice-cold *n*-pentane. The organic phase was recovered and gently removed by rotary evaporation to afford colorless liquid *trans*-**1a** (96 mg, 55% yield, purity 97% determined by GC−EI−MS).

trans-(±)-2-Methyltetrahydrofuran-3-thiol (*trans*-**1a**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, J = 6.1 Hz, 3H, CH_3), 1.58 (d, J = 7.3 Hz, 1H, SH), 1.84 (app. dtd, J = 12.65 (4a−4b), 8.3 (3−4a and 4a−5b), 6.6 (4a−5a) Hz, 1H, H4a), 2.44 (app. dtd, J = 12.65 (4a−4b), 8.0 (3−4b and 4b−5a), 5.8 (4b−5b) Hz, 1H, H4b), 2.77 (app. qt, J = 8.2 (8.0 for (2−3 and 3−4b) and 8.3 (3−4a), 7.3 (3-SH) Hz, 1H, H3), 3.59 (dq, J = 8.0 (2−3), 6.1 (2−6) Hz, 1H, H2), 3.54−

9.94:3.86 (app.td, J = 8.6 (8.3 (4a−5b) and 8.9 (5a−5b)), 5.8 (4b-5b) Hz, 1H, H5b), 3.91 (ddd, J = 8.9 (5a−5b), 8.0 4b−5a), 6.6 (4a−5a) Hz, 1H, H5a). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.20 (CH_3), 36.86 (C4), 42.66 (C3), 66.21 (C5), 83.70 (C2).

EI−HRMS (m/z): Calculated for $C_5H_{10}OS^+$, $[M]^+$, 118.0447; found 118.045. EI−MS, 70 eV, m/z (%): 74 (100), 41 (56), 84 (31), 45 (15), 56 (14), 118 (13, M^+), 43 (11), 73 (11), 55 (9), 59 (8). $LRI^{DB\text{-}5MS}$, 908. $LRI^{DB\text{-}Wax}$, 1308.

The same procedure was applied for the deprotection of **2b** (1.18 g, 5.5 mmol) to give *cis*-**1b** as a colorless liquid (399 mg, 64% yield, purity 96% determined by GC−EI−MS).

cis-(±)-2-Methyltetrahydrofuran-3-thiol (*cis*-**1b**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, J = 6.2 Hz, 3H, CH_3), 1.44 (d, J = 7.6 Hz, 1H, SH), 1.97 (dddd, J = 13.15 (4a−4b), 7.9 (4a−5b), 5.4 (4a−5a), 3.9 (3−4a) Hz, 1H, H4a), 2.46 (app. ddt, J = 13.15 (4a−4b), 8.7 (4b−5a), 6.9 (3−4b and 4b−5b) Hz, 1H, H4b), 3.40 (app. tt, J = 7.3 (7.6 (3-SH) and 6.9 (3−4b)), 4.3 (4.8 (2−3) and 3.9 (3−4a)) Hz, 1H, H3), 3.77 (app. td, J = 8.6 (5a−5b and 5a−4b), 5.4 (4a−5a) Hz, 1H, H5a), 3.98−4.07:4.02 (ddd, J = 8.6 (5a−5b), 7.9 (4a−5b), 6.9 (4b−5b) Hz, 1H, H5b), 4.03 (qd, J = 6.2 (2−6) 4.8 (2−3) Hz, 1H, H2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.91 (CH_3), 36.96 (C4), 42.04 (C3), 65.61 (C5), 77.36 (C2).

EI−HRMS (m/z): Calculated for $C_5H_{10}OS^+$, $[M]^+$, 118.0447; found 118.0480. EI−MS, 70 eV, m/z (%): 74 (100), 41 (55), 84 (29), 118 (16, M^+), 45 (16), 43 (12), 56 (12), 73 (11), 55 (9), 59 (9). $LRI^{DB\text{-}5MS}$, 950. $LRI^{DB\text{-}Wax}$, 1390.

2.8. DFT Calculations. All DFT calculations were carried out with Gaussian 16.[21] The calculation of the IR spectra began by conformational analysis of compounds (*R*,*R*)-**2** (*cis*-form) and (*S*,*R*)-**2** (*trans*-form). Preliminary conformer distribution search of (*R*,*R*)-**2** and (*S*,*R*)-**2** was performed at the molecular mechanics level of theory, employing MMFF94 force fields incorporated in GaussView 6.1 software package.[22] Ten conformers of (*R*,*R*)-**2** (11 conformers for (*S*,*R*)-**2**) were found within roughly 4 kcal/mol of the lowest energy conformer. Their geometries were optimized at the DFT level using the B3LYP functional and 6-31G** basis set, leading to four different conformers for (*R*,*R*)-**2** and (*S*,*R*)-**2**. Finally, only the three lowest energetic geometries for (*R*,*R*)-**2** and (*S*,*R*)-**2** were kept within 2.678 kJ/mol ((*R*,*R*)-**2**) and 2.232 kJ/mol ((*S*,*R*)-**2**). Vibrational frequencies and IR and VCD intensities were calculated at the same level of theory. The spectra were calculated for the isolated molecule in vacuo. For comparison to experiment, the calculated frequencies were scaled by 0.97, and the calculated intensities were converted to Lorentzian bands with a full width at half-maximum (fwhm) of 7 cm^{-1}.

2.9. IR Spectroscopy. IR spectra of **2a** and **2b** were recorded with a Thermo Nicolet Nexus 670 FTIR spectrometer at a resolution of 4 cm^{-1} by coadding 50 scans. Samples were held in a 250 μm path length cell with BaF_2 windows. IR spectra of **2a** and **2b** were measured in $CDCl_3$ at a concentration of 60 mM. The solvent absorption was subtracted out in the presented IR spectra.

2.10. GC−TOF/MS. Spectra and accurate masses of *trans*-**1** and *cis*-**1b** were obtained on an Agilent 7980A gas chromatograph coupled to a JMS-T100GCGC TOF mass spectrometer (JEOL Ltd., Akishima, Tokyo, Japan). 1 μL of sample was injected in a split/spiltless injector that was kept at 230 °C. A DB-5 capillary column (50 m × 0.22 mm i.d., 0.25 μm, Agilent J&W) was used for separation following the oven gradient: 45 °C (1 min) to 240 °C at 4 °C/min held for 5 min. Helium (6.0, Messer) was used as the carrier gas with a flow rate of 1.0 mL/min. The transfer line was kept at 250 °C. Ion source was set at 250 °C. Mass spectra were recorded in the full scan mode (45−300 m/z) with mass resolution at 5,000 using electron ionization at 70 eV. Data acquisition was performed using JEOL Mass Center software. Solutions of *trans*-**1a** and *cis*-**1b** were prepared in CH_2Cl_2 at a concentration of ∼1 mg/L.

2.11. Nuclear Magnetic Resonance Spectroscopy. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) experiments were performed using a Bruker Avance 300 spectrometer at 300 and 75 MHz, respectively, at 297 K with deuterated chloroform ($CDCl_3$) as the solvent. The spectra were referenced using the lock frequency of deuterated solvent. Chemical shifts (δ) and coupling constants (J) are expressed in ppm and Hz, respectively. NMR spectra data of synthesized *trans*-**1a**, *cis*-**1b**, **2a**, and **2b** are available in Figures S2−S7 of the Supporting Information.

2.12. Thiol Quantitation. Thiols *trans*-**1a**, 2M3FT, and FFT were quantified by a new UPLC quadrupole Orbitrap HRMS method. Sample preparation consisted of chemical derivatization described previously.[23] Liquid chromatographic separation was performed using a Vanquish system (Thermo Fisher Scientific) consisting of a binary pump, an autosampler, and a temperature-controlled column chamber. HRMS analyses were performed on a Thermo Fisher Scientific Exactive Plus Orbitrap mass spectrometer fitted with a heated electrospray ionization source (HESI-II) probe operated in the positive ion mode. Data acquisition and instrument control were managed with Xcalibur 4.3 and Tune 2.9 software (Thermo Fisher Scientific). The details of the method development and validation will be presented in a subsequent publication from our research laboratory.

2.13. ODT. ODT determination experiments were carried out in the sensory facility at ISVV. The ODT of **1** (commercial racemate) was measured in the model wine following the ISO 13301:2018 guideline.[24] The model wine solution was freshly prepared by dissolving food-grade L-(+)-tartaric acid (5 g/L) in a 12% (v/v) ethanol−water solution, and the pH was adjusted to 3.2 using NaOH pellets. To limit the change of the concentration of **1**, fresh standard solutions of **1** were prepared, stored in an inert atmosphere at −20 °C, and used for sensory analysis within 24 h. The addition of **1** to the model wine solution was performed 1 h before the sensory sessions. A panel of 25 participants (M 12, F 13, average age: 31, students and researchers at ISVV) were presented with six sets of triangular tests arranged in an ascending order in terms of the concentration of **1** (5, 15, 45, 135, 405, and 1215 ng/L). Each set consisted of three samples, in which one was different from the other two. Standard clear wine glasses were coded with a three-digit number, filled with 25 mL of the sample, and covered with lids. The participants were instructed to smell each sample within each set and to choose the sample exhibiting different aroma. The panelist was asked to provide descriptors for the aroma perceived. The collected sensory data were processed according to the group best estimated threshold procedure outlined in ISO 13301:2018.[24] ODT of *trans*-**1a** was determined in the same manner in a separate session by the same panel within the same day.

2.14. Sensory Interaction between 1 and 2M3FT. The sensory experiment was conducted in the ISVV sensory laboratory with a panel of 26 participants (M 8, F 18) recruited from ISVV. The general protocol and model wine solution preparation were identical to the ODT determination described above. Three sessions were performed. Session 1 aimed to assess the panel's ODT of 2M3FT in model wine using seven concentration levels of 0.5, 1, 2, 4, 8, 16, and 32 ng/L. Session 2 targeted the ODT of **1** in model wine with ascending concentrations of 5, 10, 20, 40, 80, 160, and 320 ng/L. For session 3, the base model wine was first supplemented with 2M3FT to reach a final concentration of 0.5 ng/L. Increasing concentration of **1** was added to the modified model wine to reach concentrations of 5, 10, 20, 40, 80, and 320 ng/L. Three sessions were conducted within the same day by the same panel. Group best estimated thresholds were obtained following the ISO 13301:2018 guideline.[24]

2.15. Statistical Analyses. Data reduction and treatment were performed using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. GC−MS/O Profile of Ag^+ SPE Extracts of Selected Wines. A total of eight red wines (vintage 2001 to 2015, aged 8 to 22 years) from the Bordeaux region (Table 1) were selected for their distinctive empyreumatic aging bouquet. To search potentially new volatile thiols, the Ag^+ SPE protocol recently developed in our laboratory[17] was used to isolate thiol fractions from red wines. Because of the trace quantity of thiols in red wines and the effluent splitting between MS and O, extra

measures were taken during sample preparation to ensure maximum MS/O detection, including using a relatively large volume of wine (one to several 750 or 1500 mL bottles) and concurrently concentrating the final Ag^+ SPE extracts to a small volume ($\leq$50 μL). This corresponded to a minimal concentration factor of 15,000. The obtained volatile thiol extracts were first analyzed by GC−MS/O and then by H/C MDGC−MS/O to screen OZs of interest by two experienced sniffers throughout this project.

A representative total ion chromatogram (TIC) of the GC−MS/O analysis of the Ag^+ SPE extract on a DB-5MS column is shown in Figure 1a. The chemical and sensorial complexity of

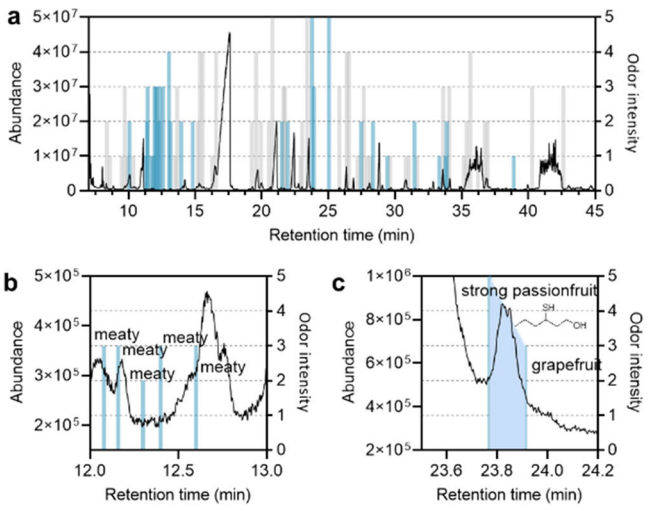

Figure 1. (a) Representative TIC overlaid with the aromagram of a Bordeaux red wine (W1) analyzed by Ag^+ SPE and GC−MS/O fitted with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm). (b, c) Expanded sections of (a). Vertical lines represent perceived OZs; in blue: OZs of interest, in gray: other OZs. Shaded area in (c) indicates that the odor was continuously perceived.

the Ag^+ SPE fraction was apparent. Twenty-nine OZs of interest exhibiting "thiol-like," "boxtree," "meaty," "roasted," "cooked food," and "grilled" notes that warranted further investigations were perceived in addition to forty-five OZs displaying disagreeable "sulfur," "rubber," "garlic," "pungent," or other aroma nuances. The detection of negative sulfurous or nonthiol OZs was related to certain sulfur-containing [e.g., 3-(methylthio)-1-propanol, 3-(methylthio)propanal], nitrogen-containing (e.g., pyrazines), and aromatic (e.g., phenylethyl alcohol) compounds which were simultaneously isolated by the Ag^+ SPE sorbent used. Our observation on the affinity of Ag^+ sorbent toward multiclass of molecules was mirrored by a recent report.[25] Nevertheless, the long-lasting odors of abundant wine volatile compounds such as acetates, alcohols, and acids were absent on GC−MS/O, showing sufficient selectivity of this sample preparation method targeting thiols.

The olfactometric profile of the thiol fractions by GC−MS/O was challenging to interpret. For instance, a total of five "meaty" OZs were subsequently noticed in an approximate 30 s elution time window (Figure 1b). The OZs of interest from GC−MS/O analysis of sample W8 on a DB-5MS column are listed in Table S1 of the Supporting Information. Each OZ was examined for their corresponding chromatographic peak and mass spectrum. Initial identification of OZs was difficult, mostly hindered by severe coelutions. Only for very limited examples can identification of known thiols based on a clearly resolved peak on GC−MS/O be made, such as for 3SH (Figure 1c). To resolve coelutions and obtain meaningful MS/O information, H/C MDGC−MS/O was necessary.

3.2. H/C MDGC−MS/O Profile of Ag^+ SPE Extracts of Selected Wines. Table S1 of the Supporting Information also presents respective OZs of interest detected after nine scheduled heart-cuts of sample W8. The first evident observation was that H/C MDGC successfully resolved persistent coelutions that were otherwise observed by conventional GC−MS/O using a single column (^{1}D−olfactometry). However, the odors detected after the second column by MDGC−MS/O (^{2}D−olfactometry) were not always consistent with those perceived on the ^{1}D. This discrepancy in perceived odors between the ^{1}D separations and ^{2}D was likely to be the results of perceptual interaction of coeluting odorants on the ^{1}D which were resolved by the ^{2}D. The next obvious point was that a number of OZs reminiscent of "meaty" and "cooked food" were detected. Although "meaty" aroma has been mentioned to contribute to the aging bouquet of fine Bordeaux red wines,[2,3,26,27] the associated molecules were limited to half a dozen known volatile thiols of which only 2M3FT exhibits a "meaty" or "roasted" odor. In another GC−MS/O study on aged red wine, FFT and 2M3FT were identified as contributing aroma compounds to the "savory" or "umami-type" aroma.[28] Our H/C MDGC−MS/O results clearly illustrated a deep involvement of "meaty" "cooked food" odors in the overall aroma space of premium Bordeaux red wines. Another interesting observation was that "meaty"/"cooked food" OZs ($LRI^{DB\text{-}WAX}$ ~ 964) occurred in all nine H/C runs despite being derived from different heart-cuts. Regardless, most resolved chromatographic peaks on the ^{2}D were still extremely small, which is not surprising for trace thiol analytes. In many instances, meaningful mass spectra for intriguing odors of interest were still not obtainable. With effortful manual peak picking and mass spectrum interpretation, the following previously known thiols 3-sulfanyl-2-methylpropan-1-ol, ethyl 2-sulfanylacetate, 3SH, 4MSP, 2M3FT, and FFT were found in the selected Bordeaux red wines. Certain detected thiols have been particularly associated with the positive aroma expression (3SH, 2M3FT, and FFT) of aged red wines[2,28] and empyreumatic notes (FFT) in various wines.[12,13,28]

3.3. Ag^+ SPE H/C MDGC−MS/O False Positive Identification. One OZ (GC−MS/O $LRI^{DB\text{-}5MS}$ ~848) exhibiting a strong "boxtree" and "thiol" odor was noted, but no identification could be concluded. Hence, LRI range ($LRI^{DB\text{-}5MS}$ 848 ± 5 = 843−857) was heart-cut, and the same odor was detected on the ^{2}D, accompanied by a resolved peak. NIST 2014 library matched it as methyl 2-sulfanylpropanoate (CAS 53907-46-3, matching score 785). The identity of this peak was confirmed by an authentic standard (MS, LRI, and odor quality) analyzed under the same analytical condition. Its tentative identification in wine by LC−HRMS was previously suggested.[29] It seemed that methyl 2-sulfanylpropanoate naturally occurred in wine, but a method blank indisputably proved that this identification was false in our wine samples. Further investigation (data not shown) showed that methyl 2-sulfanylpropanoate was an artifact that originated from the L-cysteine that was used for Ag^+ SPE elution. This false positive identification highlighted the importance of running method blanks as negative control.

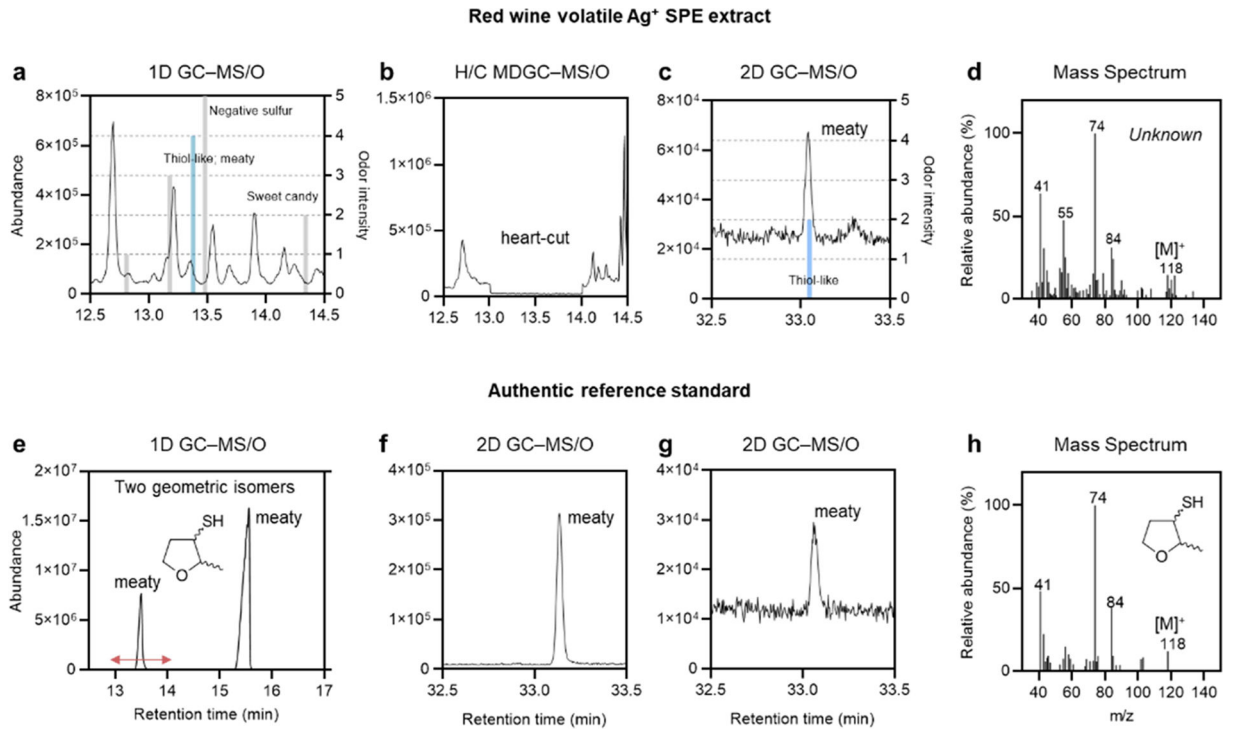

Figure 2. Compiled H/C MDGC–MS/O identification results of one isomer of 2-methyltetrahydrofuran-3-thiol **1** in a 2007 Bordeaux red wine (W3). (a) A segment (12.5–14.5 min) of GC–MS/O TIC of a wine Ag^+ SPE extract overlaid with detected OZs showing a distinct "thiol-like" "meaty" odor with an intensity level at 4. (b) A segment (12.5–14.5 min) of H/C MDGC–MS/O TIC of the same extract showing the heart-cut retention time = 13–14 min. (c) A segment (32.5–33.5 min) of H/C MDGC–MS/O TIC of the same extract showing a clearly resolved peak on the 2D. (d) Mass spectra of the peak showed in (c). (e) A segment (12.5–13.5 min) of GC–MS/O TIC of an authentic reference standard of 2-methyltetrahydrofuran-3-thiol analyzed under the identical instrument condition as that for (a). (f) A segment (32.5–33.5 min) of H/C MDGC–MS/O TIC of a high concentration of the same reference standard analyzed under the identical instrument condition as that for (c). (g) A segment (32.5–33.5 min) of H/C MDGC–MS/O TIC of a diluted authentic reference standard analyzed under the identical instrument condition as that for (c). (h) Mass spectra of the peak showed in (g).

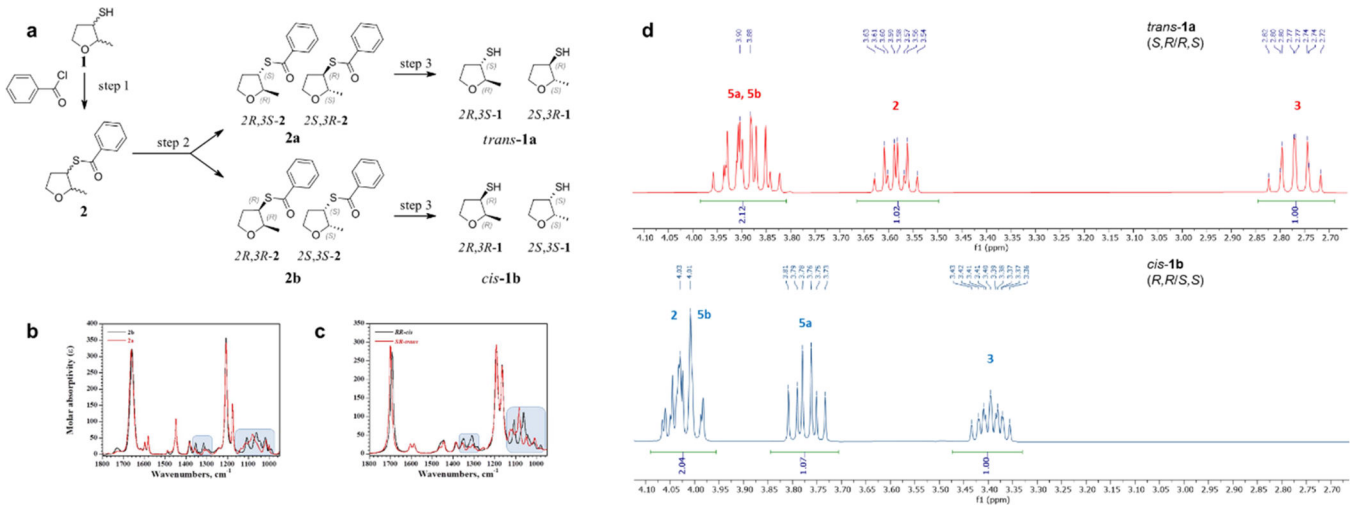

Figure 3. (a) Chemical synthesis routes to pure *trans* (*trans*-**1a**) and *cis* (*cis*-**1b**) isomers of 2-methyltetrahydrofuran-3-thiol from commercial **1**. Step 1: benzoyl chloride, K_2CO_3, acetone, rt, 3.5h. Step 2: flash chromatography (*n*-pentane/Et_2O, 1/9, v/v). Step 3: LiOH, H_2O, rt, 5h. (b) Experimental IR spectra of **2a** (in red) and **2b** (in black); shaded areas indicate spectra difference. (c) Calculated DFT spectra of *RS*-**2** (in red) and *RR*-**2** (in black); shaded areas indicate spectra difference. (d) Attribution of ^{1}H NMR spectra of H2, H3, and H5 of *trans*-**1a** and *cis*-**1b**. For atom numbering, refer to Figure S1 of Supporting Information.

3.4. Identification of 2-Methyl-tetrahydrofuran-3-thiol (1) in Wine by Ag^+ SPE H/C MDGC–MS/O. A pleasant "meaty"/"roasted meat" odor was noticed at ~$LRI^{DB\text{-}5MS}$ 900 during GC–MS/O screening (Figure 2a) of a 2007 Bordeaux red wine (W3). However, no positive identification can be reached due to coeluting peaks. Therefore, a one min heart-cut (centered at $LRI^{DB\text{-}5MS}$ 900, Figure 2b) was performed. A total of six OZs were perceived on the ^{2}D of which one had the same "cooked/roasted meat" odor that was noted on GC–MS/O. A fully resolved

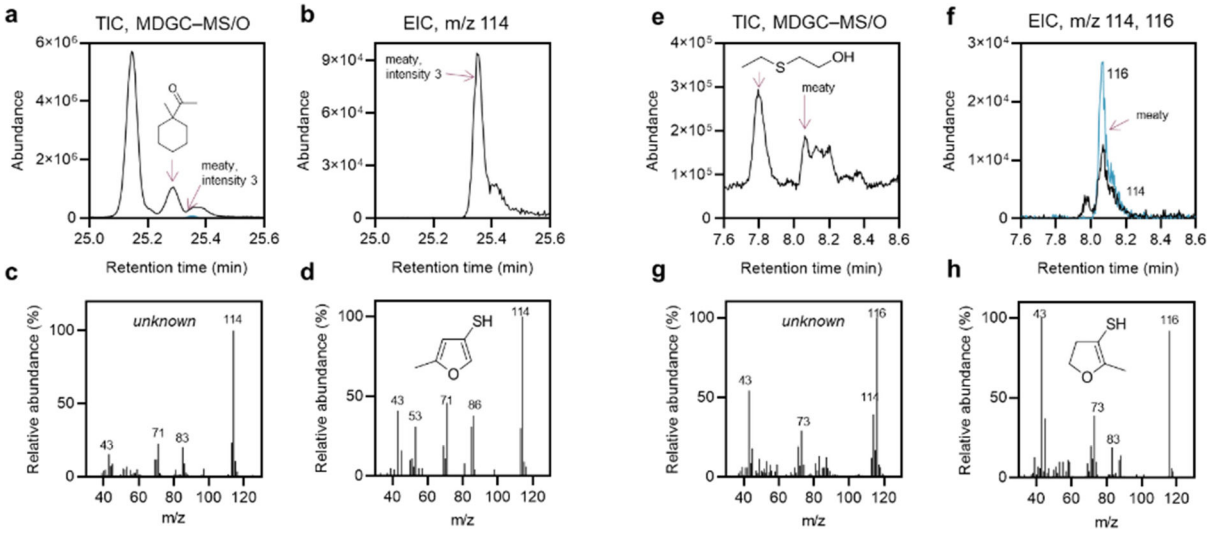

Figure 4. Chromatography and mass spectra of two unknown "meaty" smelling odors detected in the Ag^+ SPE extracts of selected Bordeaux red wines (W5 and W3) and two reference mass spectra. (a, e) TICs of the ^{2}D showing the respective retention regions; (b, f) EICs of the ^{2}D revealing the detection of ions *m/z* 114 and/or 116 during the detection of "meaty" odors; (c, g) experimental mass spectra of two unknown peaks; archived reference mass spectra (internal database from Firmenich) of (d) 5M3FT and (h) DH2M3FT.

chromatographic peak was recorded at the same retention time when this odor was detected on ^{2}D (Figure 2c). Mass spectrum search in the NIST 2014 database indicated the compound to be *cis*/*trans*-2-methyl-3-tetrahydrofuranthiol **1**. Its identity was fully confirmed by a commercial reference standard (CAS 57124-87-5, racemic mixture, Figure 2e–h). At this stage, identification was only confirmed for one isomer of **1**, not yet for the *cis*- or *trans*- form. Method blanks were conducted for both GC–MS/O and H/C MDGC–MS/O, and **1** was not detected in either run, validating its genuine occurrence in wines.

In the context of foods and beverages, **1** was mentioned in meat flavorings[30] and coffee products[31,32] in a few cases. In a previously published review,[33] it was stated that **1** had been found and quantified in wines, but this claim was questionable and cannot be backed up by the two originally cited source references.[34,35] Therefore, we believe that this is the first time that thiol **1** was positively identified in wine. When the authentic reference standard was used to confirm the presence of **1** in wine, we noticed that the identified single isomer of **1** had an $LRI^{DB\text{-}5MS}$ value very similar to that of FFT, a previously known thiofuran with extremely potent coffee-like odor (ODT 0.4 ng/L in model wine solution and 0.0084 ng/L in air[36]). In retrospective, their coelution on a classic nonpolar column during GC–O screening might partially explain why **1** was not identified in wine until now.

3.5. Preparation of *cis*-1b and *trans*-1a. **1** has two chiral centers at the C2 and C3 positions (Figure 3a), affording a pair of *cis*/*trans* stereoisomers and two pairs of enantiomers. The reference standard of **1** from a commercial supplier was a mixture of *cis*- and *trans*- isomers with an approximate ratio 1:5 or 5:1 (determined by GC–EI–MS with a DB-5MS column, Figure 2e) or 1:4.2–4.4 or 4.2–4.4:1 (later determined by the integration of SH peak on the ^{1}H NMR spectrum of commercial **1**). During our GC–MS/O and H/C GC–MS/O screening experiments, the "meaty" odor noticed only corresponded to one isomer on nonchiral GC columns DB-5MS (Figure 2a)/DB-35MS/DB-WAX. Based on available literature,[37] four enantiomers of **1** possessed distinguishable odor properties and different ODTs (~pg levels, in the unspecified matrix). Therefore, the ratio of *cis*/*trans* of **1** in the commercial racemate was determined to confirm the peak identity. NMR analysis of the commercial *cis*/*trans* mixture of **1** was carried out, but the spectrum was too difficult to interpret and did not allow confidently distinguishing between the two pairs of enantiomers (data not shown). Therefore, chemical synthesis (Figure 3a) was conducted to separate *cis*-**1b** and *trans*-**1a** from the commercial mixture. The chemical modification of the sulfhydryl group in **1** was achieved by the reaction with benzoyl chloride to form thioester **2**. The steric hindrance caused by the thioester formation facilitated the chromatographic separation of the *cis*/*trans* mixture by classical flash chromatography on silica gel to give two fractions: **2a** and **2b**. The fractions **2a** and **2b** were studied by IR spectroscopy to discriminate the two pairs of *cis* (*RR*/*SS*) and *trans* (*SR*/*RS*) diastereomers. IR spectra recorded in $CDCl_3$ solution at a concentration of 60 mM are reported in Figure 3b in the 1800–950 cm^{-1} spectral range. The IR spectra of **2a** and **2b** are similar, except around 1300 cm^{-1} and in the 1150–1000 cm^{-1} region. The band around 1300 cm^{-1} is related to the bending δOCH and wagging ωCH_2 of the furan group, whereas the bands in the 1150–1000 cm^{-1} region are related to the asymmetric ν_aCOC and symmetric ν_sCOC stretching modes of the furan group coupled with rocking CH_2 and CH_3. The bands associated with the thio-benzoyl group are not sensitive to the stereochemistry of the molecule (νC=O at 1660 cm^{-1}, ν_{8a}C=C and ν_{8b}C=C at 1598 and 1582 cm^{-1}, ν_{19a}C=C and ν_{19b}C=C at 1489 and 1449 cm^{-1}, δ_{ip}CH of phenyl at 1208 and 1177 cm^{-1}).

The predicted spectra calculated at the B3LYP/6-31G** level for the *RR*-**2** and *SR*-**2** configurations are reported in Figure 3c in the 1800–950 cm^{-1} spectral range. As observed in the experimental IR spectra, significant changes are present in the predicted DFT spectra for modes involving furan group around 1300 cm^{-1} and in the 1150–1000 cm^{-1} spectral range. Comparison of experimental and predicted spectra clearly establishes the *trans*-**2a** and *cis*-**2b** configurations.

The ^{1}H NMR analysis of **2a** and **2b** fractions was difficult because of the signal overlapping of protons at the 2, 3, and 5 positions (Figure S2 of the Supporting Information) but seemed to confirm the configuration determined by IR measurements and DFT calculations. Indeed, protons H2 and H3 are more strongly shielded for compound **2a** which is in agreement with theoretical calculations. Moreover, the proton H3 of **2a** is split to the apparent doublet of triplets (Figure S3 of the Supporting Information). Deeper analysis of this signal by the deconvolution process presented it as a ddd with ^3J = 8.9, 8.2, and 6.5 Hz which can be attributed to (2−3), (3−4a), and (3−4b) H−H coupling, respectively. According to the Karplus curve,[3] J = 8.9 Hz corresponds to the *trans* coupling of the protons at 2 and 3 positions.

The deprotection of the sulfhydryl groups of **2a** and **2b** gave *trans*-**1a** and *cis*-**1b**, respectively. The ^{1}H NMR analysis confirmed the configuration assignment as *trans*-**1a** and *cis*-**1b** (Figure 3d). The experimental vicinal coupling constants were $^3J_{2-3}$ = 8.0 Hz for *trans*-**1a** and $^3J_{2-3}$ = 4.8 Hz for *cis*-**1b**. These values are in accordance with the Karplus dependence of the H−H coupling constants on the dihedral angle, which is larger for the *trans*-isomer. In the same way as for protected forms, protons H2 and H3 were more shielded for compound *trans*-**1a.**

Therefore, the compound detected in the wine samples was confirmed as *trans*-**1a**.

3.6. Two "Meaty" OZs by Ag$^+$ SPE H/C MDGC−MS/O. Two intense and pleasant "roasted meat"/"meaty" OZs were detected during the GC−MS/O screening of Ag$^+$ SPE wine extracts. The odor qualities of these two OZs were almost indistinguishable from those of 2M3FT and *trans*-**1a**, but again, positive identification was not possible at this stage due to coelutions. Neither the OZ corresponded to known thiofurans (2M3FT, FFT, 2,5-dimethylfuran-3-thiol, and *trans*-**1a**).

The first intense (I = 5) "meaty" OZ was detected on the ^{1}D at ~ LRI$^{DB\text{-}5MS}$ 937 (rt = 15.20 min) of five bottles of WS. A 30 s heart-cut from 15 to 15.5 min was performed and resolved on the ^{2}D$^{DB\text{-}35MS}$. On the ^{2}D, two "meaty" and two "boxtree" odors were perceived. However, severe chromatographic coelutions appeared. One "meaty" odor (I = 4) was recorded in the valley region of two adjacent GC peaks both in decent abundances (Figure 4a). NIST 2014 library search suggested the first peak to be 1-(1-methylcyclohexyl)-ethanone, unlikely to correspond to any "meaty" odor. With a close inspection of the retention time region close to the beginning of the second peak, detection of m/z 114 was spotted. Extracted ion chromatogram (EIC, m/z 114) is presented in Figure 4b, which perfectly aligned with the detection of the intense "meaty" odor. The subtracted mass spectrum of this peak is given in Figure 4c, a mass spectrum very similar to that of 2M3FT. However, the identity of this peak cannot be 2M3FT as 2M3FT eluted outside this heart-cut event. Further comparison of our experimental mass spectrum to an external MS database (Firmenich) indicated a possible match to 5-methyl-3-furanthiol (5M3FT). The historically archived mass spectrum of the pure standard of 5M3FT is given in Figure 4d. 5M3FT is a constitutional isomer to 2M3FT, differing in the position of the methyl group. It was once mentioned in Maillard reaction products, but its identification (LRI^{CPWAX57CB}=1304) then was only based on mass spectrum comparison to that of 2M3FT.[19] The same authors also suggested[19] that the detected isomer to 2M3FT could also be 4-methyl-3-furanthiol (4M3FT). Elsewhere, a similar mass spectrum of a compound originated from the model Maillard reaction was proposed to be 2-methyl-4-furanthiol (2M4FT, no LRI information available),[38] another isomer to 2M3FT. Without a pure reference standard, only tentative identification can be reached. None of the suspected isomers to 2M3FT were commercially available, and several attempts to synthesize 5M3FT were made, but none was successful (data not shown).

Another strong-smelling (I = 5) "meaty" odor was detected in the Ag$^+$ SPE extract (20 μL) prepared from W3 at ~LRI$^{DB\text{-}5MS}$ 941. To enhance detectability, repeated injections (n = 5) were performed, and five heart-cuts were cryogenically focused on the head of the ^{2}D column and released at once for MDGC−MS/O analyses. TIC shows persistent coelutions (Figure 4e). A small but partially resolved peak was noticed for the "meaty" aroma (I = 4) on the ^{2}D. Ions m/z 114 and 116 were observed at the corresponding retention time (Figure 4f). A clean mass spectrum was obtained for this partially resolved peak (Figure 4g). Again, no probable hits could be retrieved from the NIST 2014 library. After comparison to the same external database (Firmenich), the peak identity was indicated as 4,5-dihydro-2-methyl-3-furanthiol (DH2M3FT). The archived reference mass spectrum of DH2M3FT is provided in Figure 4h. This thiofuran was also reported as the volatile component from Maillard reaction that had "roasted meat" aromas.[38−40] DH2M3FT was previously patented as a flavoring substance[41] and its formation pathways were proposed.[42] DH2M3FT had LRI values reported as 927[40] on a DB-1 column and 939[39] on a HP-5MS column, which was close to our data (941 on a DB-5MS column). Substantial efforts for its synthesis were devoted but eventually proved fruitless in producing this targeted thiol even at a trace quantity (data not shown). Alternatively, we investigated the possibility of generating these two thiofurans (DH2M3FT and 5M3FT) through the Maillard reaction, which was known to produce "meaty" aromas. Following a literature protocol,[19] a Maillard reaction was performed. The resulting Maillard reaction mixture exhibited extremely intense "meaty" aromas. Although the mass spectra very similar to those of suspected thiofurans (Figure 4d,h) were noticed in the Maillard reaction mixture by GC−MS/O, due to the restraint on time and resources, the isolation of targeted thiol fractions from the Maillard reaction mixture and subsequent identification were not pursued. Therefore, the structural identify of these two "meaty" smelling compounds remains to be elucidated upon obtaining pure reference standards. The access to the unavailable standards may be achieved by novel chemical synthesis by fractionating Maillard reaction mixture using Ag$^+$ SPE and preparative scale MDGC with a cryogenic fraction trap, or by other alternative means.[43] Although Ag$^+$ SPE with preparative MDGC for isolating trace thiofurans from Maillard reaction extracts is without precedent, the concept of the technique is well-known for small molecule discovery.[44] One analytical system adapting single- dimension preparative GC with the fraction collector has already allowed the separation, collection, and identification of volatile compounds in wine.[45]

3.7. Ag$^+$ SPE and GC × GC−TOF/MS for Non-Targeted Thiol Screening in Wine. As demonstrated earlier, Ag$^+$ SPE and H/C MDGC−MS/O are very effective in thiol screening due to the highly sensitive human olfactory system, but its associated data acquisition (particularly for olfactometry) and interpretation require rigorous attention from panelists/analysts. Additionally, the conventional single quadrupole mass spectrometer operated in the full scan mode in

MDGC−MS/O struggles to provide sufficient detectability needed for trace thiol analytes in complex samples. By comparison, GC × GC−TOF/MS offers higher resolution power, lower detection limits, as well as automatic data deconvolution features than H/C MDGC−MS/O. This technique has been reported for nontargeted screening of (thiols) unknowns in wine[14,15] and coffee,[15,32] in which various sample cleanup protocols were applied. Here, Ag^+ SPE coupling with GC × GC−TOF/MS as an enhanced solution for thiol screening in red wines was demonstrated. First, the sample complexity significantly impacted GC × GC−TOF/MS performance. Crude organic extract of two wines (W9, W10) and two Ag^+ SPE extracts (first CH_2Cl_2 washing and the final elute) prepared from the same wines were collected. According to previous data,[17] these fractions had various levels of complexity. Each fraction was analyzed by GC × GC−TOF/MS, and the data summarizing the number of identifications in relation to data filtering criteria are presented in Table S2 of the Supporting Information. The total number of sulfur-containing compounds (119−152 matches) was quite similar across different fractions. Approximately, half of the matches were retained after mass spectra similarity score greater than 800 was applied. ΔLRI ≤ 30 filtered out an average of 87% the initial matches. The final Ag^+ SPE extract showed more than double the number of sulfur compound identifications compared to those in other organic extracts. This trend, in a much more pronounced manner, was observed for thiols. For W9, a total of 18 thiols fitting either the mass spectra similarity score or ΔLRI criteria were seen in the final Ag^+ SPE extracts compared to just two in other fractions (Table S2 of the Supporting Information). Compared to previously reported GC × GC−TOF/MS data for similar red wine varietal,[15] thiol-rich Sauvignon blanc wine,[46] and other wines,[14] a considerably higher number of thiols reported in this study clearly suggests the superiority of applying Ag^+ SPE as sample cleanup prior to nontargeted thiol screening by GC × GC−TOF/MS.

Table S3 summarizes volatile sulfur compounds and thiols tentatively identified in the selected Premium Bordeaux red wines. It can be first concluded that GC × GC−TOF/MS achieved equal, if not better, identification performance in comparison to H/C MDGC−MS/O as nearly all thiols discovered using H/C MDGC−MS/O were captured by GC × GC−TOF/MS, including **1**. Many of these sulfur and thiol compounds exhibited "meaty" or "roasted" aromas, reflecting the aroma profile of the initial wine samples. Apart from literature-known thiols, a number of novel thiols were also tentatively uncovered based on comparison of mass spectra similarity score and LRI to commercial available databases, such as 4-propan-2-ylbenzenethiol, thiophene-2-thiol, thioguaiacol, octane-1-thiol, and 5-methyl-2-furfurylthiol. This result shed new light on thiol composition in wine. For example, thioguaiacol was recently reported in smoke-exposed wines and thought to be unique to smoke tainted wines.[47] The detection and quantitation of thiophenols in our wines (data not shown) indicate a rather ubiquitous occurrence, which seemed to be unrelated to smoke exposure but to other sources. Further details on thiophenols in wines will be presented in upcoming manuscripts from our research group.[16]

3.8. ODT. The ODT of racemic **1** and *trans*-**1a** measured in model wine solution by an untrained panel was at 71 ng/L and 55 ng/L, respectively, much higher than previously reported values (2−13 pg in unspecified matrix).[37] As detailed sensory protocol and matrix information for the previous measurements were not provided,[37] it was hard to draw meaningful comparisons here. Threshold of racemic **1** was slightly higher than that of *trans*-**1a**. The threshold of 2M3FT reported in model wine was at 4 ng/L,[26] significantly lower than that of **1** and *trans*-**1a**. 2M3FT and *trans*-**1a** (and **1**) differ in the saturation degree of the furan ring, and this might result in a difference in their perception threshold. *Trans*-**1a** had a much lower detection threshold than that (300 ng/L) of another "meat-like" "roasty" sulfur-containing furan compound 2-methyl-3-(methyldithio)furan previously identified in red wines.[48] The threshold of *cis*-**1b** was not measured.

A quick assessment for the aroma of the two isomers (*trans*-**1a** and *cis*-**1b**) was conducted with a small panel (n = 5) after transferring ∼μL of each stock solution to a cellulose smelling strip. T*rans*-**1a** possessed very similar aroma properties to 2M3FT, showing pleasant "meaty," "cooked meat," and "BBQ" aroma. Comparing the aroma qualities, *trans*-**1a** and **1** had a more rounded and pleasant meaty aroma profile than *cis*-**1b** which exhibit a hint of rubbery/pungent notes, and this observation was mirrored by previous statements.[37] Influence of structural modification and chirality on the odor quality of thiols was well documented.[36]

3.9. Sensory Interaction between 1 and 2M3FT. Intrigued by the sensorial and structural similarities between **1** and the well-known 2M3FT, we investigated the sensory interaction between these two thiofurans was investigated. A sensory panel (n = 26) first accessed the ODT of two thiofurans in model wine and found that the ODT was at 0.9 and 60 ng/L for 2M3FT and **1**, respectively. The ODT at 60 ng/L for **1** measured using a new panel was consistent with the previously determined value (71 ng/L) considering the panel variations. However, the ODT of 2M3FT at 0.9 ng/L was much lower than previously reported 4 ng/L.[26] This discrepancy could be attributed to a highly sensitive panel to 2M3FT and/or the formation of trace 2M3FT disulfide (confirmed by GC−EI−MS, data not shown) that was extremely potent with an odor threshold reported at 2 parts in 10^{14} parts of water.[49] The high reactivity of 2M3FT has been long known,[26] and it can be easily oxidized to its disulfide form.[17] Regardless, 0.5 ng/L 2M3FT (half of the group detection threshold) was supplemented to a model wine solution, to which increasing amounts of **1** were added. In the presence of 2M3FT, the ODT of **1** was measured to be at 23 ng/L, a significantly decrease from 60 ng/L, indicating a strong additive effect between two investigated thiols. 2M3FT was previously reported to have a synergistic interaction with thiol 3-methyl-3-sulfanylbutanal during the aroma extraction dilution analysis of Sauternes wines.[50] In other words, even at the subthreshold level, **1** may still have meaningful impacts on "meaty" "roasted meat" aroma in wine in the presence of other thiols.

3.10. Quantitation of *trans*-1a, 2M3FT, and FFT. The concentrations of three thiols, *trans*-**1a**, 2M3FT, and FFT, were assayed in selected wines by a newly developed UPLC quadruple Orbitrap HMRS method and are presented in Table 2. In the surveyed wines, *trans*-**1a** was detected in all samples, indicating a ubiquitous occurrence. With concentrations ranging from 1.4 to 10.3 ng/L, under its threshold (55 ng/L), *trans*-**1a** appeared to be unable to make a direct contribution to wine aroma quality. However, given the observed additive effect between **1** and 2M3FT, subthreshold concentrations of *trans*-**1a** might still impart real sensory

Table 2. Concentration (ng/L)[a] of *trans*-1a, 2M3FT, and FFT in Selected Bordeaux Red Wines

wine	*trans*-1	FFT	2M3FT
1	3.3 (1.7)	2.5 (2.2)	79.8 (3.8)
2	10.3 (0.4)	4.8 (1.1)	37.1 (1.2)
3	1.4 (2.5)	30.4 (5.0)	35.7 (1.8)
4	5.9 (0.5)	10.9 (7.2)	30.3 (6.6)
5	3.4 (2.5)	4.6 (2.1)	26.4 (1.0)
6	4.8 (0.6)	1.8 (7.0)	47.6 (2.3)
7	7.2 (1.5)	3.2 (5.5)	112.1 (8.5)
8	5.0 (3.4)	2.0 (3.1)	95.8 (1.8)
9	6.3 (0.8)	8.6 (3.0)	39.4 (3.3)
10	6.3 (1.5)	2.8 (2.2)	67.5 (3.7)

[a]Data derived from replicates (n = 3 for wine 1−7, n = 2 for wine 8−10), expressed as average values with relative standard deviation (%, RSD) given in parentheses.

impact. Moreover, in another ongoing project in our laboratory, *trans*-**1a** was noticed in quantities in wine well above its threshold (unpublished results). In a previous study, **1** (racemate) was quantitated in coffee brew samples at ∼2 ng/L.[31] Regardless of the detected isomer(s),[31] *trans*-**1a** had a more pronounced concentration in our wines than in coffee brews.

FFT ranged from 1.8 to 30.4 ng/L, lower than that of 2M3FT between 26.5 and 112.1 ng/L. Both furan thiols presented at concentrations similar to the historical data measured in Bordeaux red wines.[2,26] Out of 10 wines, 6 wine samples contained *trans*-**1a** presented at a higher concentration than that of FFT. No correlation was noticed among the three thiofurans. Considering the very limited samples that were analyzed here, a broader survey covering more wines is certainly required.

In summary, this study has reported identification of novel thiols in Bordeaux red wines with marked aging bouquet. 2-Methyltetrahydrofuran-3-thiol **1**, reminiscent of "meaty" odor, was identified. Tentative identifications of two furan thiols were proposed. This study was the first demonstration of applying Ag^+ SPE with H/C MDGC−MS/O and GC × GC−TOF/MS as effective approaches for thiol discovery. Quantitation and sensory evaluation of *trans*-**1a** were achieved. Moreover, **1** and 2M3FT exhibited an additive sensory interaction effect. Looking into the future, the following avenues may be explored. First and foremost, *trans*-**1a** needs to be surveyed in a larger set of wines of various varieties, vintages, and regions to provide more quantitative data. The enantiomeric chirality of *trans*-**1a** can be further investigated. Questions surrounding the origin, evolution, and reactivity of *trans*-**1a** in wine remain to be answered. Additional efforts could also be directed to interrogate other detected unknown "meaty" odorants for a better understanding of the aroma space of the distinctive aging bouquet of Bordeaux red wine.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c05854.

Chemical structures of *trans*-**1a**, *cis*-**1b**, **2a**, and **2b**; ^{1}H and ^{13}C NMR spectra of *trans*-**1a**, *cis*-**1b**, **2a**, and **2b**; GC−MS/O and H/C MDGC−MS/O odor screening results; nontargeted GC × GC−TOF/MS screening results of extracts with different complexities; and tentative identification based on LRIs and MSSS from GC × GC−TOF/MS analysis of Ag^+ SPE extract of wine samples (PDF)

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Notes

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