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ESSENTIAL OILS FROM NORMAL AND HAIRY ROOTS OF *VALERIANA OFFICINALIS* VAR. *SAMBUCIFOLIA*

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Key Word Index—*Valeriana officinalis* var. *sambucifolia*; Valerianaceae; *Agrobacterium rhizogenes*; hairy roots; essential oil.

Abstract—The composition of the steam-distilled oil from the roots of 9-month-old field-grown *Valeriana officinalis* var. *sambucifolia* was analysed and compared with that from *Agrobacterium*-mediated transformed roots of the same species. Capillary GC and GC–MS studies revealed that the normal oil contained bornyl acetate (13.3%) and valerenal (12.4%) and the transformed oil kessane derivatives, tentatively identified as kessyl alcohol (10.5%) and kessyl acetate (10.4%), as the main constituents.

INTRODUCTION

Valeriana officinalis L. s.l. is widely used in Western Europe for its sedative and antispasmodic properties. However, contradictory ideas still exist about the pharmacological activity of the different constituents of this species [1–6]. Most of the numerous investigations have focused on the two major groups of constituents, the valepotriates and the sesquiterpenes, the latter being steam volatile compounds.

Valeriana officinalis displays a considerable diversity in its morphology. Polyploidy occurs and there are diploid, tetraploid and octoploid types. Several varieties of this collective species are recognized by taxonomists, including *V. officinalis* L. var. *sambucifolia* Mikan fil. (syn. *V. officinalis* subsp. *sambucifolia* (Mikan fil.) Celak), which is an octoploid form.

Although the essential oil of *Valeriana* sp. has been studied extensively, little work has been published on the variety *sambucifolia*, except that of Titz *et al.* [7] on the variation of some characteristic components of the volatile oil of morphologically and karyologically defined types of *V. officinalis* s.l.

As part of an ongoing study on the secondary metabolites of hairy root cultures of *V. officinalis* var. *sambucifolia* [8, 9], we present in this paper the qualitative and quantitative analyses of the volatile constituents produced by the roots of the field-grown plants and we compare these results with those of the volatile oil produced by the transformed roots grown *in vitro*.

RESULTS AND DISCUSSION

The essential oils were obtained by steam distillation in about 0.4% (v/w) and 0.3% yield from the non-transformed roots and from the hairy roots, respectively. These yields do not differ substantially from those reported in the literature [10] for the intact plant roots of *V. officinalis* s.l. The qualitative and quantitative comparison of both essential oils is reported in Table 1. These data revealed that the oil of the transformed roots differs significantly in its composition from that of the field-grown plants.

The GC analysis of the normal roots showed that they contained 63 components and the gas chromatography–mass spectrometry (GC–MS) procedure allowed the identification of 54 of them representing 86% of the essential oil. The mixture is made up of monoterpene hydrocarbons (31%) with α-pinene (**2**), α-fenchene (**3**) and camphene (**4**) as major constituents of this class of compounds, monoterpene esters (16%), sesquiterpene hydrocarbons (17%), oxygenated sesquiterpenes (6%) and such typical valerian cyclopentanoid sesquiterpenes (15%) as valerenal (**48**), valerenyl acetate (**49**), valerenic acid (**50**), valerenol (**53**) and valerenyl isovalerate (**54**). The major components of the normal oil are bornyl acetate (**21**) (13.3%) and valerenal (**48**) (12.4%), these two compounds being considered as characteristic constituents of *V. officinalis* s.l.

The analysis of the volatile constituents in the essential oil of the hairy roots by GC and GC–MS indicated the presence of at least 60 compounds and 38 of these, amounting to 70% (w/w) of the essential oil, were identified. The mixture is mainly composed of sesquiterpene hydrocarbons and oxygenated sesquiterpenes which constitute altogether over 50% (w/w) of the compounds detected. In comparison with the normal oil, there is a marked reduction (10-fold) in the accumulation of monoterpene hydrocarbons (3.8%), whilst the spectrum of oxygenated monoterpenes is very similar in both normal and transformed oils. The reduced capacity of hairy

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roots to accumulate monoterpene hydrocarbons *in vitro* remains unexplained and requires further investigation. Typical oxygenated sesquiterpenes like kessane (**40**), valeranone (**45**), valerenyl acetate (**49**), valerenic acid (**50**), and valerenyl isovalerate (**54**) are missing in the transformed oil. Likewise, many mono- and sesquiterpene hydrocarbons as well as the 3 phenylpropanoid derivatives identified in the volatile oil of the non-transformed roots, isoeugenol (**51**), isoeugenyl acetate (**57**) and benzyl benzoate (**58**), have not been detected in the transformed roots. Furthermore, only four components (ar-curcumene (**38**), 1-hexanol (**16**), isocaryophyllene (**27**) and drimenol (**56**)) were identified in the transformed root oil and do not appear in the roots of the field-grown plants.

Table 1. Essential oil composition from hairy roots (HR) and non-transformed roots (NTR) of *Valeriana officinalis* var. *sambucifolia*

Peak	Compound (M_r)	GC-FID% HR	NTR	I_c^*
1	Tricyclene (136)	< 0.1	< 0.1	1015
2	α-Pinene (136)	0.8	7.3	1028
3	α-Fenchene (136)	1.1	8.9	1064
4	Camphene (136)	1.0	8.6	1074
5	Hexanal (100)	< 0.1	< 0.1	1089
6	β-Pinene (136)	0.3	1.8	1110
7	Sabinene (136)	< 0.1	0.3	1125
8	β-Myrcene (136)	n.d.	< 0.1	1165
9	α-Phellandrene (136)	n.d.	< 0.1	1172
10	α-Terpinene (136)	< 0.1	< 0.1	1184
11	Limonene (136)	< 0.1	1.8	1204
12	β-Phellandrene (136)	n.d.	0.8	1220
13	γ-Terpinene (136)	< 0.1	0.7	1250
14	*p*-Cymene (134)	< 0.1	0.3	1274
15	α-Terpinolene (136)	n.d.	< 0.1	1285
16	1-Hexanol (102)	< 0.1	n.d.	1348
17	δ-Elemene (204)	8.5	5.4	1479
18	Bicycloelemene (204)	n.d.	< 0.1	1490
19	β-Selinene (204)	< 0.1	0.5	1522
20	Carvacrol methyl ether (164)	n.d.	0.5	1585
21	Bornyl acetate (196)	6.6	13.3	1592
22	β-Elemene (204)	1.3	0.9	1605
23	β-Caryophyllene (204)	3.6	1.8	1619
24	α-Gurjunene (204)	2.9	2.7	1636
25	γ-Elemene (204)	0.4	< 0.1	1650
26	5-Camphenyl acetate (194)	0.2	< 0.1	1660
27	Isocaryophyllene	0.5	n.d.	1665
28	Aromadendrene (204)	n.d.	1.7	1670
29	α-Elemene (204)	n.d.	0.2	1685
30	α-Humulene (204)	3.8	< 0.1	1690
31	Myrtenyl acetate (194)	2.5	2.2	1700
32	Ledene (204)	n.d.	0.5	1707
33	Isovaleric acid (102)	0.3	0.4	1715
34	β-Cadinene (204)	0.6	0.2	1720
35	β-Cubebene (204)	n.d.	1.4	1735
36	Bicyclogermacrene (204)	1.0	1.5	1755
37	δ-Cadinene (204)	0.6	< 0.1	1770
38	Ar-curcumene (202)	1.1	n.d.	1780
39	Myrtenol (152)	3.1	< 0.1	1792
40	Kessane (222)	n.d.	0.5	1800
41	β-Ionone (192)	1.6	1.5	1954
42	Ledol (222)	n.d.	0.5	2030
43	$C_{15}H_{26}O$† (222)	1.0	0.3	2094
44	$C_{15}H_{26}O$† (222)	0.7	0.3	2108
45	Valeranone (222)	n.d.	0.9	2155
46	δ-Cadinol (222)	0.4	< 0.1	2222
47	Bisabolol (222)	n.d.	0.4	2230
48	Valerenal (218)	4.1	12.4	2240
49	Valerenyl acetate (262)	n.d.	1.0	2290
50	Valerenic acid (234)	n.d.	1.0	2305
51	Isoeugenol (164)	n.d.	< 0.1	2375
52	Kessyl acetate‡ (280)	10.4	0.5	
53	Valerenol (220)	0.5	0.6	
54	Valerenyl isovalerate (304)	n.d.	< 0.1	
55	Kessyl alcohol§ (238)	10.5	1.0	
56	Drimenol (222)	< 0.1	n.d.	
57	Isoeugenyl acetate (206)	n.d.	1.6	
58	Benzyl benzoate (212)	n.d.	< 0.1	

*Retention indices calculated as described by Van Den Dool and Kratz [18, 19]. I_c greater than 2400 (tetracosane) were not determined.

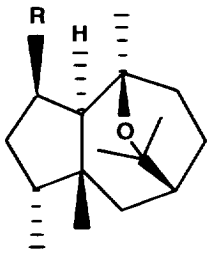
†Globulol or viridiflorol.

‡Or kessanyl acetate.

§Or kessanyl alcohol.

Ar-curcumene has been identified previously in the roots of European *V. officinalis* s.l. [11] but the three latter compounds represent new constituents of this species. Drimenol, considered as a sesquiterpene of the bicyclofarnesol series, is rare in nature and it is identified in the genus *Valeriana* for the first time. The $[M]^+$ at *m/z* 222 and characteristic peaks at *m/z* 191, 124, 109 (base peak), 98 and 81 were in good agreement with data given by Huneck [12].

The major constituents of the transformed oil were two kessane derivatives. The EI mass spectral fragmentation of the major compound (**55**) exhibited a $[M]^+$ at *m/z* 238 corresponding to the formula $C_{15}H_{26}O_2$ with major ions at *m/z* 207, 189, 124, 95, 81 and 43 (base peak), suggesting kessyl or kessanyl alcohol. The second major constituent (**52**) showed a $[M]^+$ at *m/z* 280 [$C_{17}H_{28}O_3$] and other typical fragments at *m/z* 220, 126 (base peak), 108, 93, 81 and 43, consistent with kessyl or kessanyl acetate. For both compounds, and in the absence of other spectroscopic data, it was not possible to define the positions of the hydroxyl and the acetoxyl groups on the kessane skeleton. However, the fragmentation pattern of compound **52** was in good accord with data published by Suzuki *et al.* [13]. On the basis of this observation, it seems likely that compound **52** is kessyl acetate rather than kessanyl acetate. These two compounds are only present in small amount in the oil of the normal roots (1 and 0.5%, respectively). No other kessane derivatives were detected in the mixture. The presence of kessane



Kessyl alcohol OH

Kessyl acetate OAc

derivatives in high amount in the essential oil of the transformed roots of *V. officinalis* is of great importance because it is generally considered that these derivatives are typical of Indian and Japanese valerian or kesso. These compounds have been reported in *V. officinalis* var. *angustifolia* Miq. [14] and *V. fauriei* Briq. [13]. The other predominant compounds were δ-elemene (**17**) (8.5%), bornyl acetate (**21**) (6.6%), valerenal (**48**) (4.1%) and α-humulene (**30**) (3.8%). Important differences were also noticed in the percentages of compounds α-pinene (**2**), α-fenchene (**3**), camphene (**4**) and valerenal (**48**) between the normal and the transformed oils.

It is well known that the volatile constituents of the Valerianaceae are synthesized and stored as oily droplets in roots and rhizomes. In order to check if the difference in oil profiles could be explained by the morphology of the two types of roots, transverse sections of hairy and normal roots were examined by microscopy. It was found that their anatomical features were similar. In particular, the structures of the hypodermis and the contiguous parenchyma of the cortex in which the valepotriates and the essential oil are stored were identical in the two types of roots.

From the results of this investigation, it is obvious that the composition of the essential oils of the normal and the transformed roots of *V. officinalis* var. *sambucifolia* is complex. Numerous differences have been noticed, in particular the high content of kessyl alcohol (10.5%) and kessyl acetate (10.4%) in the hairy roots, whereas these compounds are present in low amounts in the normal oil. A similar investigation has been reported by Kennedy *et al.* [15] on the volatile oil extracted from hairy roots of *Artemisia absinthium* and the comparison with the oil produced *in vivo* by the normal roots of the field-grown plant. Significant qualitative and quantitative differences were also reported in the oil profiles. Up to now, no satisfactory explanation has been put forward to account for such differences between the normal and the transformed essential oils. It would be of great interest to develop *in vitro* tissue cultures for studying the biosynthetic pathways of the compounds and the system of gene regulation involved.

EXPERIMENTAL

Plant material. The seeds of *Valeriana officinalis* var. *sambucifolia* were provided by the Station Fédérale de Recherches Agronomiques, Centre d'Arboriculture et d'Horticulture des Fougères (Conthey, Switzerland). The plants grown in the field for 9 months were identified by comparison with the authentic herbarium specimens of the Conservatoire et Jardin Botaniques in Geneva. A voucher specimen is deposited in the authors' laboratory.

Establishment and cultivation of the hairy roots. The establishment of the hairy roots was previously described [8]. The hairy roots were cultured in half strength Gamborg B5 [16] liquid medium supplemented with 2% sucrose. The medium was hormone-free and adjusted to pH 5.9 before autoclaving. The cultures were maintained in darkness at 25° on a gyratory shaker at 80 rpm for 40 days.

Extraction procedure. Fresh roots (either hairy roots or non-transformed roots) (400 g) were steam distilled for 5 hr using the apparatus as specified in the *European Pharmacopoeia* [17]. The distillate was collected in *n*-pentane (Fluka, Switzerland), dried over Na_2SO_4 and finally adjusted to 1 ml with the same solvent.

Analysis of essential oils. GC–FID measurements were performed on a HP 5890 gas chromatograph equipped with a Supelcowax 10 column (60 m × 0.25 mm i.d. and 0.25 μm film thickness). The temp. prog. was isothermal at 60° for 5 min, 60–230° at 5° min^{-1}, isothermal at 230° for 20 min. Both injector and detector temps were maintained at 250°. Carrier gas: He at 140 kPa. Injected volume: 0.04 μl in splitless mode.

For GC–MS analysis, the HP 5890 gas chromatograph was coupled with a HP 5989A mass spectrometer operating in EI mode at 70 eV (ion source temp.: 160°). Injected volume: 1 μl in split mode (split ratio 1:20) on a Supelcowax 10 column. The other operating conditions were as described in GC part.

Identification of components. The identification of the compounds was achieved on the basis of the mass spectra library of Firmenich Co. (Geneva, Switzerland) and by comparison of the retention indices, calculated according to Van Den Dool and Kratz [18, 19], with literature data. Eighteen normal paraffins, C_7 to C_{24} (Fluka, Switzerland), were used as the reference series.

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