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Note

Comparative study of the essential oils of *in vivo* and *in vitro* grown *Valeriana officinalis* L. and *Centranthus macrosiphon* Boiss. by coupled gas chromatography–mass spectrometry

C. VIOLON*, W. SONCK and A. VERCRUYSSE

Dienst Farmacognosie, Fytochemie en Toxicologie, Vrije Universiteit Brussel, Laerbeeklaan 103, 1090 Brussels (Belgium)

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The major valerian secondary metabolites can be subdivided into two groups: valepotriates* and essential oils. Studies dealing with *in vitro* grown plants[2–5] indicated that the most efficient valepotriate production was provided by differentiated root tissue cultures of *Centranthus macrosiphon* Boiss.[4,5]. In order to study the extent of essential oil production under *in vitro* conditions we have analysed both *in vivo* and *in vitro* grown plant materials from *Valeriana officinalis* L. and *Centranthus macrosiphon* Boiss., to determine their essential oil contents. That the *in vitro* cultures contained volatile oil was strongly suggested by the presence in them of microscopic droplets resembling the lipidic, volatile oil-containing droplets described for *V. officinalis* roots[4]. We also demonstrated valepotriates to be present in these droplets[4]. The composition of the essential oil of *Valeriana officinalis* L. roots has been studied extensively[6–11] and that of the essential oil of *Valeriana celtica* L. was recently reported[12]. The former essential oil is made up of mono- and sesquiterpene hydrocarbons, esters, carbonyl compounds, ethers, alcohols and carboxylic acids[9]. Regarding *Centranthus* species, only lower fatty acids have hitherto been identified in the oil obtained by steam distillation of *Centranthus ruber* DC. roots[13]. The essential oils present in both *in vivo* and *in vitro* grown were isolated using a micro steam distillation–extraction apparatus which required only small amounts of starting materials. The qualitative and comparative study of the essential oils of *Centranthus macrosiphon* Boiss. and *Valeriana officinalis* L. thus obtained was carried out by coupled gas chromatography–mass spectrometry (GC–MS), a convenient procedure widely used for investigating complex mixtures of natural origin.

* Valepotriates are triesters of freely unstable polyhydroxycyclopenta[*c*]pyrans with isovaleric (R_1), acetic (R_2) and β-acetoxyisovaleric acid (R_3) and they contain an epoxide. Valtrate ($2 \times R_1$, $1 \times R_2$), isovaltrate ($2 \times R_1$, $1 \times R_2$) and acevaltrate ($1 \times R_1$, $1 \times R_2$, $1 \times R_3$) are conjugated dienes, which form blue cyclopentapyrrilium salts with hydrochloric acid. Didrovaltrate ($2 \times R_1$, $1 \times R_2$) is a monoene valepotriate[1].

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EXPERIMENTAL

Plant material

Valeriana officinalis L. and *Centranthus macrosiphon* Boiss. were grown in the greenhouse from seeds. *V. officinalis* seeds were obtained from the Hortus Centralis Cultura Herbarum Medicarum, Brno, Czechoslovakia (1981) and *C. macrosiphon* seeds from the National Botanical Garden, Meise, Belgium (1981). *In vitro* cultures of *V. officinalis* and *C. macrosiphon* were started from sterile seedlings obtained from the same seeds and grown aseptically on solid media. In order to study in these cultures the influence of plant hormones on growth, morphological behaviour and secondary metabolite production, cultures of the two plants were initiated on several media, differing in plant hormone content. The cultures thus initiated were subcultured every 5 weeks on their own medium during 1 year. In this way we obtained stable callus cultures of both plants and root differentiated tissue cultures of *C. macrosiphon*. The harvested plant material was dried below 40°C. The (iso)valtrate content (*i.e.*, the major valepotriate representative in these plants) of the plant material used for essential oil analysis is given in Table I. An extensive description of the growth conditions and valepotriate analysis has been published elsewhere[3–5].

Isolation of essential oil

A 2-g sample of dry plant material was steam distilled and ether extracted for 3 h using a micro apparatus[11]. The organic phase was evaporated *in vacuo* with a Rotavapor and the remaining essential oil taken up in absolute ethanol. In order to control the efficiency of this steam distillation–extraction procedure we prepared a standard sample of essential oil of *V. officinalis* roots grown *in vivo* by steam distillation according to the Dutch Farmakopeia VI. The essential oils resulting from both procedures were then compared by GC–MS (see Results and discussion). The amounts of essential oils in the different plant materials are given in Table I.

GC–MS study

The GC–MS analyses were performed on a Hewlett-Packard Model 9592 GC–MS instrument, fitted with a fused-silica capillary column (50 m × 0.23 mm I.D.) coated with CP Sil 5 CB (film thickness 0.12 μm). The GC column temperature

TABLE I

(ISO)VALTRATE (*a*) AND TOTAL ESSENTIAL OIL CONTENT (*b*) (GRAMS PER 100 g OF DRY MATERIAL) OF THE PLANT MATERIAL USED FOR QUALITATIVE ESSENTIAL OIL ANALYSIS

Plant	*Root, grown in vivo*		*Grown in vitro*			
			Callus		*Root differentiated tissue*	
	a	*b*	*a*	*b*	*a*	*b*
V. officinalis	0.75	0.7	2.50	0.9	–	
C. macrosiphon	3.50	1.4	0.10	<0.1	5.0	1.6

programme was 7 min at 70°C, then increased to 290°C at 4°C/min, with 2 min at 290°C. The injection port temperature was held at 300°C (falling needle system) and the carrier gas (helium) flow-rate was 0.7 ml/min.

The standard compounds used were α-pinene, isoborneol, borneol, bornyl acetate, isobornyl acetate, β-caryopyllene and isobornyl isovalerate, which were obtained from Roth (Karlsruhe, F.R.G.), and valeranone, which was kindly given by Dr. H. Hendriks (Groningen, The Netherlands). These compounds are all characteristic constituents of *V. officinalis* essential oil[1,6–11], and a chromatogram of their mixture is given in Fig. 1A.

RESULTS AND DISCUSSION

The gas chromatograms of samples of essential oil of *V. officinalis* roots grown *in vivo* and prepared by steam distillation–ether extraction and by steam distillation according to the Dutch Farmakopeia VI displayed essentially identical separation patterns, as confirmed by MS data. A gas chromatogram of the essential oils obtained by steam distillation according to the Dutch Farmakopeia VI is shown in Fig. 1B. The following peaks could be identified by comparison with standard compounds and MS library data[14]: camphene [retention time (r.t.) = 10.2 min], β-pinene (r,.t. = 11.8 min), limonene (r.t. = 14.9 min), terpinolene (r.t. = 18.9 min), isoborneol

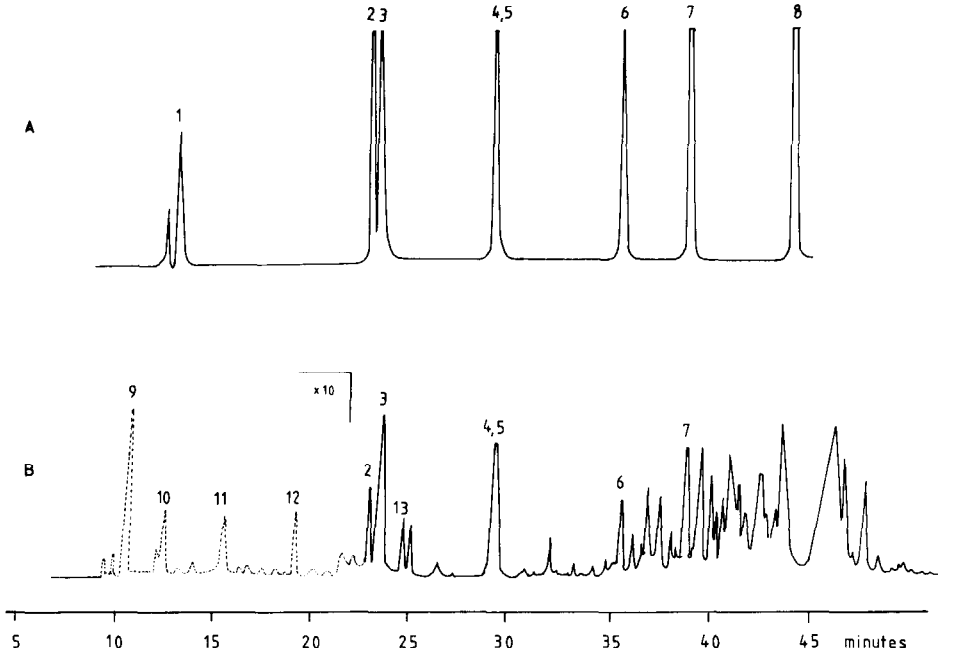

Fig. 1. (A) Gas chromatogram of the mixture of standard compounds: 1 = α-pinene; 2 = isoborneol; 3 = borneol; 4 and 5 = bornyl acetate and isobornyl acetate; 6 = β-caryophyllene; 7 = isobornyl isovalerate; 8 = valeranone. (B) Gas chromatogram of the essential oils of *V. officinalis* roots, grown *in vivo*: 2 = isoborneol; 3 = borneol; 4 and 5 = bornyl acetate and isobornyl acetate; 6 = β-caryophyllene; 7 = isobornyl isovalerate; 9 = camphene; 10 = β-pinene; 11 = limonene; 12 = terpinolene; 13 = α-terpineol.

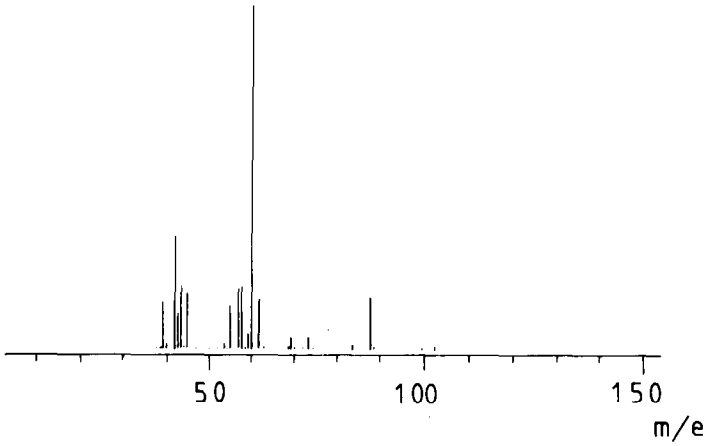

Fig. 2. Mass spectrum of compound X.

(r.t. = 23.0 min), borneol (r.t. = 23.6 min), α-terpineol (r.t. = 24.2 min), isobornyl acetate (r.t. = 29.5 min), β-caryophyllene (r.t. = 35.6 min) and isobornyl isovalerate (r.t. = 39.1 min). There was only one peak in the steam distillated–ether extract which did not appear in the steam distillate; this was due to 2,6-di-*tert.*-butyl-4-methylphenol (BHT), present as a stabilizer in the diethyl ether used. The major identified constituents were borneol, isobornyl acetate, isobornyl isovalerate and β-caryophyllene.

GC–MS of the essential oil of *C. macrosiphon* roots grown *in vivo* allowed the identification of large amounts of isovaleric acid and valeric acid, BHT and peak X, the mass spectrum of which is shown in Fig. 2. Peak X, eluting just after valeric acid, is probably also a lower fatty acid. We have evidence that it could represent a thermal degradation product of valepotriates, as pure valepotriate standards [(iso)valtrate, didrovaltrate, acevaltrate] gave rise to the same peak X when injected under our GC conditions.

The steam distillate–extracts of all our *in vitro* cultures (callus of *C. macrosiphon* and *V. officinalis*, root differentiated tissue cultures of *C. macrosiphon*) proved to contain exclusively lower fatty acids (isovaleric acid, valeric acid, peak X) plus BHT.

In summary, the essential oil of *in vivo* grown *V. officinalis* roots, obtained by the micro steam distillation–extraction procedure has a qualitative composition corresponding to that described in the literature. On the other hand, the essential oil of *in vivo* grown *C. macrosiphon*, prepared in the same way, contains only lower fatty acids and no other vaolatile oil components. Finally, both essential oils isolated from *in vitro* cultures of *V. officinalis* and *C. macrosiphon* also contain lower fatty acids and no other volatile oil components. The lower fatty acids occurring in the steam distillate–extracts from *in vivo* roots of *C. macrosiphon* and also from our *in vitro* cultures probably arise from thermal degradation of valepotriates during steam distillation.

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