Essential Oil Constituents of *Valeriana italica* and *Valeriana tuberosa*. Stereochemical and Conformational Study of 15-Acetoxyvaleranone

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Valeriana italica, *V. tuberosa*, Essential Oil, 15-Acetoxyvaleranone

The chemical composition of the essential oils obtained by hydrodistillation of roots, stems with leaves and inflorescences of *Valeriana italica* Lam. and *Valeriana tuberosa* L. were studied by GC/MS. Seventy-three and fourty-one constituents were identified from each plant, respectively. The major constituent of the oil obtained from the roots of *V. italica* was isolated and identified as 15-acetoxyvaleranone. Its stereochemistry and conformation has been studied using NMR spectroscopy and molecular modelling. The oils obtained from *V. tuberosa* completely lacked the characteristic valerane or kessane sesquiterpenes.
Running title: Essential oils of *Valeriana italica* and *V. tuberosa*

Introduction

The crude drug Valerianae radix and the valerian-derived phytomedicines that are widely used as mild sedatives in Europe, have a great contribution in the pharmaceutical industry of the medicinal plants. Valerian ranks at the 8th place among the top-selling herbal supplements (Blumenthal, 2001), making very interesting the research about the chemical constituents of the species belonging to the same genus. The *Valeriana* genus contains 20 species found in Europe (Ockendon, 1976). Two of them, *Valeriana tuberosa* L. and especially *V. italica* Lam. (syn. *V. dioscoridis* Sibth & Sm), are common in southern Greece (Strid and Tan, 1991), where *V. officinalis* is absent.

The pharmacological activity of the Valerianae radix has been attributed to two major groups of constituents, the valepotriates and the sesquiterpenes. The latter are volatile compounds found in the essential oil of the roots and some of them such as α-kessyl alcohol or kessanol have a sedative effect comparable to imipramine (Oshima *et al.*, 1995). In addition, according to the European Pharmacopoeia, the crude drug Valerianae radix must contain not less than 0.5% (v/m) of essential oil.

To our best knowledge, no previous phytochemical works have been recorded for the two aforementioned *Valeriana* species found in Greece. As a part of the research on the essential oils of plants of the Greek flora (Magiatis *et al.*, 1999), we report herein on the chemical constituents of the essential oils obtained by hydrodistillation of roots, stems and inflorescences of *V. tuberosa* and *V. italica.*

Results and Discussion

The chemical composition of the oils was analysed using various gas chromatography-mass spectrometric GC/MS techniques (EI and CI). Qualitative and quantitative analytical results are listed in Table I along with the retention indices of the identified compounds. The identification of components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (Massada, 1976) and those described by Adams (2001), as well as on comparison of their retention indices (Van den Dool, 1963) with literature values (Adams, 2001).

The analysis of the essential oils of *V. tuberosa* led to the identification of fourty-one constituents. The major constituents of the essential oil of the roots were *trans*-caryophyllene (72.7%), caryophyllene oxide (10.9%) and camphene (5.8%), of the stems and leaves were phytol (16.2%), eicosanoic acid

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Table I. Chemical composition (%) of the essential oils of *V. italica* and *V. tuberosa*[a].

	V. italica			*V. tuberosa*			
	Roots	Stems and leaves	Inflorescences	Roots	Stems and leaves	Inflorescences	RI
Furfural	–	1.02	3.01	–	1.51	3.84	830
Isovaleric acid	1.01	4.05	15.76	–	6.28	0.51	833
n-Hexanol	–	–		–	–	3.72	867
Thujene	0.22	–	–	–	–	–	931
α-Pinene	7.47	–	–	–	–	–	937
3-Methyl valeric acid	–	–	2.99	–	–	5.03	947
α-Fenchene	6.21	–	–	–	–	–	950
Camphene	–	–	–	5.83	–	–	953
β-Pinene	3.04	–	–	–	–	–	981
Myrcene	0.41	–	–	–	–	–	991
α-Terpinene	0.15	–	–	–	–	–	1017
p-Cymene	3.04	–	–	–	–	–	1024
Limonene	1.35	–	–	–	–	–	1029
cis-Ocimene	1.42	–	–	–	–	–	1037
Benzenacetaldeyde	–	–	1.15	–	–	0.65	1043
trans-Ocimene	3.54	–	–	–	–	–	1049
γ-Terpinene	2.03	–	–	–	–	–	1064
Terpinolene	0.39	–	–	–	–	–	1088
Nonanal	–	–	–	–	–	1.34	1097
Linalool	–	–	0.20	–	–	–	1098
1,4-Dimethoxy benzene	–	–	–	–	–	11.30	1163
Terpin-4-ol	0.38	–	–	–	–	–	1175
Naphthalene	–	–	–	–	1.07	0.46	1179
α-Terpineol	0.30	–	–	–	–	–	1189
Decanal	–	–	–	–	–	0.50	1197
Thymol methyl ether	4.50	–	–	–	–	–	1234
Carvacrol methyl ether	1.02	–	–	–	–	–	1246
Bornyl acetate	0.80	–	–	2.42	–	–	1285
Thymol	1.63	–	–	–	–	–	1289
Carvacrol	0.42	–	–	–	–	–	1298
α-Cubenene	0.18	–	–	–	–	–	1353
Eugenol	–	–	–	–	–	0.47	1358
α-Copaene	0.15	–	–	–	–	–	1377
trans-β-Damascenone	–	–	–	–	1.03	0.35	1379
β-Elemene	0.32	–	–	–	–	–	1389
Methyl eugenol	–	–	–	–	–	0.45	1401
α-Gurjunene	0.35	–	–	–	–	–	1408
trans-Caryophyllene	0.19	1.14	–	72.70	–	2.04	1418
β-Gurjunene	6.62	–	–	–	–	–	1434
α-Guaiene	0.22	–	–	–	–	–	1438
α-Humulene	1.17	–	–	5.77	–	–	1454
allo-Aromadendrene	1.81	–	–	–	–	–	1462
γ-Muurolene	0.32	–	–	–	–	–	1479
Germacrene-D	1.40	–	–	–	–	–	1485
β-Selinene	0.52	–	–	–	–	–	1490
Zingiberene	–	–	–	–	–	0.51	1494
Valencene	0.21	–	–	–	–	–	1496
Bicyclogermacrene	1.01	–	–	–	–	–	1499
α-Muurolene	0.23	–	–	–	–	–	1500
Pentadecane	–	–	2.88	–	–	–	1500
trans, *trans*-α-Farnesene	–	–	0.55	–	–	13.28	1506
α-Bulnesene	0.34	–	–	–	–	–	1508
γ-Cadinene	0.15	–	–	–	–	–	1512
7-*epi*-α-Selinene	0.27	–	–	–	–	–	1518
δ-Cadinene	0.83	–	–	–	–	–	1524
Germacrene-D-4-ol	5.90	–	–	–	–	–	1574
Globulol	0.38	–	–	–	–	–	1580

Table I. (cont.)

	V. italica			*V. tuberosa*			
	Roots	Stems and leaves	Inflorescences	Roots	Stems and leaves	Inflorescences	RI
Caryophyllene oxide	–	–	–	10.90	–	–	1582
Viridiflorol	0.56	–	–	–	–	–	1591
Isospathulenol	0.68	–	–	–	–	–	1639
α-Muurolol	1.24	–	–	–	–	–	1646
α-Cadinol	2.32	–	–	–	–	–	1654
Valerenol	0.51	–	–	–	–	–	1655
Valeranone	4.39	–	–	–	–	–	1672
Pentadecanal	–	–	–	–	0.40	1.78	1715
Benzyl benzoate	–	–	0.61	–	–	0.52	1763
Tetradecanoic acid	–	–	–	–	0.48	–	1767
Octadecane	–	–	–	–	–	0.52	1800
Neophytadiene	–	1.60	1.18	–	2.48	0.32	1837
6,10,14-trimethyl-2-pentadecanone	–	1.84	4.91	–	0.74	8.93	1845
Ethyl linoleolate	–	–	–	–	–	0.54	1891
Phytol	–	12.10	3.72	–	16.20	–	1943
Hexadecanoic acid	–	4.47	1.08	–	3.85	–	1966
15-Acetoxyvaleranone	14.50	–	–	–	–	–	2035
Heneicosane	–	1.62	1.35	–	1.59	1.41	2100
Octadecanoic acid methyl ester	–	–	–	–	–	1.94	2128
Docosane	–	–	0.40	–	0.70	–	2200
Tricosane	–	3.09	21.49	–	4.77	2.86	2300
Kessyl alcohol	0.31	–	–	–	–	–	–
Eicosanoic acid methyl ester	–	–	–	–	15.00	7.15	–
Eicosanoic acid	–	1.13	3.41	–	–	–	–
Tetracosane	–	–	2.24	–	0.88	0.61	–
Heneicosanoic acid methyl ester	–	–	–	–	–	0.43	–
Pentacosane	–	3.99	14.35	–	2.62	5.96	–
Docosanoic acid methyl ester	–	1.77	4.48	–	13.46	–	–
Docosanoic acid	–	–	–	–	–	5.31	–
9-Hexacosene	–	–	–	–	0.48	–	–
Hexacosane	–	–	0.39	–	0.48	–	–
Eptacosane	–	4.71	5.20	–	2.1	3.68	–
Tetracosanoic acid methyl ester	–	–	1.01	–	1.33	–	–
Octacosane	–	1.09	0.33	–	–	–	–
Cyclotetracosane	–	2.8	–	–	–	–	–
Nonacosane	–	50.7	3.77	–	4.80	0.74	–
Total	86.4	97.12	96.46	97.62	82.25	87.15	

[a] Compounds listed in order of elution from a HP-5 MS column. RI over 2300 were not determined.

methyl ester (15.0%), docosanoic acid methyl ester (13.5%) and of the inflorescences were *trans*, *trans*-α-farnesene (13.3%), 1,4-dimethoxy benzene (11.3%) and 6,10,14-trimethyl-2-pentadecanone (8.9%). It is interesting to point out that all the essential oils of *V. tuberosa* were lacking of the characteristic valerane or kessane sesquiterpenes.

The analysis of the essential oils of *V. italica* afforded seventy-three constituents. The major constituents of the essential oil of the roots were 15-acetoxyvaleranone (14.5%), α-pinene (7.5%) and β-gurjunene (6.6%), of the stems and leaves were nonacosane (50.7%), phytol (12.1%) and isovaleric acid (5.00%) and of the inflorescences were tricosane (21.5%), isovaleric acid (15.8%) and pentacosane (14.4%).

The major constituent of the oil obtained from the roots of *V. italica* could not be directly identified by GC/MS. It was isolated with column chromatography and identified by ^{1}H-NMR, ^{13}C-NMR and 2D NMR spectroscopy as 15-acetoxyvaleranone (**1**). This compound has also been described as a constituent of Indian valerian oil (*V. wallichi*) (Kulkarni *et al.*, 1964), which is used in a similar way as *V. officinalis* (Lewis, 1977), but its high resolution ^{1}H-NMR, ^{13}C-NMR and MS data as well as its retention index have never been reported. Moreover, the stereochemistry of **1** has never been studied spectroscopically, but only with chemical correlation reactions (Kulkarni *et al.*, 1964). In order to investigate the stereochemistry and the conformation of **1**, a detailed study including 2D NOESY and molecular modelling experiments has been performed. In the NOESY experiment, the strong correlation between the methyl protons of position 14 and the methylene protons of position 15 made clear that the carbon skeleton was indeed a *cis*-fused decalin. The orientation of the isopropyl side chain was unequivocally found to be equatorial since the splitting pattern and the coupling constant of H-6_{ax} (t, J = 12.3 Hz) revealed the presence of an axial proton at position 7. With these two basic observations, two possible relative stereochemistry structures could be assigned: **1** and **2** (or their enantiomers), which can be depicted as the conformers **1a** and **2a** (Fig. 1). The other possible conformers e. g. **1b** in which the side chain is axially oriented are excluded due to the already discussed coupling constant of H-6ax. The possibility of non-chair conformers was excluded using molecular mechanics calculations. As expected the lowest energy conformations **1a** and **2a** were much more stable than any other conformer including non-chair rings. The discrimination between **1a** and **2a** was based on the NOESY spectrum which showed that H-6_{ax} was correlated with H-1_{ax} and H-3_{ax} and not with H-14 or H-15. This observation was consistent only with **1a** confirming the proposed relative stereochemistry (Kulkarni *et al.*, 1964). As far as it concerns the

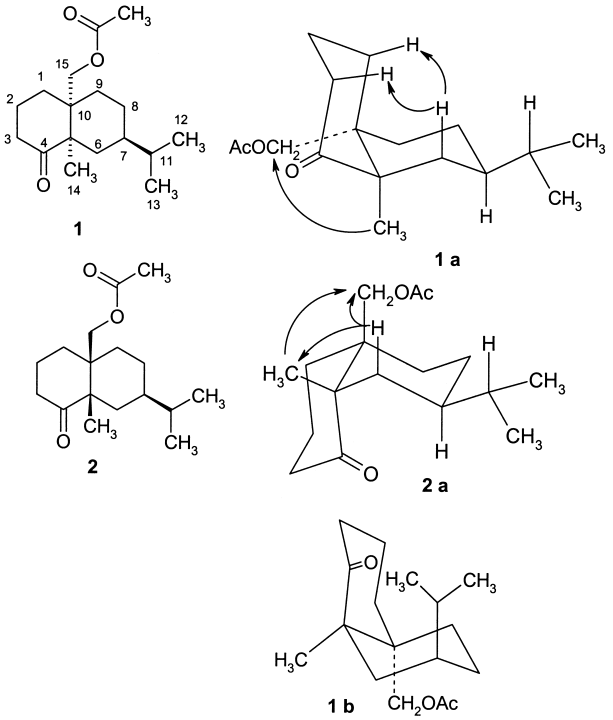

Fig. 1. Observed NOE correlations for **1a** and expected NOE correlations for **2a**.

absolute stereochemistry, in our hands **1** showed the same optical rotation and the same cotton effect as described (Kulkarni *et al.*, 1964). The strong negative cotton effect (Kulkarni *et al.*, 1964; Narasimha, 1971) is indeed consistent only with the absolute stereochemistry as depicted in **1**. Consequently **1a** depicts the real structure of 15-acetoxyvaleranone.

In conclusion, the presence of significant amounts of valerane sesquiterpenes (*ca.* 20%), in the essential oil of the roots of *V. italica* indicates a new useful and abundant source, which can be further exploited and developed.

Experimental

General experimental procedures

GC-MS analysis was obtained from a Hewlett-Packard 6890–5973 system operating on EI mode, equipped with a capillary column HP-5 MS 30 m × 0.25 mm; film thickness: 0.25 μm; temperature program: 60 °C (5 min) to 280 °C at a rate of 3 °C/min; inj. temp. 200 °C. GC-MS analysis was also performed on a Finnigan GCQ *Plus* ion-trap mass spectrometer with an external ion source in both the EI and chemical ionization (CI) modes at a flow rate of 1.0 ml/min using CH_4 as the CI ionization reagent. NMR spectra were recorded on Bruker spectrometer [^1H (500 MHz) and ^{13}C (100 MHz)]. Molecular calculations were performed using the MM+ force field. The Polak-Ribiere (conjugate gradient) minimization method with an energy convergence criterion of 0.04 KJ mol^{-1} was used for geometry optimization.

Plant material

V. tuberosa was collected in Lakonia at Parnon mountain (1400–1600 m height) in May 2001. *V. italica* was collected in Attiki at Imitos mountain (1000 m height) in April 2001. A voucher specimen of each plant (KL129, NEK018) is deposited in the herbarium of the Division of Pharmacognosy, University of Athens.

Distillation of the oils and isolation of 15-acetoxyvaleranone

The fresh plant material was separated in roots, stems with leaves and infloresceces (200 g of each part) and submitted for 3 h to hydrodistillation with 3 l H_2O. *V. tuberosa* afforded 0.18 ml (0.09%), 0.16 ml (0.08%) and 0.23 ml (0.12%) of essential oil from each part respectively. *V. italica* afforded 0.42 ml (0.21%), 0.20 ml (0.10%) and 0.24 ml (0.12%) of essential oil from each part respectively. The essential oils were dried over anhydrous sodium sulfate and stored at 4–6 °C. A portion of the essential oil of *V. italica* was submitted to column chromatography using flash Si gel 60 Merck (40–63 μm) with CH_2Cl_2/MeOH: 99:1 as the eluent to afford **1**.

15-Acetoxyvaleranone (**1**), $[\alpha]_D$ −40°(*c* 0.2, CH_2Cl_2); ^1H NMR ($CDCl_3$/TMS, 400 MHz, δ ppm, J in Hz): 0.88 (3H, d, J = 6.8 Hz, H-12), 0.90 (3H, d, J = 6.8 Hz, H-13), 1.10 (3H, s, H-14), 1.22 (1H, dd, J = 12.3, 3.1 Hz, H-6_{eq}), 1.36 (1H, m, H-7), 1.36 (1H, m, H-8_{eq}), 1.45 (1H, m, H-11), 1.48 (1H, m, H-1_{eq}) 1.50 (2H, m, H-9), 1.68 (1H, m, H-8_{ax}), 1.76 (1H, t, J = 12.3 Hz, H-6_{ax}), 1.92–2.00 (2H, m, H-2), 2.00 (3H, s, CH_3), 2.30 (1H, ddd, J = 15.6, 5.1, 1.6 Hz, H-3_{eq}), 2.36 (td, J = 13.9, 5.6 Hz, H-1_{ax}), 2.69(1H, ddd, J = 15.6, 13.2, 8.3 Hz, H-3_{ax}), 3.81 (1H, d, J = 11.4 Hz, H-15b), 3.98 (1H, d, J = 11.4 Hz, H-15a); ^{13}C NMR ($CDCl_3$/TMS, 100 MHz, δ ppm): 210.00 (C-4), 165.00 ($\underline{C}OCH_3$), 70.51 (C-15), 50.89 (C-3), 41.36 (C-10), 38.48 (C-7), 37.63 (C-6), 36.40 (C-3), 32.69 (C-11), 30.96 (C-9), 27.96 (C-1), 23.91(C-8), 21.37 (C-2), 20.71 ($CO\underline{C}H_3$), 19.71 (C-CH_3), 19.59 (C-CH_3), 16.28 (C-CH_3); MS-EI: m/z = 280 [M^+] (35), 238 (8), 207 (100), 189 (15), 149 (40).

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Adams R. (2001), Identification of essential oil components by gas chromatophy/ quadrupole mass spectroscopy, Allured Publishing Co, Carol Stream, USA.

Blumenthal M. (2001), Herb sales down 15 percent in mainstream market. HerbalGram **59**, 69.

Kulkarni K. S., Paknikar S. K. and Bhattacharyya S. C. (1964), Structure and stereochemistry of hydroxyvaleranone and acetylhydroxyvaleranone. Tetrahedron **20**, 1289–1300.

Lewis W. (1977), Medical Botany. Plants Affecting Man's Health. John Wiley & Sons, New York, p. 168.

Magiatis P., Melliou E., Skaltsounis A. L., Chinou I. and Mitaku S. (1999), Chemical composition and activity of the essential oils of *Pistacia lentiscus* var. *chia*. Planta Med. **65**, 749–751.

Massada Y. (1976), Analysis of Essential Oil by Gas Chromatography and Spectrometry. John Wiley & Sons, New York.

Narasimha R. P. (1971), Conformation of valerane. J. Org. Chem. **36**, 2426–2434.

Ockendon D. J. (1976), Flora Europaea, Vol. **4**. Cambridge University Press, London, p. 52–55.

Oshima Y., Matsuoka S. and Ohizumi Y. (1995), Antidepressant principles of *Valeriana fauriei* roots. Chem. Pharm. Bull. **43**, 169–170.

Strid A. and Tan K. (1991), Mountain Flora of Greece, Vol. **2**. Edinburgh University Press, Edinburgh, p. 344.

Van den Dool H. and Kratz P. D. (1963), A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chrom. **11**, 463–471.