

Analysis of the Seasoning-like Flavour Substances of a Commercial Lovage Extract (*Levisticum officinale* Koch.)

Imre Blank

Nestec Ltd, Research Centre, Vers-chez-les-Blanc, PO Box 44, CH-1000 Lausanne 26, Switzerland

Peter Schieberle

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, W-8046 Garching, Germany

The acidic fraction of a commercial lovage extract (*Levisticum officinale* Koch.) revealed the characteristic seasoning-like principle of the herb. The volatile components were analysed by gas chromatography-olfactometry. Aroma extract dilution analysis resulted in six odorants having high sensory relevance. They were identified as 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), (*E*)- β -damascenone, 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-methylbutanoic acid, and acetic acid. These odorants have not yet been reported in the literature as components of lovage. Sotolon was the key aroma compound of the acidic fraction of lovage extract due to its characteristic seasoning-like flavour and high flavour dilution factor. The concentration of sotolon was determined by a stable isotope dilution assay.

KEY WORDS *Levisticum officinale* Koch. Lovage Umbelliferae 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (Sotolon) Gas chromatography-olfactometry Aroma extract dilution analysis Stable isotope dilution assay.

INTRODUCTION

Lovage (*Levisticum officinale* Koch.) is a herb exhibiting a pronounced seasoning-like flavour. It is also called 'Maggi herb' due to this characteristic aroma note. Lovage extracts are used as raw material in the production of condiments to impart seasoning-like flavour quality.

A detailed study of the chemical composition of different lovage extracts (root, seed, leaf) has been published by Toulemonde and Noleau.¹ They identified more than 190 volatiles, mainly monoterpene hydrocarbons and phthalides, by means of GC-MS and GC-FTIR. However, no data have been reported concerning the compounds responsible for the typical seasoning-like flavour of lovage.

Gas chromatography in combination with olfactometry methods (GC-O) has been shown to be a valuable technique to detect odour-active com-

pounds of an aroma extract.^{2,3} The odour-activity of eluting compounds can be determined by GC-O of serial dilutions of the aroma extract. One such procedure called Aroma Extract Dilution Analysis (AEDA) was applied to different food systems and helped to focus the identification experiments on sensory relevant compounds (review in Reference 4).

The aim of the present study was to identify the odour-active compounds responsible for the seasoning-like flavour note of lovage extract by GC-O.

EXPERIMENTAL

Material

Lovage extract. The lovage extract was supplied by Vitoreco Ltd. (Kempttal, Switzerland). The juice of the whole plant was concentrated under vacuum to 70°Brix. Chemicals. The following compounds corresponding to those in Table 2 were obtained commercially: nos. 1, 2, 3, 5

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(Aldrich, Steinheim, Germany) and no. 4 (Givaudan, Dübendorf, Switzerland). Further reference compounds were either commercially available (butanoic acid, (*E*)-non-2-enal, vanillin, from Aldrich) or gifts from Ph. Duby (Comp. Arômes, Vevey, Switzerland), i.e. hex-1-en-3-one, oct-1-en-3-one, and (*E*)- β -damascenone (no. 6 in Table 2).

Synthesis of [¹³C]-3-Hydroxy-4,5-dimethyl-2(5H)-furanone([¹³C]-Sotolon)

Condensation of diethyl 2-methyl-3-oxobutanedioate and [¹³C]-acetaldehyde followed by lactonization and subsequent decarboxylation of the cyclic intermediate under strong acidic conditions resulted in [¹³C]-sotolon as recently described.⁵

Isolation of Acidic Compounds

To achieve lyophilization of the viscous material, the lovage extract (150 g) was mixed with distilled water (300 ml) and freeze-dried using Lyolab B (Secfroid, Lausanne, Switzerland). The final product (37 g) was stored under nitrogen at -20°C . A suspension of the lyophilizate (10 g) in diethyl ether (300 ml) was stirred for 6 h. The solvent was changed every 2 h to increase the extraction efficiency. The combined supernatants were concentrated on a Vigreux column (50 cm \times 1 cm) to 100 ml. The acidic compounds were extracted with sodium carbonate (0.5 mol/l, pH 11.2, 4 \times 300 ml). The aqueous solution was then acidified (5 mol/l HCl) to pH 2.0 and the acidic compounds were extracted with diethyl ether (3 \times 150 ml). The organic layer was dried over sodium sulphate (1 h, + 4°C) and finally concentrated by microdistillation⁶ to 0.5 ml.

Capillary Gas Chromatography (HRGC)

HRGC was performed as described^{7,8} with some modifications. A Carlo Erba Mega 2 Series equipped with an automatic cold 'on-column' injector and a flame ionization detector (FID) was used (Fisons Instruments, Switzerland). Fused-silica capillaries of different polarity were employed coated with FFAP and OV-1701, respectively, both 30 m \times 0.32 mm i.d. with a film thickness of 0.25 μm (J&W capillaries; Fisons Instruments from Brechbühler, Zurich, Switzerland). The flow rate of the carrier gas helium was 2.0 ml/min. The oven temperature was pro-

grammed as follows: 35°C (2 min), $40^{\circ}\text{C}/\text{min}$ to 50°C (2 min), $6^{\circ}\text{C}/\text{min}$ to 180°C , $10^{\circ}\text{C}/\text{min}$ to 230°C (10 min). At the end of the capillary, the effluent was split 1:1 into a FID and a sniffing port (patent held by Firmenich, Geneva, Switzerland, commercialized by Brechbühler, Zurich, Switzerland) using deactivated and uncoated fused-silica capillaries (50 cm \times 0.25 mm i.d.). The splitter (Gerstel, Germany) was flushed with helium to accelerate the split flow to 10 ml/min. The FID and sniffing-port were held at 230°C . Nitrogen was used as make-up gas for the FID. Linear retention indices (RI) were calculated from the retention times using a PC program based on cubic spline interpolation.^{9,10}

Gas Chromatography-Olfactometry (GC-O)

Aliquots of the aroma extract (acidic fraction) were separated by HRGC and the odorants were perceived at the sniffing port.^{3,7} The sensory significance of each odorant was evaluated by AEDA and expressed as the flavour dilution (FD) factor as recently described.¹¹ Odour thresholds in air were determined by GC-O^{3,7} using the FFAP capillary for polar odorants.⁸

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were performed by means of a Finnigan MAT 8430 double focusing mass spectrometer (Bremen, Germany) connected to an HP-5890 gas chromatograph and by using the FFAP capillary and the same HRGC conditions as described above. Electron impact spectra (MS-EI) were generated at 70 eV.

Quantification by Stable Isotope Dilution Assay^{11,12}

Sample preparation and quantitative analysis by mass chromatography using [¹³C]-sotolon as the internal standard were performed as recently described.⁵ The calibration factor was calculated from model mixtures containing definite amounts of the unlabelled and the labelled component.¹³

Sensory Evaluation

Sensory evaluation (nasal perception) was performed by seven assessors. The fractions obtained from lovage extract (samples C and D in Table 1)

Table 1. Odour profiles of lovage extract and samples prepared from lovage extract

Sample	Odour description ^a	Seasoning note
A Lovage extract	cooked vegetables, celery-like, caramel-like, spicy, maple-like, seasoning-like, honey-like, sweet, acidic (intense flavour)	+
B Lyophilizate of A	caramel-like, seasoning-like, celery-note, sweet, less vegetable/herb-like (intense flavour)	+
C Acidic fraction of B	seasoning-like, caramel-like, maple-like, celery-like, sweet, acidic (intense flavour)	+
D Neutral fraction of B	honey-like, flowery, fatty, herb-like, sweet (weak flavour)	-

^a Samples were judged by seven assessors. Odour qualities of the samples were described individually and spontaneously.

were concentrated to 1 ml for sensory evaluation of the overall aroma. The panel members were instructed to describe the odour quality individually and spontaneously by sniffing the head space of the samples. Descriptive terms were reduced by deletion of rarely used descriptors and combination of synonymous terms.

RESULTS AND DISCUSSION

Sample Preparation and Sensory Evaluation

To localize compounds responsible for the seasoning-like flavour of the lovage extract, a sensory directed chemical analysis procedure was applied. Each isolation and analytical step was accompanied by sensory evaluation. Lovage extract was separated into different fractions whose overall flavours were described by seven assessors. As shown in Table 1, the acidic fraction of the lyophilisate (sample C) obtained from the lovage extract represented the seasoning-like flavour note. Consequently, this fraction was further analysed by GC-O.

GC-O of the Acidic Fraction

Twenty two odour-active regions were detected by GC-O of the acidic fraction characterized by their RI and odour qualities perceived at the sniffing-port. A very intense seasoning-like odour, similar to the overall flavour of the acidic fraction, was detected at RI = 1350 on the OV-1701 capillary. However, only a small peak was observed in this region of the gas chromatogram

(data not shown). The sensory relevance of the odour-active components was then estimated by AEDA indicating fourteen odorants in the FD-factor range of 3 to 300. The odour quality of the compounds showing FD factors higher than 10 were described as seasoning-like, honey-like, caramel-like and rancid.

AEDA was used as a screening method to focus the identification experiments on potent odorants which were indicated by high FD-factors. The chemical identity of each compound was verified by comparing it with the reference substance on the basis of RI values, MS spectra and/or odour quality. As shown in Table 2, six odorants showing high sensory relevance (FD \geq 10) were identified as acetic acid (no. 1), 3-methylbutanoic acid (no. 2), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDF, no. 3), 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (homofuraneol, no. 4), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon, no. 5), and (*E*)- β -damascenone (no. 6). All these odour-active compounds are reported for the first time as constituents of lovage.

The main odour qualities describing the odour profile of the acidic fraction of lovage extract were seasoning-like, sweet/caramel-like/honey-like and rancid/fatty/musty. The rancid/acidic flavour note of 3-methylbutanoic acid and acetic acid was enhanced by butanoic acid and the lipid degradation products hex-1-en-3-one, oct-1-en-3-one and (*E*)-non-2-enal (data not shown in Table 2). The sweet note of homofuraneol, HDF and (*E*)- β -damascenone was additionally influenced by vanillin and two unknown compounds (RI = 1510 and 1570 on the OV-1701 capillary).

Table 2. Odour-active compounds of the acidic fraction of lovage extract

No.	Compound	Retention index ^a		Odour quality ^b	FD-factor ^c (FD ≥ 10)
		OV-1701	FFAP		
1	Acetic acid ^d	785	1455	sourish, pungent	10
2	3-Methylbutanoic acid ^d	1025	1670	rancid, sweaty	30
3	4-Hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone (HDF) ^d	1235	2045	caramel-like	10
4	2-Ethyl-4-hydroxy-5-methyl-3(2 <i>H</i>)-furanone (homofuraneol) ^d	1315	2100	caramel-like	30
5	3-Hydroxy-4,5-dimethyl-2(5 <i>H</i>)-furanone (sotolon) ^d	1350	2215	seasoning-like	300
6	(<i>E</i>)β-Damascenone ^e	1500	1825	honey-like, flowery	100

^a The retention indices were determined on the capillaries OV-1701 and FFAP.

^b Odour description assigned during aroma extract dilution analysis.

^c The flavour dilution (FD) factors were evaluated using the FFAP capillary.

^d The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index on two capillaries, mass spectrum and the odour quality perceived at the sniffing port.

^e The MS signals of the compound were too weak for an interpretation; the compound was identified by comparing it with the reference substance on the basis of the remaining criteria reported in footnote *d*.

Sotolon

The main aroma quality of the acidic fraction was seasoning-like. This flavour note was imparted by sotolon which was the key aroma compound owing to its characteristic seasoning-like flavour and high FD-factor (FD = 300, Table 2). Its concentration was determined by stable isotope dilution assay showing the relatively high amount of 7.6 mg/kg lovage extract. The high sensory relevance of sotolon in lovage extract found by AEDA is in good agreement with the high concentration and low odour threshold of 0.3 µg/l water⁵ (detection). Thus, the concentration of sotolon was about 25×10^3 times higher than its detection threshold.

Sotolon has been identified in different food systems, e.g. raw cane sugar,¹⁴ sake,¹⁵ flor sherry wine,¹⁶ roasted coffee,^{8,17} and fenugreek seeds¹⁸ as an important contributor to the overall flavour. Kobayashi¹⁹ reported that the aroma characteristic of sotolon changes from seasoning-like and curry-like at high concentrations to a caramel-like aroma at low concentrations. According to Hodge,²⁰ the cyclic structure containing a planar enoloxo group is responsible for this caramel-like note. Further aroma-active compounds identified in lovage extract which belong to the group of cyclic enolones are HDF and homofuraneol.

Sotolon was the only odorant in the acidic fraction of lovage extract which smelt seasoning-like. In agreement with our previous investigation,⁵ we did not detect its 5-ethyl homologue, 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (EHMF, also called abhexon or 'Maggi lactone') by GC-O.

This potent flavour compound has been reported as the flavour principle of seasonings prepared from plant protein hydrolysates.²¹ EHMF has recently been identified and quantified in coffee,⁸ liquid seasonings and soya sauce.⁵

Odour Thresholds

The odour thresholds in air of acetic acid, 3-methylbutanoic acid and homofuraneol were evaluated using GC-O. The values are summarized in Table 3 including the thresholds of other polar odour-active compounds detected in the acidic fraction of lovage extract. Significant differences were found in the group of enoloxo components. The lowest threshold was determined for sotolon, followed by homofuraneol (10 times

Table 3. Odour threshold of polar compounds identified in the acidic fraction of lovage extract

No.	Compound ^a	Threshold ^b (ng/l; air)
5	Sotolon	0.01–0.02
4	Homofuraneol	0.1–0.2
3	4-Hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone	0.5–1.5
2	3-Methylbutanoic acid	1–2
1	Acetic acid	30–90

^a The numbers of the compounds refer to Table 2.

^b The odour thresholds were determined on an FFAP capillary. The threshold range was established by the lowest and the highest value found in triplicates.

higher) and then HDF (about 70 times higher). The threshold of 3-methylbutanoic acid was in the same range as that of HDF and about 40 times lower compared to acetic acid.

CONCLUSION

According to the results obtained by GC-O, sotolon is mainly responsible for the seasoning-like note of lovage extract. The overall flavour is shown to be rounded off by additional sweet aroma qualities (caramel-like, honey-like). The results of this study are also of interest with respect to the processing of lovage. The drying and concentration processes should be performed in such a way that only small losses of sotolon occur.

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REFERENCES

1. B. Toulemonde and I. Noleau in *Flavors and Fragrances, A World Perspective* (Proceedings of the 10th International Congress of Essential Oils, Fragrances and Flavors), ed. B. M. Lawrence, B. D. Mookherjee and B. J. Willis, p. 641, Elsevier Science Publishers, Amsterdam (1988).
2. T. E. Acree, J. Barnard and D. G. Cunningham, *Food Chem.*, **14**, 273 (1984).
3. F. Ullrich and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **184**, 277 (1987).
4. W. Grosch, *Chemie in unserer Zeit*, **24**, 82 (1990).
5. I. Blank, P. Schieberle and W. Grosch in *Progress in Flavour and Precursor Studies*, ed. by P. Schreier and P. Winterhalter, p. 103. Allured Publishers, Wheaton, IL, USA (1993).
6. J. M. H. Bemelmans in *Progress in Flavour Research*, ed. D. G. Land and H. E. Nursten, p. 79, Applied Science, London (1979).
7. I. Blank, K.-H. Fischer and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **189**, 426 (1989).
8. I. Blank, A. Sen and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **195**, 239 (1992).
9. W. A. Halang, R. Langlais and E. Kugler, *Anal. Chem.*, **50**, 1829 (1978).
10. K. Ebert and H. Ederer in *Computeranwendung in der Chemie*, p. 298, Verlag Chemie, Weinheim (1983).
11. W. Grosch and P. Schieberle in *Characterization, Production and Application of Food Flavours* (Proceedings of the 2nd Wartburg Aroma Symposium), p. 139, Akademie Verlag, Berlin (1988).
12. P. Schieberle and W. Grosch, *J. Agric. Food Chem.*, **35**, 252 (1987).
13. A. Sen, G. Laskawy, P. Schieberle and W. Grosch, *J. Agric. Food Chem.*, **39**, 757 (1991).
14. Y. Tokitomo, A. Kobayashi, T. Yamanishi and S. Muraki, *Proc. Japan Acad.*, **56B**, 457 (1980).
15. K. Takahashi, M. Tadenuma and S. Sato, *Agr. Biol. Chem.*, **40**, 325 (1976).
16. B. Martin and P. X. Etievant, *J. High Resolut. Chromatogr.*, **14**, 133 (1991).
17. I. Blank, A. Sen and W. Grosch in *14th International Conference on Coffee Science, ASIC 91*, San Francisco, p. 117 (1992).
18. P. Girardon, Y. Sauvaire, J. C. Baccou and J. M. Bessiere, *Lebensm. Wiss. Technol.*, **19**, 44 (1986).
19. A. Kobayashi in *Flavour Chemistry. Trends and Developments* (ACS Symp. Ser. 388), ed. R. Teranishi, R. G. Buttery and F. Shahidi, p. 49, American Chemical Society, Washington DC (1989).
20. J. E. Hodge in *The Chemistry and Physiology of Flavours*, ed. H. W. Schultz, E. A. Day and L. M. Libbey, p. 465, AVI Publishing Company, Westport, CT (1967).
21. H. Sulser, M. Habegger and W. Büchi, *Z. Lebensm. Unters. Forsch.*, **148**, 215 (1972).

