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THE ESSENTIAL OIL OF *NEPETA NUDA*. IDENTIFICATION OF A NEW NEPETALACTONE DIASTEREOISOMER

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Key Word Index—*Nepeta nuda*; Lamiaceae; essential oil; 4aβ,7α,7aα-nepetalactone.

Abstract—The essential oils of nine specimens of *N. nuda* were prepared by steam distillation, and analysed by GC and GC/MS. Thirty-seven substances were identified, accounting for 91.6–99% of the oils. They all contained varying amounts of 1,8-cineole, a mixture of nepetalactones and germacrene-D as chief components. In seven samples the new 4aβ, 7α, 7aα-nepetalactone was found. It was isolated, and identified by ^{1}H and ^{13}C NMR.

INTRODUCTION

Few of the *ca* 280 species of the large genus *Nepeta* [1] have been studied thoroughly. Some were used in folk medecine, e.g. *N. cataria* (catnip) which was recommended in the 17th century as a fortifier, a disinfectant and a cure against colds, and was cultivated in many herbal gardens. Even now most books about medicinal plants contain a paragraph about it. Extracts of *N. kopetdaghensis* were found to be active as a bacteriostatic, a diuretic and a cure against eczema-type skin disorders [2]. Alcoholic preparations of *N. hindostana* decreased the level of serum lipids and lipoproteins, and might thus supplement remedies against atherosclerosis [3].

Most interest however, was generated by the feline attractant properties of several *Nepeta* species. It was found that nepetalactones were responsible for this characteristic [4–7]. The structure and absolute configuration of nepetalactone (1) [4aα, 7α, 7aα-nepetalactone or 5,9*S*-dimethyl-3-oxa-(6*S*, 10*R*) bicyclo-(4, 3, 0)-4-nonen-2-one], which is the major component of the essential oil of *N. cataria*, and of the accompanying epimer epi-nepetalactone (2) (4aα, 7α, 7aβ-nepetalactone), were determined by degradation and partial syntheses [8–12]. In 1980 a third nepetalactone diastereoisomer (4aβ, 7α, 7aβ-nepetalactone) (3) was isolated from the essential oil of *N. mussinii*. Its structure and stereochemistry were deduced by degradation and by comparison of its ^{13}C NMR and ^{1}H NMR spectra with those of the known **1** and **2** [13].

Because of the dearth of information about essential oil compositions of *Nepeta* (only *N. nepetella* was thoroughly investigated recently [14]), and of the possibility that this knowledge might help simplify the complex, often ambiguous classification of this diversified, species-rich genus (the last complete revision dates back as far as 1848 [15]), this work was started.

RESULTS AND DISCUSSION

Plants were raised from seeds obtained from various botanical gardens in Europe. But, although many were tagged with different names, morphological analysis of the full-grown, flowering and fruiting plants showed that quite a number of individuals were either *N. nuda* L. or *N. grandiflora* M. Bieb. The positive side of this fact was, that it would allow a crude evaluation of the variation of essential oil composition within a species.

Nepeta nuda is a perennial plant with many erect subglabrous stems. Lower leaves petiolate, middle and upper leaves subsessile, 10 × 3.5 cm, densely short-haired and gland-dotted, base cordate to truncate. Inflorescence of several-many subremote, pedunculate, many-flowered verticillasters with bracteate, short, linear-subulate bracts. Flower stalks very short; calyx 4–5 mm long, ovoid when fruiting, straight, with five equal teeth reaching up to half of the tube length; corolla 8–9 mm long, pale violaceous or white. Nutlets 1.5–2.2 × 1–1.2 mm, ellipsoid, three-angled, brownish, smooth or with a few flattened tubercles, top always with at least a few short hairs.

The species is widespread from central and southeast Europe over Turkey and the southwest Soviet Union toward southwest and central Asia. Within this large geographical range, a considerable amount of variability can be observed in calyx and corolla colour, indumentum, inflorescence and calyx morphology. Attempts have been published to formalise these patterns into a nomenclatural framework, but a satisfactorily complete survey has not yet come to our attention [16–18].

The essential oils of nine plants (Tables 1 and 2) were prepared in low to moderate yield (0.03–0.3%) by steam distillation. They were analysed by GC-MS directly, and after separation into an apolar and a polar fraction by liquid chromatography (LC), and/or separation by preparative gas chromatography. Most plants produce a herbaceous, mildly menthole-like odour, which may be attributed to the overall presence of 1,8-cineole (Table 1). A second feature of all the oils is a moderate to high germacrene-D (0.2–23.0%) and nepetalactone mixture content (13.0–84.5%). Apart from the simultaneous occurrence of the three known nepetalactones **1**–**3**, the outstanding characteristic of seven oils is the presence of a fourth nepetalactone diastereoisomer, eluting before the

Table 1. Composition (in %) of the essential oil of different *N. nuda* specimens

Component	Kováts index on OV-1	Plant 1	2	3	4	5	6	7	8	9
α-Pinene	929	tr	tr	—	tr	0.2	0.2	0.5	0.7	tr
Camphene	940	—	—	—	—	—	—	—	tr	—
Sabinene	964	0.2	0.1	—	—	0.1	0.4	0.8	1.3	tr
β-Pinene	968	0.5	0.5	0.1	0.2	0.7	1.6	2.1	2.9	0.5
3-Octanone	973	0.2	0.1	0.3	—	tr	tr	tr	tr	0.1
Myrcene	983	0.1	0.1	tr	0.2	tr	0.2	0.3	0.7	tr
p Cymene	1010	—	0.1	—	—	tr	tr	tr	—	—
1,8-Cineole (+ Limonene)	1018	11.2	10.1	1.8	3.4	4.3	22.9	38.0	37.4	6.7
Cis-β-ocimene	1027	—	tr	—	—	—	tr	tr	0.7	tr
Trans-β-ocimene	1037	tr	0.2	tr	—	tr	0.1	0.7	3.8	0.3
γ-Terpinene	1045	—	—	—	—	—	tr	tr	—	—
Cis-sabinene hydrate	1053	0.1	tr	—	—	tr	0.2	0.7	—	—
Linalol	1085	—	tr	—	—	tr	tr	tr	—	—
Trans-sabinene hydrate	1096	0.2	0.1	tr	—	tr	tr	0.2	—	—
δ-Terpineol	1147	0.4	0.2	tr	—	tr	0.7	0.5	0.5	tr
Terpinen-4-ol	1161	tr	0.3	—	—	tr	0.1	0.4	0.2	tr
2-Methyl-2,3-dihydroindole(?)* α-Terpineol	1169 1171	1.0	0.5	tr	0.6	0.3	5.3	1.0	1.2	tr
4aβ, 7α, 7aα-Nepetalactone (**4**)	1314	18.4	—	3.9	0.7	tr	3.4	—	0.9	tr
4aα, 7α, 7aα-Nepetalactone (**1**)	1322	6.0	4.6	4.8	2.8	3.6	6.0	1.5	0.9	7.1
4aα, 7α, 7aβ-Nepetalactone (**2**)	1351	36.6	60.3	74.4	22.6	76.6	26.5	18.9	6.7	70.9
4aβ, 7α, 7aβ-Nepetalactone (**3**)	1355	1.7	2.1	1.4	54.8	1.2	3.2	1.6	4.5	3.5
α-Copaene	1372	0.6	0.6	0.4	2.5	1.8	0.4	0.9	0.4	0.5
β-Bourbonene	1380	4.5	1.4	1.6	0.8	0.7	0.7	1.8	2.5	2.7
β-Elemene	1385	0.9	0.7	0.2	tr	0.2	0.2	0.4	0.3	0.1
*n*Tetradecane	1400	—	tr	tr	—	tr	0.1	tr	tr	—
β-Caryophyllene	1414	0.8	0.6	2.7	1.3	2.4	2.4	0.4	1.8	2.2
γ-Muurolene (?)*	1424	0.7	0.5	0.2	tr	0.1	0.2	0.2	0.3	0.3
Unidentified sesquiterpene	1437	0.6	0.2	0.1	tr	tr	tr	tr	tr	0.2
β-Farnesene	1446	0.4	1.0	0.6	0.7	1.2	0.7	2.4	1.4	0.4
Alloaromadendrene (?)*	1455	0.3	—	tr	—	tr	0.2	—	0.2	0.2
Dehydronepetalactone	1460	0.2	0.2	tr	—	tr	tr	—	tr	tr
Germacrene-D	1472	4.9	10.0	4.6	0.4	0.2	13.5	17.2	23.0	1.7
α-Elemene	1486	—	—	—	—	—	0.6	tr	0.9	—
α-Farnesene	1494	—	0.3	tr	tr	tr	0.2	tr	0.2	—
β-Bisabolene	1496	0.4	0.9	tr	tr	0.6	tr	1.6	0.3	—
δ-Cadinene	1512	0.3	0.8	0.1	1.0	0.7	0.3	1.7	0.9	0.1
Caryophyllene oxide	1565	0.4	—	0.6	6.4	1.9	2.1	tr	2.4	1.5

*(?) = tentative, based solely on the mass spectrum; tr = $< 0.05\%$; — = $< 0.02\%$

Table 2. Origin and registry numbers of the *N. nuda* plants used for the preparation of the essential oils of Table 1

Plant	Origin	Hort. Bot. Univ. Gandavensis	Collection P. Goetghebeur
1	Cult. H. B. Giessen	83–1166	5406
2	Cult. H. B. Krefeld	83– 639	5403
3	Wild (Armenia, USSR)	*83–1071	5413
4	Wild (Armenia, USSR)	*83–1075	5409
5	Cult. H. B. Berlin	85–1033	5517
6	Cult. H. B. Essen	80–5575	5430
7	Cult. H. B. Berlin	83– 941	5405
8	Cult. H. B. Giessen	83–1165	5414
9	Wild (Armenia, USSR)	*83–1071	5412

others from the GC columns (Ip on OV-1 1314). The substance was isolated from the essential oil of plant 1 (Tables 1 and 2) by LC on florisil using *n*-pentane and dichloromethane as eluants, followed by repeated preparative GC of the dichloromethane fraction on Apiezon L. The mass spectrum was almost identical with those of **1**–**3** and this suggested structure **4** (4aβ, 7α, 7aα-nepetalactone). Indeed, in all as yet identified nepetalactones and related substances, C-7 has the *S*-conformation [9, 12, 13], so that only four diastereoisomers are possible, and **4** would thus be the remaining isomer, with a *trans*-ring junction.

The proposed structure was confirmed by the NMR spectra. In the ^{13}C NMR spectrum of **4** (Table 3), the chemical shift of δ20.4 for C-9 is essentially the same as in nepetalactone **1**, indicating a *trans*-position of C-9 to the carbonyl. A *trans*-position of the C-3/C-4 double bond to the carbonyl on the other hand results in a high value of δ120.6 for C-4 (as in nepetalactone **2**). In the ^{1}H NMR spectrum, the *trans*-position of the carbonyl to the C-9 methyl group, and to the C-3/C-4 double bond leads to relatively high δ-values for the C-9 and C-8 methyl groups and for the olefinic proton, thus combining features of **1** and **2** (Table 4).

On analysis of the essential oils on home-made OV-1 columns [19], a fair separation of the four nepetalactones was obtained. However, the GC-traces always showed an ungainly, broad, asymmetric peak between **1** (Ip 1322) and **2** (Ip 1351) (Fig. 1A), the intensity and shape of which could change during the normal life span of the columns. By careful and continuous monitoring of the mass spectra over the whole range of the peak, only nepetalactone signals were detected, which ruled out decomposition. Using an SE-30 coated fused silica capillary column, no broad peak was present, but **2** and **3** coalesced, precluding quantitation. On a column coated with a mixed stationary phase (OV-1 + CW 20M; 1:1), the broadening was reduced, but in this case **2** and **3** overlapped the peaks of β-bourbonene and β-elemene. FFAP columns gave well-resolved signals for the nepetalactones, but broad peaks for the terpenes (Fig. 1B). Combination of the analyses on the OV-1 and FFAP columns were then used for quantitation. The origin of the supplementary peak is uncertain but may be the result of a partial isomerisation of **2** into **1** during GC. In fact during preparative GC on a packed Apiezon L column, repeated injections of the nepetalactone fraction of an essential oil rich in **2** (Table 1, plant 2) led to a steady increase of **1** at the cost of **2**. Similar phenomena were not observed with nepetalactones **1**, **3** and **4**. Sakan *et al.* [20] described the isomerisation of **2** into **1** by boiling the substance in xylene in the presence of potassium carbonate. Moreover, when analysing *N. cataria* essential oil on an Apiezon L column, Regnier *et al.* [21] observed the occurrence of a nepetalactone peak between **1** and **2**, which they tentatively identified as neonepetalactone. However, neonepetalactone elutes after β-farnesene (Ip 1446) on apolar columns [14], which could mean that the tentatively identified substance was in fact the isomerisation product.

Concerning the use of the essential oil composition for chemotaxonomic purposes, drawing any conclusion would be premature when one notes the varying nepeta-

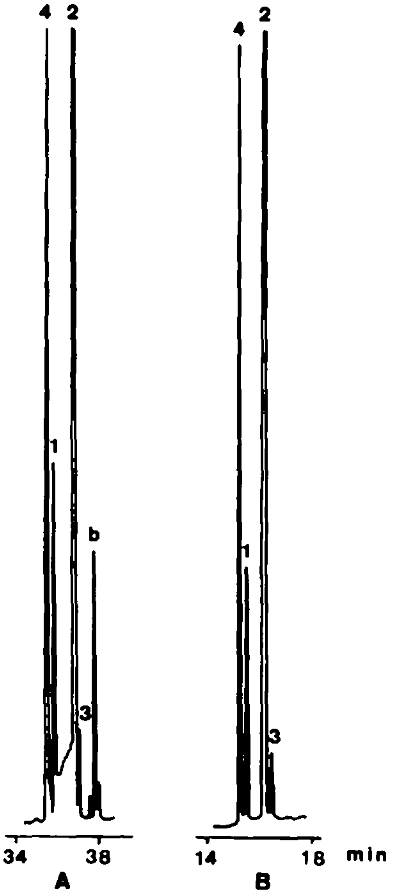
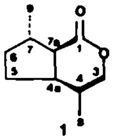


Fig. 1. GC traces of the essential oil of *N. nuda* plant 1. The nepetalactone segment using different capillary columns. A = glass, OV-1; B = fused silica, FFAP. 1 = 4aα, 7α, 7aα-; 2 = 4aα, 7α, 7aβ-; 3 = 4aβ, 7α, 7aβ-; 4 = 4aβ, 7α, 7aα-nepetalactone; b = β-bourbonene.

Table 3. ^{13}C NMR Chemical shifts (ppm) of Nepetalactones 1–4 [cf. 13]*

Carbon	Multiplicity	1	2	3	4
1	*s*	170.5	169.9	169.8	171.4
3	*d*	133.4	135.7	134.0	136.3
4	*s*	115.1	120.4	115.4	120.6
4a	*d*	(40.7)	(37.3)	(39.4)	(41.9)
5	*t*	(30.9)	(26.1)	(30.4)	(25.7)
6	*t*	(33.0)	(30.0)	(32.7)	(31.7)
7	*d*	(39.7)	(32.1)	(38.3)	(32.5)
7a	*d*	49.3	49.0	46.2	52.5
8	*q*	15.4	14.3	14.7	13.9
9	*q*	20.3	17.6	17.2	20.4

*Values in parentheses indicate tentative assignments, because interconversion between 4a and 7, and between 5 and 6 is possible.



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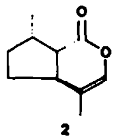

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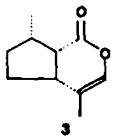

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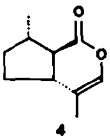

4

Table 4. Representative ^{1}H NMR chemical shifts (ppm) of Nepetalactones **1**–**4** [cf. 13]

Proton	**1**	**2**	**3**	**4**
H-3 (olefin)	6.15 (*m*)	6.23 (*m*)	6.18 (*m*)	6.27 (*m*)
H-8 (allylic CH_3)	1.64 (*m*)	1.71 (*m*)	1.59 (*m*)	1.69 (*m*)
H-9 (CH_3)	1.19 (*d*; $^3J = 6$Hz)	1.11 (*d*; $^3J = 6$Hz)	0.99 (*d*; $^3J = 7$Hz)	1.18 (*d*; $^3J = 5.5$Hz)

lactone content and the variation of the content of the individual nepetalactones in the different oils (e.g. plant 4, high in **3**).

EXPERIMENTAL

Seeds of *N. nuda* were obtained from the Botanical Gardens of Giessen, Krefeld, Berlin, Essen (FRG) and Yerevan (USSR). Seedlings grown in the greenhouses of the Botanical Garden of the University of Gent (Belgium) were moved to a sheltered plot in the open. Voucher specimens were deposited in the herbarium GENT (Laboratory of Plant Systematics, State University of Gent, Belgium) (Table 2); duplicates are available for exchange.

Isolation of the essential oils. These were prepared from air-dried flowering plant material by treatment with steam (generated in a separate boiler) for 3 hr, followed by exhaustive extraction of the steam distillate with CH_2Cl_2.

Analytical GC. This was performed as described before on an OV-1 glass capillary column (40 m × 0.5 mm) [19, 22], an SE-30 fused silica column (30 m × 0.53 mm) and a FFAP fused silica column (15 m × 0.53 mm). The mixed OV-1/CW 20M phase (1:1) was applied by the static method [23] on a glass capillary column pre-treated by the $BaCO_3$ procedure [24] (20 m × 0.5 mm).

GC/MS analyses. These were obtained with a Varian MAT 112 instrument as before [19, 22] and with an HP 5970A mass selective detector, coupled to an HP 5890 gas chromatograph fitted with an SE-30 fused silica capillary column (30 m × 0.53 mm). Substances were identified by comparison of their mass spectra and Kovâts indices [25] with those of reference substances which were purchased, isolated from or identified in essential oils of known composition (δ-terpineol in *Rosmarinus officinalis* essential oil [26]).

4aβ, 7α, 7aα-nepetalactone. Mass spectrum (GC-MS, HP 5970A, 70 eV) m/z (rel. int.): 166$[M]^+$ (56), 41 (100), 69 (91), 81 (87), 95 (80), 123 (80), 67 (62), 109 (52), 55 (50), 80 (45), 53 (40).

Preparative GC. This was performed with a Varian 1420 instrument equipped with a catharometer and a 1 m × 2.5 mm glass column packed with 15% Apiezon L on Chromosorb W-AW. Operating conditions were as before [19].

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