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The volatile components of the aerial parts of *Melittis melissophyllum* L. subsp. *melissophyllum* gathered in Spain

Arturo Velasco-Negueruela (*), Jesús Sanz (**), María José Pérez-Alonso (*) & Jesús Palá-Paúl (*)

Resumen: Velasco-Negueruela, A.; Sanz, J.; Pérez-Alonso, M. J. & Palá-Paúl, J. 2004. Los aceites esenciales obtenidos de las sumidades floridas de *Melittis melissophyllum* L subsp. *melissophyllum* recolectada en España. *Bot. Complut. 28: 133-137.*

Los componentes volátiles obtenidos por cohobación y desorción térmica a partir de las sumidades floridas frescas y secas de *Melittis melissophylum* subsp. *melissophyllum* fueron estudiados por cromatografía de gases (CG) acoplada a espectrometría de masas (EM). El aceite esencial de la planta fresca obtenida por cohobación estaba constituido por ácido 8,11,14-eicosatrienoico (31,4%), ácido hexadecanoico (10,9%), biciclogermacreno (8,1%), germacreno D (7,1%) y β-cariofilleno (7,0%) como componentes mayoritarios y por cantidades menores de 1-octen-3-ol (3,9%) y fitol (2,8%), mientras que el aceite esencial obtenido por el mismo método a partir de la planta seca tenía como componentes mayoritarios ácido 8,11,14-eicosatrienoico (29,1%), ácido hexadecanoic (21,4%), fitol (16,0%) y espatulenol (5,2%). En los aceites volátiles obtenidos por desorción térmica (DT) directa de la planta tanto fresca como seca, la cumarina (2H-1-benzopiran-2-ona) (82,6 y 81,1%) se detectó como el componente más importante junto con fitol (1,5 y 6,3%) y hexacosano (6,4 y 4,3%).

Palabras clave: *Melittis melissophyllum* L. subsp. *melissophyllum*, *Labiatae*, aceite esencial, cumarina, desorción térmica, España.

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The volatiles obtained by hydrodistillation and thermal desorption (TD) from the aerial parts of *Melittis melissophylum* subsp. *melissophyllum* were studied by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The oil from the fresh plant was shown to contain 8,11,14-eicosatrienoic acid (31.4%), hexadecanoic acid (10.9%), bicyclogermacrene (8.1%), germacrene D (7.1%) and β-caryophyllene (7.0%) as main constituents and minor amounts of 1-octen-3-ol (3.9%) and phytol (2.8%), whereas the oil from the air dried plant had 8,11,14-eicosatrienoic acid (29.1%), hexadecanoic acid (21.4%), phytol (16.0%) and spathulenol (5.2%) as major components In the volatiles obtained by direct thermal desorption of both fresh and dried plant, coumarin (2H-1-benzopyran-2-one) (82.6 y 81.1%) was shown to be the most important constituent together with phytol (1.5 y 6.3%) and hexacosane (6.4 y 4.3%).

Key words: *Melittis melissophyllum* L. subsp. *melissophyllum*, *Labiatae*, essential oil, coumarin, termical desorption, Spain.

INTRODUCTION

Melittis melissophyllum L., bastard balm, toronjil silvestre, belongs to the plant family *Lamiaceae* and is a perennial herb with big axillary white, pink, purple or even variegated flowers inhabiting shady places in W., S. and C. Europe extending eastwards to Lithuania and N.C. Ukraine. Ball (1972) recognised three subspecies:

subsp. *melissophyllum* extending W.C. Europe, and grows in dark forests of the mid North of the Iberian Peninsula (García Rollán, 1999).

subsp. *carpatica* (Klokov) P. W. Ball in E.C. Europe extending eastwards to W. Ukraine and S.W. White Russia.

subsp. *albida* (Guss.) P.W. Ball extending S. Italy, Sicilia and Balkan Peninsula.

The essential oil from the aerial parts of *M. melissophyllyum* subsp. *albida* obtained by steam-distillation and analysed by Skaltsa-Diamantidis *et al* (1991) was found to contain chrysanthenyl acetate (12.3%), α-terpineol (17.1%) and caryophyllene oxide (10.8%) as main components. According to Hegnauer (1966) coumarin (2H-1-benzopyran-2-one) was the only volatile constituent of *M. melissophyllum.* This compo-

* Departamento de Biología Vegetal I (Botánica), Facultad de Biología, Universidad Complutense 28040-Madrid, España. AVN44@bio.ucm.es.

** Instituto de Química Orgánica, CSIC, C/ Juan de la Cierva 3, 28006-Madrid, España iqojs02@iqog.csic.es.

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nent probably arises upon hydrolysis of an unknown heteroside, with 2-hydroxy-(Z)-cinnamic acid as a aglycone that spontaneously lactonizes to coumarin. Skrzypczak and Skrzypczak (1993) studied by thin layer chromatography (TLC) the butanolic extracts of ground *M. melissophyllum* grown *in vitro* and found flavonoids and phenolic derivatives.

In this work we present the results of a study of the volatile composition of the aerial parts of *M. melissophyllum* subsp. *melissophyllum*, carried out by GC, GC-MS and thermal desorption (TD) directly coupled to gas chromatography - mass spectrometry (TD-GC-MS). As far as we know there is no other previous report on the *M. melissophyllum* subsp. *melissophyllum* essential oil analysis.

MATERIAL AND METHODS

Plant material

One sample of a *M. melissophyllum* population was gathered at flowering in 'Siete Revueltas', Segovia, Spain (6/6/2003, 30TVL1419). A voucher specimen, MACB 85301 was deposited at the Herbarium of Biology Faculty, Complutense University, Madrid, Spain.

Extraccion and Isolation

The aerial parts of *M. melissophyllum* were left to dry at room temperature (sample MMD1) and 27 g of the plant material were coarsely minced and placed in a flask containing 100 mL of water and hydrodistilled for 8h in a Clevenger-type apparatus according to the method recommended in the Real Farmacopea Española (1997). Fresh aerial parts (250 g sample MMF1) were ground and placed in a flask containing 500 mL of water and steam distilled in a Clevenger-type apparatus according to the same method. Both essential oils were dried over anhydrous magnesium sulphate and stored at 4 °C in the dark. Essential oil yield based on dry plant material was for MMD1 0,38% and for MMF1 0,25%. Dried *M. melissophyllum* (5 mg Sample MMD2) and 10 mg of fresh frozen *M. melissophyllum* (Sample MMF2) were analyzed using the TD-GC-MS method.

Analyses Gas Chromatography (GC)

A Varian GC 3300 fitted with a fused silica capillary column (coated with polymethylsiloxane DB-1 as stationary phase (50 m x 0.25 mm i.d., 0.25 (m film thickness) was used for GC analysis. Oven temperature was programmed from 95 °C to 240 °C at 4 °C/min. Injection was performed at 250 .°C using a 1:100 split ratio. A flow of 1.5 mL/min carrier gas (N_2) was used. Detection was performed by FID at 300 °C. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS of essential oils was carried out in an *Agilent* 6890 GC coupled to an *Agilent* 5973 Mass Detector. A fused silica SE-30 capillary column (25 m x 0.25 mm i.d., 0.25 (m film thickness) was programmed from 70 °C to 250 °C at 4 °C/min. Mass spectra were recorded in the EI mode at 70 eV.

Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS)

A thermal desorption system from *ATAS* (Optic 2) was directly coupled to the GC-MS equipment previously described. Volatiles were desorbed from samples (5-10 mg) at 180 °C during 2 min, using He as carrier gas. Column was kept at 40 °C during desorption and then programmed at 20 °C min to 70 °C and from 70 °C to 250 °C at 4.°C/min according to the methods previously published, Esteban *et al* (1993, 1996).

Component identification

Most constituents were identified by gas chromatography by comparison of their GC retention indices (I) with those of literature (Dev *et al,.* 1986; Adams, 1995; Jennings & Shibamoto, 1980; Libbey, 1991; Swigar & Swilverstein, 1981; Joulin & König, 1998; Davies, 1998) or with those of standards purchased, synthesized or identified in oils of known composition. Further identification was confirmed when possible by comparison of their mass spectra with those stored in the MS databases (NIST and WILEY libraries) or with mass spectra from literature (Dev *et al.,* 1986; Adams, 1995; Jennings & Shibamoto, 1980; Libbey, 1991; Swigar & Silverstein, 1981; Joulain & König, 1998). Relative component concentrations were obtained directly from GC peak areas.

RESULTS AND DISCUSSION

The components of the oil from the aerial fresh and dried parts of *M. melissophyllum* subsp. *melissophyl-*

lum, their retention indices, their percentage composition and identification methods are given in Table 1 in which the components are listed in order of their elution on the DB-1 column. The major components of the oil from the fresh sample were found to be 8,11,14-eicosatrienoic acid (31.4%), hexadecanoic acid (10.9%), bicyclogermacrene (8.1%), germacrene D (7.1%) and β−caryophyllene (7.0%). The major components detected in the oil from the dried plant were 8,11,14-eicosatrienoic acid (29.1%), hexadecanoic acid (21.4%), phytol (16.0%) and spathulenol (5.2%). More detailed results are shown in Table 1 in which the complete list of the identified components is given. It is worth noting that although coumarin was

Table 1
Percentage composition of the essential oils from the aerial parts of *Melittis melissophyllum* subsp. *melissophyllum*

Component[a])	I[b])	MMF1(%)[c])	MMD1%[d])	Method[e])
(*E*)-2-Hexenal	845	t	-	MS, I_1
n-Hexanol	862	t	-	MS, I_2
α-Thujene	920	t	t	MS, I_2
α-Pinene	934	t	t	MS, I_2
1-Octen-3-one	962	0.1	t	MS, I_1
1-Octen-3-ol	970	3.9	t	MS, I_2
Sabinene	975	t	0.3	MS, I_2
Myrcene	985	t	t	MS, I_2
3-Octanol	990	t	t	MS,I_1
α-Terpinene	1011	t	t	MS, I_2
p-Cymene	1014	0.1	t	MS, I_2
Limonene	1024	0.1	t	MS, I_2
δ3-Carene	1028	t	t	MS, I_2
(*Z*)-β-Ocimene	1038	t	t	MS, I_2
γ-Terpinene	1050	t	t	MS, I_2
m-Cymenene	1075	0.1	t	MS, I_1
Terpinolene	1079	0.1	t	NS,I_1
Linalool	1086	0.4	t	MS, I_2
Terpinen-4-ol	1168	0.3	0.1	MS, I_2
α-Terpineol	1180	0.1	t	MS, I_2
α-Copaene	1375	0.4	0.1	MS, I_1
β-Bourbonene	1383	0.5	0.1	MS, I_1
β-Elemene	1386	0.2	t	MS, I_1
Coumarin	1391	t	t	MS, I_1
β-Caryophyllene	1418	7.0	1.7	MS, I_2
β-Gurjunene	1426	0.1	t	MS, I_1
α-Humulene	1450	2.6	0.8	MS, I_2
Germacrene D	1477	7.1	0.8	MS, I_1
Bicyclogermacrene	1492	8,1	1.9	MS, I_1
(*E*)-β-Ionone	1500	0.1	t	MS, I_2
(*E,E*)-α-Farnesene	1505	t	t	MS, I_1
γ-Cadinene	1507	t	t	MS, I_1
δ-Cadinene	1512	0.5	0.2	MS, I_1
(*Z*)-Nerolidol	1546	0.6	0.3	MS, I_2
Spathulenol	1566	0.5	5.2	MS, I_1
Caryophyllene oxide	1572	0.9	1.8	MS, I_2
Viridiflorol	1584	0.2	0.1	MS, I_1
Hexadecanoic acid	1957	10.9	21.4	MS, I_1
Phytol	2097	2.8	16.0	MS, I_1
8,11,14-Eicosatrienoic acid	2135	31.4	29.1	MS, I_1
Docosane	2200	0.3	0.3	MS, I2
Tricosane	2300	1.0	1.1	MS, I_2
Tetracosane	2400	0.5	1.1	MS, I_2
Pentacosane	2500	1.2	1.3	MS, I_2
Hexacosane	2600	0.2	0.2	MS, I_2
Heptacosane	2700	2.6	3.6	MS, I_2

[a]) Components listed in order to their elution on DB_1 column. [b]) Kováts retention indices. [c]) Sample MMF1. [d]) Sample MMD1. [e])Identification Methods: MS = Mass spectra; I_1 = Kováts index according to literature values, I_2 = Kováts index according to authentic standards. t = traces (<0.1%).

Table 2
Volatiles (%) of *Melittis melissophyllum* subsp. *melissophyllum* by Thermal *Desorption –Mass Spectrometry(TD-GC-MS)*

Component[a])	I[b])	MMF2(%)[c])	MMD2(%)[d])	Method[e])
α-Pinene	934	t	t	MS, I_2
1-Octen-3-ol	970	t	t	MS, I_2
3,4-Dihydrocoumarin	1337	t	t	MS, I_1
Coumarin	1391	**82.6**	**81.1**	MS, I_1
β-Caryophyllene	1418	0.5	t	MS, I_2
Germacrene D	1477	0.9	t	MS, I_1
γ-Muurolene	1472	t	t	MS, I_1
Bicyclogermacrene	1492	1.0	t	MS, I_1
(*Z*)-β-Ionone	1505	t	t	MS, I_1
Spathulenol	1567	0.6	1.0	MS, I_2
Caryophyllene oxide	1572	t	t	MS, I_2
Hexadecanoic acid	1957	t	6.6	MS, I_1
Phytol	2097	1.5	6.3	MS, I_1
8,11,14-Eicosatrienoic acid	2135	t	0.7	MS, I_1
Hexacosane	2600	6.4	4.3	MS, I_2
Heptacosane	2700	4.3	0.1	MS, I_2

[a]) Components listed in order to their elution on silicon column. [b]) Kováts retention indices. [c]) Sample MMF2. [d]) Sample MMD2. [e])Identification Methods: MS = Mass spectra; I_1 = Kováts index according to literature values, I_2 = Kováts index according to authentic standards. t= traces (<0.1)

identified in both essential oils only was found in trace amounts (<0.1%). In other plant species such as 'Sweet Clover' (*Melilotus officinalis* (L.) Pallas; 'Sweet woodruff' (*Galium odoratum* (L.) Scop); 'Tonka Beans' (*Dipterys odorata* (Aublet) Willd.; *Lavandula dentata* L; *L. angustifolia* Miller subsp. *angustifolia*; *L. latifolia* Medicus and some *Poaceae* as *Anthoxanthum odoratum* L., coumarin is also generated.

Since *M. melissophyllum* produces a pleasant fragrant characteristic aroma of new-mown hay of coumarin, a precursor of this compound could be present as a part of an unknown heteroside with 2-OH-(*Z*)-cinnamic acid as an aglycone that could spontaneously lactonize. Since coumarin is very soluble in hot water, 1g dissolves in 50mL boiling water (The Merck Index 2001) this could be the explanation of the small amounts of coumarin in both essential oils studied, whereas the volatiles obtained by Thermal Desorption (Table 2) were shown to contain coumarin (82.6-81.1%) as main constituent of both the fresh frozen and dried *M. melissophyllum*.

BIBLIOGRAFÍA

Adams, R. P. 1995. *Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy*. Allured Publishing Co., Illinois, Il.

Ball, P. W. 1972. *Melittis* L. In *Flora Europaea*, Tutin *et al.* (Eds.), 3: 143, Cambridge.

Davies, N. W. 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr.* 503: 1-24.

Dev, S.; Narula, A. P. S. & Yadav, J. S. 1986. *Handbook of Terpenoids*. CRC Press, Boca Ratón, FL.

Esteban, J. L.; Martínez Castro, I. & Sanz, J. 1993. Evaluation and optimization of the automatic thermal desorption (ATD) method in the gas chromatographic determination of plant volatile compounds. *J. Chromatogr. A,* 657, 155.

Esteban, J. L. Martínez Castro, I. Sanz, J. 1996. Rapid identification of volatile compounds in aromatic plants by ATD/GC/MS *Chromatographia,* 43, 63-72.

García Rollán, M. 1999. Atlas clasificatorio de la flora de España Peninsular y Balear. Volumen I. Ministerio de Agricultura, Pesca y Alimentación, 646.

Hegnauer, R. 1965. *Chemotaxonomie der Pflanzen.* 4: 331, Birkhauser Verlag, Baseer und Sttutgart.

Jennings, W. & Shibamoto, T. 1980. *Qualitative Analysis of Flavour and Fragrance Volatiles by Capillary Gas Chromatography.* Academic Press. New York.

Joulain, D. &. König, W. A. 1998. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*. EB.-Verlag, Hamburg.

Libbey, L. M. 1991. A Paradox data base for GC/MS data on Components of Essential Oils and Other Volatiles. *J. Essent. Oil Res*., 3: 192-194.

Real Farmacopea Española. 1997. Ministerio de Sanidad y Consumo, Madrid.

Skaltsa-Diamantidis, H.; Tsitsa-Tzardi, E.; Tzakou, O. & Argiriadou, N. 1991. Analysis of the essential oil of *Melittis melissophyllum* L. subsp. *abida* Guss. *J. Ess. Oil Res.,* 3:367-368.

Skrzypczak, E. & Skrzypczak, L. 1993. The tissue culture and chemical analysis of *Melittis melissophyllum* L. *Acta Horticulture* 330: 263-265.

Swigar, A. A. & Silverstein, R. M. 1981. *Monoterpenes*. Aldrich Chem. Co., Milwaukee, Wisconsin.

The Merck Index. 2001. 13th Edition, Merck Research Laboratories, Whitehouse Station, M. J.