Analytical Methods

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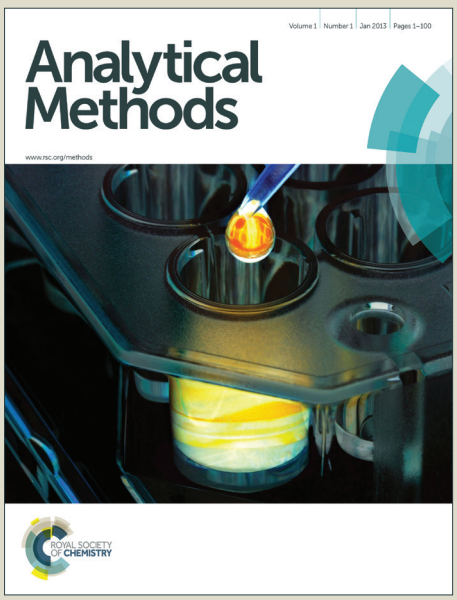

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Oxidation of hydroxymethylpyrazines and hydroxylated phenyl compounds in a gas chromatography inlet

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Large proportions of aldehydes were formed when hydroxymethylpyrazines, and benzyl- and cinnamyl alcohols were analysed by gas chromatography-mass spectrometry using a heated inlet with a previously used glass inlet liner. To avoid false reporting of non-natural compounds, any identification of aldehydes potentially formed in a GC inlet requires verification.

1. Introduction

Gas chromatography-mass spectrometry (GC-MS) is a routine method in many laboratories worldwide for the analysis of volatile natural products, such as compounds involved in chemical communication. Analysing underivatised samples of natural extracts is often preferred in order to facilitate the identification of new natural products, and is essential when using biological detectors such as electroantennogram detectors (EAD).

Pyrazines as natural products are involved in chemical communication in numerous biological systems, most commonly in insects.[1-10] In the food industry many pyrazine-derived compounds are used as flavouring agents, and some are formed *in situ* from amino acids in Maillard reactions when food is heated.[11-18] Recently, pyrazines, along with more complex derivatives, were discovered in bacterial odours and as products from microorganism reactions.[19-31] We have recently investigated the pollination chemistry of sexually deceptive *Drakaea* orchids and their thynnine wasp pollinators. In several species the key compounds were identified as hydroxymethylpyrazines – a largely new group of natural products.[32-35] In the process of identifying these compounds, we also found various amounts of the corresponding aldehyde compounds in some analyses. When the compounds were prepared synthetically in the laboratory, and after purification with semi-preparative high pressure liquid chromatography (HPLC), and subsequent analyses by GC-MS, various amounts of aldehydes were still present. These findings led us to investigate the oxidation of primary alcohols to aldehydes in the gas chromatograph.

It is well known that chemical reactions, polymerisations, and rearrangements can take place in GC inlets, especially when the inlet liners have been used for an extended period of time. For example, artificial peaks were detected when underivatised methamphetamine hydrochloride was analysed employing injector temperatures exceeding 200 °C. [36] Isoprene has been proven to form dimers in a hot PTV inlet, [37] and dehydroalanine was formed from mimosine and cysteine in another study,[38] to name a few. In our own unrelated work, we have previously observed the oxidation of 4-hydroxymethylquinoline to the corresponding aldehyde by GC-MS, during the identification of fungal metabolites.[39] However, to the best of our knowledge, apart from one report of methanol being oxidised to formaldehyde,[40] there are no specific studies reporting the problem of formation of aldehydes from easily oxidisable primary alcohols when using GC in the literature.

The aims of this work were (i) to investigate under which conditions the oxidation reaction from hydroxymethylpyrazine to corresponding aldehyde occurs, and (ii) to investigate whether the same problem affects other similar oxidisable hydroxymethyl compounds that are more commonly found, exemplified by benzyl alcohol and cinnamyl alcohol.

2. Materials and methods

Compounds **1**-**6** were available from previous work in the laboratory (for preparations see [32-35]), and **7**-**8** from Sigma-Aldrich Australia. For structures of compounds **1**-**8** see Fig.1, and for retention times and mass fragments of **1**-**4** and **6** see Supplementary data.

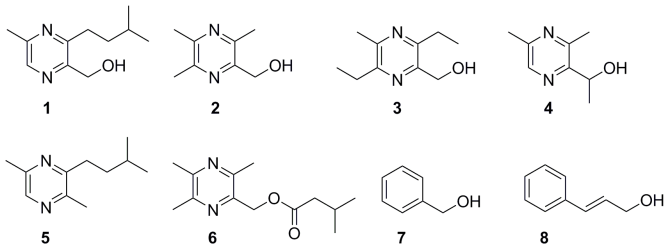


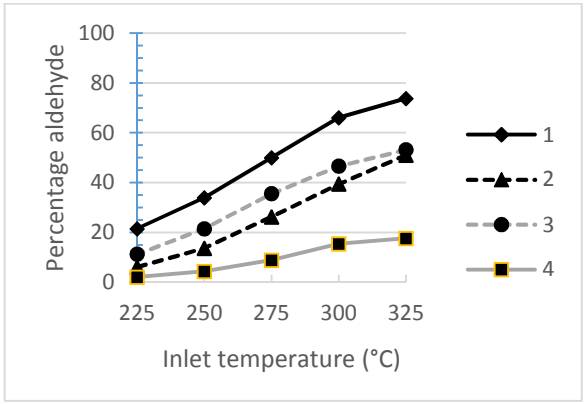
Fig. 1. Structures of pyrazines **1**-**6**, benzyl alcohol **7** and cinnamyl alcohol **8**, used in this study.

The purity of all compounds were confirmed to >98% by HPLC, and these compounds are known to be stable when stored as solutions in acetonitrile or dichloromethane at room temperature for weeks (unpublished data). The identities of the corresponding oxidised products of compounds **1**-**4** were confirmed by comparison of their mass spectra and retention times, and ultimately coinjection, with synthetic aldehydes prepared by oxidising small amounts of the alcohols with pyridinium chlorochromate or purchased from Sigma-Aldrich Australia.

EI-MS (70 eV) were recorded on an Agilent 5973 mass detector connected to an Agilent 6890 GC equipped with a BPX5 column [(5% phenyl dimethylpolysiloxane), 30 m × 0.25 mm × 0.25 µm film thickness, SGE Australia], using helium as a carrier gas with a constant flow rate of 1.0 mL/min. A scan range of *m/z* 39 – 300 and a solvent delay of 5 min were used. Injections were carried out with an Agilent 7683 autoinjector; injection volume 1.0 µL, splitless injection for 1.0 min. The compounds were prepared in HPLC grade acetonitrile at 0.1 M concentration. The ion source was set to 230 °C, and the transfer line temperature to 250 °C. The oven temperature program was 50 °C, ramped at 15°C /min to 280 °C, and held for 5 min. Two inlet liners (SGE, single tapered, quartz wool p/n 092019), one new and one previously used for ca. 100 injections were used, and the injector temperature was varied from 200 °C to 325 °C (200 °C to 300 °C for **7** and **8**) in the experiments. The previously used liner had been exposed to solvent extracts (acetonitrile or dichlromethane) of orchid flowers as described in previous work.[34]

3. Results and discussion

Using the previously used injection liner, all of the four tested hydroxymethylpyrazines **1**-**4**, benzyl alcohol **7**, and cinnamyl alcohol **8**, were oxidised to the corresponding aldehydes when they were injected at all temperatures within the range of 200-325 °C (Fig. 2). The two control compounds, alkylpyrazine **5** and compound **6** (i.e. an ester of **2**), were all intact with >98% purity observed at all injector temperatures.



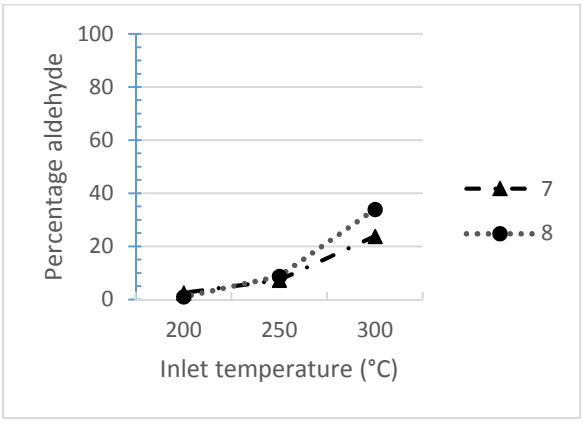


Fig. 2. Oxidation of hydroxymethylpyrazines **1**-**4** (upper panel), benzyl alcohol **7** and cinnamyl alcohol **8** (lower panel) in GC injector at different temperatures, measured as the peak area of aldehyde divided by the combined peak area of aldehyde and corresponding alcohol (100% aldehyde = all alcohol oxidised to aldehyde).

At an injector temperature of 250 °C, over 4% aldehyde was formed from compounds **1**-**4** and **7**-**8**, with a maximum of 34% aldehyde being formed at this temperature for compound **1**. At 300 °C the corresponding formation of aldehydes from the parent alcohols ranged from 15 – 66% (Fig. 2). Furthermore, for the pyrazine compounds, primary alcohols were more prone to oxidation compared to secondary alcohols (Fig. 2 and Supplementary data). In addition, greater amounts of aldehydes were observed with the pyrazine alcohols compared to the benzyl and cinnamyl alcohols (Fig. 2). Relevant areas of two representative chromatograms, showing compounds **2** and **8** at 300 °C, are shown in Fig. 3. It is also noteworthy that no dehydration products were observed in the chromatogram, nor were further oxidised products such as carboxylic acids, but these may not have eluted properly without derivatisation. The structures of all formed aldehydes were confirmed to be identical with authentic synthetic standards by co-injection, in that synthetic standards of corresponding aldehydes were co-injected with their respective alcohols and the retention times, GC peak shapes, and mass spectra of these synthetic standards were confirmed to be identical to those of the compounds formed during injection.

Lowering the injector temperature did not eliminate the formation of oxidation products, although as would be expected the peak abundance of both aldehydes and alcohols decreased considerably when the injector temperature was lowered. At 200 °C the size of the peak areas were between 25 – 66% of those obtained at injection temperatures of 325 °C (See supplementary data). It should also be noted that in preliminary experiments, oxidation reaction products were also observed when split mode (1:10) was used.

When the used inlet liner was replaced with an identical new, unused liner, all alcohols were confirmed to be of >97 % purity (Fig. 3).When the used liner was re-installed, oxidation products were again detected, thereby confirming that the liner was facilitating the oxidation during injection. It is unknown how this oxidation occurs, but it is possible that oxidising impurities or activated sites could be present in the used liner, coupled with trace amounts of oxygen that can be present from leakage of the septum when the sample is injected.

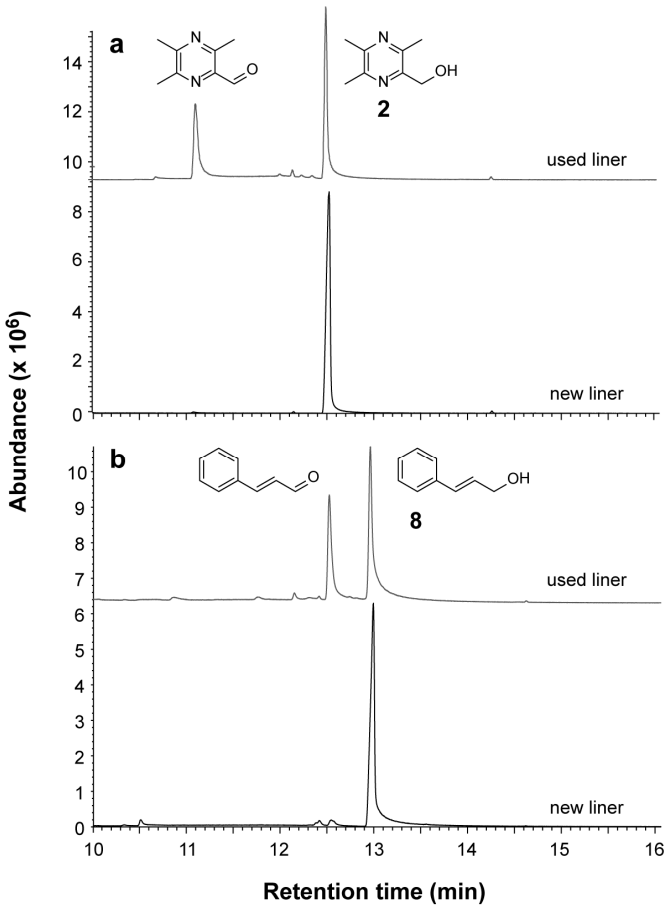


Fig. 3. Representative chromatograms of a) pyrazine **2** and b) cinnamyl alcohol **8** with used and new inlet liners at an injector temperature of 300 °C.

With the increasing identifications of hydroxymethylpyrazines as important semiochemicals, prior knowledge of potential GC inlet oxidations that can occur during analysis is of paramount importance. Apart from being important semiochemicals involved in *Drakaea* orchid pollination, hydroxymethylpyrazines have also been found in Australian *Caladenia* orchids,[41] and in multiple species of thynnine wasps (unpublished data). In many applications, such as natural product identifications, purification of extracts prior to GC analysis is difficult, as the compounds of interest commonly are highly volatile and present in minute amounts, which makes the awareness of oxidation reactions even more important.

Benzyl alcohols and cinnamyl alcohols are also common semiochemicals known from many natural systems [42] and have been identified in numerous contexts, with over 500 examples of benzyl alcohol and benzaldehyde having been simultaneously detected in single samples by gas chromatography according to literature sources. It is quite plausible that benzaldehyde could be an artefact in many of the reported cases. In our own work we have found that aldehydes of the orchid semiochemicals **1**-**4** are biologically active, although when the samples were re-analysed using a new inlet liner, it was evident that they were not natural products (unpublished data). Ideally, complementary analytical techniques such as NMR and/or IR should be used when easily oxidisable alcohols are analysed, however these techniques generally require purification of the compound in question. Alternatively, cool on-column injection or derivatisation protocols (e.g. trimethylsilylation or acetylation of the alcohol) could be employed to validate the presence of the aldehydes. In addition, control samples such as benzyl alcohol could be used to check the activity of the inlet liner.

4. Conclusions

We have shown that it is critical to verify that no oxidation reactions are taking place in the inlet of gas chromatographs when unknown samples such as natural extracts are analysed. Lowering the temperature of the injection port is not sufficient to suppress oxidation, and can also drastically reduce the sensitivity of the analysis. Before aldehydes can be confirmed as natural sample constituents, control experiments or alternative analytical methods should be employed to verify these aldehydes have not simply been derived from an easily oxidisable alcohol.

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Notes and references

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1. J.J. Brophy and G.W.K. Cavill, *Heterocycles* 1980, **14**, 477.
2. J.J. Brophy, G.W.K. Cavill and R.K. Duke, Insect Biochem. 1983, **13**, 503.
3. J.J. Brophy, G.W.K. Cavill, J.A. McDonald, D. Nelson and W.D. Plant, Insect Biochem. 1982, **12**, 215.
4. J.J. Brophy, G.W.K., Cavill and W.D. Plant, *Insect Biochem.* 1981, **11**, 307.
5. W.V. Brown and B.P. Moore, *Insect Biochem.* 1979, **9**, 451.
6. G.W.K. Cavill and E. Houghton, *Aust. J. Chem.* 1974, **27**, 879.
7. T. Chuman, P.J. Landolt, R.R. Heath and J.H. Tumlinson*, J. Chem. Ecol.* 1987, **13**, 1980.
8. J.H. Cross, R.C. Byler, U. Ravid, R.M. Silverstein, S.W. Robinson, P.M. Baker, J.S. de Oliviera, A.R. Jutsum and J.M. Cherrett*, J. Chem. Ecol.* 1979, **5**, 187.
9 R.M. Duffield, M.S. Blum and J.W. Wheeler, *Comp. Biochem. Physiol.* 1976, **54**, 439.
10. R.M. Duffield, M. Shamin, J.W. Wheeler and A.S. Menke, *Comp. Biochem. Physiol.* 1981, **70B**, 317.
11. H.-K. Chun and C.-T. Ho*, J. Food Lipids* 1997, **4**, 239.
12. W.M. Coleman III and T.J. Steichen, *J. Sci. Food Agric.* 2006, **86**, 380.
13. M. Czerny, R. Wagner and W. Grosch, *J. Agric. Food Chem.* 1996, **44**, 3268.
14. D.P. De Schutter, D. Saison, F. Delvaux, G. Derdelinckx, J.-M. Rock, H. Neven and F.R. Delvaux, *J. Agric. Food Chem.* 2008, **56**, 246.
15. J.A. Maga, *Food Rev. Int*. 1992, **8**, 479.
16. J.A. Maga and C.E. Sizer, *J. Agric. Food Chem*. 1973, **21**, 22.
17. M.A. Majcher and H.H. Jelén, *J. Agric. Food Chem.* 2005, **53**, 6432.

18. O.G. Witzthum amd P. Werkhoff, *J. Agric. Food Chem.* 1975, **23**, 510.
19. H. Achenbach, W. Kohl and B. Kunze, *Chem. Ber.* 1979, **112**, 1841.
20. A. Adams and N. De Kimpe, *Food Chem.* 2007, **101**, 1230.
21. R.L. Buchanan and W.M. Houston, *J. Food Sci.* 1982, **47**, 779.
22. M. Bungert, T. Jahns and H. Becker, *Flavour Frag. J.* 2001, **16**, 329.
23. T.-B. Cheng, G.A. Reineccius, J.A. Bjorklund and E. Leete, *J. Agric. Food Chem.* 1991, **39,** 1009.
24. C.A. Citron, P. Rabe and J.S. Dickschat, *J. Nat. Prod.* 2012, **5**, 1765.
25. J.S. Dickschat, H. Reichenbach, I. Wagner-Döbler and S. Schulz, *Eur. J. Org. Chem.* 2005, 4141.
26. J.S. Dickschat, I. Wagner-Döbler and S. Schulz, *J. Chem. Ecol.* 2005, **31**, 925.
27. J.S. Dickschat, S. Wickel, C. Bolten, J., T. Nawrath, S. Schulz and C. Wittmann, *Eur. J. Org. Chem.* 2010, 2687.
28. C.-M. Ryu, M.A. Farag, C.-H. Hu, M.S. Reddy, J.W. Kloepper and P.W. Paré, *Plant physiol.* 2004, **134**, 1017.
29. C.-M. Ryu, M.A. Farag, C.-H. Hu, M.S. Reddy, H.-X. Wei, P.W. Paré, and J.W. Kloepper, *Proc. Natl. Acad. Sci. U.S.A* 2003, **100**, 4927.
30. S. Schulz and J.S. Dickschat, *Nat. Prod. Rep.* 2007, **24**, 814.
31. S. Schulz, J. Fuhlendorff and H. Reichenbach, *Tetrahedron* 2004, **60**, 3863.
32. B. Bohman, L. Jeffares, G. Flematti, L.T. Byrne, B.W. Skelton, R.D. Phillips, K.W. Dixon, R. Peakall and R.A. Barrow, *J. Nat. Prod.* 2012, **75**, 1589.
33. B. Bohman, L. Jeffares, G. Flematti, R.D. Phillips, K.W. Dixon, R. Peakall and R.A. Barrow, *Org. Lett.* 2012, **14**, 2576.
34. B. Bohman, R.D. Phillips, M.H.M. Menz, B.W. Berntsson, G.R. Flematti, R.A. Barrow, K.W. Dixon and R. Peakall, *New Phytol.* 2014, **203**, 939.
35. B. Bohman and R. Peakall, *Insects* 2014, **5**, 474.
36. T.-L. Li, Y.-S. Giang, J.-F. Hsu, S.-G. Cheng, R.H. Liu and S.-M. Wang, *Forensic Sci. Int.* 2006, **162**, 113.
37. Y. Estevez, C. Gardrat, K. Berthelot, E. Grau, B. De Jeso, S. Ouardad and F. Peruch, *J. Chrom. A* 2014, **1331**, 133.
38. Y.M. Kim, T.O. Metz, Z. Hu, S.D. Wiedner, J.S. Kim, R.D. Smith, W.F. Morgan and Q. Zhang, *Rapid Commun. Mass Spectrom.* 2011, **25**, 2561.
39. F. Vinale, E. Ghisalberti, G. Flematti, R. Marra, M. Lorito and K. Sivasithamparam, *Lett. Appl. Microbiol.* 2010, **50**, 380.
40. C. Leong Keea, C. Sing Lai, X. Ge, M. Yong Low, L. A. Ciolino, *J. Chrom. A.* 2012, **1257**, 158.
41. B. Bohman, R.D. Phillips, G.R. Flematti, R. Peakall and R.A. Barrow, *Nat. Prod. Comm.* 2013, **8**, 701.
42. A.M. El-Sayed, http://www.pherobase.com (accessed July 2014).