Determination of Methoxypyrazines in Red Wines by Stable Isotope Dilution Gas Chromatography–Mass Spectrometry

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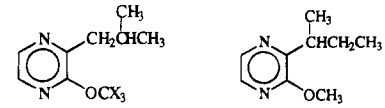
Quantitative analysis of methoxypyrazines in a range of red wines is reported for the first time. By stable isotope dilution gas chromatography–mass spectrometry, 2-methoxy-3-(2-methylpropyl)-pyrazine was determined in 12 Australian and New Zealand Cabernet Sauvignon wines (mean concentration, 19.4 ng/L) and in 6 Bordeaux wines that are blends, to varying degrees, of Cabernet Sauvignon, Merlot, and Cabernet Franc (mean concentration, 9.8 ng/L). These findings suggest that the concentration of the methoxypyrazine in wines of these varieties is ca. 30 times lower than that indicated in a previous study. In these 18 wines, the concentration of the methoxypyrazine exceeded its sensory detection threshold in water, consistent with its contributing to wine flavor. The concentration in two French Pinot noir wines was below the sensory detection threshold. In all wines, the level of 2-methoxy-3-(1-methylpropyl)pyrazine was less than 2 ng/L.

Keywords: *Methoxypyrazine; red wine; quantitation; gas chromatography–mass spectrometry; isotope dilution; deuterium labeling; Vitis vinifera; Cabernet Sauvignon*

Grapes of *Vitis vinifera* cv. Cabernet Sauvignon provide wines that frequently have a vegetative, herbaceous, grassy, or green aroma. This can provide complexity and varietal character to the wine aroma when in balance with other flavor components, but at high levels it becomes overpowering. The aroma has been attributed to 2-methoxy-3-(2-methylpropyl)pyrazine (**1**) (Figure 1), a potent flavorant that contributes to the characteristic aroma of some vegetables such as peas and bell peppers (Murray et al., 1970; Murray and Whitfield, 1975). The extremely low sensory detection threshold of 2 ng/L in water for methoxypyrazine **1** (Buttery et al., 1969; Seifert et al., 1970) shows that specialized isolation and analytical techniques may be required to detect the component in wine. Such levels are well below the limits of HPLC (Heymann et al., 1986), and gas chromatography–mass spectrometry (GC–MS) was required for the tentative identification of **1** in grapes of the cultivars Cabernet Sauvignon (Bayonove et al., 1975) and Sauvignon blanc (Augustyn et al., 1982). Nevertheless, the first reported attempt to use GC–MS to identify the component in a red wine proved unsuccessful (Slingsby et al., 1980), suggesting that attention to selectivity of isolation and analysis may be crucial.

Quantitative analysis of ultratrace components is particularly demanding. Development of a definitive method is best achieved by isotope dilution mass spectrometry, using a stable isotope-labeled analog of the analyte as an internal standard (Giovannini et al., 1991; Boyd, 1993). This technique has been used to quantify methoxypyrazine **1** and its isomer 2-methoxy-3-(1-methylpropyl)pyrazine (**2**) in Sauvignon blanc grapes and wines, using 2-($^2H_3$)methoxy-3-(2-methylpropyl)-pyrazine (**3**) as an internal standard (Figure 1) (Harris et al., 1987). This approach confirmed not only that methoxypyrazine **1** contributes to Sauvignon blanc wine aroma (Allen et al., 1991) but also that its concentration, even in wines with strikingly evident herbaceous/vegetative aroma, can be less than 20 times its sensory detection threshold in water (Harris et al., 1987; Lacey et al., 1991). The likelihood of a similar trace concentration in wines of Cabernet Sauvignon and related varieties is indicated by determination of 20 ng/L of **1** in Cabernet Sauvignon grape must immediately after alcoholic fermentation (Allen et al., 1990a,b) and by determination of 17 and 21 ng/kg in an Italian Cabernet Sauvignon wine and a Cabernet Franc wine, respectively (Calò et al., 1991). However, these concentrations in the low nanograms per liter range contrast with a study of a single Bordeaux red wine, by other isolation and analytical techniques, which estimated the concentration of **1** to be 500 (± 70) ng/L (Boison and Tomlinson, 1990).



1 X = ^1H 2

3 X = ^2H

Figure 1. Endogenous wine methoxypyrazines **1** and **2** and deuterium-labeled internal standard **3**.

The discrepancy highlights the need for definitive determination of methoxypyrazine **1** in a range of red wines, including Bordeaux red wines. The present paper reports a study using stable isotope dilution GC–MS to ensure rigor of quantitative analysis. The investigation provides quantitative data on methoxypyrazines in a range of red wines, including Bordeaux wines, for the first time.

EXPERIMENTAL PROCEDURES

Wines. With one exception, Australian and New Zealand wines were commercial wines selected from the 1987 National Wine Show, Canberra, and the grape variety Cabernet Sauvignon contributed not less than 80% of the grape origin of

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each wine. One additional wine was selected for its particularly intense herbaceous/vegetative aroma; it was a 100% Cabernet Sauvignon wine from young (9-year-old) vines in a cool, maritime region of Australia (Mornington Peninsula). The wines were 2–4 years old at the time of analysis. The eight French wines were selected from the teaching wine cellar, Charles Sturt University; they were 9–10 years old at the time of analysis. Prior to analysis, either wines were stored in unopened bottles at 10–14 °C or a quantity (240 mL) was transferred to a glass bottle with a Teflon-lined cap (250 mL, Schott) and stored in the dark at −20 °C under carbon dioxide headspace.

Chemicals and Glassware. Methoxypyrazine **2** was purchased from Pyrazine Specialities, Inc., Atlanta, GA; it was found to be >98.5% pure by GC–MS. The synthesis of **1** and **3** has been described previously (Harris et al., 1987). Dichloromethane was of HPLC grade (Millipore), shaken repeatedly with concentrated H_2SO_4, washed (H_2O, 5% aqueous $NaHCO_3$, then H_2O), dried (Na_2SO_4), glass distilled from P_2O_5, and stored at −20 °C in an amber container fitted with a Teflon-lined cap. For toxicity reasons, operations with dichloromethane were conducted in a fume hood. For the methoxypyrazine isolation steps that follow distillation, the glassware was cleaned with chromic acid (24 h, ca. 25 °C; caution: corrosive and oxidizing agent), 3 M HCl (3 h, 80 °C), 5% $NaHCO_3$ (24 h, ca. 25 °C), and high purity (ca. 18 MΩ conductivity) H_2O. Ion-exchange resin (Bio-Rad, AG50WX4 AR) was used as received.

Methoxypyrazine Isolation. Internal standard **3** (2.065 ng/μL in dichloromethane, 12.0 μL) was added to wine samples (240 mL) by calibrated microliter syringe (Hamilton, 25 μL), with measurement of the volume between air interfaces, and use of a 5-μL solvent plug to displace the standard solution from the barrel and needle during transfer. After time was allowed for any equilibration (1–2 h), the sample was adjusted to pH 6 (10% NaOH) and then distilled at atmospheric pressure; 50 mL of distillate was collected, stirred with ion-exchange resin (200 mg, 1 h, in the dark), and filtered (glass sinter), and the filtered resin was washed with water (2 × 1 mL). A suspension of the washed resin in water (1 mL) was adjusted to pH 10 by dropwise addition of 10% NaOH solution. After 5 min, the supernatant solution was removed, the resin was washed with water (0.5 mL), and the aqueous solutions were combined. The resin was washed with dichloromethane (0.5 mL), and the aqueous solution was extracted consecutively with the dichloromethane wash and a further portion of dichloromethane (0.5 mL). If not immediately evaporated, the combined dichloromethane extracts were sealed into glass ampules and stored at −20 °C in the dark. The dichloromethane extracts (1 mL) were concentrated to ca. 20 μL by slow evaporation (ca. 6 h) in the dark, at room temperature, under a nitrogen atmosphere, in tapered Reacti-vials (Pierce, 1 mL, then 100 μL) with frequent rinsing of the vial walls by remaining solution. Concentrates were transferred into glass capillaries that were sealed and then stored in the dark at −20 °C until mass spectrometric analysis.

Mass Spectrometry. Australian and New Zealand wines were analyzed by capillary GC–MS on a VG Micromass 70–70F mass spectrometer using a fused silica capillary column of 5% phenyl and 95% dimethyl silicone (SGE, BP5, 50 m × 0.32 mm i.d., 1.0-μm film thickness) as previously described in detail (Harris et al., 1987). French wines were analyzed with a VG Trio 2 capillary GC–quadrupole MS system using a HP5890 Series II gas chromatograph. Chromatography was performed with fused silica capillary columns of dimethyl silicone (J&W, DB-1, 60 m × 0.32 mm i.d., 1.0-μm film thickness), 6% phenyl and 6% cyanopropyl dimethyl silicone (J&W, DB-1701, 30 m × 0.32 mm i.d., 1.0-μm film thickness), or Carbowax (J&W, DB-Wax, 30 m × 0.32 mm i.d., 0.5-μm film thickness). Columns were directly coupled to the ion source. Injections were made by a syringe with silica needle into a HP 19245C temperature-programmable cool on-column capillary inlet with electronic pressure control, at 30 °C, using a deactivated fused silica retention gap (J&W, 3 m × 0.32 mm i.d.) to reconcentrate components at the beginning of the stationary phase. The oven was programmed to provide a 2-min delay at 30 °C followed by a temperature rise of either (i) 25 °C/min to 90 °C then 5 °C/min to 200 °C (DB-1), or (ii) 25 °C/min to 110 °C, 5 °C/min to 150 °C, 25 °C/min to 210 °C, then held at 210 °C for 5 min (DB-1701 and DB-Wax). Helium was used as carrier gas at a constant (69 kPa, DB-Wax and DB-1701) or programmed (69 kPa for 0.5 min, then 138 kPa/min to 138 kPa, DB-1) inlet pressure.

Mass spectra and selected ion monitoring were recorded in positive-ion mode at an electron energy of either 70 (EI) or 50 eV (CI), emission current of 200 (EI) or 700–800 μA (CI), and source temperature set to 180 (EI) or 150 °C (CI). Ammonia (anhydrous) was used as reagent gas at a source envelope pressure of 3×10^{-3} Pa. The ions monitored were m/z 167.1 and 170.1 (CI) or 124.1 and 127.1 (EI) with a dwell time of 0.10 (DB-1) or 0.08 s (DB-1701, DB-Wax) and a span of 0.2 u. Scan spectra were recorded over the range 60–200 u, with scan time of 0.5 s and interscan delay of 0.1 s. Relative ion abundances were assessed from peak areas.

Instrumental Calibration. The VG Trio-2 GC–MS system was calibrated by analysis of mixtures of **1** and **3** prepared at seven $^2H_0/^2H_3$ mole ratios (mole ratio range, 0–2.271, at 650 pg/μL of **3**) using 1-μL injections. Each calibration standard was prepared in duplicate, in dichloromethane, using amber Reacti-vials and Mininert valves (Pierce), by serial dilution using calibrated microliter syringes (Hamilton). The response was determined as the weighted linear regression of measured $^2H_0/^2H_3$ area ratio, as ordinate, on $^2H_0/^2H_3$ mole ratio, as abscissa, using the reciprocal of the variance of the area ratio as weighting (Miller, 1991). Variances were estimated from replicate analyses at high ($^2H_0/^2H_3$ mole ratio = 0.757, six replicates) and low ($^2H_0/^2H_3$ mole ratio = 0.00942, seven replicates) levels of undeuterated material. Limits of detection were calculated as the intercept plus three standard deviations.

Standardization. Each day, the VG Trio 2 GC–MS system was mass calibrated in static (50–650 u) and dynamic (60–200 u) modes (EI, heptacosa) prior to methoxypyrazine determination. Source parameters were optimized in positive CI mode with *n*-amyl acetate (m/z 131: m/z 148 = 1:10), and the instrument response was determined with a standard solution (1 μL) of **1** and **3** (200 pg/μL) and **2** (20 pg/μL). Quantitative analysis was performed only if the response with the standard solution was within 10% of the mean of previous satisfactory determinations. Solvent blank injections were made before and after standards to check system cleanliness and standard carryover.

Quantitative Analysis. This was performed by GC–MS analysis in CI mode. Samples of methoxypyrazine concentrates (3–9 μL) were applied using, as necessary, multiple injections within a few minutes onto the cold column (Lacey and Sanders, 1992) to allow the mass spectrometer vacuum system to cope with the injected solvent and to avoid the flooded zone exceeding the retention gap. Mole ratios ($^2H_0/^2H_3$) of components were calculated from the standard response determined that day, with correction for the trace of $^2H_0$ component in the $^2H_3$ internal standard that was measured during instrumental calibration. The limit of detection was taken to be the greater of either (i) three times the signal to noise ratio or (ii) the limit of detection determined from instrumental calibration, after modification to allow for the variation of internal standard quantity or response from that of calibration [$LD^{sample} = LD^{Cal} \times area(^2H_3)^{Cal}/area(^2H_3)^{sample}$, where LD^{sample} is the determined limit of detection, LD^{Cal} is the limit of detection in instrumental calibration, $area(^2H_3)^{Cal}$ is the peak area arising from **3** in calibration, and $area(^2H_3)^{sample}$ is the peak area arising from **3** in sample].

Statistical Analysis. Weighted linear regressions were calculated according to the procedure of Miller (1991); the unweighted linear regression was calculated with Excel version 4 (Microsoft Corp.).

RESULTS AND DISCUSSION

Eight French, nine Australian, and three New Zealand wines were analyzed. The grape variety Cabernet Sauvignon provided 80% or more of the grape varietal composition of the Australian and New Zealand wines. The French wines were from the Médoc region of

Table 1. Concentration of Methoxypyrazines 1 and 2 in French Wines[a-c]

wine	origin	region	methoxypyrazine (ng/L) 2-methylpropyl 1	methoxypyrazine (ng/L) 1-methylpropyl 2
1	1982, Léoville-Las Cases[d]	St. Julien	14.9	0.23
2	1983, Léoville-Las Cases[d]	St. Julien	10.6	0.18
3	1983, Cantemerle[e]	Haut Médoc	9.3	1.9
4	1982, Conseillante[f]	Pomerol	9.8	0.06
5	1982, Canon[g]	St. Émilion	7.1	0.05
6	1982, Magdelaine[h]	St. Émilion	7.1	nd
7	1983, Gevrey Chambertin[i]	Burgundy	<0.7[j]	0.26
8	1983, Morey St. Denis[i]	Burgundy	<0.4[j]	0.12

[a] CIMS, DB-1 column. [b] nd, not detectable. [c] Superscripts d-h indicate vineyard compositions as reported by Parker (1991). [d] 67% Cabernet Sauvignon, 17% Merlot, 13% Cabernet Franc, and 3% Petit Verdot. [e] 40% Cabernet Sauvignon, 40% Merlot, 18% Cabernet Franc, and 2% Petit Verdot. [f] 45% Merlot, 45% Cabernet Franc, and 10% Malbec. [g] 55% Merlot, 45% Cabernet Franc. [h] 90% Merlot, 10% Cabernet Franc. [i] 100% Pinot noir. [j] Upper limit due to coelution.

Table 2. Concentration of Methoxypyrazine 1 in Australian and New Zealand Cabernet Sauvignon-Based Wines and Mean January Temperature (MJT) of the Growing Region

wine	region[a]	methoxypyrazine 1[b,c] (ng/L)	MJT[d] (°C)
1	Hunter Valley, NSW	3.6	22.7
2	Griffith, NSW	6.2	23.6
3	Leeton, NSW	7.6	23.6
4	Seymour, Vic	9.1	21.2
5	McLaren Vale, SA	11.2	19.8
6	Frankland, WA	12.3	20.0
7	Mudgee, NSW	17.1	22.7
8	Yarra Valley, Vic	26.1	18.0
9	Hawkes Bay, NZ	27.6	18.8
10	Auckland, NZ	27.6	17.9
11	Hawkes Bay, NZ	28.6	18.8
12	Mornington Peninsula, Vic[e]	56.3	18.0

[a] NSW, New South Wales; Vic, Victoria; SA, South Australia; WA, Western Australia; NZ, New Zealand. [b] CIMS, BP5 column. [c] In order of increasing methoxypyrazine concentration to facilitate comparison with MJT. [d] Data from Jackson and Spurling (1988), Dry and Smart (1984), Smart (1988), and Smart and Dry (1980). [e] 100% Cabernet Sauvignon wine selected for its particularly intense herbaceous/vegetative aroma.

Bordeaux, in which Cabernet Sauvignon is important and often dominant; the St. Émilion/Pomerol region of Bordeaux, where Merlot, Cabernet Franc, and, to a lesser extent, Cabernet Sauvignon are important; and Burgundy, where Pinot noir is the red grape variety. Reported Bordeaux vineyard compositions (Parker, 1991) are indicated in footnotes to Table 1.

Analysis by GC-MS in CI mode provided lower detection limits and greater freedom from signals of other sample matrix components than analysis in EI mode. Instrumental calibration showed excellent response linearity for methoxypyrazine **1** (VG Trio 2, r = 0.999949) and a detection limit into the femtogram range (0.2 pg). The calibration intercept was consistent with 0.1% of component **1** in the deuterated internal standard **3**.

Quantitative Analysis of 2-Methoxy-3-(2-methylpropyl)pyrazine (1). The concentrations of **1** are presented in Tables 1 (French wines) and 2 (Australian and New Zealand wines). In the two Pinot noir wines (Table 1, wines 7 and 8), coelution with other components allowed only an upper limit to be specified for the concentration of **1**, but the upper limits were below the ca. 2 ng/L sensory detection threshold of the component (Buttery et al., 1969; Seifert et al., 1970) and well below the concentrations found in the other wines.

Evidence from three sources confirmed the identity of the measured endogenous component as methoxypyrazine **1**. First, the component was coincident (±0.01 min) with synthetic **1** on three stationary phases of widely differing polarity (DB-1, DB-1701, and DB-Wax), and its concentration determined with each stationary phase provided consistent values (Table 1, wine 1, analysis with DB-1, DB-1701, and DB-Wax; determined concentrations 14.9, 14.2, and 15.6 ng/L, respectively). Second, the peak for the component was symmetrically enhanced with no increase in peak width at half-height, upon co-injection with comparable quantities of synthetic **1** (Table 1, wine 2, DB-1701 column). Finally, it was possible to measure a weak mass spectrum in both EI and CI modes [EI: Table 1, wine 3, DB-1701 column, m/z (relative intensity) 151 (13), 124 (100), 94 (18). CI: Table 1, wine 1, DB-1701 column, m/z (relative intensity) 167 (100), 124 (41)]. The spectra agree with those of synthetic **1**.

Comparison with Related Published Data. The very low concentration of methoxypyrazines in wine places stringent demands on the techniques used for their isolation, detection, and quantitation. Consequently, there have been few reports of their detection or quantitative analysis in red wine. The first reported attempt to detect methoxypyrazine **1** (Slingsby et al., 1980) involved extraction of a large quantity (170 L) of a Cabernet Sauvignon wine, but the attempt was unsuccessful. With specialized apparatus for solvent extraction and two stages of careful low-temperature, high-vacuum evaporation, Boison and Tomlinson (1990) were able to use a much smaller wine volume and reported that the concentration of **1** was 500 (±70) ng/L in a study of a single Bordeaux wine. However, for quantitative analysis of methoxypyrazines, their strategy had a major drawback in that they sought to identify all components present. Consequently, selectivity in isolation or quantitative analysis was not used, increasing the likelihood of interference from coeluting components. Their extraction and evaporation steps did not incorporate an internal standard and relied upon an estimated recovery based upon studies with components other than methoxypyrazines, in model solutions, at concentrations in the micrograms per liter range. For GC-MS analysis, acetophenone and a homologous series of n-alkanes were used as internal standards, but this fails to meet the minimum requirement, for ultratrace analysis, of a standard that is the same chemical class as the analyte of interest (Giovannini et al., 1991; Boyd, 1993). Indeed, it has been found that an isomeric methoxypyrazine, with a different alkyl side chain, may not act reliably as an internal standard for an endogenous methoxypyrazine (Allen and Boyd, 1994).

For ultratrace quantitative analysis, stringent use of internal standards is essential (Millard, 1977), and it is now well-established that the closest approach to a

definitive method is stable isotope dilution mass spectrometry (Giovannini et al., 1991; Boyd, 1993). In this process, the analyte itself, isotopically labeled, is added as an internal standard prior to analysis and is carried through all isolation, evaporation, and analytical steps, compensating for losses in isolation and evaporation and for variation in injection volumes and detection efficiency. This technique has been used to determine the concentration of **1** (20 ng/L) in Cabernet Sauvignon grape must immediately after alcoholic fermentation (Allen et al., 1990a,b). Subsequently, using a variation of the method of Harris et al. (1987), but with a nonisotopically labeled internal standard, the concentration of **1** was determined to be 17 ng/kg in an Italian Cabernet Sauvignon wine and 21 ng/kg in an Italian Cabernet Franc wine (Calò et al., 1991).

The present strategy used stable isotope dilution GC–MS to ensure rigor of quantitative analysis. It also incorporated selectivity for basic components in the isolation procedure and exerted selectivity in GC–MS analysis through chemical ionization with ammonia as reagent gas. In all of the 18 Cabernet Sauvignon or Cabernet Sauvignon/Merlot/Cabernet Franc wines (Table 1, wines 1–6; Table 2, all wines), the determined concentration of **1** was above the ca. 2 ng/L sensory detection threshold in water (Buttery et al., 1969; Seifert et al., 1970) but far lower than that reported by Boison and Tomlinson (1990), even in the wine selected for its unusually intense herbaceous/vegetative aroma (Table 2, wine 12). The concentration of methoxypyrazine **1** in the six Bordeaux wines of the present investigation (7.1–14.9 ng/L; Table 1, wines 1–6) can be compared with the known (ca. 2 ng/L) sensory detection threshold for **1** in water (Buttery et al., 1969; Seifert et al., 1970), the concentrations that significantly influenced white wine aroma and herbaceous/vegetative aroma (1 and 8 ng/L, respectively) when **1** was added to a methoxypyrazine-free white wine (Allen et al., 1991), and the flavor and aroma thresholds (2 and 16 ng/L) reported for the addition of **1** to a red wine that lacked methoxypyrazines (Maga, 1990). These data are consistent with the proposal that methoxypyrazine **1** provides these Bordeaux wines with the restrained level of herbaceous/vegetative flavor and aroma that is characteristic of prestigious wines of that region.

The concentration of **1** in the 18 Cabernet Sauvignon and Cabernet Sauvignon/Merlot/Cabernet Franc wines (3.6–56.3 ng/L; Table 1, wines 1–6; Table 2, all wines) is similar to that previously determined (0.6–38.1 ng/L) in 22 French, Australian, and New Zealand wines from the grape cultivar Sauvignon blanc (Lacey et al., 1991), a grape variety that also provides wines with a herbaceous/vegetative aroma.

Quantitative Analysis of 2-Methoxy-3-(1-methylpropyl)pyrazine (2). The profile of the mass channel for the $[M + H]^+$ ions of **1** and **2** (Figure 2) was remarkably free of ions of other matrix components, allowing tentative identification of very low concentrations of **2**. Deuterated isomer **3** eluted close to **2** (Δt = 0.21 min, DB-1; 0.35 min, DB-Wax) allowing the former to be used as an internal standard for quantitative analysis of the latter. The natural component was confirmed to be **2** by coincidence (±0.01 min) with authentic material on two stationary phases of widely differing polarity (Table 1, wines 1 and 7, DB-1 and DB-Wax), and its concentration determined with each stationary phase provided consistent values (DB-1 and DB-Wax: Table 1, wine 1, 0.23 and 0.14 ng/L, respectively; Table 1, wine 7, 0.26 and 0.28 ng/L, respectively). Methoxypyrazine **2** could be determined in seven of the French wines. Analysis of the Australian and New Zealand wines indicated a concentration less than 1 ng/L in all cases. Signal intensities were too close to the detection limits for more detailed identification.

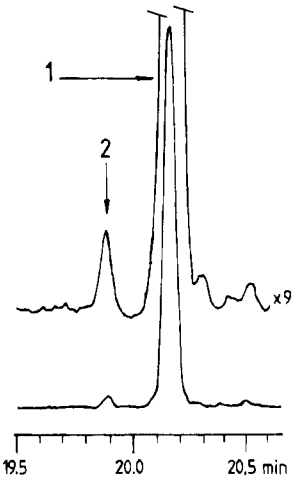

Figure 2. Profile of the m/z 167 mass channel of the $[M + H]^+$ ions of methoxypyrazines **1** and **2** (Table 1, wine 1; CI mode, DB-1 column).

Factors Influencing the Concentration of Methoxypyrazine 1. In the two Pinot noir wines of Burgundy (Table 1, wines 7 and 8), the concentration of **1**, an upper limit due to coelution, was below the sensory detection threshold. The relative absence of **1** in these two wines and its occurrence in all other wines may reflect a dependence of the occurrence of **1** on grape cultivar. Further work is required to clarify this.

It is recognized viticulturally that Cabernet Sauvignon wines from warm areas usually have lower vegetative/herbaceous aroma than wines of this grape variety from cool areas. If methoxypyrazine **1** is responsible for this aroma, a corresponding variation of its concentration would be expected. Table 2 relates the concentration of **1** to the mean January temperature (MJT) of the growing region, an indicator of average temperature conditions in the southern hemisphere growing season (Smart and Dry, 1980; Dry and Smart, 1984). Although other factors are also likely to be important, such a relationship is evident (linear regression, $R = 0.754$, $p < 0.005$) confirming behavior similar to that found previously with Sauvignon blanc grapes and wines (Lacey et al., 1991).

Comparative Methoxypyrazine Abundances. In all wines, except the two Pinot noir wines (Table 1, wines 7 and 8) which contained very low levels of both **1** and **2** and one Bordeaux wine (Table 1, wine 3), the abundance of methoxypyrazine **2** was typically only 2% of that of **1** and its concentration less than 1 ng/L, a situation comparable with that found in Sauvignon blanc wines (Lacey et al., 1991). As the sensory detection threshold for **2** (1 ng/L) (Murray et al., 1970) is close to that for **1** (2 ng/L) (Buttery et al., 1969; Seifert et al., 1970) these low concentrations of **2** suggest that the component will seldom contribute significantly to the sensory character of the wines. Nevertheless, in one Bordeaux wine (Table 1, wine 3) the concentration of **2** (1.9 ng/L) slightly exceeded the component's sensory detection threshold. Among the wines investigated, this wine contained an unusually high proportion of **2** (20% of the concentration of **1**), and a contribution of **2** to the aroma and flavor of this wine cannot be excluded.

Conclusion. Quantitative methoxypyrazine analysis of a range of red wines is reported for the first time. A definitive method, stable isotope dilution GC–MS,

identified 3.6–56.3 ng/L (mean, 16.2 ng/L) of methoxypyrazine **1** in 18 Cabernet Sauvignon and Cabernet Sauvignon/Merlot/Cabernet Franc wines, including 6 Bordeaux wines and 1 wine selected for its particularly intense herbaceous/vegetative aroma. The concentration is ca. 30 times lower than a value previously reported for a single Bordeaux red wine using less rigorous techniques. In the 18 Cabernet Sauvignon and Cabernet Sauvignon/Merlot/Cabernet Franc wines, the concentration of **1** exceeded the component's sensory detection threshold in water, consistent with the methoxypyrazine having the potential to influence the wine aroma and flavor. In two Burgundy Pinot noir wines, coelution restricted the determination of **1** to an upper limit. As this limit (<0.7 ng/L) is below the component's sensory detection threshold in water, the methoxypyrazine is unlikely to have a sensory effect in these wines. Although methoxypyrazine **2** was almost invariably a comparatively minor contributor, its concentration in one Bordeaux wine did exceed its sensory detection threshold in water, indicating that this component may also contribute to methoxypyrazine aroma or flavor in some wines.

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