INTRODUCTION

The essential oil of *C. nepeta* (L.) Savi ssp *glandulosa* (Req.) P. W. Ball grown from seeds in the Botanical Garden of the State University of Gent (Belgium) is particularly rich in the oxides of piperitone and piperitenone, and is free of pulegone, menthones and menthols [1]. *C. nepeta* (L.) Savi ssp. *nepeta* on the other hand mainly contains the latter substances [2, 3]. Because the morphological characteristics of these subspecies as described in *Flora Europaea* tend to overlap [4], it appeared possible that the composition of the essential oils might be used to separate them. However, it is clear that before drawing any such conclusions, more specimens, preferably collected in the wild, had to be studied.

RESULTS AND DISCUSSION

The first indication that the composition of the essential oil of *C. nepeta* ssp. *glandulosa* prepared from plants raised in our Botanical Garden [1] might be an extreme case, was gleaned when a new specimen, grown from the original supply of fruits, afforded a more complex oil, containing the menthones, pulegone and the oxides of piperitone and piperitenone (Table 1A).Further plant material was then collected in the wild. Referring to the earlier findings [1-3], GC analysis gave rather unexpected results: six samples, identified as *C. nepeta* ssp. *nepeta* yielded oils which not only contained pulegone, the menthones and menthols, but were also rich in the oxides of piperitone and piperitenone (Table 1C-H). The essential oil of the last sample (Table 1B), identified as *C. nepeta* ssp. *glandulosa*, has a composition which, save for the presence of some piperitone and piperitenone oxides, is identical to the published composition of *C. nepeta* ssp. *nepeta* [2, 3]. This shows quite clearly that the same oils can be produced by both subspecies, and this rules out their use for chemotaxonomic purposes.

EXPERIMENTAL

Essential oils were prepared by hydrodistillation in a semi-micro Likens-Nickerson apparatus, and analysed by GC and GC-MS on WCOT and FSOT SE-52 columns in circumstances as described before [1]. Plant material was collected in Tanneron (Alpes Maritimes, France; sample B), near Le Lauzet-Ubaye (samples C-F), the Réservoir de Serre-Ponçon (Les Demoiselles Coiffées; sample G) and La Fresquière (sample H) (Alpes de Provence, France). Voucher specimens were deposited in the herbarium Gent: A = PG5463; PGB = 5616; C-F = PG 5609-5612; G = PG 5608; H = PG 5607.

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CARYOPHYLLENE DERIVATIVES FROM *PULICARIA ARABICA*

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Key Word Index—*Pulicaria arabica*; Compositae; sesquiterpenes; caryophyllene derivatives.

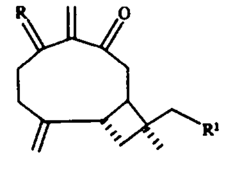
Abstract—The aerial parts of *Pulicaria arabica* afforded in addition to known caryophyllene derivatives seven new ones. The configuration at C-11 of previously reported derivatives has to be corrected as followed from the observed NOEs.

INTRODUCTION

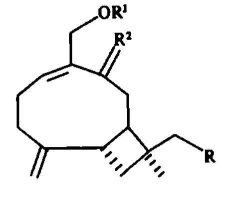
The relatively large genus *Pulicaria* (Compositae, tribe Inuleae, subtribe Inulinae) has been studied by several groups. In addition to widespread compounds two species gave diterpenes [1, 2], two others unusual caryophyllene derivatives [3, 4] and one species, which has been placed in the *Francoeuria*, afforded different types of sesquiterpene lactones [5]. We now have reinvestigated *P. arabica* (L.) Cass.; the results are discussed in this paper.

RESULTS AND DISCUSSION

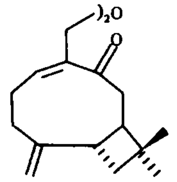
Pulicaria arabica has been investigated previously and from the aerial parts several flavones and flavone glyco-



	1	**2**	**3**	**4**	**5**
R	β-OH,H	β-OMe,H	β-OMe,H	α-OMe,H	α-OH,H
R¹	OH	OH	OAc	OAc	OH



	6	**7**	**8**	**9**	**10**	**11**	**12**
R	H	H	OH	OAc	OAc	OAc	OAc
R¹	H	Ac	Ac	H	Ac	H	Me
R²	O	O	O	O	O	α-OH,H	O
						Δ^5E	Δ^5E



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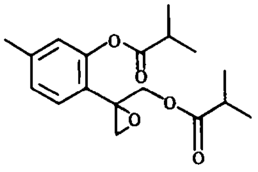

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sides and reported [6]. Our investigation gave the thymol derivative **14**, also present in other *Pulicaria* species [3, 4] and 13 caryophyllene derivatives, the known ketones **1** and **6–10** [3, 4] as well as seven new ones, the $\Delta^{6(14)}$-derivatives **2–4**, the corresponding dihydro derivative **5**, the Δ^5-derivatives **11** and **12** and the dimeric compound **13**.

Comparison of the ¹H NMR spectrum of **2** (Table 1) with that of **1** [3] clearly showed that the corresponding 5-*O*-methyl ether was present. In the spectrum of **3** (Table 1) only the methylene doublets of the oxygen bearing carbon were shifted down field and an acetoxy singlet was present, therefore, the structure was also clear. However, NOE difference spectroscopy clearly indicated that the configuration at C-11 has to be reversed. Clear effects were observed between H-13, H-9 (10%) and H-10α (8%). H-5 gave a NOE with OMe (10%), but not with H-9, which is an indication that a β-methoxy group was present. This also followed from the coupling if models were inspected. Furthermore the ¹H NMR data of **4** (Table 1) clearly indicated that this methyl ether was the 5α-epimer of **3**. Accordingly, the couplings of H-5 were altered and now clear NOEs were observed between H-5, H-1 (7%), H-8β (4%) and OMe (5%), between H-12, H-1 (8%), H-8β (5%), H-10β (4%) and H-13 (5%) as well as between H-13, H-9 (10%) and H-10α (5%).

Inspection of the ¹H NMR spectrum of **5** (Table 1) showed that this ketone was a 6,14-dihydro derivative. Accordingly, a methyl doublet at δ1.14 had replaced the exomethylene signals of H-14. The observed NOEs between H-12, H-1 (7%) and H-8β (10%), between H-6 and H-5 (10%), between H-13 and H-10β (5%) as well as between H-1 and H-5 (10%) established the configurations at C-5, C-6 and C-11.

The ¹H NMR spectrum of **11** (Table 1) again showed that a diol was present. However, a low field broadened double doublet at δ5.52 indicated a Δ^5 double bond and an acetoxy singlet together with a pair of doublets a 11-acetoxy methylene group. Spin decoupling allowed the assignment of nearly all signals. The resulting sequences required the proposed structure and the NOEs indicated a *cis*-relationship between H-7 and H-1, between H-5 and H-14 as well as between H-13 and H-9. The ¹H NMR spectrum of **12** (Table 1) was in part close to that of **11**. However, the absence of a H-7 signal and the down field shift of H-5 indicated a conjugated Δ^5-ketone. The E-configuration followed from the chemical shift of H-5. A reinvestigation of the configuration at C-11 by NOE difference spectroscopy in the ketones **8–10** showed that all have the same one. Thus, the preliminary proposed stereochemistry at C-11 has to be reversed (compounds **1–3**, **5–7**, **12** and **13** in lit. [3]). Typical for caryophyllenes with an oxygen function at C-12 or C-13 are the chemical shifts of the methylene protons which are always at higher fields in 13-hydroxy derivatives. This was observed also in the isomeric 12- and 13-hydroxycaryophyllenepoxides [7] prepared by microbiological transformation of caryophyllene.

The ¹H NMR spectrum of **13** (Table 1) was nearly identical with that of **6**. However, no $[M]^+$ could be detected in the EI mass spectrum. The highest fragments were at *m/z* 233 and 217. CI mass spectrometry gave a clear $[M+1]^+$ peak at *m/z* 451. Accordingly, **13** was the corresponding ether formed from two molecules of **6**.

EXPERIMENTAL

Air-dried aerial parts (300 g, voucher deposited in the Herbarium of the University of Zagazig) were extd with Et_2O–MeOH–petrol (1:1:1). The ext. obtained was treated with MeOH to remove long chain saturated compounds affording 9 g of sol. material. CC (silica gel) gave four fractions (1: Et_2O–petrol (1:3), 2: Et_2O–petrol (1:1), 3: Et_2O, 4: Et_2O–MeOH (9:1). TLC of fraction 1 (silica gel, PF 254, Et_2O–petrol, 1:20) gave thymol (12 mg), thymolisobutyrate (10 mg), stigmasterol (12 mg), sitosterol (30 mg) and dammradienyl acetate (14 mg). TLC of fraction 2 (Et_2O–petrol, 1:1) gave **14** (19 mg) and **7** (9 mg). TLC of fraction 3 (Et_2O–petrol, 1:1) afforded **6** (15 mg) and TLC of fraction 4 (Et_2O) gave **13** (4 mg), **11** (2 mg), **5** (2 mg), **1** (5 mg), **8** (5 mg) **10** (3 mg) and two crude fractions (A and B). Fraction A gave by HPLC (RP 8, *ca* 100 bar) (MeOH–H_2O 13:7), **9** (2 mg) (R_t 7.5 min), **2** (3 mg) (R_t 8.5 min.), **4** (9 mg), (R_t 13 min) and **3** (8 mg) (R_t 14.5 min) and fraction B (MeOH–H_2O, 7:3) **12** (2 mg) (R_t 10 min). Known compounds were identified by comparing their 400 MHz ¹H NMR spectra with those of authentic material.

12-Hydroxy-5β-methoxy-6(14)-dehydro-5,6-dihydrocaryophyllen-7-one (**2**). Colourless oil: IR $\nu_{max}^{CCl_4}$, cm⁻¹: 3600, 1690; MS: *m/z* (rel. int.): 264.173 $[M]^+$ (4) (calc. for $C_{16}H_{24}O_3$: 264.173), 249 (4), 235 (8), 147 (62), 119 (78), 93 (83), 91 (100); $[\alpha]_D^{24°}$ − 9 ($CHCl_3$; *c* 0.3).

12-Acetoxy-5β-methoxy-6(14)-dehydro-5,6-dihydrocaryophyllene-7-one (**3**). Colourless oil; IR $\nu_{max}^{CCl_4}$, cm⁻¹: 1745, 1690; MS *m/z* (rel. int.): 306.183 $[M]^+$ (21) (calc. for $C_{18}H_{28}O_4$: 306.183), 277 (18), 247 (10), 246 (7), 147 (77), 119 (94), 93 (78), 91 (100); $[\alpha]_D^{24°}$ − 90 ($CHCl_3$; *c* 0.2).

Table 1. ^{1}H NMR spectral data of **2**–**5** and **11**–**13** ($CDCl_3$, 400 MHz, δ-values)

H	**2**	**3**	**4**	**5***	**11***	**12**	**13**
1	2.73 *br ddd*	2.71 *br ddd*	2.74 *m*	2.95 *m*	2.43 *br ddd*	2.48 *br ddd*	2.38 *br ddd*
5	4.19 *br dd*	4.18 *br dd*	4.34 *br dd*	4.42 *br s*	5.52 *br dd*	6.45 *br dd*	5.89 *br dd*
8	3.35 *dd*	3.14 *dd*	2.69 *dd*	2.99 *dd*	2.04 *m*	2.97 *dd*	2.91 *dd*
8'	2.52 *dd*	2.46 *dd*	2.77 *dd*	2.43 *dd*	1.67 *m*	2.52 *br d*	2.32 *br d*
9	2.00 *m*	1.98 *m*	2.15 *m*	2.05 *m*	1.74 *m*	1.86 *br dd*	1.73 *br dd*
10	2.04 *dd*	2.04 *dd*	2.10 *dd*	2.14 *dd*	1.86 *dd*	2.01 *dd*	1.89 *dd*
10'	1.78 *dd*	1.82 *dd*	1.75 *dd*	1.73 *dd*	1.51 *dd*	1.57 *dd*	1.57 *dd*
	3.69 *dd*	4.15 *d*		3.68 *dd*	4.14 *d*	4.10 *d*	
12	3.62 *dd*	4.03 *d*	4.10 *s*	3.63 *dd*	4.04 *d*	4.06 *d*	1.04 *s*
13	1.09 *s*	1.13 *s*	1.15 *s*	1.09 *s*	1.10 *s*	1.12 *s*	1.04 *s*
14	5.95 *br s*	5.89 *br s*	5.96 *br s*	1.14 *d*	4.33 *br d*	4.01 *br d*	4.24 *br d*
14'	5.81 *br s*	5.81 *br s*	5.68 *br s*		4.17 *br d*	3.95 *d*	4.10 *d*
15	4.83 *brs*	4.83 *br s*	4.84 *br s*	4.77 *br s*	4.98 *br s*	5.03 *br s*	5.04 *br s*
15'	4.73 *br s*	4.73 *br s*	4.71 *br s*	4.61 *br s*	4.84 *br s*	4.97 *br s*	4.95 *br s*
OR	1.48 *t* (OH)	2.08 *s*	2.10 *s*	1.47 *t* (OH)	2.09 *s*	2.09 *s*	
	3.29 *s* (OMe)	3.29 *s*	3.30 *s*			3.28 *s*	

*H-6 2.92 *dq*; H-7 4.23 *dd*. J[Hz]: 1,9 – 1, 10 = 1, 10′ ≈ 9; 10,10′ = 11; compounds **2** and **3**: 4,5 = 4′,5 = 8; 8,8′ = 10.5; 8,9 = 12; 8′,9 = 5; 12,12′ = 11.5; 12 OH = 5; compound **4**: 4,5, = 4; 4,5′ = 11; 8,8′ = 8′9 = 12; 8,9 = 4.5; compound **5**: 5,6 = 2.5; 6,14 = 7; 8,8′ = 11; 8,9 = 12.5; 8′,9 = 3; 12,12′ = 11.5; 12, OH = 5; compound **11**: 4,5 = 6; 4′,5 = 10.5; 7,8 = 6; 7,8′ = 10; 12,12′ = 11; 14,14′ = 12; compounds **12** and **13**: 4,5 = 4; 4′,5 = 12; 8,8′ = 15; 8,9 = 12; 14,14′ = 12; compound **13**: 12,12′ = 11.5.

12-*Acetoxy-5α-methoxy-6(14)-dehydro-5,6-dihydrocaryophyllen-7-one* (**4**). Colourless oil; IR $v_{max}^{CCl_4}$, cm^{-1}: 1750 (OAc), 1690 (C=CC=O); MS *m/z* (rel. int.): 306.183 $[M]^+$ (28) (calc. for $C_{18}H_{26}O_4$: 306.183), 277 (25), 247 (12), 246 (5), 147 (66), 119 (80), 93 (78), 91 (100); $[\alpha]_D^{24°}$ + 39 ($CHCl_3$; *c* 0.2).

5α,12-*Dihydroxy-5,6-dihydrocaryophyllen-7-one* (**5**). Colourless oil; IR $v_{max}^{CCl_4}$, cm^{-1}: 3600 (OH), 1710 (C=O); MS *m/z* (rel. int.): 252 $[M]^+$ (0.8), 234 (2), 93 (58), 57 (100).

12-*Acetoxy-7α,14-dihydroxy-5E-caryophyllene* (**11**). Colourless oil; IR $v_{max}^{CCl_4}$, cm^{-1} 3600, 1750; MS *m/z* (rel. int.): 276 $[M - H_2O]^+$ (1), 234 $[M - HOAc]^+$ (2.6), 221 (2), 105 (64), 93 (100), 91 (90); $[\alpha]_D^{24°}$ – 10 ($CHCl_3$; *c* 0.2).

12-*Acetoxy-14-methoxy-5E-caryophyllen-7-one* (**12**). Colourless oil; IR $v_{max}^{CCl_4}$, cm^{-1}: 1750, 1690; MS *m/z* (rel. int.): 306 $[M]^+$ (11), 246 (7), 147 (63), 93 (100); $[\alpha]_D^{24°}$ – 90 ($CHCl_3$; *c*0.2).

Bis-[5Z-7-oxo-caryophyllene]-14-O-ether (**13**). Colourless oil; IR $v_{max}^{CCl_4}$, cm^{-1}: 1700; MS *m/z* (rel. int.): 233 $[C_{15}H_{21}O_2]^-$ (8), 217 $[C_{15}H_{21}O]^+$ (10), 205 (8), 189 (10), 147 (44), 119 (36), 93 (84), 69 (100).

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