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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 (IBMP); **4b**) and iso-butyl (2-methoxy3-iso-butylpyrazine (IBMP); **4c**) as alkyl chain, they contribute to the aroma of important foodstuffs¹ 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 Jones⁶ 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 Spoerri⁷ introduced the more accessible hydrohalides of the amides, and furthermore, described in detail the conversion of the preliminary hydroxypyrazines 1 to the 2chloro derivatives 2 using phosphorous oxychloride⁸ (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 IR¹⁴ or NMR¹⁵ 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 [²H₃]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 d₃-3a-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 d₃-3a-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; d₃-**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 ¹H-NMR spectra of commercial SBMP **4b** and its deuterated isotopologue d₃-4b from route A and of 3b and its deuterated isotopologue d₃-3b 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 CH₃-groups in different positions in compounds 4b and 3b. The pattern of the other signals of d₃-4b is comparable and in accordance with published data. 11,12,20 In the 13C-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 (¹H-¹³C- and ¹H-¹⁵N-HMBC) experiments (Figures S-3 and S-4). This way, the ¹⁵N 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 [2H3]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 d₃-4c. In their mass spectral interpretation, this methyl group (or [2H₃]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 = 151and 154 in the case of the deuterated compound. In our hands, such fragmentation was only observed for compounds d₃-3b and c (Figure S-5) and not for d₃-4b and c (Table S-1). Interestingly, in a thesis conducted at the same institution as Gerritsma's earlier work,²² Chen¹¹ 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 Spoerri⁷ (A) was followed to obtain deuterated MPs d₃-4. However, commenting their earlier work¹⁶ 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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