

## From Wine to Pepper: Rotundone, an Obscure Sesquiterpene, Is a Potent Spicy Aroma Compound

CLAUDIA WOOD,<sup>†,§,‡</sup> TRACEY E. SIEBERT,<sup>†,‡</sup> MANGO PARKER,<sup>†,‡</sup>  
 DIMITRA L. CAPONE,<sup>†</sup> GORDON M. ELSEY,<sup>†</sup> ALAN P. POLLNITZ,<sup>\*,†</sup>  
 MARCUS EGGERS,<sup>||</sup> MANFRED MEIER,<sup>||</sup> TOBIAS VÖSSING,<sup>||</sup> SABINE WIDDER,<sup>||</sup>  
 GERHARD KRAMMER,<sup>⊥</sup> MARK A. SEFTON,<sup>†</sup> AND MARKUS J. HERDERICH<sup>†</sup>

The Australian Wine Research Institute (AWRI), P.O. Box 197, Glen Osmond (Adelaide),  
 SA 5064, Australia; School of Agriculture, Pontificia Universidad Católica de Chile,  
 Casilla 306 Correo 22, Santiago, Chile; and Symrise Scent and Care Division and Symrise Flavor and  
 Nutrition Division, Mühlenfeldstrasse 1, D-37603 Holzminden, Germany

An obscure sesquiterpene, rotundone, has been identified as a hitherto unrecognized important aroma impact compound with a strong spicy, peppercorn aroma. Excellent correlations were observed between the concentration of rotundone and the mean 'black pepper' aroma intensity rated by sensory panels for both grape and wine samples, indicating that rotundone is a major contributor to peppery characters in Shiraz grapes and wine (and to a lesser extent in wine of other varieties). Approximately 80% of a sensory panel were very sensitive to the aroma of rotundone (aroma detection threshold levels of 16 ng/L in red wine and 8 ng/L in water). Above these concentrations, these panelists described the spiked samples as more 'peppery' and 'spicy'. However, approximately 20% of panelists could not detect this compound at the highest concentration tested (4000 ng/L), even in water. Thus, the sensory experiences of two consumers enjoying the same glass of Shiraz wine might be very different. Rotundone was found in much higher amounts in other common herbs and spices, especially black and white peppercorns, where it was present at ~10000 times the level found in very 'peppery' wine. Rotundone is the first compound found in black or white peppercorns that has a distinctive peppery aroma. Rotundone has an odor activity value in pepper on the order of 50000–250000 and is, on this criterion, by far the most powerful aroma compound yet found in that most important spice.

**KEYWORDS:** Grape; wine; Shiraz; Syrah; rotundone; pepper; *Piper nigrum*; aroma; spice; GC-MS-O

### INTRODUCTION

Pepper is one of the most potent spices added to food and is found on almost every dinner table. Black pepper (*Piper nigrum*) has been valued for its aroma and flavor for millennia. It was the principal spice imported by the Roman Empire (1, 2). When Alaric and the Visigoths laid siege to Rome, they demanded 3000 pounds of pepper as part of the ransom (1). Pepper was so important to the European economy in the 15th century that demand inspired an age of exploration, beckoning sailors to embark on voyages of discovery (2, 3). Just as the lure of pepper established the spice route (2) and led Columbus to discover the New World, our research into pepper aroma in Australian red wine has led us to identify an important impact odorant in peppercorns and other plant products.

*P. nigrum* is a vine belonging to the Piperaceae family; it is indigenous to the southwestern (Malabar) coast of India and today is extensively cultivated in Southeast Asia and Brazil. Unripe fruits can be preserved as green peppercorns or fungally fermented to produce the black peppercorns that are highly valued for their delicious aroma and hot taste. White pepper is produced when the fruit (pericarp) is removed from the ripe red peppercorns. About three-fourths of all pepper sold is black pepper, with white pepper accounting for most of the remainder (3).

Wine, made from grapes from the vine *Vitis vinifera*, has also been enjoyed since ancient times. The aroma of wine is complex and reflects the grape variety and maturity, site, and winemaking, with hundreds of volatile compounds present. Some of these aroma differences can be related to individual aroma impact compounds. For example, methoxy-pyrazines are responsible for green capsicum (bell pepper) aromas in some cool climate wines (4, 5), and thiols such as 3-mercaptohexanol, 4-mercapto-4-methylpentan-2-one, and 3-mercaptohexyl acetate are responsible for passionfruit and tropical guava aromas in Sauvignon Blanc and other varieties of wine (6–8).

\* Corresponding author (fax +61 8 8303 6601; e-mail ajpollni@senet.com.au).

<sup>†</sup> The Australian Wine Research Institute.

<sup>§</sup> Pontificia Universidad Católica de Chile.

<sup>‡</sup> These authors contributed equally to this work.

<sup>||</sup> Symrise Scent and Care Division.

<sup>⊥</sup> Symrise Flavor and Nutrition Division.

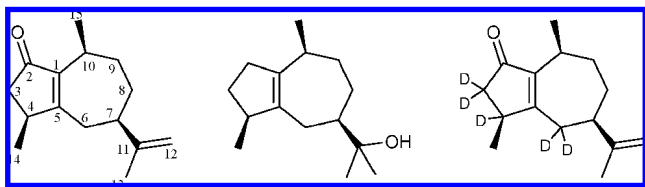


Figure 1. Structures of (–)-rotundone, guaiol, and  $d_5$ -rotundone.

Shiraz is a major red wine grape variety. Australian Shiraz is a medium- to full-bodied wine, usually oaked, encompassing a flavor spectrum from blackberry, plum, and mulberry to spicy, licorice, cigar box, eucalyptus, and black pepper. Shiraz is grown in Australia in a range of climates from hot sunny regions, which tend to produce full-flavored plum, spice, blackberry, and chocolate flavors, to cooler wetter regions, which sometimes produce more of the distinctive black pepper aroma, depending on the year of vintage (9, 10). Some of Australia's most famous "icon" wines are made from Shiraz grapes, for example, Penfolds Grange and St. Henri, Henschke Hill of Grace and Mt. Langi Ghiran Langi Shiraz. Various studies on the compositional and sensory analysis of Shiraz, which is also known as Syrah or Hermitage, have been published (9–12). However, as for pepper itself, the compound(s) responsible for the distinctive 'black pepper' aroma in Shiraz grapes and wine has (have) remained unidentified.

## MATERIALS AND METHODS

**Rotundone (Figure 1)** was synthesized from the sesquiterpene guaiol (Figure 1). Guaiol was extracted from guaiac wood oil (an essential oil from the guaiac tree *Guajacum officinale*) using an *n*-hexane/acetone mixture (2:1). After acetylation of guaiol using acetic anhydride, pyridine, and 4-dimethylaminopyridine (DMAP) in toluene, an allyl oxidation step was performed with either cobalt acetate, cobalt naphthenate, or cobalt(II) or cobalt(III) acetyl acetonate. Rotundone was then obtained via acetate pyrolysis and purified using column chromatography with silica gel 60 (70–230 mesh). From 1 kg of guaiac wood oil a main fraction of 15.6 g was obtained after chromatography yielding 42% rotundone (6.55 g), which was purified by further chromatography: EIMS,  $m/z$  (rel intensity) 219 (17), 218 ( $[M]^+$ , 100), 204 (14), 203 (89), 189 (10), 177 (11), 175 (27), 163 (38), 162 (28), 161 (50), 147 (37), 137 (39), 135 (19), 133 (25), 121 (18), 120 (27), 119 (37), 107 (21), 105 (32), 95 (17), 93 (20), 91 (30), 79 (18), 77 (18), 67 (15), 55 (11), 41 (17);  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ),  $\delta$  4.72 (1H, app quint,  $J = 0.9$ ,  $\text{H}_{12a}$ ), 4.69 (1H, app quint,  $J = 1.8$ ,  $\text{H}_{12b}$ ), 2.98 (1H, ddq,  $J = 11.2$ , 3.4 and 7.3,  $\text{H}_{10}$ ), 2.69 (1H, app quint,  $J = 7.2$ ,  $\text{H}_4$ ), 2.58 (1H, dd,  $J = 18.6$  and 6.6,  $\text{H}_{3\alpha}$ ), 2.56 (1H, dd,  $J = 15.6$  and 12.0,  $\text{H}_{6\alpha}$ ), 2.33 (1H, dt,  $J = 15.6$  and 1.8,  $\text{H}_{6\beta}$ ), 2.00 (1H, m,  $\text{H}_7$ ), 1.95 (dt,  $J = 18.6$  and 1.6,  $\text{H}_{3\beta}$ ), 1.81–1.74 (3H, overlapping m,  $\text{H}_{8,9\alpha}$ ), 1.75 (3H, br s,  $\text{H}_{13}$ ), 1.51 (1H, m,  $\text{H}_{9\beta}$ ), 1.10 (3H, d,  $J = 7.2$ ,  $\text{H}_{14}$ ), 0.99 (3H, d,  $J = 7.2$ ,  $\text{H}_{15}$ );  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ),  $\delta$  208.1, 176.9, 151.0, 145.4, 109.0, 46.1, 43.0, 37.9, 36.8, 32.6, 30.7, 26.9, 20.2, 19.2, 17.5.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , run on a Varian Unity Inova spectrometer, using Varian vnmr6.1c software) and  $^{13}\text{C}$  NMR spectra (150 MHz,  $\text{CDCl}_3$ ) of the reference compound were in excellent agreement with those reported (13) except that the  $^1\text{H}$  NMR signal for  $\text{H}_{15}$  was not given in that paper. We obtained an optical rotation that was in the same direction and similar to the published value (13) of  $[\alpha]_D^{25} -76.5^\circ$  ( $\text{CHCl}_3$ ;  $c$  0.09). Retention indices (RI), 1685 (DB-1), 1700 (DB-5), 1890 (DB-1701), and 2280 (Wax), were in agreement with the Symrise GC-MS database and the literature (14).

**$d_5$ -Rotundone (Figure 1)** was synthesized and characterized as described in the following paper (15).

**Samples.** The red wine used as nonpeppery control was a young (<12 months old) cask ("bag-in-box") wine, predominantly Cabernet Sauvignon variety. No rotundone was detected by GC-MS (i.e., <0.5 ng/L) in this control red wine used for sensory analyses. Shiraz grape samples were obtained from vineyards in South Australia and Victoria over two vintages, and the important sensory attributes of the grape

samples, including the aroma descriptor 'pepper', were rated by a sensory panel as described in Parker et al. (9). Black and white peppercorns, other spices, and other natural products were picked fresh as local produce or purchased (usually in the form of dried leaves) from local suppliers in Adelaide, Australia. For dried spices other than pepper, we verified that pepper had not been added through demonstrating the absence of piperine (the major component of pepper at approximately 5% v/v) (16). All solvents were verified for purity by GC-MS prior to use. Water was purified by the Milli-Q system.

**Enrichment of Rotundone in Pepper Extracts.** Peppercorns (250 g) were ground (Moulinex coffee grinder) and immersed in acetone for 1 week at 25 °C, after which the extract was filtered and the solvent was removed (under vacuum at 30 °C), yielding crude pepper extract (ca. 6.5% w/w) that, when left overnight, formed crystals (predominantly piperine). Removal of these crystals typically left ca. 3.5% w/w of crude extract, further cleaned up by coarse and fine silica gel chromatography as follows.

**Coarse Silica Chromatography.** The crude extract (ca. 8.8 g) was dissolved in *n*-pentane (250 mL) and swirled in contact with silica gel 60 (70–230 mesh, 90 g), after which the *n*-pentane was discarded. The silica was similarly washed again with *n*-pentane (250 mL) and then with 250 mL aliquots of solvent system *n*-pentane/ethyl acetate of increasing polarity ratio as follows: 95:5, 80:20, 50:50. The 80:20 fraction had a strong peppery aroma, so it was further concentrated to 1 mL by evaporation of the solvent (Vigreux column packed with Fenske helices) followed by evaporation under a gentle nitrogen flow until dry, yielding ca. 0.43 g of crude pepper extract (0.17% w/w of the original peppercorns).

**Fine Silica Column Chromatography.** This crude pepper extract was dissolved in *n*-pentane, loaded onto a silica column [7 g of silica gel 60 (70–230 mesh)], and eluted at atmospheric pressure with 100 mL aliquots of solvent system *n*-pentane/ethyl acetate of increasing polarity ratio as follows: 2 × 100:0 (*n*-pentane), 96:4, 94:6, 92:8, 90:10. For the last three solvent systems the fractions were collected in 20 mL vials, so that there were five fractions for each of the last three eluent solvent systems. Fractions that exhibited strong pepper aroma were set aside for further purification by high-performance liquid chromatography (HPLC), and the solvent was removed, yielding a combined total mass of 0.164 g (ca. 0.07% w/w of the original peppercorns).

**HPLC.** HPLC was performed (on the peppery fractions from the fine silica column chromatography above) using an Agilent 1100 series HPLC fitted with a Luna 5  $\mu$  silica 100A column of dimensions 250 × 2 mm. Solvent A was hexane/ethyl acetate (98:2), and solvent B was ethyl acetate. Gradient elution was done as follows: 0–5 min, solvent A; 5–10 min, increase to 2% of solvent B; 10–20 min, increase to 5% solvent B; 20–25 min, 5% solvent B; 25–30 min, increase to 10% solvent B; 30–35 min, increase to 50% solvent B; 35–40 min, 50% solvent B; 40–45 min, decrease to 0% solvent B; 45–55 min, solvent A. Pump pressure was 44 bar, and solvent flow was 0.4 mL/min. All samples were filtered prior to analysis. Linalool,  $\beta$ -caryophyllene, and caryophyllene oxide were used as standard markers for the elution of rotundone. From 18 to 25 min, fractions (200  $\mu$ L) were collected every 30 s. Fractions exhibiting the pepper aroma were combined to form an enriched extract used to confirm the presence of rotundone by GC-MS, GC-MS-O, and co-injections with the synthesized compound.

**Enrichment of Rotundone in Grape Extracts.** Australian Shiraz grapes (typically 1 kg) were destemmed, homogenized, blended, and stored at –20 °C overnight (typically in 250 g subsamples). Following thawing at room temperature, the mixture was poured into 50 mL Falcon tubes, sonicated for 10 min, and then centrifuged at 4000 rpm at 20 °C for 10 min. The combined supernatant was filtered through Whatman no. 1 filter paper, and the juice obtained (approximately 650 mL from 1 kg grapes) was stored at –20 °C before use. Prior to extraction, the juice was diluted 1:1 with water.

**Large Scale Solid-Phase Extraction (SPE) Cleanup.** Strata SDB-L bulk sorbent (10 g) was used to set a 17.5 cm SPE column. The column was conditioned with *n*-pentane/ethyl acetate (4:1) (200 mL) followed by methanol (100 mL). A pH 3.2 buffer (200 mL aqueous potassium hydrogen tartrate and tartaric acid) was then loaded onto the column, followed by the juice sample, then water (200 mL). The column was

eluted with 50 mL aliquots of solvent system *n*-pentane/ethyl acetate of increasing polarity ratio as follows: 1 × 100:0(*n*-pentane), 1 × 98:2, 4 × 90:10, 2 × 80:20. The 90:10 fractions (exhibiting pepper aroma) were combined, and the solvent was removed and further purified by the HPLC method, as described above.

**Confirmation of Rotundone.** The presence of rotundone was confirmed in both the enriched pepper and enriched grape extracts by GC-MS-O and co-injections with increasing amounts of the synthesized compound, which gave symmetrical peak enhancement, a matching mass spectrum, and the distinctive pepper aroma only at the correct retention indices on all three GC column phases (DB-5, DB-1701, and Wax).

**Quantitative Determination of Rotundone.** Rotundone concentration was determined by stable isotope dilution analysis solid-phase extraction—solid-phase microextraction gas chromatography—mass spectrometry (SIDA SPE-SPME-GC-MS) as described by Siebert et al. (15), except where noted below.

**Sample Preparation.** Grape and wine samples were prepared as described in Siebert et al. (15). For analysis of spices and other products, the product (e.g., ~5 g of finely crushed dried leaves, weighed exactly) was soaked in ethanol (50 mL) for 72 h. Into a 100 mL volumetric flask was measured 10 mL of this ethanol extract and made up to volume with water. *d*<sub>5</sub>-Rotundone (12.5 ng in 100 μL of ethanol) was added as internal standard (I.S.) to each sample.

**GC-MS Analysis.** An Agilent Technologies 6890 gas chromatograph (GC) was equipped with a Gerstel MPS2 multipurpose sampler and coupled to an Agilent 5973N mass selective detector. The GC was also fitted with a Gerstel olfactory detection port (ODP). The instrument was controlled with Agilent G1701CA ChemStation software in conjunction with the Gerstel MASTer software (version 1.81). The data were analyzed with Agilent G1701CA ChemStation software. The GC was fitted with one of the following three J&W capillary columns: DB-Wax, DB-5MS, or DB-1701, each of dimensions approximately 30 m × 0.25 mm, 0.25 μm film df. The carrier gas was helium (ultrahigh purity), and the flow rate was 1.0 mL/min in constant flow mode. We also used a 60 m DB-Wax column for some particularly difficult separations.

**Detection of Rotundone by Gas Chromatography–Mass Spectrometry–Olfactometry (GC-MS-O).** Our general methodology for GC-MS in combination with an olfactory (sniff) detector (GC-MS-O) and advantages and limitations thereof have been described elsewhere (19–22). An Agilent inlet was fitted with a resilanized borosilicate glass liner, 6.5 mm o.d., 4 mm i.d., 78.5 mm long, tapered at the column interface with a plug (2–4 mm) of resilanized glass wool. Injector temperature was 240 °C. The sample (2 μL) was injected in pulse splitless mode. The oven temperature was started at 80 °C, held at this temperature for 1 min, then increased to 220 at 3 °C/min, and finally increased to 240 at 40 °C/min and held at 240 °C for 20 min. The MS transfer line was held at 240 °C. Similar temperature programs were used for the DB-5 and DB-1701 GC columns, but with higher injector, final oven, and MS interface temperatures of 250 °C. The mass spectrometer quadrupole temperature was set at 150 °C, and the source was set at 260 °C. For scan runs, positive ion electron impact spectra at 70 eV were recorded in the range of *m/z* 35–350 at 2.36 scans/s. The *n*-alkane series used to determine retention indices was run in the same manner (21).

**Sensory Analyses: Aroma Detection Thresholds.** The aroma detection thresholds of rotundone in wine and water were determined according to the American Society of Testing and Materials (ASTM) method E 679-79 (22, 23). The sensory testing took place over approximately 3 weeks. Most panelists had previous experience with difference testing and with sensory evaluation of wine. The judges (*n* = 49 for water, 47 for red wine, 45 common to both) were AWRI staff and students of various ethnic origins, aged between 21 and 56, with similar numbers of males and females. Solutions of rotundone were prepared from stock solutions with concentration verified by SPE-SPME-GC-MS analysis (15). The wine or water samples were presented as 20 mL portions in covered, randomly coded glasses (as part of a triangle test) in ascending order of rotundone concentration. Those panelists who successfully detected all concentrations were retested at lower levels, whereas panelists who were unsuccessful at detecting the

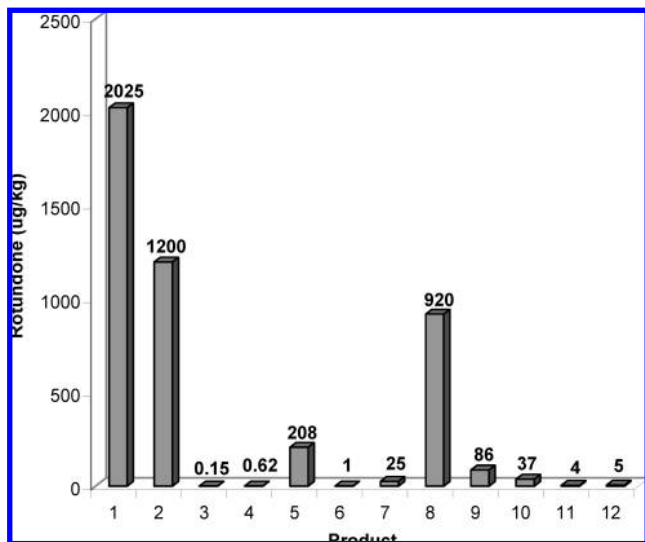
highest concentration were retested at higher levels. The concentrations employed were 2, 5, 16, 49, 146, and 437 ng/L. Retest concentrations were 2, 0.6, and 0.2 ng/L (low end) or 437, 1310, and 4000 ng/L (high end). The samples were prepared by adding an ethanol solution (<1 mL) of rotundone to 1 L of wine or water. An equal volume of ethanol was added to the corresponding blank samples. The samples in each individual “triangle” were presented in random order and identified only by three-digit random numbers. The sample that was different from the other two was always the spiked sample. Assessments were conducted orthonasally (sniffing) without tasting. Following sensory assessments we verified, by reanalyzing the samples, that the concentration of rotundone present in the wine and water samples was as originally added; that is, there was no significant loss of rotundone due to evaporation, lack of solubility, or adsorption onto the glass surface (24). Each panelist was assigned a best estimate threshold (BET) value, being the geometric mean of the highest concentration missed and the next higher concentration tested. For all panelists who successfully detected rotundone at a concentration below 4000 ng/L the geometric mean of the individual BETs was then calculated to give a subgroup threshold value.

## RESULTS AND DISCUSSION

With our first sensory experiments we determined that Shiraz grapes were the source of the ‘black pepper’ aroma, which varied considerably between samples from different vineyards and vintages (9). Subsequent studies resulted in our obtaining fractions from Shiraz grapes, which contained a single component that gave a strong peppery aroma when assessed by GC-MS-O. From the small amounts we were able to extract from grapes, we could determine a putative molecular ion (*m/z* 218), which was indicative of sesquiterpenes, and one major fragment ion (*m/z* 203). In addition, we obtained retention indices for the pepper aroma (and corresponding ions of *m/z* 218 and 203) on three different GC column phases, enabling us to target the unknown compound in other plant matrices.

It became clear from our GC-MS-O work that the aroma associated with the peppery component in the grape fractions bore a striking resemblance to the aroma of commercial pepper. We therefore analyzed a crude extract of ground white pepper by GC-MS-O and found that the compound responsible for the grape peppery character was apparently present in the white pepper extract and, importantly, at much higher concentration than in the grape samples. It then became clear from searching the literature that, despite numerous high-quality publications on the composition of white and black pepper (16, 25–31), no one had identified this key compound in these products, nor had its importance been recognized. In the absence of a recognized compound responsible for such distinctive ‘black pepper’ aroma, the sensory impact of pepper has, up to now, been mainly attributed to complex interactions of many odorants (29, 30) or to piperine and related alkaloids, which impart ‘heat’ in the mouth (3, 15).

From the mass spectra, retention data, and distinctive aroma it was now clear to us that the compound responsible for the main aroma of black and white peppercorns and the compound responsible for the peppery aroma in Shiraz grapes were one and the same. We therefore isolated the unknown target compound from peppercorn extracts in which it was present at far higher concentration than in grape extracts and eventually obtained a clear mass spectrum of our target compound that coincided with the matching peppery aroma (by GC-MS-O) at consistent retention indices (RIs) on three different GC columns, which matched one compound in the Symrise GC-MS database: the sesquiterpene rotundone. We confirmed that rotundone was indeed our target compound with a reference sample synthesized from the sesquiterpene guaialol (Figure 1) that gave a matching



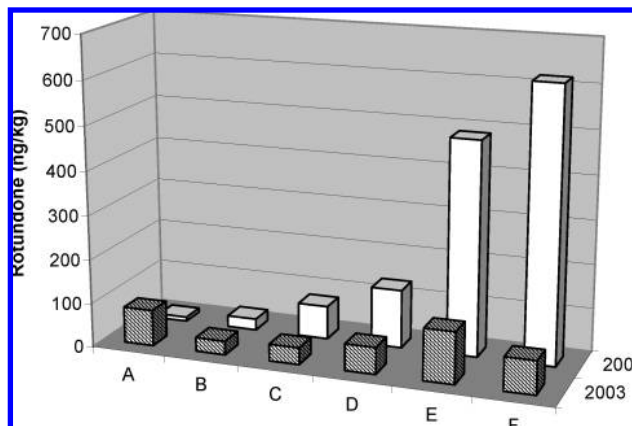
**Figure 2.** Concentration of rotundone found in various plants and plant products ( $\mu\text{g}$  of rotundone/kg of product, duplicate extraction and analysis, mean result shown): 1, white pepper (*Piper nigrum*); 2, black pepper (*P. nigrum*); 3, wine (*Vitis vinifera* cv. Shiraz); 4, grapes (*V. vinifera* cv. Shiraz); 5, marjoram (*Origanum majorana*); 6, oregano (*Origanum vulgare*); 7, geranium (*Pelargonium alchemilloides*); 8, nut grass (*Cyperus rotundus*); 9, rosemary (*Rosmarinus officinalis*); 10, saltbush (*Atriplex cinerea*); 11, basil (*Ocimum basilicum*); 12, thyme (*Thymus vulgaris*).

mass spectrum coinciding with the same RIs and the peppery aroma (by GC-MS-O) on the three GC columns. Co-injection experiments gave symmetrical peak enhancement and a matching mass spectrum on the three GC column phases when standard additions of synthetic rotundone were made to enriched pepper and grape extracts.

To determine the concentration of rotundone in wine, grapes, and other matrices (e.g., spices) we prepared  $d_5$ -rotundone (**Figure 1**) for use as an internal standard and developed and validated a stable isotope dilution analysis (SIDA) method using GC-MS (15). The method is accurate and precise with a low limit of quantitation ( $<1$  ng/L) (15).

This GC-MS technique allowed us to identify and quantify rotundone in a range of natural products and spices (**Figure 2**.) Our analyses show rotundone is widespread in nature and is a key aroma component of several common herbs and spices. In this preliminary survey its concentration was highest in peppercorn extracts and nut grass weed (*Cyperus rotundus*), the plant from which it was first isolated and identified (32). However, some other plant samples (e.g., marjoram) had surprisingly high amounts of rotundone.

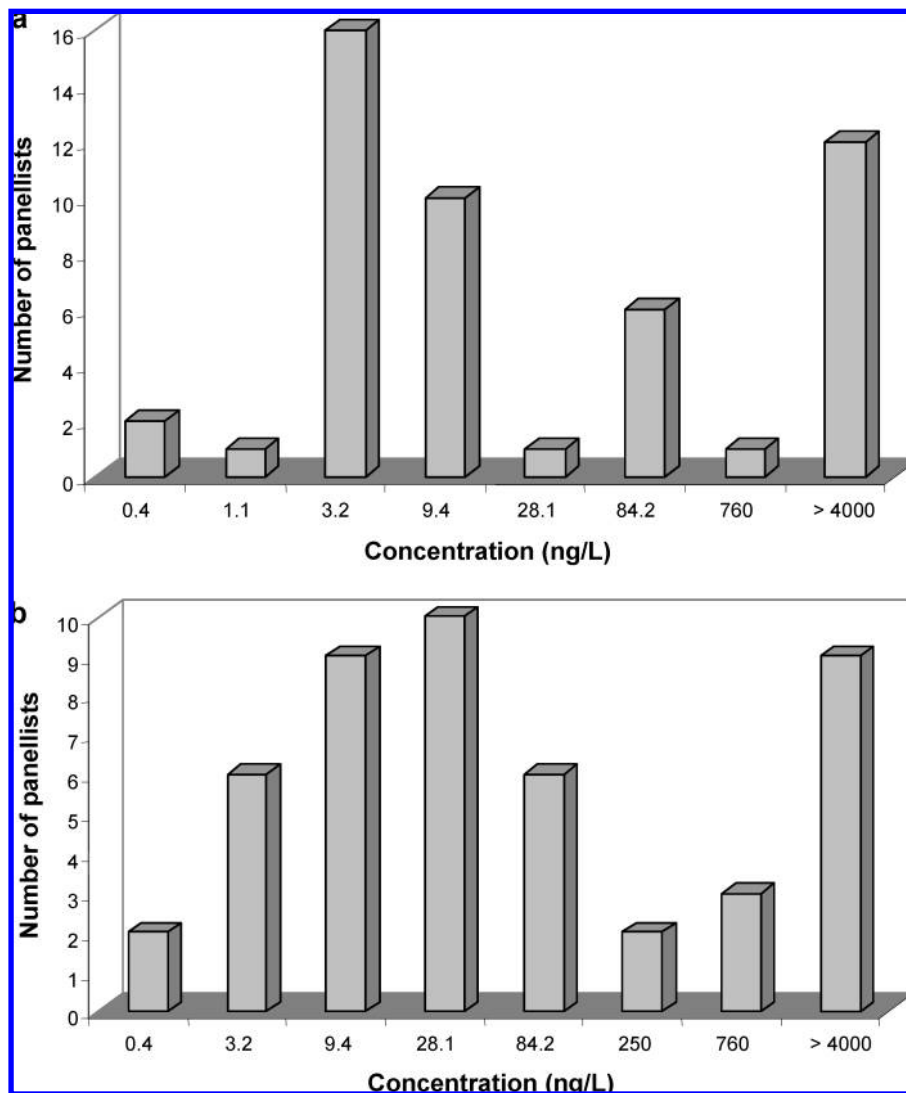
Even though there are numerous publications on sesquiterpenoids in the literature, rotundone has been described in only a few instances. Previously, rotundone had been found in the grassy weed *C. rotundus* (32, 33) (which is one of the most invasive weeds known and has various uses in traditional medicine), in tubers of nagarmotha (*Cyperus scariosus*) (34) (used for treatment of inflammation and stomach disorders in Indian medicine), and in agarwood (13, 14) [Jinkoh in Japanese and widely used as a traditional Japanese incense and a Chinese medicine (13, 14)]. Rotundone was tentatively identified as one of 29 odorants in cooked lentils, based on GC retention indices on two columns and aroma description 'cooked lentil, spicy' (35). However, apart from this one exception, in none of the papers was comment made of the aroma of rotundone, perhaps because, in neat form, rotundone has a harsh, burnt aroma that becomes pepper-like only on dilution.



**Figure 3.** Concentration of rotundone (ng/kg) in Shiraz grapes from six vineyards (A–F) over two vintages (2002 and 2003). Vintage 2002 was widely regarded as being a more ‘peppery’ year than vintage 2003 for Shiraz grapes in several wine regions in Australia.

To further characterize the relevance of rotundone for the aroma of red wine, we studied its presence in Shiraz grapes and red wines and determined its aroma detection threshold. We analyzed 18 Shiraz grape homogenates, which had previously undergone panel sensory rating for pepper aroma and flavor intensity (9). Grapes were collected over two vintages, 2002, a year when distinct pepper aromas had been observed in many grape samples, and 2003, which was considered to yield grapes predominantly low in pepper character. In addition, some grapes were from locations renowned for producing wines with the ‘black pepper’ character, whereas others were sourced from regions not linked to ‘peppery’ wines. **Figure 3** shows data for grape samples from the same locations in six vineyards from both the 2002 and 2003 vintages. Our SIDA GC-MS analysis (15) demonstrated that rotundone concentration in the grape samples ranged from 10 to 620 ng/kg. The samples with the highest rotundone concentrations were also those rated as highest in ‘pepper’ aroma and flavor intensity by the sensory panel. These samples were from locations renowned for producing peppery wines and from the 2002 vintage. The wine made from the same vineyard as the most peppery grape sample had relatively high rotundone concentrations of 145 ng/L (2002 vintage) and 29 ng/L (2003 vintage), in agreement with the corresponding grape samples (vineyard F in **Figure 3**). There was good correlation between the rotundone levels and the pepper intensity sensory scores for all vineyards except for vineyard A, which was also the only one that had a considerably lower rotundone concentration in 2002. In summary, for the Shiraz grape samples analyzed, the results confirmed anecdotal evidence linking certain vineyards and vintages to grapes high in pepper aroma and flavor and demonstrated a relationship between the concentration of rotundone in grapes and the intensity of the ‘peppery’ grape aroma and flavor.

We also analyzed 20 red wines (10 Shiraz and 10 Cabernet Sauvignon), which had previously undergone panel sensory rating for 25 aroma descriptors including ‘pepper’ aroma intensity. These wines had been assessed as part of a consumer preference study (36) and were not from regions renowned for producing peppery wines, apart from one wine that was rated the highest in pepper intensity and also had the highest concentration of rotundone (75 ng/L). Although no rotundone was detected in the three Shiraz wines rated lowest in pepper aroma by the sensory panel, it could be identified in the remaining seven Shiraz wines, with wines of moderate pepper aroma intensity containing around 20 ng/L of rotundone. For



**Figure 4.** Distribution of best estimate threshold concentration (ng/L) for rotundone in water (a) or red wine (b). In both matrices about 20–25% of panelists could not smell the compound at 4000 ng/L (the highest level tested).

the Shiraz samples there was excellent correlation ( $R = 0.990$ ,  $P < 0.001$ ) between the concentration of the compound measured by GC-MS and the mean sensory 'peppery' intensity rated by the panel. Rotundone was detected in 3 of the 10 Cabernet Sauvignon wines, albeit in low concentration (up to 20 ng/L in a cool climate Cabernet Sauvignon/Merlot/Cabernet Franc blend). We also analyzed several other premium red wines known for peppery aroma. The concentration of rotundone ranged from 29 ng/L (a Shiraz from a "nonpeppery" year) to 145 ng/L (the same Shiraz in a "peppery" year). Two non-Shiraz wines also had high levels of rotundone: 134 ng/L rotundone in a Mourvedre and 128 ng/L rotundone in a Durif. Taken together, the red wine survey and sensory data demonstrated the widespread presence of rotundone in red wine and established, as observed for grapes, a close relationship between the concentration of rotundone and the intensity of the 'peppery' aroma in red wine.

Finally, we wished to confirm that this apparent relationship between the concentration of rotundone and the peppery aroma of grapes or red wine was not based on simple cocorrelation, but that rotundone at these low concentrations was sufficiently potent to cause the observed 'peppery' aromas. Hence, we determined the aroma detection threshold of rotundone in water and in a red wine that had been previously shown by GC-MS analysis to contain no detectable amount of this compound (i.e.,

<0.5 ng/L). Three fourths of the panelists (75%) were very sensitive to rotundone (subgroup threshold of 8 ng/L in water and 16 ng/L in red wine, see Materials and Methods). Above these concentrations, these panelists described spiked samples as more 'peppery' and 'spicy'. **Figure 4** shows the distribution of the sensory panelists' best estimate thresholds in water or red wine. A fourth of the panel, however, was relatively insensitive and not able to smell this compound at concentrations typically found in most red wines. Nevertheless, these threshold studies show that rotundone is in fact one of the most powerful aroma compounds found so far (4–8, 37, 38) and that such small nanograms per liter concentrations are sufficient to bring about the 'peppery' character of grapes or wine. Furthermore, these limits of aroma detection are far below the concentrations found in most of the herbs and spices we examined (**Figure 2**). Much larger amounts of rotundone were measured in other products, compared to grapes and wine (**Figure 2**).

The aroma potency of a compound in a product is sometimes defined by its odor activity value (OAV), the ratio of concentration to odor threshold (30, 39). The most intense aroma compounds previously found in black or white pepper were linalool (floral aroma, OAV = 1300–3300) and limonene (citrus-like, OAV = 810–1800) (30). These OAVs were based on thresholds determined in starch solution rather than in water or red wine. On the basis of the thresholds we determined in water

and red wine matrices, rotundone, which has a spicy peppery aroma, would have an OAV in pepper on the order of 50000–250000 and is, on this criterion, by far the most powerful aroma compound yet found in that most important spice.

Whereas most of the sensory panelists were sensitive to rotundone, approximately 20% could not detect this compound, even in water, at the highest concentration tested (4000 ng/L). Thus, the sensory experience of two consumers enjoying the same glass of Shiraz wine or sharing the same meal seasoned with pepper might be very different. The variation in individual sensitivity to rotundone suggests that the way wines containing this compound are assessed by consumers or wine judges could vary substantially from one person to another. Similarly, the flavor perception of ground pepper might vary considerably among consumers. This is supported by the common practice of pepper being placed on the table or offered to individuals by restaurant waiters. The dose-dependent changes to rotundone's odor quality and/or the observation that a sizable group of people are not very sensitive or completely anosmic might explain why the sensory relevance of rotundone had been overlooked for so many years.

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