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ORIGINAL PAPER

Identification of the components of the essential oil from *Trollius europaeus* flowers

Ewa Witkowska-Banaszczak

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Abstract The essential oil of *Trollius europaeus* flowers obtained by hydrodistillation was analyzed by gas chromatography coupled with mass spectrometry (GC–MS). The compounds giving fragrance of essential oils commonly used in perfumery 3,7-dimethyl-1,6-octadien-3-ol, nonanal, 3-methyl-2-pent-2-enyl-cyclopent-2-enone and oxacycloheptadec-8-en-2-one, rare in the Plant Kingdom, were tentatively identified. In the analyzed essential oil, the saturated fatty acids hexadecanoic acid (7.54 %), tetradecanoic acid (4.24 %), dodecanoic acid (3.10 %) and unsaturated fatty acids 9,12,15-octadecatrienoic acid (3.47 %), hydrocarbons, namely eicosane (20.03 %), hexadecane (8.63 %) and 1,2-benzenedicarboxylic acid (2.39 %), were also found.

Keywords *Trollius europaeus* · Essential oil · Gas chromatography · Mass spectrometry

Introduction

Trollius europaeus L. belongs to the Ranunculaceae family, *Trollioidae* subfamily. This species is widely distributed in Europe and in areas of western Siberia. In folk medicine, it has been used in the treatment of scurvy because of the presence of vitamin C (Gruenwald et al. 2004).

Our earlier studies have indicated the occurrence of the flavonoids, namely 7-methylapigenin 4′-rhamnopyranoside (1 → 2) xylopyranoside and orientin, vitexin, isoorientin, isovitexin, 2″-arabinopyranoside of orientin and vitexin, orientin 2″-glucopyranoside, vitexin 2″-galactopyranoside, orientin 2″-xylopyranoside (adonivernith), and phenolic acids caffeic, chlorogenic, γ-resorcylic, *p*-coumaric, vanillic, sinapic, ferulic, syringic and *p*-hydroxyphenylacetic ones in the leaves of *T. europaeus* (Maciejewska-Rutkowska et al. 2007).

Ranunculin and magnoflorine, characteristic for the Ranunculaceae family, also occur in *T. europaeus* (Gruenwald et al. 2004). The carotenoids neoxanthin and xanthophyll epoxides are responsible for the yellow color of *Trollius* flowers (Egger and Dubbagh 1970; Baumeler and Eugster 1992; Gruenwald et al. 2004). In the petals of the crown, neoflor and epineoflor have also been found (Deli et al. 1998; Marki-Fischer and Eugster 2004). Pollen contains the aminoacid proline and the growth substance abscisic acid (Lipp 1991). Phytoecdysteroids occur in trace amounts in this plant (Dinan et al. 2002). The content of volatile organic compounds has so far been determined in fresh anthers or sepals of *T.europaeus*. The aim of this study was to determine the components of the essential oil obtained from whole dried flowers.

Experimental part

Plant materials and oil extraction

Trollius europaeus L. was cultivated in an experimental field of the Department of Botany of Poznań University of Life Sciences. The flowers were collected in May 2010, and dried.

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E. Witkowska-Banaszczak (✉)
Department of Pharmacognosy, Poznan University of Medical Sciences, ul. Święcickiego 4, 60-781 Poznań, Poland
e-mail: banaszcz@ump.edu.pl

The essential oil was obtained by hydrodistillation of the dried flowers using a Deryng apparatus. 30 g of the material was poured over with 500 ml of distilled water and heated for 3 h (counted from the moment when the content of the flask started to boil). The heating intensity was regulated in such a way as to make about 3 ml of the liquid flow to the receiver every minute. 30 min after the distillation was finished, the volume of the essential oil was read. The extraction was performed three times (Polish Pharmacopeia 2002).

GC–MS analyses

The identification of the essential oil compounds was performed using a Varian 4000 GC–MS instrument equipped with a Varian VF-5 ms silica column (30 mm × 0.25 mm × 0.39 mm, No. CP8944). Helium was used as the carrier gas at a flow rate of 1.0 ml/min with a split ratio equal to 1:50. The oven temperature was raised from 40 to 280 °C at a rate of 10 °C/min. The injector temperatures were kept at 220 °C. The detector temperatures were as follows: ion trap 220 °C, manifold 50 °C, and transfer line 280 °C.

The compounds were identified by comparing their retention time and EI (70 eV) mass spectra with standards from a NIST Mass Spectra Library. Semi-quantitative data were obtained from relative peak area percentages.

Results and discussion

Essential oil of a yellow color and a nice, flowery smell was obtained from the flowers of *T. europaeus* by hydrodistillation. The content of the essential oil in the dried flowers was 0.3 % v/w. On the basis of the GC–MS analysis, the compounds found in the essential oil from *T. europaeus* flowers were tentatively identified as 3,7-dimethyl-1,6-octadien-3-ol, nonanal, 3-methyl-2-pent-2-enyl-cyclopent-2-enone, oxacycloheptadec-8-en-2-one, 9,12,15-octadecatrienoic acid, dodecanoic acid, tetradecanoic acid with the match factor of 800–900 and hexadecanoic acid, hexadecane, 1,2-benzenedicarboxylic acid, eicosane with the match factor of >900 (Table 1; Fig. 1). The identified compounds accounted for 57.4 % of the ingredients of the oil fraction.

These are compounds belonging to different chemical groups. Some of them are common in the Plant Kingdom (e.g. linalool, nonanal), others are rare (e.g. oxacycloheptadec-8-en-2-one). Plants produce them in order to attract pollinating insects, or deter them (for example (R)-(−)-linalool, nonanal) acting in defense against destructive effects of, for example, insect larvae, including the larvae of the main pollinator, *Chiastocheta* flies (Gallet et al. 2007; Ibanez et al. 2010). The compounds also exhibit antimicrobial activity. Essential oils and/or their components are widely used by humans in medicine, cosmetics, food industry, and agriculture (Dekker et al. 2011; Logan et al. 2010; Carroll et al. 2011).

3,7-Dimethyl-1,6-octadien-3-ol, nonanal, 3-methyl-2-pent-2-enyl-cyclopent-2-enone and oxacycloheptadec-8-en-2-one are known compounds, giving fragrance of essential oils, widely used in perfumery. The content of these compounds in the essential oil of *T. europaeus* flowers amounted to 2.43, 1.95, 1.55, and 2.08 %, respectively. 3,7-Dimethyl-1,6-octadien-3-ol (linalool) is aliphatic monoterpene alcohol with the scent of lily of the valley, commonly occuring in the Plant Kingdom, especially in the family Lamiaceae, e.g. in

Table 1 Compounds tentatively identified in the essential oil from the flowers of *T. europaeus* using GC–MS

No.	Name	Retention time (min:s)	% of total amount	Match factor	Molecular mass (g/mol)	Molecular formula
1	3,7-Dimethyl-1,6-octadien-3-ol	9.156	2.43	890	154	$C_{10}H_{18}NO$
2	Nonanal	9.218	1.95	813	142	$C_9H_{18}O$
3	3-Methyl-2-pent-2-enyl-cyclopent-2-enone	12.160	1.55	878	164	$C_{11}H_{16}O$
4	Dodecanoic acid	13.419	3.10	876	200	$C_{12}H_{24}O_2$
5	Tetradecanoic acid	14.957	4.24	897	228	$C_{14}H_{28}O_2$
6	Hexadecanoic acid	16.359	7.54	914	256	$C_{16}H_{32}O_2$
7	1,2-Benzenedicarboxylic acid	16.413	2.39	914	166	$C_8H_6O_4$
8	Hexadecane	17.255	8.63	910	226	$C_{16}H_{34}$
9	Oxacycloheptadec-8-en-2-one	17.470	2.08	821	252	$C_{16}H_{28}O_2$
10	9,12,15-Octadecatrienoic acid	17.516	3.47	857	278	$C_{18}H_{30}O_2$
11	Eicosane	18.429	20.03	951	282	$C_{20}H_{42}$
	Total identified		57.41			

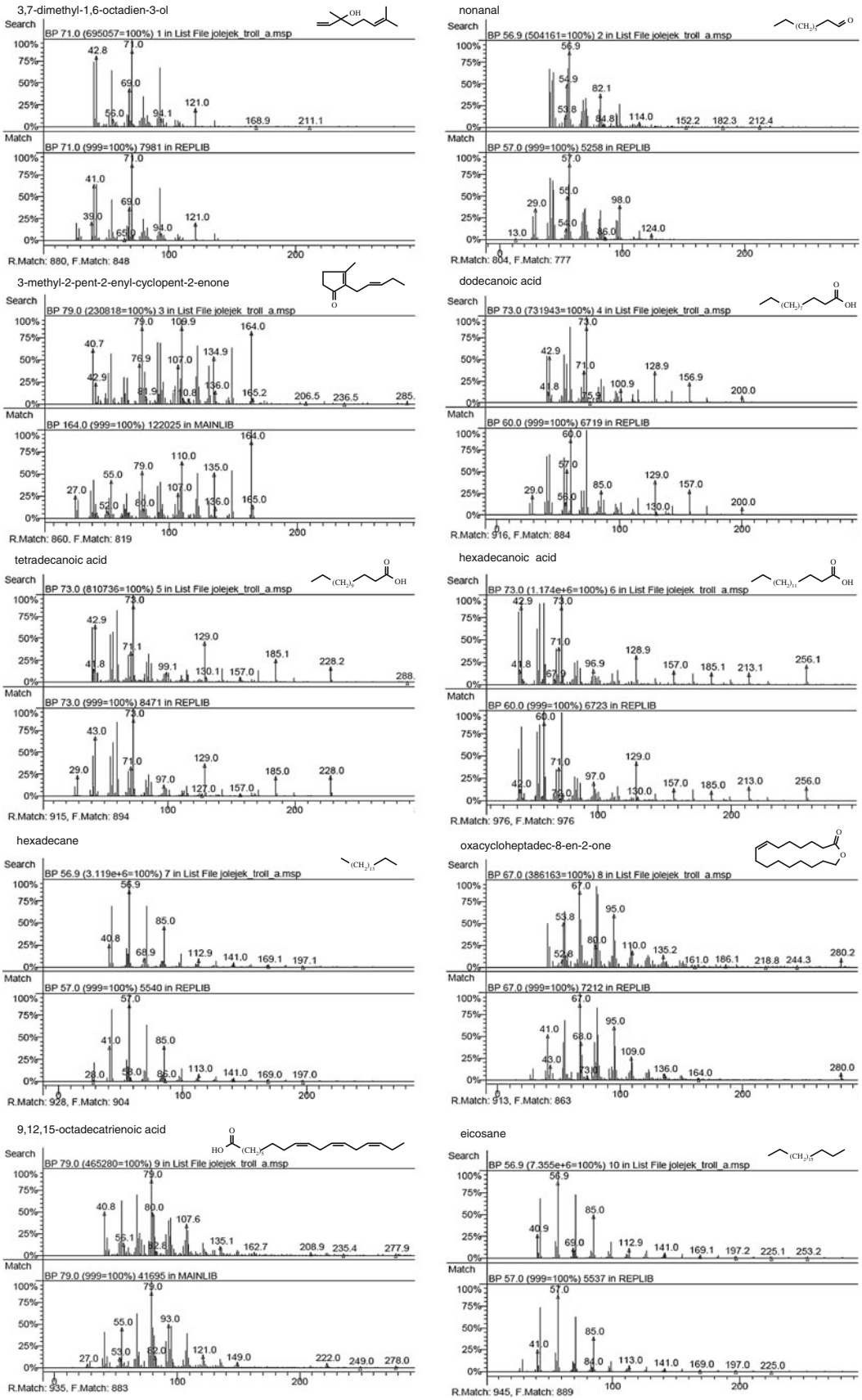

Fig. 1 GC-MS profiles of the main essential oil compounds from the flowers of *T. europaeus*

Lavandula spp. (25–38 %) (Bruneton 1999), *Salvia officinalis* (Meshkatalsadat and Asadi 2010; Gruenwald et al. 2004), *Thymus vulgaris*, *Mentha species*, *Hyssopus officinalis*, and *Ocimum basilicum* (Gruenwald et al. 2004). In the species from the Ranunculaceae family, it has so far been found only in *Aquilegia glandulosa*, *Ranunculus acris*, *R. gramines*, and *Trollius europaeus* (Jűrgen and Dőtterl 2004; Ibanez et al. 2010). Linalool has repellent properties (e.g. for *Aedes aegypti* causing yellow fever) (Dekker et al. 2011). Nonanal-alkyl aldehyde, a common component of essential oils, was present in the oils extracted from *Olea europaea* L. fruits (Jiménez et al. 2004), the essential oil of *Taraxacum officinale* flowers (Bylka et al. 2010), and the peel of *Citrus limon* and *C. sinensis* (Gruenwald et al. 2004). It also occurs in the following Ranunculaceae species: *Trollius europaeus*, *Anemone sylvestris*, *Aquilegia canadensis*, *A. chrysantha*, *A. glaudulosa*, *Caltha palustris*, *Pulsatilla rubra*, *P. vulgaris*, *Ranunculus acris*, *R. gramineus*, *and R. platnifolius* (Jűrgen and Dőtterl 2004; Ibanez et al. 2010). Nonanal has a strong fruity and floral odor, and is also considered a wine odoriferous substance (Culleré et al. 2011). Nonanal is active against *Trichophyton mentagrophytes*, which causes athlete's foot and other skin infections (Wood and Szewczak 2007). It can also be used as a mosquito repellent (Logan et al. 2010). 3-Methyl-2-pent-2-enyl-cyclopent-2-enone (jasmon), derived from cyclopentane, occurs in *Trollius europaeus*, *Ranunculus gramineus*, *Caltha palustris* (Jűrgen and Dőtterl 2004; Ibanez et al. 2010), and Japanese green tea (Sen-cha) (Jumtee et al. 2011). Oxacycloheptadec-8-en-2-one (cyclohexadecen-7diole, macrocyclic musk ambrette) is characterized by a sweet odor (like musk), rarely found in plants. It occurs in the seeds of *Abelmoschus moschatus* (Gruenwald et al. 2004). Among the fatty acids identified in the analyzed oil, hexadecanoic acid (palmitic acid) occurred in the largest amount, 7.54 %. In the species from the Ranunculaceae family, it has been identified before in *Aquilegia canadensis*, *A. chrysantha*, *A. glandulosa*, *A. vulgaris*, *Caltha palustris*, *Pulsatilla rubra*, *P. vulgaris*, *Ranunculus gramineus*, *R. acris*, *R. platanifolius*, as well as in *Trollius europaeus* (Jűrgen and Dőtterl 2004). Tetradecanoic acid (myristic acid) and dodecanoic acid (lauric acid) constituted 4.24 and 3.10 % of the total amount of the essential oil, respectively. These fatty acids occur in large quantities in the oils of *Cocos nucifera*, *Elaeis guineensis*, and *Theobroma cacao* (Coimbra and Jorge 2012; Gruenwald et al. 2004). Dodecanoic acid occurs as the major compound in the *Laurus nobilis* seed oil (Ozcan et al. 2010) and in *Phoenix dactylifera* L., together with tetradecanoic acid (Juhaimi et al. 2012). It was shown that lauric acid had antimicrobial activity against *Propionibacterium acnes*, *Staphylococcus aureus*, and *S. epidermidis* (Coimbra and Jorge 2012; Nakatsuji et al. 2009). Palmitic acid inhibits HIV-1 infection and is safe for tissues and probiotic bacteria (Paskaleva et al. 2010; Lin et al. 2011). This compound was identified in the essential oil of *Viola tianshanica* (Yang et al. 2011), *Scutellaria orientalis* ssp. *alpina*, *S. utriculata* (Formisano et al. 2011), and *Astragalus species* (Gruenwald et al. 2004). In the investigated essential oil, 9,12,15-octadecatrienoic acid (α-linolenic acid) was identified; its content was 3.47 %. This compound belongs to the group of omega-3 fatty acids, commonly occurring in flaxseed, canola oil, soybeans, and walnut oils (Simopoulos 2004; Gruenwald et al. 2004). Oils rich in α-linolenic acid are used to prevent and treat heart diseases, an elevated level of cholesterol and triglycerides in blood, and hypertension (Fiaccavento et al. 2006; Brouwer et al. 2004). 1,2-Benzenedicarboxylic acid–phthalic acid was identified in fresh, white and red *Panax ginseng* Abd El-Aty et al. (2008), as well as in crude extracts of *Alpinia galanga* (L.) Willd (Rao et al. 2010). The hydrocarbons hexadecane and eicosane, commonly found in essential oils (*Taraxacum officinale*, *Coriandrum sativum*, *Hypericum hircinum*), occurred in the greatest amount, i.e. 20.03 % of the total amount of the oil (Kumar Dwivedi et al. 2010; Bylka et al. 2010; Maggi et al. 2010).

Only four of the compounds identified in the essential oil from whole dried flowers of *T.europaeus* in this study were found in the anther and sepals, namely, nonanal, jasmone, hexadecane, and linalool. Nonanal and (E,E)-α-farnesene were the main volatile compounds determined in fresh thecae (Jűrgen and Dőtterl 2004), whereas in the oil from whole dry flowers, the hydrocarbons eicosane and hexadecane were dominant. (E,E)-α-Farnesene was not found in the investigated oil. Oxacycloheptadec-8-en-2-one, rare in the plant world, as well as hexadecanoic, tetradecanoic, dodecanoic, α-linolenic, and 1,2-benzodicarboxylic acids were identified and determined in the oil from *T. europaeus* for the first time.

Author contribution Ewa Witkowska-Banaszczak collected the material to be tested, obtained the essential oil and developed the results of GC–MS analysis. The author prepared the documentation and described the results of the research in this paper.

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