Methoxypyrazines in Red Wines: Occurrence of 2-Methoxy-3-(1-methylethyl)pyrazine

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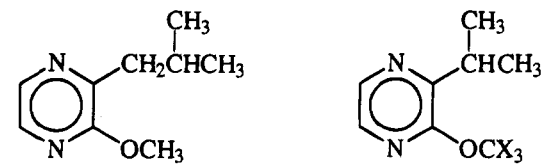
Methoxypyrazine quantitative analysis by stable isotope dilution gas chromatography–mass spectrometry has identified a wine in which the concentration of 2-methoxy-3-(1-methylethyl)pyrazine (isopropylmethoxypyrazine) in one bottle (9.7 ng/L) was comparable with the concentration of 2-methoxy-3-(2-methylpropyl)pyrazine (isobutylmethoxypyrazine) (9.3 ng/L). At this concentration, sensory detection threshold data suggest that isopropylmethoxypyrazine may contribute to the aroma of the wine more strongly than isobutylmethoxypyrazine. Comparatively low levels of isopropylmethoxypyrazine were found in other wines studied and in other bottles of the same wine. This suggests that isopropylmethoxypyrazine can occur in wine from sources other than the grape berry and normal winemaking processes.

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

Methoxypyrazines are potent flavorants that occur in some *Vitis vinifera* grapes and their wines. Quantitative analysis of these compounds has been achieved through the combination of gas chromatography–mass spectrometry (GC–MS) and stable isotope dilution methodology. Such analysis has demonstrated the occurrence of 2-methoxy-3-(2-methylpropyl)pyrazine (isobutylmethoxypyrazine) **(1)** (Figure 1), and this component has been found in all Sauvignon blanc and Cabernet Sauvignon grapes and wines examined so far, typically at a concentration of 2–30 ng/L (Harris et al., 1987; Allen et al., 1990, 1994; Lacey et al., 1991). In many of these analyses, 2-methoxy-3-(1-methylethyl)-pyrazine (isopropylmethoxypyrazine) **(2)** (Figure 1) was also identified (Harris et al., 1987; Lacey et al., 1991), although at a concentration that was always much lower than that of isobutylmethoxypyrazine. The lower concentration of isopropylmethoxypyrazine, coupled with the similar sensory detection thresholds (ca. 2 ng/L) of the two methoxypyrazines in water (Buttery et al., 1969a,b; Murray et al., 1970; Seifert et al., 1970) and in white wine (Allen et al., 1991), suggests that isobutylmethoxypyrazine will be the dominant contributor to vegetative/herbaceous methoxypyrazine aroma in wine. Nevertheless, a study of methoxypyrazine addition to red wine (Maga, 1990) has indicated a higher odor threshold for isobutylmethoxypyrazine than for isopropylmethoxypyrazine; this suggests that even comparatively low isopropylmethoxypyrazine concentrations may still have sensory importance in red wine. Isopropylmethoxypyrazine would take on particular sensory importance if its concentration in wine were to exceed its sensory detection threshold and also approach or exceed the concentration of isobutylmethoxypyrazine. This study identifies an occurrence of this situation that has implications not only for the aroma of the wine but also for the origin of the elevated level of isopropylmethoxypyrazine in this wine.



1 **2** X = ^{1}H **3** X = ^{2}H

Figure 1. Wine isobutylmethoxypyrazine **1**, wine isopropylmethoxypyrazine **2**, and deuterium-labeled internal standard **3**.

EXPERIMENTAL PROCEDURES

Wines, Chemicals, and Glassware. Twelve Australian and New Zealand red wines and eight French red wines were studied; their origin, grape varietal composition, age, and storage have been described in detail previously (Allen et al., 1994). Isopropylmethoxypyrazine was purchased from Pyrazine Specialities, Inc., Atlanta, GA, and was found to be >99.8% pure by GC–MS. Detailed procedures have been reported previously for the purification of dichloromethane and the cleaning of critical glassware (Allen et al., 1994), for the synthesis of isobutylmethoxypyrazine (Harris et al., 1987), and for the synthesis of 2-($^2H_3$)methoxy-3-(1-methylethyl)pyrazine **(3)** (Figure 1) (Lacey et al., 1991). Ion-exchange resin (Bio-Rad, AG50WX4 AR) was used as received.

Methoxypyrazine Isolation and Analysis. A detailed procedure has been published recently (Allen et al., 1994) for the methoxypyrazine isolation, mass spectrometric analysis, instrumental calibration, and quantitative analysis. That procedure was modified as follows to allow analysis of isopropylmethoxypyrazine **2** instead of isobutylmethoxypyrazine **1**. During methoxypyrazine isolation, **3** (1.48 ng/μL in dichloromethane, 12.0 μL) was added as internal standard to the wine samples. Mass spectrometry monitored ions of either *m/z* 153.1 and 156.1 (CI) or 137.1 and 140.1 (EI). Instrumental calibration used mixtures of isopropylmethoxypyrazine **2** and its trideuterated analog **3** prepared at seven $^2H_0/^2H_3$ mole ratios (mole ratio range: 0–0.220 at 484 pg/μL of **3**). For

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Table 1. Concentration of Isopropylmethoxypyrazine 2 in French Wines Including its Percentage Abundance Relative to That of Isobutylmethoxypyrazine 1

			concentration of isopropylmethoxypyrazine 2[a]	
wine	origin	region	ng/L	as % (w/w) of isobutylmethoxypyrazine 1[b]
1	1982, Léoville-Las Cases	St. Julien	<0.4	<2.7
2	1983, Léoville-Las Cases	St. Julien	<0.4	<3.8
3	1982, Ch. Conseillante	Pomerol	<0.1	<1.0
4	1982, Ch. Canon	St. Émilion	<0.4	<5.6
5	1982, Ch. Magdelaine	St. Émilion	<0.5	<7.0
6	1983, Ch. Cantemerle	Haut Médoc	9.7	104
			10.1[c]	104[c]
7	1983, Ch. Cantemerle[d]	Haut Médoc	0.92	12.2
8	1983, Ch. Cantemerle[e]	Haut Médoc	0.97	12.8

[a] Positive-ion CI-MS, DB-1 column, except where indicated otherwise. [b] Determined during the same GC–MS analysis according to the method of Allen et al. (1994) which reports the data on wines 1–6. [c] Mean of four determinations provided by positive-ion CI-MS (DB-1 and DB-1701 columns) and positive ion EI MS (DB-1 and DB-1701 columns). [d,e] Analysis of a second and third bottle of the wine, respectively.

calibration, linear regression weightings were estimated from replicate analyses of isopropylmethoxypyrazine **2** and its trideuterated analog **3** at $^2H_0/^2H_3$ mole ratios of 0.07329 (six replicates) and 0.000913 (seven replicates). Standardization of the instrument response was determined each day of quantitative analysis with a standard solution (1 μL) of isopropylmethoxypyrazine **2** and its trideuterated analog **3** (both 200 pg/μL).

RESULTS AND DISCUSSION

Except where indicated, analysis of isopropylmethoxypyrazine was performed by mass spectrometry in positive-ion CI mode. This provided a lower detection limit and greater freedom from intrusive signals of other matrix components than positive-ion EI mode. In positive-ion CI mode, instrumental calibration with mixtures of isopropylmethoxypyrazine **2** and its trideuterated analog **3** showed excellent response linearity (VG Trio 2, r = 0.999937) and a detection limit for isopropylmethoxypyrazine of 0.4 pg. The calibration intercept was consistent with 0.11% of undeuterated isopropylmethoxypyrazine in the deuterated internal standard.

The mass channel for the quantitative analysis of isopropylmethoxypyrazine (m/z 153.1; $[M + H]^+$) showed greater intrusion by ions of other matrix components than had been found (Allen et al., 1994) with the corresponding mass channel for isobutylmethoxypyrazine. Furthermore, as intrusion of coeluting components was more severe with these red wines than with white wines, unequivocal measurement of isopropylmethoxypyrazine in the 12 Australian and New Zealand red wines of the present study was inhibited by coeluting components despite the eminent suitability of the experimental strategy to previous analyses of Sauvignon blanc wines (Lacey et al., 1991). This intrusion of other components necessitated good column resolution for the quantitative analysis of isopropylmethoxypyrazine in the French red wines. Furthermore, it also required a signal-to-noise ratio adequate for critical assessment of the width and shape of the relevant peak, and it was necessary to establish consistency of the peak width, peak shape, and determined methoxypyrazine concentration with columns of differing polarity and under different mass spectrometry ionization conditions.

Two of the eight French wines of the present study were Pinot noir wines from Burgundy. Signals from intrusive components limited the determination of isopropylmethoxypyrazine in these wines to an upper limit of 0.5 and 1.9 ng/L. As the level of isobutylmethoxypyrazine in these two wines was also low (Allen et al., 1994) and below the component's aroma detection threshold of ca. 2 ng/L in water (Buttery et al., 1969a,b; Seifert et al., 1970), the wines were not examined further. The remaining six French wines were from the Bordeaux regions of Médoc and St. Émilion/Pomerol. They were examined more rigorously as they had shown clearly measurable isobutylmethoxypyrazine levels in the range 7.1–14.9 ng/L (Allen et al., 1994). In five of these wines, the isopropylmethoxypyrazine concentration was very low (Table 1, wines 1–5). Coeluting components again restricted the determination of isopropylmethoxypyrazine to an upper limit, but this limit was 0.5 ng/L or less, a value well below its aroma detection threshold of ca. 2 ng/L in water (Murray et al., 1970; Seifert et al., 1970) and in wine (Maga, 1990; Allen et al., 1991). Furthermore, this upper limit of the isopropylmethoxypyrazine concentration was only 1–7% (Table 1) of the isobutylmethoxypyrazine concentration identified in these wines (Allen et al., 1994). This low level of isopropylmethoxypyrazine, compared to isobutylmethoxypyrazine, is consistent with results of a previous study of Sauvignon blanc wines (Lacey et al., 1991).

Evidence for Isopropylmethoxypyrazine. In contrast to the other wines, the concentration of isopropylmethoxypyrazine in one wine (Table 1, wine 6) slightly exceeded that of isobutylmethoxypyrazine. The assignment of the former component as isopropylmethoxypyrazine was confirmed by the following evidence. First, the ion current signal for the component displayed a symmetrical peak shape and the expected peak width (Figure 2), and it maintained these characteristics across two GC stationary phases of widely differing polarity (DB-1 and DB-1701) and, with each stationary phase, across both modes of ionization (EI and CI). Second, the retention time of the component was identical (±0.01 min) to that of synthetic isopropylmethoxypyrazine on each of the contrasting stationary phases (DB-1, t_R = 17.45 min; DB-1701, t_R = 10.44 min) with, in each case, selected ion monitoring in both CI and EI modes to capitalize on the different mass channels of the base peaks (CI, m/z 153.1; EI, m/z 137.1). Third, the difference between the elution time of the component and that of the internal standard **3** was identical (±0.01 min) to that determined with synthetic isopropylmethoxypyrazine. Fourth, the relative insignificance of coeluting impurities was demonstrated by the consistency of the determined concentration across the four combinations of stationary phase and ionization mode (DB-1/CI, 9.7 ng/L; DB-1/EI, 9.7 ng/L; DB-1701/CI, 10.6

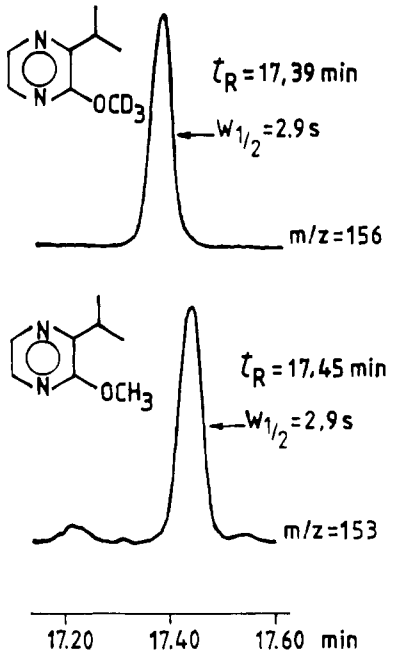

Figure 2. Peak shape and width at half-height ($W_{1/2}$) of [M + H]$^+$ ion of wine isopropylmethoxypyrazine **2** (m/z 153.1) in comparison with that of its trideuterated internal standard **3** (m/z 156.1) (Table 1, wine 6, CI mode, DB-1 column).

ng/L; DB-1701/EI, 10.5 ng/L). Finally, the EI mass spectrum (m/z 60–200) of the component, although weak, displayed the characteristic fragmentation pattern of isopropylmethoxypyrazine [wine component, DB-1 column, m/z (relative intensity) 152 (37), 137 (100), 124 (29); synthetic isopropylmethoxypyrazine, m/z (relative intensity) 152 (30), 137 (100), 124 (25)].

Previous attempts to identify isopropylmethoxypyrazine in grapes and wines have been hampered *inter alia* by its very low concentration (Augustyn et al., 1982; Harris et al., 1987; Lacey et al., 1991), by lack of an internal standard and poor reproducibility of retention times (Augustyn et al., 1982), by use of only a single GC stationary phase (Augustyn et al., 1982; Harris et al., 1987; Lacey et al., 1991), and by use of only one ionization mode (Augustyn et al., 1982; Lacey et al., 1991). Thus, the data of the present study significantly strengthen the evidence for the occurrence of isopropylmethoxypyrazine in wine.

Both isopropylmethoxypyrazine and isobutylmethoxypyrazine have a reported sensory detection threshold of ca. 2 ng/L in water (Buttery et al., 1969a,b; Murray et al., 1970; Seifert et al., 1970) and white wine (Allen et al., 1991), suggesting that the influence of isopropylmethoxypyrazine on the aroma of wine 6 (Table 1) may be comparable with that of isobutylmethoxypyrazine. Furthermore, a study of methoxypyrazine addition to a red wine that contained no endogenous methoxypyrazines (Maga, 1990) has shown that although both methoxypyrazines exhibited the same flavor threshold (2 ng/L), isobutylmethoxypyrazine had a much higher aroma threshold (16 ng/L) than isopropylmethoxypyrazine (2 ng/L). If these findings are typical of the influence of methoxypyrazines in other red wines, the contribution of isopropylmethoxypyrazine to the aroma of wine 6 (Table 1) can be expected to exceed that of isobutylmethoxypyrazine.

The comparative level of isopropylmethoxypyrazine and isobutylmethoxypyrazine in this wine contrasts with previous quantitative studies of wines and grape juices which have found an isopropylmethoxypyrazine concentration that was invariably less than 23%, and usually less than 10%, of the concentration of the isobutyl compound (Harris et al., 1987; Allen et al., 1990; Lacey et al., 1991). This prompted analysis of two further bottles of the wine. In both cases (wines 7 and 8, Table 1), analysis showed that the isopropylmethoxypyrazine concentration was well below the isobutylmethoxypyrazine concentration and that it was also below the sensory detection threshold of isopropylmethoxypyrazine of ca. 1–2 ng/L in water (Murray et al., 1970; Seifert et al., 1970) or white wine (Allen et al., 1991), a situation typical of the other red wines analyzed. This bottle variation confirms that the comparatively high level of isopropylmethoxypyrazine found in wine 6 (Table 1) is not intrinsic to the wine itself. Isopropylmethoxypyrazine is known to be produced by some microorganisms (Leete et al., 1992), sometimes in relatively high concentrations (Gallois et al., 1988). This raises the possibility that the unusually high level of isopropylmethoxypyrazine in wine 6 (Table 1) may arise from bottle-specific microbial contamination, perhaps associated with the cork. Further work is in progress to investigate this aspect.

Conclusion. The relatively high level of isopropylmethoxypyrazine in wine 6 (Table 1) has allowed the evidence for its occurrence in wine to be significantly strengthened. Furthermore, stable isotope dilution GC–MS analysis determined its concentration to be comparable with that of isobutylmethoxypyrazine, providing evidence that isopropylmethoxypyrazine may sometimes have a significant sensory effect in wine. However, the ten-fold lower level of isopropylmethoxypyrazine in other bottles of the same wine suggests that the high level is not endogenous to the wine prior to bottling but may arise from an extraneous source such as microbial contamination, possibly associated with corks.

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