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# THE VOLATILES OF *CALAMINTHA NEPETA* SUBSP. *GLANDULOSA*

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**Key Word Index**—*Calamintha nepeta* subsp. *glandulosa*; Lamiaceae; volatiles; essential oils; headspace; piperitenone oxide; piperitone oxide; 4-hydroxypiperitone [6-hydroxy-3-methyl-6-(methylethyl)-cyclohex-2-enone]; artifact formation.

**Abstract**—The volatiles of *C. nepeta* subsp. *glandulosa* were studied by analysis of the essential oil and of the headspace (after concentration on Tenax GC). Amongst the 27 compounds identified in the essential oil, up to 92% consisted of piperitone oxide and piperitenone oxide, the relative concentrations of which depended on the maturity of the plants. The weak fragrance of the intact plant originated mainly from limonene and piperitone oxide. During the preparation of the essential oil, *trans*-sabinene hydrate and piperitone oxide isomerized in part into terpinen-4-ol and 4-hydroxypiperitone, respectively.

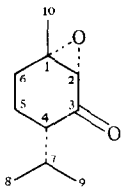
## INTRODUCTION

The genus *Calamintha* has been investigated very sparingly. As far as the volatiles are concerned, only the composition of the essential oil of *C. nepeta* (L.) Savi (Syn. *Satureja calamintha* Scheele) has been determined. Its notably mint-like character was found to originate from pulegone, menthone and isomenthone [1, 2], and this characteristic was proposed to help differentiate between *Calamintha* and *Satureja* on chemotaxonomical grounds [3]. In this connection, and within the broader framework of investigations in our laboratory on aromas and flavours, we were struck by the odour of the closely related *C. nepeta* (L.) Savi subsp. *glandulosa* (Req.) P. W. Ball, several specimens of which grow in the Botanical Garden of the University of Gent. Indeed both plant and essential oil smell distinctly musty and very different from *C. nepeta* subsp. *nepeta*, and thus it seemed worthwhile to study their volatile composition.

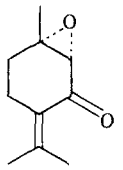
## RESULTS AND DISCUSSION

The GC and GC/MS analyses of the yellow, slightly viscous oil of *C. nepeta* subsp. *glandulosa*, prepared by steam distillation (0.1–0.3% fresh weight), showed the presence of two major compounds (Table 1). These could not be identified immediately because of the lack of corresponding mass spectra in the commonly used tables [4–6] at our disposal, and by the absence of the relevant Kováts indices [7] on standard columns in the literature. After isolation and purification by preparative GC on Apiezon L, spectroscopic analysis ($^1$H NMR, $^{13}$C NMR and IR) revealed the first compound (Kováts index 1231 on OV-1) to be piperitone oxide (**1**). It is noteworthy that **1** has two diastereotopic [8] methyl groups (C-8 and C-9), which give separate signals in the $^1$H NMR and $^{13}$C NMR spectra (see Experimental). The second substance (Kováts index 1333) was found to be piperitenone oxide (**2**).

The presence of high concentrations of compounds **1** and **2** in the essential oil of *C. nepeta* subsp. *glandulosa* explains why its odour is completely different from that of




**1**




**2**

*C. nepeta* as **1** and **2** smell, respectively, sweetish, slightly camphoraceous and dihydrocarvone-like.

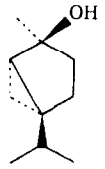
The composition of the headspace of the fresh plant differs quantitatively from the composition of the oil. By their relatively higher concentration, more compounds participate in the formation of the fragrance of the intact plant. Among them are limonene, piperitone oxide, dihydrocarvyl acetate and β-caryophyllene (and relatively little piperitenone oxide, possibly due to its high solubility in water [9]) and this leads to a difference in odour between the oil and the fresh plant.

A further difference between headspace and essential oil was the absence of terpinen-4-ol (**4**) and an unknown (Kováts index 1280) with $[M]^+$ at $m/z$ 168 (an isomer of piperitone oxide, **1**) in the former. In order to determine whether the two substances were natural or artifacts, a freshly prepared sample of the essential oil (Table 1; October 1984 A) was mixed with the thoroughly dried, residual, oil-free plant material, and submitted to a second steam distillation. This treatment did not lead to a change in the content of the unknown, but increased the amount of terpinen-4-ol (**4**) (Table 1; October 1984 B; Kováts index 1164) at the cost of *trans*-sabinene hydrate (**3**) (Kováts index 1056), thus making **4** a probable artifact. This isomerization has already been observed during the preparation of the essential oil of *Mentha candicans* [10], and more thoroughly investigated by Koedam *et al.* [11, 12]. It may account for the absence of terpinen-4-ol

Table 1. Composition (in %) of the headspace and of essential oils of *C. nepeta* subsp *glandulosa*

| Compound | Kováts index on OV-1 | Head-space | Essential oils | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | August 1983 | June 1984 | September 1984 | October 1984 A | B | C |
| Methyl 2-methylbutanoate | 765 | 0.7 | | | | | | |
| α-Thujene | 921 | 1.2 | tr | tr | tr | tr | — | — |
| α-Pinene | 927 | 4.4 | 0.1 | 0.1 | 0.4 | 0.4 | — | 0.2 |
| Camphene | 940 | 0.6 | tr | tr | tr | tr | — | — |
| Sabinene | 963 | 3.5 | 0.1 | 0.2 | 0.4 | 0.3 | tr | 0.1 |
| β-Pinene | 966 | 2.7 | 0.1 | 0.2 | 0.4 | 0.4 | tr | 0.3 |
| Myrcene | 982 | 1.9 | 0.2 | 0.4 | 0.4 | 0.5 | 0.2 | 0.3 |
| α-Phellandrene | 992 | 4.4 | 0.6 | 2.4 | 1.4 | 1.4 | 1.0 | 1.6 |
| Octan-3-ol | 993 | | | | | | | |
| α-Terpinene | 1000 | 1.2 | tr | tr | tr | — | — | — |
| *p*-Cymene | 1011 | 0.7 | 0.1 | 0.3 | 0.3 | 0.4 | 0.5 | 1.5 |
| 1,8-Cineole | 1018 | 0.1 | tr | tr | tr | tr | tr | — |
| Limonene | 1020 | 15.3 | 0.7 | 4.3 | 2.6 | 2.6 | 0.8 | 3.0 |
| γ-Terpinene | 1048 | 2.3 | 0.5 | 0.3 | 0.6 | 0.9 | 0.3 | 1.0 |
| *trans*-Sabinene hydrate | 1056 | 1.2 | 0.6 | 1.7 | 1.2 | 1.7 | 0.4 | — |
| Terpinolene | 1077 | 0.8 | tr | 0.3 | tr | 0.2 | 0.3 | 0.2 |
| *cis*-Sabinene hydrate | 1089 | 1.0 | 0.3 | 1.2 | 0.9 | 0.6 | 0.7 | 0.9 |
| Terpinen-4-ol | 1164 | — | 0.9 | 2.6 | 3.3 | 2.8 | 3.5 | 4.9 |
| Dihydrocarvone | 1172 | 0.6 | tr | 0.1 | tr | 0.2 | 0.5 | — |
| α-Terpineol | 1177 | — | tr | 0.4 | 0.3 | 0.2 | 0.4 | 0.5 |
| Piperitone oxide | 1231 | 25.4 | 40.5 | 30.7 | 57.6 | 72.3 | 74.2 | 57.7 |
| 4-Hydroxypiperitone | 1280 | — | 0.6 | 1.4 | 2.0 | 1.5 | 1.5 | 15.9 |
| Dihydrocarvyl acetate I | 1292 | 3.4 | 1.3 | 0.5 | 2.6 | 1.0 | 2.0 | 3.6 |
| Dihydrocarvyl acetate II | 1310 | 0.8 | tr | 0.6 | 1.2 | 0.3 | 0.9 | — |
| Piperitenone oxide | 1333 | 1.6 | 52.0 | 42.4 | 21.4 | 5.2 | 6.0 | 4.7 |
| β-Caryophyllene | 1413 | 5.4 | 0.7 | 2.3 | 1.6 | 1.8 | 1.0 | 1.3 |
| α-Humulene | 1446 | 0.7 | tr | 0.1 | tr | 0.1 | tr | tr |
| Germacrene-D | 1473 | — | 0.4 | 0.8 | 0.9 | 1.2 | 0.1 | 0.1 |

tr = trace, < 0.1%; — = < 0.02%. In addition to the above compounds, 11 unknowns were detected.

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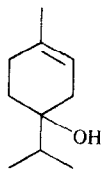


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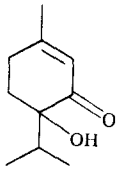


5

(**4**) in the headspace of *C. nepeta* subsp. *glandulosa*. On the other hand, as the concentration of the unknown (Kováts index 1280) was small when compared with that of its possible precursor piperitone oxide (**1**), it seemed to indicate that its presence was due to the 'reaction time', as expressed by the period of actual contact of the substances with steam. When a sample of plant material was first boiled with water for 3 hr before isolation of the oil by steam distillation, the amount of the unknown increased notably, at the expense of **1** (Table 1; October 1984 C). Later it was found that this transformation could be effected efficiently by stirring a solution of **1** in 0.1 M sodium carbonate at ambient temperature for 24 hr. Analysis of the purified substance showed it to be 4-hydroxypiperitone (**5**), identical with material synthesized recently from piperitone [13]. As in the case with piperitone oxide (**1**), **5** also has two diastereotopic [8] methyl groups, which give separate signals in the $^1$H NMR and $^{13}$C NMR spectra (see Experimental).

The number of plants producing piperitone oxide (**1**) and piperitenone oxide (**2**) seems to be rather limited. Up to now **1** and **2** were known to occur in high concentration in different *Mentha* sp. [14–19], while smaller amounts were found in *Cannabis sativa* [20], *Plectranthus rugosus* (Lamiaceae) [21] and *Satureja odora* [22]. Outside of *Mentha*, and apart from *C. nepeta* subsp. *glandulosa* (Table 1), only *Satureja parvifolia* contains **1** and **2** as its main volatile components (41% **1**, 19% **2** and 13% piperitone) [23]. However, there is a distinct analogy between the described composition of the oils of *S.*

*parvifolia* [23] and *C. nepeta* subsp. *glandulosa* on the one hand, and of *C. nepeta* [1, 2] and *S. odora* [22] on the other. Bearing in mind the obvious difficulties encountered in classifying *Satureja* species, many of which are known under different names, often of different genera, one might wonder if all four are not *Calamintha* sp. This would be in accordance with Adzet and Passet [3] who proposed to distinguish between *Satureja* and *Calamintha* on the basis of the presence of high concentrations of phenols in the oil of the former (although later on they discovered a linalol-chemotype [24]), and of high concentrations of C-3 ketones of the *p*-menthane series in the latter.

According to the literature, when **1** and/or **2** are the major components of a *Mentha* oil, one or the other is usually present in a large excess (sometimes to the exclusion of the other [9]), and only two examples have been described of a *M. suaveolens* chemotype [25] and of *M. royleana* [26], where the oils contain almost equal amounts of **1** and **2**. However, as follows from the results in Table 1, this variation might be due to the degree of maturity of the plants used in the former investigations, because in the case of *C. nepeta* subsp. *glandulosa* the quantitative composition of the oil depends on the collection time. The content of piperitone oxide (**1**) steadily increases, while that of piperitenone oxide (**2**) decreases from a maximum of 52% to 5%. No explanation can be given for this observation, because nothing is known at the moment about the efficiency or the specificity of the enzymes responsible for the formation of **1** and **2** according to currently accepted biotransformation schemes [27, 28].

An intriguing fact about the volatiles of *C. nepeta* subsp. *glandulosa* is the presence of small to relatively large quantities of dihydrocarvone and dihydrocarvyl acetates, because the genetics of essential oil biosynthesis in *Mentha* require the former compounds to be formed under the direction of the dominant gene 'C', while piperitenone synthesis is controlled by the recessive gene 'c' [27, 29–31]. The concurrent syntheses of dihydrocarvone and piperitenone might be explained by the presence of a deviating chemotype (e.g. 'Cc') amongst a majority of 'cc'-type plants. Analysis of the oil of the individual plants (still in cultivation and at our disposal) and comparison of the oil of different parts of the same plant should give a better insight into the observed phenomenon.

Finally, from a practical point of view, and as can be deduced from Table 1, the use of the headspace technique presents some interesting features including the following. First, it allows good detection of low-boiling substances which are sometimes lost during the preparation of essential oils by steam distillation, or are only present in minor concentration as compared with higher boiling, less volatile compounds. Second, it may yield indications about artifact formation during the preparation of an essential oil. It gives a better image of what is actually smelled, and last, but not least, it is fundamentally non-destructive during sampling, which allows time-course experiments over longer periods of time with the same living organism [32–34].

## EXPERIMENTAL

Seeds of *C. nepeta* subsp. *glandulosa* (nomenclature according to ref. [35]) were collected in the wild near Chaumont (France). Seedlings grown in the greenhouses of the Botanical Garden of the University of Gent (Belgium), were moved to a sheltered plot in the open, where they are permanent specimens of the living collection, under reg. no. 80/47. The essential oils were prepared by steam distillation, followed by extraction of the distillate with $CH_2Cl_2$, from plant material collected in July 1983, June and September 1984.

*Headspace sampling.* Freshly collected stalks (40 cm from the top with leaves and flowers attached; total weight 50–100 g; July 1984) were put in 15 l. desiccators, which were then flushed with air at 150 ml/min for 15 min. A Tenax GC adsorption tube (i.d. 12 mm; 1.8 g of adsorbent) was then attached to the outlet of the desiccator, and sampling was carried out for a further 5–10 min at an air rate of 150 ml/min. For GC and GC/MS, the compounds were thermally desorbed as described earlier [33, 34].

*Analytical GC and GC/MS.* These procedures were performed as described before [36] on an OV-1 glass capillary column (i.d. 0.5 mm; l. 40 m) treated with hexamethyldisilazane or bis-(trimethylsilyl)-trifluoro acetamide, giving a Kováts index for β-caryophyllene of 1413 [36]. Minor components were identified by comparison of their mass spectrum and Kováts index with those of reference substances, which were purchased, isolated from ref. [36] or identified in essential oils of known composition (the sabinene hydrates in oil of *Majorana hortensis* [37]). Germacrene-D was isolated from *Nepeta grandiflora* [H. De Pooter, unpublished], and identified by its $^1$H NMR, $^{13}$C NMR and mass spectra [38, 39].

*Prep. GC.* This was carried out on Apiezon L as in ref. [36].

*Artifact formation tests.* Plant material (30 g) collected in the first week of October 1984 and stored in the freezer until needed, was submitted to combined steam distillation–extraction [40] in a semi-micro apparatus [41] for 2 hr. The oil (36 mg; Table 1; October 1984 A) was then thoroughly mixed with the dried plant residues, and again submitted to steam distillation, yielding 34 mg of oil (Table 1; October 1984 B). A second batch of plant material was boiled with water for 3 hr, after which the oil was isolated by steam distillation–extraction as above (Table 1; October 1984 C).

*Piperitenone oxide* (**2**) [15]. MS (GC/MS) *m/z* (rel. int.): 166 $[M]^+$ (23), 41 (100), 67 (100), 68 (86), 43 (81), 39 (79), 138 (56), 53 (48), 55 (34), 69 (28), 79 (28), 109 (26). $^1$H NMR (Varian T60; $CDCl_3$): δ 1.44 (H-10, *s*, 3H), 1.80 (H-8, slightly broadened *s*, 3H), 2.08 (H-9, *br s*, 3H), 1.7–2.2 (H-5 and H-6, *m*, 4H), 3.13 (H-2, *s*, 1H). $^{13}$C NMR (Varian FT80-20MHZ; $CDCl_3$): δ 21.7 (*q*, C-10), 23.0 (*t*, C-5), 23.0 (*q*, C-8), 23.0 (*q*, C-9), 28.0 (*t*, C-6), 62.9 (*s*, C-1), 63.2 (*d*, C-2), 127.7 (*s*, C-4), 148.5 (*s*, C-7), 197.6 (*s*, C-3). IR $\nu_{max}^{neat}$ cm$^{-1}$: 1675 and 1600 (vs, α,β-unsatd ketone); 1230, 845, 765 (s, epoxide).

*Piperitone oxide* (**1**). MS (direct) *m/z* (rel. int.): 168 $[M]^+$ (6), 69 (100), 41 (96), 55 (92), 97 (70), 70 (64), 43 (60), 139 (60), 71 (48), 126 (36), 39 (30), 125 (28), 98 (22). $^1$H NMR ($CDCl_3$): δ 0.79 (H-8 or H-9, *d*, *J* = 7 Hz; 3H), 0.90 (H-8 or H-9, *d*, *J* = 7 Hz, 3H), 1.39) (H-10, *s*, 3H), 1.40–2.60 (H-4, 5, 6, 7, *m*, 6H), 3.05 (H-2, *s*, 1H). $^{13}$C NMR ($CDCl_3$): δ 16.9 (*t*, C-5), 18.2 (*q*, C-8 or C-9), 20.0 (*q*, C-8 or C-9), 21.8 (*q*, C-10), 28.5 (*t*, C-6), 28.6 (*d*, C-7), 51.9 (*d*, C-4), 61.2 (*s*, C-1), 62.2 (*d*, C-2), 207.5 (*s*, C-3). IR $\nu_{max}^{neat}$ cm$^{-1}$: 1705 (vs, ketone); 1210, 840, 780 (*s*, epoxide).

*4-Hydroxypiperitone* **5** [13]. Piperitone oxide (**1**) (110 mg) was stirred in 25 ml 0.1 M $Na_2CO_3$ at ambient temp. for 24 hr. After addition of 5 g $(NH_4)_2SO_4$, the reaction mixture was extracted with $CH_2Cl_2$ (4 × 15 ml) and the extract dried over $MgSO_4$. The solvent was distilled off and the residual oil was separated by prep. GC on Apiezon L, yielding 70 mg of **5** (60%) as a colourless oil with a musty odour. MS (direct) *m/z* (rel. int.): 168 $[M]^+$ (8), 82 (100), 43 (30), 71 (25), 125 (23), 41 (22), 39 (17), 97 (16), 126 (15), 54 (14), 140 (11). $^1$H NMR ($CDCl_3$): δ 0.69 (H-8 or H-9, *d*, *J* = 7 Hz, 3H), 0.99 (H-8 or H-9, *d*, *J* = 7 Hz, 3H), 1.93 (H-10, *br s*,

3H), 1.6–2.5 (H-5, 6, 7, *br m*, 5H), 3.62 (OH, *br s*, 1H), 5.81 (H-2, *br s*, 1H). $^{13}$C NMR ($CDCl_3$): $\delta$ 16.2 (*q*, C-8 or C-9), 16.5 (*q*, C-8 or C-9), 23.3 (*q*, C-10), 30.0 (*t*, C-5 or C-6), 30.1 (*d*, C-7), 32.3 (*t*, C-5 or C-6), 76.3 (*s*, C-4), 123.2 (*d*, C-2), 162.9 (*s*, C-1), 202.7 (*s*, C-3). IR $\nu_{max}^{Neat}$ cm$^{-1}$: 3480 (br, vs, OH); 1665, 1630 (sh) (br, vs, $\alpha,\beta$-unsatd ketone).

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