Journal of Chromatographic Science, Vol. 42, May/June 2004

β-Eudesmol, a New Sesquiterpene Component in Intact and Organized Root of Chamomile (*Chamomilla recutita*)

Éva Szöke[1,*], Emoke Máday[1], Jonathan Gershenzon[2], John L. Allen[2], and **Éva Lemberkovics[1]**

[1]Semmelweis University, Department of Pharmacognosy, Üllöi út 26, H-1085 Budapest, Hungary and [2] Max Planck Institute of Chemical Ecology, Tatzendpromenade 1a, D-07745 Jena, Germany

Abstract

Gas chromatography (GC) and GC–mass spectrometry are used to identify a new sesqiterpene, β-eudesmol, which seems to be a characteristic essential oil component of the intact and in vitro organized root of chamomile [*Chamomilla recutita* (L.) Rauschert]. It is identified on three types of stationary phases by GC. The confirmation of identity is carried out by comparison of mass spectra with those reported in the literature and measured from a reference compound. The percentage evaluation of the oil component is made by area normalization, on the basis of three parallel measurements. Among the cultivated and wild chamomile species examined, the wild species from the areas of Szeghalom contain the highest quantity of β-eudesmol (9.25% in the total essential oil).

Introduction

Several groups of chamomile compounds have been identified and shown to be of medical importance. It has been previously examined that the production of terpenoids and polyins in chamomile tissue cultures (1). For analysis, gas chromatography (GC) and GC–mass spectrometry (MS) methods were used. High-performance liquid chromatography (HPLC) and LC–MS methods are not so fashionable for investigations of volatile sesquiterpenes because the retention times are too long and, therefore, special HPLC conditions have to be used (2).

β-Eudesmol has been previously identified in the essential oil of propolis (3). It was separated and detected on packed columns: (*i*) 3% OV-17 Gas Chrom Q (3-m × 2.3-mm i.d.) and (*ii*) 1.5% Sp 2250 + 0.95% Sp1401 Supelcoport (3-m × 3.4-mm i.d.); carrier gas, N_2 (30 mL/min); column temperature, 60–230°C (8°C/min); and detector, flame ionization detector (FID). Its structure was confirmed by GC–MS. It must be stated that on both stationary phases, β-eudesmol eluted with the same retention time as (–)-α-bisabolol, but this did not disturb the detection of β-eudesmol because it was not present in the propolis samples.

Gong (4) identified β-eudesmol in the essential oil of *Cinnamomi cortex*, also on an OV-17 stationary phase, but a capillary column (30-m × 0.25-mm i.d.) was used. The column temperature was programmed from 60°C to 270°C at the rate 15°C/min.

Four eudesmol isomers were identified by GC and GC–MS in *Helichrysum doerfleri* (Asteraceae): 10-epi-γ-eudesmol, α-eudesmol, β-eudesmol, and γ-eudesmol. The GC analyses were performed on the capillary column DB-5 (30 m × 0.32 mm). The initial temperature of the column was 60°C, and it was then heated to 280°C with a 3°C/min rate. Mass spectra were obtained from a GC–MS system operating on electron impact (EI) mode (HP 5MS 30-m × 0.25-mm × 0.25-µm film thickness capillary column). The thermal program was the same as that used for the GC analyses (5).

β-Eudesmol occurs as a main component in the rhizome of the Japanese *Atractylodes lancea* (6). GC analysis was carried out using a DB-WAX column (30-m × 0.25-mm i.d., 0.25-µm film thickness), the temperatures of the injector and FID detector were held constant at 240°C and 250°C, respectively. The column temperature was programmed from 120°C (1 min) to 240°C (5 min) at 5°C/min. In studying the composition of essential oil of this plant, others (7) have used the following GC parameters: capillary column, DB-17 (30-m × 0.53-mm i.d., 1-µm film thickness); carrier gas, He (1.5 mL/min); column temperature, 50°C (1 min); 50–170°C (20°C/min), 170–190°C (3°C/min), 190–230°C (8°C/min), 230–250°C (10°C/min), and 250°C (2 min); and detector, FID.

For pharmacological effects of β-eudesmol—a major com-

* Author to whom correspondence should be addressed: email szokee@drog.sote.hu.

ponent of *A. lanceae* rhizoma—the authors (8) have investigated and proved the inhibition of sodium–potassium–ATPase activity because of the interaction with the enzyme in the sodium E_1 state. In addition, the extract of the rhizome and root was also used for treatment of urinary and colon diseases (9). Miyazawa (10) established that (+)β-eudesmol possessed antimutagenic activity, similar to paenol from *Dioscorea japonica*. Its potentiating effect on succinylcholine-induced neuromuscular blockade was proven by animal tests (11).

Experimental

Plant materials

Chamomilla recutita (L.) Rauschert (syn.: *Matricaria recutita* L., *Matricaria chamomilla* L.; Asteraceae) was used. Intact (grown in soil) cultivated Degumil and BK-2 chamomile types were obtained from the field of Kerepes (Hungary), and wild populations were obtained from the areas of Szeghalom, Hortobágy, and Szabadkígyós (Hungary) at the end of June, at the time of flowering.

In vitro cultures

Organized plants were grown from sterile seeds of the Degumil and Szabadkígyós types. The seeds were first immersed for 1 min in 70% ethanol and then sterilized with a diocide solution (ethanol–mercury chloride and methylpyridine–chloride, 1:100) 3 times each for 6 min (12). Afterwards the seeds were rinsed three times in sterile distilled water. After two weeks, the plantlets were transplanted to Murashige-Skoog hormone-free media with 3% sucrose (13). The juvenile plants were subcultured every 6–8 weeks on solid MS media under light (2500 Lux, 16 h) at 26°C.

Table I. GC Parameters

GC	GC 8000 (Fisons, Milan, Italy)
Capillary column	30-m × 0.32-mm i.d.
Stationary phases	DB-1701 and β-DEXm, 0.25-µm film thickness
Oven temperature	programmed from 60°C to 230°C, at 8°C/min
Detector	flame ionization, 240°C
Carrier gas	nitrogen
Inlet pressure and flow rate	0.05 MPa, 6.8 mL/min
Injector temperature	200°C
Injection mode	splitless for 10 s, then split ratio 1:10
Injector volume	0.4 µL of a 1:1000 dilution of the oil in chloroform
Evaluation	Chrom-Card computer program (Thermo Electro)

Chemicals

The standards originated from Fluka Chemie AG (Buchs, Switzerland) and Carl Roth GmbH (Karlsruhe, Germany) firms (purum; 97–99%, GC). The β-eudesmol standard was sent and structure identified by Professor Hisayuki Kanamori (Physics

Table II. GC–MS Parameters of System A

GC	Hewlett-Packard model 6890 (Palo Alto, CA)
Capillary column	30-m × 0.25-mm i.d.
Stationary phase	DB-WAX polyethylene glycol ester; 0.25-µm film thickness (J&W Scientific, Milwaukee, WI)
Column temperature program	isothermal at 40°C for 3 min, then programmed in following steps: 40–45°C at 1.5°C/min 45–80°C at 3°C/min 80–180°C at 5°C/min 180–240°C at 10°C/min
Carrier gas	helium
Inlet pressure and flow rate	0.20 MPa, 1.0 mL/min
Injector temperature	220°C
Mode of operation	splitless
Transfer line temperature	250°C
MS	HP Model 5973 mass-selective detector
MS source temperature	230°C
Quadrupole temperature	150°C
Mode of operation	electron impact mode; electron energy: 70 eV;
Scanning range	10–400 *m/z*

Table III. GC–MS Parameters of System B

GC	Finnigan GCQ (San Jose, CA)
Capillary column	30-m × 0.22-mm i.d.
Stationary phase	BPX-5 (5% phenyl methyl silicone); 0.25-µm film thickness
Column temperature program	Programmed from 60°C to 230°C at 8°C/min
Carrier gas	helium
Inlet pressure and flow rate	0.20 MPa, 1 mL/min
Injector temperature	200°C
Mode of operation	splitless for 6 s, then split 1:10
MS	Finnigan MS
Start	3 min after sample injection
Mode of operation	electron impact mode; electron energy: 70 eV
Scanning range	40–650 *m/z*
Evaluation	Finnigan GCQ 2.0 computer program

and Chemistry Division, Hiroshima Prefectural Institute of Public Health and Environment, Hiroshima, Japan). The standard and investigation samples were stored in cooling apparatus at + 5°C.

Organic solvents (*n*-hexane, chloroform) were of analytical quality and supplied by Reanal (Budapest, Hungary). Carbon dioxide (95–96%, w/w, pure) was purchased from Messer Griesheim Hungaria (Miskolc, Hungary).

Preparation of the essential oil samples

The essential oil of the roots was extracted in a steam-distilling apparatus according to the Pharmacopoea Hungarica (Ph.Hg.VII.) and the oil content was measured gravimetrically (14). The essential oil compounds were identified by GC and MS, procedures which were carried out by validated conditions.

GC parameters

The GC analysis were carried out on a FISONS GC 8000 (Fisons, Milan, Italy). The conditions are listed in Table I. For the evaluation Chrom-Card computer program was used (Thermo Electro, Milan, Italy).

For the identification of each component we used authentic reference compounds and essential oils of known composition. The concentration of the oil components was established by area normalization, on the basis of three parallel measurements. The deviation from the average was (±) 6–8% of each compound.

GC–MS parameters

The GC-MS analyses were carried out on Hewlett-Packard (system A) and Finnigan GCQ (system B) instruments. The conditions are listed in Tables II–III.

Results and Discussion

The total essential oil content and the percentage composition of the oils were determined in cultivated and wild chamomile populations. The highest amount of the total essential oil was obtained from the roots of the cultivated BK-2 chamomile types (0.13% m/m). A similar result was observed in the roots of the wild Szabadkígyós population (0.12%, m/m). The roots of in vitro-cultivated chamomile culture synthesized the higher amount of essential oil than in the same chamomile type concerning the roots from intact parent plants.

Among the components characteristic of the intact chamomile root oil, all cultivated and wild chamomile populations examined contained a new sesquiterpene–alcohol, β-eudesmol. Figure 1 shows the chromatogram obtained. Its retention time corresponded to that of (–)-α-bisabolol, occurring in the inflorescence (flowers) of the plant (15,16), on β-DEXm and BPX5 stationary phases (GC and GC–MS system B). In root cultures, β-eudesmol was identified by a GC–MS method (system A) on a DB-WAX stationary phase. The relative retention data are presented in Table IV. According to latter GC parameters, the retention time (t_R) of β-eudesmol was 32.79 min, behind that of (–)-α-bisabolol (t_R of 32.60 min). The resolution factor between the two compounds was 3.8. Thus, we could establish that these GC para-

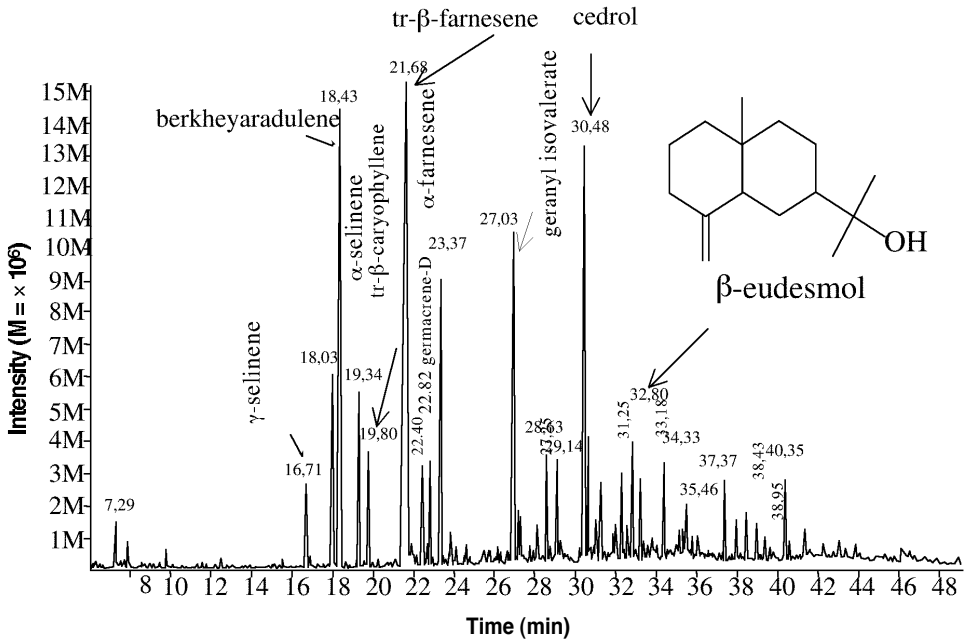

Figure 1. Total ionic chromatogram of essential oil from in vitro root (system A: 30-m × 0.25-mm i.d., 0.25-µm; DB-WAX; 40°C 3 min, 1.5°C/min to 45°C, 3°C/min to 80°C, 5°C/min to 180°C, 10°C/min to 240°C, 240°C for 3 min; and He, 0.20 MPa, 1.0 cm^3/min).

meters provided a good separation for (–)-α-bisabolol and β-eudesmol.

The two compounds were distinguished according to their mass spectra too; the characteristic fragments [*m/z* (relative intensity)] were the following: α-bisabolol, 6-methyl-2-(3-methyl-cyclohex-3-enyl)-hept-5-en-2-ol (Mw 222, $C_{15}H_{26}O$), 222 $[M]^+$ (0.5), 204(50), 189(7), 161(20), 147(3), 139(10), 134(15), 119(98), 109(100), 105(18), 93(40), 69(65), and 55(16).

Table IV. Relative Retentions (to *trans*-β-Farnesene) of β-Eudesmol and (–)-α-Bisabolol on Three Starionary Phases

Compounds	Relative retentions		
	β-DEXm	DB-WAX	BPX-5
(–)-α-Bisabolol	1.32	1.50	1.23
β-Eudesmol	1.32	1.52	1.23

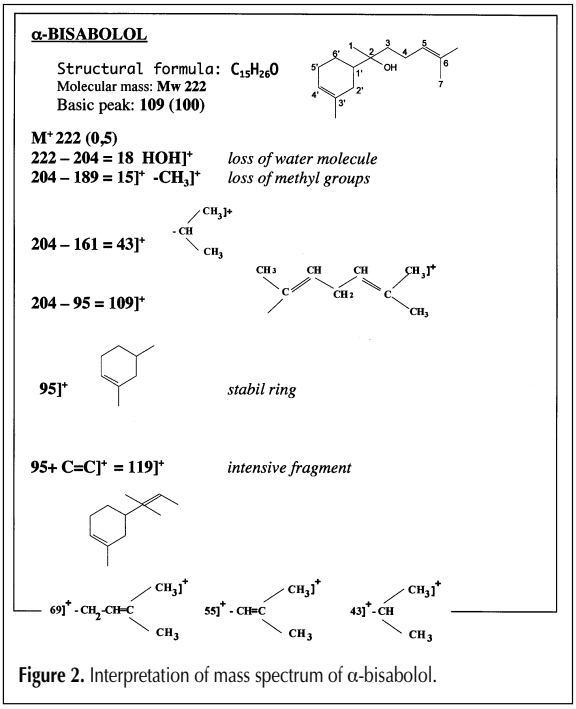

Figure 2. Interpretation of mass spectrum of α-bisabolol.

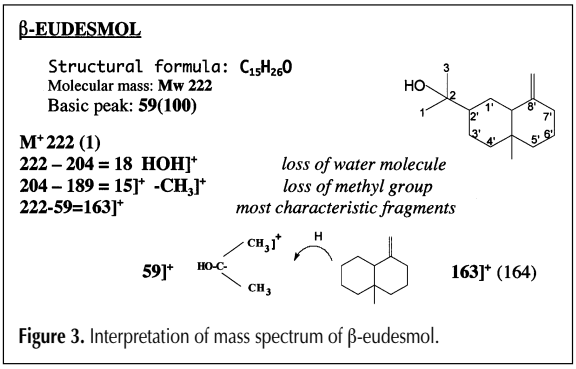

Figure 3. Interpretation of mass spectrum of β-eudesmol.

β-Eudesmol, 2-(4a-methyl-8-methylene-decahydro-naphthalen-2-yl)-propan-2-ol (Mw 222, $C_{15}H_{26}O$): 222 $[M]^+$ (1), 204(4), 189(5), 164(25), 149(60), 133(22), 123(34), 109(50), 93(52), 81(47), 67(18), 59(100), 53(18). The fragmentation of the two molecules verified the different chemical structures (Figures 2 and 3).

Studying the percentage composition of essential oil samples, we established that the main component of the root oil was *trans*-β-farnesene both in intact and organized plants. The significantly highest amount of β-eudesmol was attributed to Degumil (8.23%) among the cultivated species and the wild population from Szeghalom (9.25%), respectively (Figure 4). It was interesting that the higher β-eudesmol contents belonged to the samples containing lower *trans*-β-farnesene. Although β-eudesmol seemed a characteristic component of the intact roots, in sterile roots of Degumil and Szabadkígyós types it was also present (Table V). In the highest amount it was found in the organized root of Degumil (2.81%), which was still much less than in intact roots.

Conclusion

Regarding the numerous pharmacological tests proving the biological activity of β-eudesmol, the aim was to investigate plants in Hungary that contain β-eudesmol as the oil constituent. For identification of the new component of β-eudesmol, GC and GC–MS methods were used. We have established that the intact chamomile root oils contained this compound in a significantly high amount (between 1% and 10%), and in vitro it was also synthesized but in lower quantity. At the same time, it was not detectable in the oils of the flowers, but these oils were in general rich in (–)-α-bisabolol.

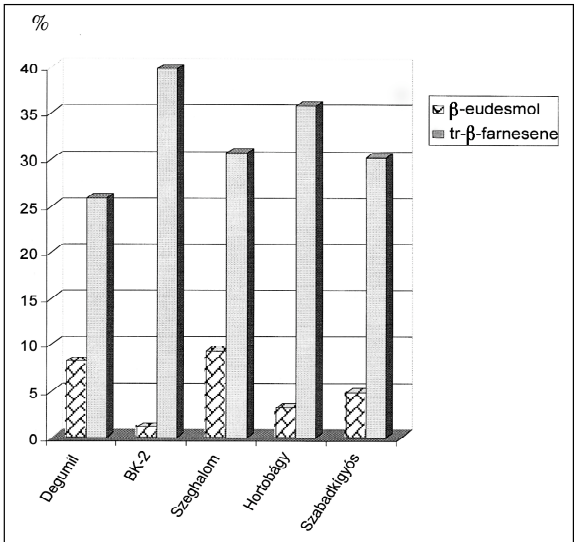

Figure 4. Concentration of β-eudesmol and *trans*-β-farnesene in the root oils of cultivated (Degumil, BK-2) and wild (Szeghalom, Hortobágy, Szabadkígyós) chamomile populations.

Table V. Concentration (%) of the Essential Oil Components in the In Vivo (Intact) and In Vitro (Organized) Roots of Cultivated (Degumil) and Wild (Szabadkígyós) Chamomiles*

Compounds	Cultivated chamomile		Wild chamomile	
	Intact root	Org. root	Intact root	Org. root
4-(2',4',4'-Trimethyl-bicyclo[4.1.0] hept-2'-en-3'-yl)-3-buten-2-one	0.18	3.20	0.60	1.84
Berkheyaradulene	0.83	11.80	1.75	4.17
α-Selinene	0.21	2.41	0.59	1.25
β-Caryophyllene	0.13	1.80	0.40	1.25
trans-β-Farnesene	25.80	26.19	30.20	33.57
Germacrene-D	0.11	0.52	0.73	0.50
α-Muurolene	0.74	–	2.98	–
α-Farnesene	1.20	3.93	1.17	0.82
α-Cadinene	0.07	–	0.15	–
Geranyl isovalerate	3.37	6.50	1.48	9.63
Spathulenol	0.56	–	0.73	–
Cedrol	24.90	8.71	17.54	1.40
Bisabolol oxide B	0.70	traces	2.63	0.24
β-Eudesmol	**8.23**	**2.81**	**4.87**	**1.23**
Bisabolone oxide	0.26	traces	0.22	1.00
cis-En-in-dicycloether	13.85	0.67	16.10	0.25
Chamomilla ester	0.55	traces	0.25	2.00
trans-En-in-dicycloether	4.03	traces	3.10	traces

* "Traces" means less than 0.10%.

Acknowledgments

We acknowledge the help of Professor Hisayuki Kanamori (Physics and Chemistry Division, Hiroshima Prefectural Institute of Public Health and Environment, Hiroshima, Japan) for providing the standard β-eudesmol. Also, the assistance of Zsolt Muskáth (Department of Pharmaceutics, Semmelweis University, Budapest, Hungary) in the GC–MS analyses is appreciated. Many thanks for the financial support of Hungarian National Scientific Foundation (OTKA T030034).

References

1. E. Máday, É. Szöke, Zs. Muskáth, and É. Lemberkovics. A study of the production of essential oils in chamomile hairy root cultures. *Eur. J. Drug Metabol. and Pharmacokinetics* **24:** 303–308 (1999).
2. L. Márk, R. Ohmacht, S. Glazl, and G. Reznicek. Separation of sesquiterpenes from *Achillea* species by HPLC-MS on non-porous columns. *Chromatographia* (in press).
3. M. Földvári. Composition and microbiological value of the essential oil of propolis in Hungary. Ph.D. Thesis, Semmelweis University, Budapest, Hungary, 1984.
4. F. Gong, Y.Z. Liang, Q.S. Xu, and F.T. Chau. Gas chromatography-mass spectrometry and chemometric resolution applied to the determination of essential oils in *Cortex cinnamomi*. *J. Chromatogr. A* **905:** 193–205 (2001).
5. V. Roussias, M. Tsoukatou, P.V. Petrakis, I. Chinou, M. Skoula, and J.B. Harborne. Volatile constituents of four Helichrysum species growing in Greece. *Biochem. Syst. Ecol.* **28:** 163–75 (2000).
6. Y. Nakai, T. Kido, K. Hashimoto, Y. Kase, J. Sakakibara, M. Higuchi, and H. Sasaki. Effect of the rhizomes of *Atractylodes lancea* and its constituents on the delay of gastric emptying. *J. Ethnopharmacology* **84:** 51–55 (2003).
7. H. Kanamori, I. Sakamoto, M. Katoh, T. Doi, and A. Mochiike. Studies on the evaluation of Atractylodes lancea rhizome. *Bull. Hiroshima Pref. Inst. Public Health* **39:** 27–30 (1992).
8. K. Satoh, F. Nagai, K. Ushiyama, I. Yasuda, K. Akiyama, and I. Kano. Inhibition of sodium-potassium-ATPase activity by β-eudesmol, a major component of *Actractylodes lanceae* rhizome, due to interaction with enzyme in the sodium E1 state. *Biochem. Pharmacol.* **44:** 373–78 (1992).
9. H. Tamai and J. Yamahara. β-Eudesmol for treatment of colon disease. Jpn. Kokai Tokkyo Koho JP 04013629 A2 Heisei, Japan, Reg. patent, 1992.
10. M. Miyazawa. Antimutagenic activity of (+)β-eudesmol and Paenol from *Dioscorea japonica*. *J. Agric. Food Chem.* **44:** 1647–50 (1996).
11. S. Yano and M. Tanaka. Structural components of β-eudesmol essential for its potentiating effect on succinylcholine-induced neuromuscural blockade in mice. *Biol. Pharm. Bull.* **17:** 1232–40 (1994).
12. É. Szöke, A.L. Shavarda, and I.N. Kuzovkina. Effect of the culturing conditions on essential oil formation in callus tissue of wild chamomile inflorescences. *Sov. Plant Physiology* **25:** 579–84 (1979).
13. T. Murashige and F. Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15:** 473–79 (1962).
14. Pharmacopea Hungarica. *Pharmacopoea Hungarica Editio VII*, Vol. 1 and 3, Publisher Medicina, Budapest, Hungary, 1986, pp. 395–98,1503–1506.
15. É. Szöke, E. Máday, G. Marczal, and É. Lemberkovics. Analysis of biologically active essential oil components of Chamomiles in Hungary. *Acta Hort.* **597:** 275–84 (2003).
16. É. Szöke, E. Máday, E. Tyihák, I. N. Kuzovkina, and É. Lemberkovics. New terpenoids in cultivated and wild chamomile (in vivo and in vitro). *J. Chromatogr. B* **800:** 231–38 (2004).

Manuscript accepted February 5, 2004.