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New insights into the synthesis and characterization of 2-methoxy-3-alkylpyrazines and their deuterated isotopologues†

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A previously described synthetic route for preparation of 2-methoxy-3-alkylprazines (MPs) based on condensation of glyoxal with an α-amino acid amide, followed by methylation with iodomethane yields 3-alkyl-1-methyl-1H-pyrazin-2-ones (*N*-methyl derivatives), rather than the designated 2-methoxy-3-alkylpyrazines (*O*-methyl derivatives). Despite similar nuclear magnetic resonance and mass spectral properties, gas chromatographic (GC) retention indices differ significantly, indicating chemical difference. With the example of 3-sec-butyl-1-methyl-1H-pyrazin-2-one and its 3-sec-butyl-1-[$^2H_3$]methyl-1H-pyrazin-2-one isotopologue, the position of the methyl group introduced could be assigned unambiguously, using heteronuclear multiple bond correlation (HMBC) NMR experiments. For future characterization, the spectroscopic (NMR, EI$^+$MS) as well as GC retention index data on two stationary phases of the most aroma relevant MPs and their deuterated isotopologues are summarized.

Keywords: methoxypyrazines; synthesis; characterization; NMR; GC-MS; linear retention indices

Introduction

2-Methoxy-3-alkylpyrazines (MPs; **4**) are a class of important and powerful flavor compounds. Owing to the extremely low flavor threshold values of some of the MPs, in particular those with iso-propyl (2-methoxy-3-iso-propylpyrazine (IPMP); **4a**), sec-butyl (2-methoxy-3-sec-butylpyrazine (SBMP); **4b**) and iso-butyl (2-methoxy3-iso-butylpyrazine (IBMP); **4c**) as alkyl chain, they contribute to the aroma of important foodstuffs[1] such as bell pepper or peas[2,3] and certain *Vitis vinifera* varieties.[4,5]

Published methods for the synthesis of 2-methoxy-3-alkylpyrazines vary. A common approach to generate the heterocyclic ring system follows the route originally described by Jones[6] for the synthesis of hydroxypyrazines **1**, in which an α-aminoacid amide is condensed with a 1,2-dicarbonyl compound (Figure 1). Later, Karmas and Spoerri[7] introduced the more accessible hydrohalides of the amides, and furthermore, described in detail the conversion of the preliminary hydroxypyrazines **1** to the 2-chloro derivatives **2** using phosphorous oxychloride[8] (Figure 1, route A). The reaction of these 2-chloro derivatives with ethanolic sodium ethoxide, forming the ethyl ethers (ethoxypyrazines) has also been described in this early work and was used in subsequent studies for the synthesis of various 2-alkoxy-3-alkylpyrazines.[9–12]. Buttery and co-workers used an alternative approach for converting the preliminary hydroxypyrazines to 2-methoxy-3-alkylpyrazines **4** by derivatization with diazomethane[2,13] (Figure 1, route B). However, they observed a by-product in a ratio of 2:1 (in favor of the by-product), which they described as 1,2-dihydro-3-alkyl-1-methyl-2-pyrazinone (or 3-alkyl-1-methyl-1H-pyrazin-2-one) **3**. The reason for this result was supposed to be that hydroxypyrazines **1** exist largely in the pyrazinone **1*** form in neutral solution, which could be confirmed by IR[14] or NMR[15] experiments.

In a more recent publication, Gerritsma *et al.*[16] described a method using sodium hydride and iodomethane to convert the originally formed hydroxypyrazines **1** to the targeted 2-methoxy-3-alkylpyrazines **4** (Figure 1, route C; later ascertained as a misconception, since this route leads to compounds **3** as outlined below). These authors also described the synthesis of the deuterated isotopologues (using [$^2H_3$]iodomethane instead of iodomethane). Such compounds are important reference substances for trace level analysis of 2-methoxy-3-alkylpyrazines **4**,

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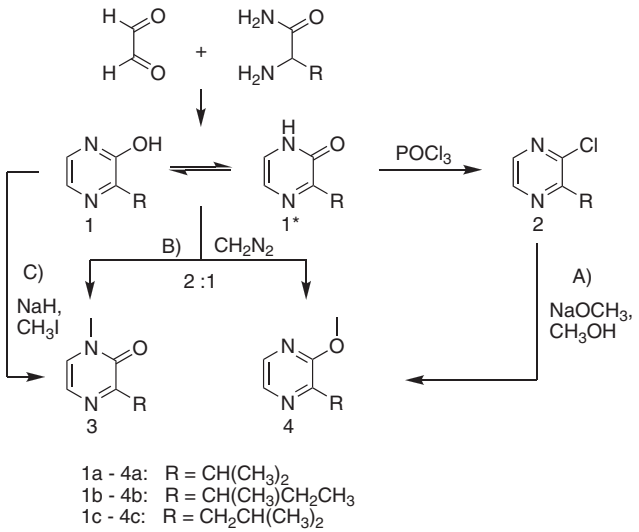

Figure 1. Routes to synthesize MPs as described in the literature.

following the approach of stable isotope dilution analysis (SIDA) for quantification.[17]

In the work described here, we synthesized the desired deuterated 2-[$^2H_3$]methoxy-3-alkylpyrazines d_3-**4a–c** for wine aroma analysis. Considering the published methods described for MP synthesis, we first followed the method described by Gerritsma *et al.*[16] (Figure 1, route C) as it seemed to be the most convenient approach. However, the products obtained showed different gas chromatographic (GC) properties compared with reference MPs **4a–c**. This paper clarifies some of the previously published results and summarizes the chromatographic and spectroscopic characterization of compounds obtained by the method described by Gerritsma *et al.*[16] and MPs, respectively their deuterated isotopologues.

Results and discussion

In our hands, the deuterated derivatives obtained after the approach of Gerritsma *et al.*[16] (Figure 1, route C) were not the designated MPs d_3-**4a–c**, as they showed considerably higher retention indices after GC analysis, than those for the commercial non-deuterated MP reference substances. We obtained substances, which could later be assigned to the structures of d_3-**3a–c**. Incorporation of deuterium into an organic molecule leads to a somewhat lowered retention index in gas liquid chromatography, despite its higher absolute molecular mass. This effect is thus called *inverse isotope effect*[18] and has been attributed to the different binding-length of C–H and C–D as summarized by Matucha *et al.*[19] The unexpected and drastically higher retention indices of more than 200 (on a 5% phenylmethylpolysiloxane stationary phase) or 600 (on a polyethylene glycol stationary phase) units for d_3-**3a–c** and **4a–c** were an indication for a differing chemical nature of the substances. Unfortunately, Gerritsma and co-workers provided only MS and NMR data, but MS data acquisition had obviously been achieved without prior GC separation. A full listing of retention and MS data can be found in the experimental supplementary section (Table S-1).

To verify synthesis procedures, deuterated sec-butyl pyrazine derivative was synthesized according to the originally described pathway (Figure 1, route A; d_3-**4b**) as well as the non-deuterated compound (later assigned as **3b**) according to Gerritsma *et al.*[16] (Figure 1, route C). GC and GC-MS data of the compound obtained from route A are consistent with those of the reference compound SBMP **4b** from Sigma-Aldrich (Table S-1). Considering earlier results,[2,13] 1,2-dihydro-3-sec-butyl-1-methyl-2-pyrazinone (or 3-sec-butyl-1-methyl-1H-pyrazin-2-one) **3b** was considered a possible product obtained from route C. Direct comparison of the ^{1}H-NMR spectra of commercial SBMP **4b** and its deuterated isotopologue d_3-**4b** from route A and of **3b** and its deuterated isotopologue d_3-**3b** showed disappearance of the methyl-singlets at 3.96 and 3.49 ppm, respectively (full spectral listings are in the experimental supplementary information section). This chemical shift difference of almost 0.5 ppm and a remarkable upfield shift of the aromatic protons by about 1 ppm for **4b** clearly demonstrate the success of incorporating the CH_3-groups in different positions in compounds **4b** and **3b**. The pattern of the other signals of d_3-**4b** is comparable and in accordance with published data.[11,12,20] In the ^{13}C-NMR spectra of compound **3b** and of commercial MP **4b** (Figure S-2), the signal of the heteroatom bound methyl group is shifted about 16 ppm from 37.3 ppm in **3b** to 53.3 ppm in **4b**. The positions of this methyl groups were unambiguously assigned by heteronuclear correlation NMR (^{1}H-^{13}C- and ^{1}H-^{15}N-HMBC) experiments (Figures S-3 and S-4). This way, the ^{15}N chemical shifts were estimated for both nitrogen atoms in **3b** with −217 and −54 ppm. This is comparable with literature values for the 1H-pyrazin-2-one with −198 and −36 ppm.[21] It could be shown (Figure S-3) that the introduction of the methyl group (3.5 ppm) is at the nitrogen in the amide position (−217 ppm), whereas the CH hydrogen (3.3 ppm) of the sec-butyl group shows a coupling to the nitrogen in 4 position (−54 ppm) as expected. This demonstrates that the synthesis pathway suggested by Gerritsma *et al.* (route C) lead to the 3-alkyl-1-methyl-1H-pyrazin-2-ones **3**, and not to the desired MPs **4**. Such finding underlines the significance of simple retention index information for characterization of compounds amenable to GC, as spectral data can be similar.

Furthermore, a closer investigation of the mass spectra of deuterated **3b (3c)** and **4b (4c)** revealed a loss of 15 and 18 mass units (representing a methyl or a [$^2H_3$]methyl group, respectively) which can also be used for differentiation of the two structures. Gerritsma *et al.*[16] described the loss of either fragments from the molecular ion ($M = 169$) on the example of what they believed to be d_3-**4c**. In their mass spectral interpretation, this methyl group (or [$^2H_3$]methyl group) loss could have come either from the methoxy position or the alkyl side chain, as they had found fragment ions at both $m/z = 151$ and 154 in the case of the deuterated compound. In our hands, such fragmentation was only observed for compounds d_3-**3b and c** (Figure S-5) and not for d_3-**4b and c** (Table S-1). Interestingly, in a thesis conducted at the same institution as Gerritsma's earlier work,[22] Chen[11] observed this discrepancy in mass spectra from compounds synthesized according to route A (modified according to Masuda *et al.*[10]) as well. Unfortunately, no clear statement had been given that the earlier described synthesis obviously leads to wrong compounds **3** rather than the desired MPs **4**. This is the more surprising, as the same group recently published (as co-authors) a work on quantitative analysis of MPs in juice and wine using SIDA.[12] The original synthetic route of Karmas and Spoerri[7] (A) was followed to obtain deuterated MPs d_3-**4**. However, commenting their earlier work[16] had not been an option. With regard to the

shortcomings found in literature, full MS spectra are supplied in Figure S-5 for compounds d_3-**3a–c** resulting from synthetic route C (shown in Figure 1) in addition to the text listings in Table S-1.

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