ORIGINAL INDIRECT IDENTIFICATION OF 3-SULFANYLHEXAN-1-OL DIMER \( (3,3'\text{-DISULFANEDIYLHEXAN-1-OL}) \) IN SAUTERNES BOTRTYISED WINES

E. Sarrazin\(^1\), S. Shinkaruk\(^2\), C. Thibon\(^3\), P. Babin\(^4\), B. Bennetau\(^4\), T. Tominaga\(^1\), and PH. DARRIET\(^1\)

\(^1\) Faculté d’Œnologie, Institut des Sciences de la Vigne et du Vin, UMR 1219, INRA, Université Bordeaux II, 351 cours de la Libération, F-33405 Talence Cedex, France
\(^2\) Ecole Nationale d’Ingénieurs des Travaux Agricoles de Bordeaux, 1 cours du Général de Gaulle, CS 40201, F-33175 Gradignan Cedex, France
\(^3\) SARCO, BP 40, F-33015 Bordeaux Cedex, France
\(^4\) Institut des Sciences Moléculaires, Université Bordeaux 1, UMR 5802, CNRS, 351 cours de la Libération, F-33405 Talence Cedex, France

Abstract

A four-step purification method was developed to isolate a citrus-reminiscent compound that seemed to be specific to Sauternes botrytised wines. Combining multidimensional gas chromatography coupled with mass spectrometry and sniffing detection, with exact mass measurement, the fragmentation pattern of the odorant and its elemental formula \((\text{C}_{6}\text{H}_{12}\text{O}_{2}\text{S})\) were determined. Based on these data, the unusual structure of 3-propyl-1,2-oxathiolane was identified and characterised for the first time. The specific occurrence of 3-propyl-1,2-oxathiolane in Sauternes wine extracts was demonstrated to be an indirect proof of the presence of 3-sulfanylhexan-1-ol dimer \( (3,3'\text{-disulfanediylhexan-1-ol}) \). Although 3-sulfanylhexan-1-ol has already been shown to undergo oxidative dimerisation in natural products, this mechanism has never been established in wine. The tentative identification of 3,3'-disulfanediylhexan-1-ol is thus decisive to enhance our understanding of the way 3-sulfanylhexan-1-ol, a major wine odorant, evolves under mild oxidative conditions, such as wine ageing.

Introduction

Sauternes wine aroma has recently been studied by gas chromatography-olfactometry (GC-O) (1). Among the volatile compounds found in these specialty wines, six were reminiscent of citrus. Five were volatile thiols and have been identified as 3-sulfanylhexan-1-ol, 4-methyl-4-sulfanylpentan-2-one, 3-sulfanylpentan-1-ol, 3-sulfanylheptan-1-ol and 2-methyl-3-sulfanylbutan-1-ol (2, 3). The last citrus zone did not correspond to a volatile thiol. As this odorant was not detected in dry white wines made from the same grape varieties \( (i.e. \) Semillon and Sauvignon blanc), it seemed to be specific to botrytised wines. However, it was detected by GC-sniffing using a 230 °C-injection mode, but not using a 45°C-injection mode. It was thus thought to be formed thermally in the injector from another compound that might be present specifically in botrytised wines. The aim of this research was to identify the citrus odorant, as well as to understand its thermal formation from botrytised wine extracts in the injector.
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Experimental

Purification method. A 4.5 L sample of Sauternes wine was extracted with two successive additions of 600 mL of dichloromethane. The combined organic phases were washed with a 1 mM aqueous solution of sodium p-hydroxymercuribenzoate in 0.2 M Trizma® base [2-amino-2-(hydroxymethyl)-1,3-propanediol], pH 10 (2 x 120 mL for 10 min each time). The organic phase obtained was dried over anhydrous sodium sulphate and concentrated using a rotary evaporator to 2 mL. The concentrate was then purified by reverse-phase HPLC, using the method developed by Pineau et al. (4). The fractions with remarkable odours were extracted again with dichloromethane, as described by Pons et al. (5), and analysed by MDGC-MS-sniffing.

Heart-Cut Multidimensional Gas Chromatography-Olfactometry-Mass Spectrometry-Sniffing (MDGC-MS-sniffing). The MDGC separation was performed on two GC ovens, as described by Pons et al. (5). Preseparation was performed using a non polar SPB1 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The second column was a BPX5, a BPX50 or a BPX70 (SGE, 50 m, 0.25 mm i.d., 1.0 μm film thickness). The Agilent 5973 MS detector was functioning in electronic impact (70 eV) or chemical ionization modes (CH₄ reactant). Mass spectra were taken over the m/z 40-300 range.

Synthesis of 3-propyl-1,2-oxathiolane. 3-Propyl-1,2-oxathiolane was synthesised from 3-sulfanylhexan-1-ol using an optimized procedure similar to that proposed by Davis and Whitham (6). ¹H and ¹³C NMR spectra were recorded with a Bruker AC-300 FT (¹H: 300 MHz, ¹³C: 75 MHz), using TMS as an internal standard. Chemical shifts (δ) are expressed in ppm. NMR ¹H (CDCl₃) δ 0.93 (t, CH₃-8); 1.32 (m, 2H, CH₂-7); 1.35-1.87 (m, 2H, CH₂-6); 1.87 (m, 1H, CH-4b); 2.33 (m, 1H, CH-4a); 4.00 (m, 2H, CH₂-5); 4.14 (m, 1H, CH-S). NMR ¹³C (CDCl₃) δ 14.0 (CH₃-8); 22.5 (CH₂-7); 37.0 (CH₂-4); 38.5 (CH₂-6); 53.5 (CH-3); 75.1 (CH₂-5).

Results and Discussion

Identification of 3-propyl-1,2-oxathiolane. The volatile compounds from a total of 4.5 L Sauternes botryised wine were isolated by liquid-liquid extraction with dichloromethane. Acidic compounds as well as volatile thiols were removed by washing the organic extract with an alkaline mercury solution. As the citrus compound could not be accurately identified from this extract by MDGC-MS-sniffing, a further purification step was undertaken to avoid coelution. This was performed by HPLC with a C₁₈ reversed-phase column and led to 25 fractions. The samples containing the citrus odorant were extracted again and analysed by MDGC-MS-sniffing. Combining MS detection on electronic impact and chemical ionization modes, the molecular mass of the citrus odorant was established as M = 132 ([M + H]⁺ = 133) (Figure 1). MDGC-MS-sniffing analysis was repeated for the same extract on BPX70 and BPX50 columns (oven II), confirming this result. Linear retention indices on these two capillaries were respectively 1592 and 1236.

Exact mass measurement was then used to determine the elemental formula as C₆H₁₂OS (132.0605 Da) on a Waters GCT Premier (Waters, Manchester, UK). Based on these data, the unusual structure of 3-propyl-1,2-oxathiolane was hypothesized. This compound was synthesised and its structure was fully characterised for the first time (¹H and ¹³C NMR, 2D NMR, IR). As mass spectrum and linear retention indices of the synthetic pure substance were identical to data obtained from wine samples, the identification of 3-propyl-1,2-oxathiolane was confirmed.
Origin of 3-propyl-1,2-oxathiolane. One of Sauternes wine key-odorants is 3-sulfanylhexan-1-ol (I) (2, 3). This compound is also found in passion fruit where it is known to oxidize by dimerisation to form the disulfide (II) (7), which can then oxidize to the sultine (III) (8) (Figure 2). 3-propyl-1,2-oxathiolane (IV) was synthesised but was shown to be very unstable and to oxidize quickly to III. On the basis of the sulphur oxidation state, IV was thought to be an intermediary oxidizing product, and an hypothetical mechanism was suggested in agreement with the pathways already proposed for other kinds of organic sulphur-containing compounds (9, 10) (Figure 2).

To validate this hypothesis, synthetic II and III were injected by GC-MS at 45°C and 230°C. As IV was only detected when II was injected at 230°C, the thermal formation of the oxathiolane IV from the disulfide II was confirmed. This oxidation pathway of 3-sulfanylhexan-1-ol is thus experimentally demonstrated for the first time. The same result was obtained for botrytised wine samples: the GC-sniffing analysis revealed the presence of the interesting compound only using a-230°C injection mode. As a consequence, the identification of 3-propyl-1,2-oxathiolane (IV) in Sauternes botrytised wines is an indirect evidence of the presence of the disulfide II. As far as we know, this dimer is tentatively identified in wine for the first time.
However, 3-propyl-1,2-oxathiolane was detected in Sauternes botrytised wines, but not in dry white wines made from the same grape varieties. Therefore, these results suggested a specific composition of botrytised wines which made possible the formation of the disulfide during wine ageing.

Conclusion

This research reported the tentative identification of 3-sulfanylhexan-1-ol disulfide (3,3'-disulfanediyldihexan-1-ol) in Sauternes botrytised wines. 3-Sulfanylhexan-1-ol has a high olfactory impact in botrytised wines. Therefore, it is challenging to extend our knowledge of its reactivity and stability, particularly under mild oxidative conditions, such as Sauternes wine ageing in oak barrels.

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References