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ISOLATION AND IDENTIFICATION OF VALERENANE SESQUITERPENOIDS FROM *VALERIANA OFFICINALIS**

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Key Word Index—*Valeriana officinalis*; Valerianaceae; cyclopentane sesquiterpenoid acids; valerenal; valerenol; valerenyl esters; EI and negative ion chemical ionization GC/MS; IR; ^{1}H NMR; ^{13}C NMR.

Abstract—Seven new valerenane sesquiterpenoids were isolated from a dichloromethane extract of *Valeriana officinalis*. The NMR, IR and mass spectral data of the isolated compounds, including the valerenic acids, are given in this paper.

INTRODUCTION

Valerenal (1), valerenic acid (2), hydroxy valerenic acid (3) and acetoxyvalerenic acid (4) are known constituents of *Valeriana officinalis* L.s.l. [1, 2]. In addition to these compounds, valerenol (5) and a series of valerenyl esters (6–9) were identified, for the first time, in our investigation of *V. officinalis*.

RESULTS AND DISCUSSION

The esters were identified as a pair of acetates and a pair of (iso) valerates by negative ion chemical ionization (NICI) mass spectrometry [3, 4]. The NICI and EI mass spectra were almost identical within each pair. Significant differences, however, were found in their ^{1}H and ^{13}C NMR spectra. In particular, the signals of the isobutenyl part differed in the same way as in known spectra of *E*- and *Z*-2-substituted unsaturated alcohols and their acetates [5–7].

In accordance with the combined spectral data we have found *E-valerenol* (5), *E*- and *Z*-valerenyl acetate (6a/b), *E*- and *Z*-valerenyl isovalerate (7a/b), valerenyl *n*-valerate (8) and valerenyl *n*-hexanoate (9). Characteristic values for the chemical shifts in the ^{1}H and ^{13}C NMR spectra of the *E*- and *Z*-isomers are given in Tables 1 and 2. By NICI-MS, we have identified two minor components as (*Z* or *E*)-valerenyl valerate and (*Z* or *E*)-valerenyl hexanoate, in which the hexanoic acid part may be either linear or branched. During the NICI-GC/MS experiment ferulic acid, bornyl acetate and α-terpenyl acetate, all three known components of *Valeriana*, were observed, while three previously unknown constituents were tentatively identified, as citronellyl isovalerate, citronellyl hexanoate and methyl citronellate.

EXPERIMENTAL

Dried, powdered roots (30 kg) of a commercial strain of *V. officinalis* L.s.l. (V.N.K., Elburg The Netherlands) were extracted with about 100 l CH_2Cl_2. The extract was concd to 3 l and extracted (× 2) with the same vol. of an aq. 2% NaOH soln. The combined aq. layers were acidified and extracted (× 2) with 2 l

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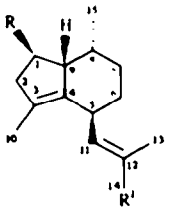

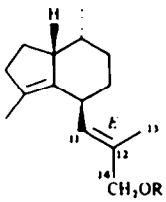

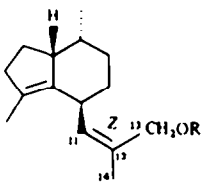

	R^1	R^2	
1	CHO	H	Valerenal
2	COOH	H	Valerenic acid
3	COOH	OH	Hydroxyvalerenic acid
4	COOH	OAc	Acetoxyvalerenic acid

5	R=H	Valerenol
6a	R=Ac	*E*-Valerenyl acetate
7a	R=CO-CH_2-CH(Me)$_2$	*E*-Valerenyl isovalerate
6b	R=Ac	*Z*-Valerenyl acetate
7b	R=CO-CH_2-CH(Me)$_2$	*Z*-Valerenyl isovalerate

8 R=CO-C_4H_9 Valerenyl *n*-valerate
9 R=CO-C_5H_{11} Valerenyl hexanoate

Table 1. ^{1}H NMR data for compounds 5, 6a/b and 7a/b (TMS = 0)

E	5	6a	7a	*Z*	6b	7b
=CH-11	5.73		5.76	=CH-11	5.72	
Me-13	1.72		1.70	Me-14	1.75	
CH_2-14	3.99	4.4	4.46	CH_2-13	4.64	4.64

Table 2. ^{13}C NMR data for compounds 5, 6b and 7a/b (TMS = 0)

E	5	7a	*Z*	6b	7b
C-11	127.6	131.0	C-11	132.3	132.3
C-12	135.2	135.0	C-12	134.9	
C-13	13.7	13.9	C-14	63.2	63.0
C-14	69.2	70.0	C-13	21.4	

petrol (bp < 40°)–Et_2O (2:1), giving a soln of the organic acids (soln A). The remaining CH_2Cl_2 extract was washed with H_2O until neutral and was concd by means of a rotary evaporator. The residue was redissolved in 3 l petrol (bp < 40°) and filtered (soln B). After evaporation of the solvent from soln B, 500 g of a residue containing the terpenoid compounds was obtained. This was diluted with an equal vol. of petrol (bp < 40°) and transferred onto a silica gel column (Merck 7734, 125 × 12 cm.). CC was performed, using 20 l petrol, 10 × 5 l of petrol–Et_2O mixtures (99:1 to 90:10 in 1% steps), 5 l petrol–Et_2O (3:1), 5 l petrol–Et_2O (1:1) and 10 l Et_2O.

Fractions of about 1 l each were collected; after GC analysis, fractions 26–34 were combined, concd and submitted to EI- and NICI-GC/MS [3, 4]. *Z*-Valerenyl acetate and *E*-/*Z*-valerenyl isovalerate were isolated by means of prep. TLC (hexane–Et_2O, 4:1) followed by prep. GC. After evaporation of the solvent, soln A yielded about 500 g of a residue which was redissolved in 1 l of pentane and stored at −20° for 24 hr. The ppt contained ferulic acid, valerenic acid and hydroxy valerenic acid. This ppt was submitted to CC (silica gel Merck 7734, 40 × 4 cm) using petrol (bp < 40°)–Et_2O mixtures from 10 upto 100% Et_2O in 10% steps. The 20% Et_2O fraction contained valerenic acid while ferulic acid and hydroxyvalerenic acid were obtained after elution with 100% Et_2O. These compounds were isolated by prep. TLC (hexane–Et_2O, 1:4). Acetoxy valerenic acid was obtained by prep. TLC of the remaining pentane using hexane Et_2O (3:2).

MS. Finnigan 9500/3300/6110 GC/MS computer system, modified for negative ion operation [8], fused silica capillary column (25 m × 0.32 mm CP sil 5, Chrompack, Middelburg, The Netherlands), temp. programme 60–300° at 6°/min, carrier gas He. Reactant gas mixture for negative ion CI: approximately 1:1 CH_4-nitrous oxide [3]. Cycle time during acquisition of mass spectra: 1 sec.

Capillary GC. Packard Instruments 430 dual flame. Temp. programme 60–250° at 4°/min, fused silica capillary column (25 m × 0.22 mm CP sil 5 Chrompack, Middelburg, The Netherlands), carrier gas N_2, 1 ml/min, injector and detector temp. 250°.

Prep. GC. F & M 700 and F & M 720 kath. OV 17 column (2 m × 1 cm i.d.), 200° isothermal, carrier gas He, 50 ml/min.

NMR. Jeol FX-100 operating at 99.55 MHz for ^{1}H NMR and at 25.0 MHz for ^{13}C NMR.

TLC. Prep. silica gel plates (20 × 20 cm, 1 mm G1510 LS 254, Schleicher & Schuell, Dassel, W. Germany).

Valerenal (1). EI-GC/MS *m/z* (rel. int.): 39 (40), 41 (79), 43 (30), 51 (12), 53 (31), 55 (53), 65 (25), 67 (25), 69 (13), 77 (57), 78 (18), 79 (68), 80 (12), 81 (37), 91 (100), 92 (15), 93 (50), 94 (11), 95 (31), 103 (12), 105 (74), 106 (19), 107 (55), 108 (22), 109 (26), 115 (22), 117 (25), 119 (38), 120 (11), 121 (33), 122 (12), 128 (11), 129 (13), 131 (21), 133 (44), 134 (14), 135 (17), 145 (30), 147 (63), 148 (28), 149 (13), 160 (13), 161 (57), 162 (13), 175 (96), 176 (15), 185 (71), 186 (10), 189 (32), 203 (60), 218 [M]$^+$ (35); IR ν_{max}^{neat} cm^{-1}: 2702 (aldehyde), 1695 and 1685 (C=O, α,β-unsaturated aldehyde), 1638 (C=C), 832 (trisubstituted C=C); ^{1}H NMR ($CDCl_3$, TMS int. standard: δ0.81 (*d*, 3H, *J* = 6.8 Hz), 1.65 (*m*, 3H), 1.79 (*d*, 3H, *J* = 1.3 Hz), 2.94 (*m*, 1H), 3.71 [*dm*, 1H, *J* = 9.4 Hz (*d*)], 6.73 [*dq*, 1H, *J* = 9.4 Hz (*d*) resp. 1.3 Hz (*q*)], 9.37 (*s*, 1H); ^{13}C NMR ($CDCl_3$, TMS int. standard): δ9.1 *q* (C-13), 11.9 *q*, 13.4 *q*, 24.4 *t*, 25.3 *t*, 28.7 *t*, 32.9 *d* (C-8), 34.6 *d* (C-5), 37.3 *t* (C-2), 47.4 *d* (C-9), 131.8 *s*, 132.5 *s*, 137.3 (C-12), 155.7 *d* (C-11), 195.5 *d* (C-14). The ^{1}H and ^{13}C NMR data were identical to the spectral data of synthetic valerenal [9].

Valerenic acid (2). EI-GC/MS *m/z* (rel. int.): 39 (34), 41 (69), 43 (23), 45 (10), 51 (10), 53 (26), 55 (46), 65 (26), 67 (20), 69 (12), 77 (51), 78 (15), 79 (54), 80 (13), 81 (36), 91 (100), 92 (15), 93 (52), 94 (15), 95 (39), 103 (10), 105 (82), 106 (26), 107 (96), 108 (23), 109 (17), 115 (22), 116 (12), 117 (30), 119 (42), 121 (38), 122 (79), 123 (11), 128 (10), 129 (11), 131 (28), 132 (11), 133 (75), 134 (12), 135 (10), 145 (27), 146 (10), 147 (41), 148 (39), 149 (16), 159 (18), 160 (21), 161 (81), 162 (13), 173 (15), 179 (12), 189 (34), 191 (14), 201 (14), 219 (12), 234 [M]$^+$ (71), 235 (12); IR ν_{max}^{KBr} cm^{-1}: 3600–2400 (COOH), 1675 (C=O, α,β-unsaturated acid), 1630 (C=C); ^{1}H NMR ($CDCl_3$, TMS int. standard): δ0.76 (*d*, 3H), 1.62 (*br s*, 3H), 1.88 (*s*, 3H), 2.10 (*t*, 2H), 2.90 (*br s*, 1H), 3.50 (*br d*, 1H), 7.16 (*d*, 1H), 12.48 (*s*, 1H); ^{13}C NMR ($CDCl_3$, TMS int. standard): identical with published data [10].

Hydroxyvalerenic acid (3). EI-GC/MS *m/z* (rel. int.): 39 (44), 41 (100), 43 (65), 53 (35), 55 (67), 65 (25), 67 (23), 69 (32), 77 (53), 79 (50), 81 (35), 91 (76), 93 (42), 95 (31), 105 (69), 107 (43), 109 (34), 115 (18), 117 (12), 119 (42), 120 (53), 129 (12), 131 (41), 135 (13), 143 (19), 145 (40), 146 (20), 147 (28), 157 (10), 158 (14), 159 (47), 161 (55), 171 (19), 172 (11), 179 (25), 187 (30), 188 (10), 199 (18), 217 (16), 232 (60), 233 (10), 250 [M]$^+$ (8); IR ν_{max}^{KBr} cm^{-1}: 3600–2400 (COOH), 3340 (OH), 1676 (C=O, α,β-unsaturated acid), 1635 (C=C); ^{1}H NMR ($CDCl_3$, TMS int. standard): δ0.74 (*d*, 3H, Me-15, *J* = 6.8 Hz), 1.66 (*br s*, 3H, Me-10), 1.88 (*d*, 3H, Me-13, *J* = 1.3 Hz), 2.7 (*br s*, 1H), 3.5 (*br d*, 1H), 4.17 [*dt*, 1H, *J* = 2.5 (*t*) and 6.8 (*d*) Hz], 5.6 (*br*, 2H, OH and COOH), 7.11 [*dq*, 1H, *J* = 1.5 (*q*) and 6.8 (*d*) Hz]; ^{13}C NMR ($CDCl_3$, TMS int. standard): identical with published data [10].

Acetoxyvalerenic acid (4). EI-GC/MS *m/z* (rel. int.): 39 (10), 41 (21), 43 (57), 45 (10), 51 (13), 52 (25), 53 (10), 55 (14), 59 (10), 77 (14), 79 (45), 91 (26), 105 (36), 107 (11), 117 (10), 119 (15), 120 (100), 121 (13), 131 (28), 143 (13), 145 (25), 146 (13), 147 (11), 159 (31), 171 (13), 187 (20), 217 (10), 232 (58), 233 (13), 292 [M]$^+$ (0); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500–2400 (COOH), 1735 (C=O, acetate), 1692 (C=O, α,β-unsaturated acid), 1635 (C=C); ^{1}H NMR ($CDCl_3$, TMS int. standard): δ0.80 (*d*, 3H, *J* = 6.9 Hz), 1.66 (*d*, 3H, *J* = 1.8 Hz), 1.90 (*d*, 3H, *J* = 1.5 Hz), 2.04 (*s*, 3H, acetate methyl), 2.86 (*s*, 1H), 3.55 (*br d*, 1H, *J* ~ 9.6 Hz), 5.04 (*br d*, 1H, *J* = 7.1 Hz), 7.14 (*dd*, 1H, *J* = 9.6 and 1.5 Hz), 9.6 (*br s*, 1H, COOH); ^{13}C NMR ($CDCl_3$, TMS int. standard): δ12.0 *q* (C-13), 12.7 *q* (C-15), 13.2 *q* (C-10), 21.3 *q* (Me acetate), 25.4 *t*, 28.5 *t*, 31.2 *d* (C-8), 34.4 *d* (C-5), 44.6 *t* (C-2), 54.7 *d* (C-9), 75.7 *d* (C-1), 125.9 *s*, 128.6 *s*, 131.6 *s*, 145.0 d (C-11), 171.2 *s* (C=O of acetate), 173.7 *s* (C-14).

Valerenol (5). EI-GC/MS *m/z* (rel. int.): 39 (32), 41 (78), 43 (63), 51 (10), 53 (30), 55 (66), 57 (10), 65 (21), 67 (27), 69 (22), 77 (53), 78 (15), 79 (65), 80 (13), 81 (57), 91 (100), 92 (16), 93 (62), 94 (22), 95 (57), 103 (12), 105 (93), 106 (23), 107 (91), 108 (23), 109 (22), 110

(10), 115 (19), 117 (22), 119 (61), 122 (22), 121 (41), 122 (29), 128 (10), 129 (11), 131 (33), 132 (10), 133 (45), 134 (12), 135 (17), 145 (48), 146 (11), 147 (57), 148 (17), 149 (22), 159 (16), 160 (28), 161 (27), 162 (26), 163 (12), 187 (44), 189 (85), 190 (12), 202 (12), 220 $[M]^+$ (65). 221 (11); IR ν_{max}^{neat} cm^{-1}: 3320 (OH), 1008 (C–O, α,β-unsaturated primary alcohol); ^1H NMR ($CDCl_3$, TMS int. standard): δ0.76 (*d*, 3H, J = 6.8 Hz), 1.63 (*d*, 3H, J = 0.7 Hz), 1.72 (*d*, 3H, J = 0.7 Hz), 2.19 (*t*, 2H, J = 7.2 Hz), 2.9 (*br s*, 1H), 3.4 (*br d*, 1H, J = 9.4 Hz), 3.99 (*s*, 2H), 5.73 (*d*, 1H, J = 9.4 Hz); ^{13}C NMR ($CDCl_3$, TMS int. standard): δ12.1 *q*, 13.4 *q*, 13.7 *q* (C-13), 24.6 *t*, 26.2 *t*, 28.7 *t*, 33.2 *d* (C-8), 33.4 *d* (C-5), 37.5 *t* (C-2), 47.4 *d* (C-9), 69.2 *t* (C-14), 127.6 *d* (C-11), 129.1 *s*, 133.1 *s*, 135.2 *s* (C-12).

Z-Valerenyl acetate (**6b**). EI-GC/MS *m/z* (rel. int.): 39 (34), 41 (37), 43 (100), 53 (15), 55 (36), 67 (14), 77 (24), 79 (31), 81 (23), 91 (51), 93 (27), 95 (19), 105 (58), 106 (13), 107 (52), 108 (11), 109 (12), 115 (11), 117 (17), 119 (33), 120 (10), 121 (14), 131 (43), 132 (14), 133 (22), 145 (65), 146 (17), 147 (31), 149 (14), 159 (23), 160 (51), 161 (14), 187 (88), 188 (14), 202 (43), 203 (10), 262 $[M]^+$ (0); NICI-MS: 59 (100), 201 (21), 261 $[M-1]^-$ (58); IR ν_{max}^{neat} cm^{-1}: 1740 (C=O, acetate), 1235 (C–O, acetate); ^1H NMR ($CDCl_3$, TMS int. standard): δ0.75 (*d*, 3H, J = 6.8 Hz), 1.62 [*dt*, 3H, J = 1.8 (*d*) and 1.0 (*t*) Hz], 1.75 (*d*, 3H, J = 1.2 Hz), 2.06 (*s*, 3H, Me-acetate), 2.9 (*br s*, 1H), 3.48 (*br d*, $J \sim 9$ Hz), 4.64 (*s*, 2H, CH_2-13), 5.72 (*br d*, CH-11, $J \sim 9$ Hz). Trace of the *E*-isomer, with a signal at δ4.4 (CH_2-14). ^{13}C NMR ($CDCl_3$, TMS int. standard): No off-resonance spectrum was run as only a small sample was available. In the methyl-region there is a possible impurity. The intensity of the carbonyl-signal was too low to be observed. δ12.1, 13.2, 20.9, 21.4, 21.7, 24.7, 27.0, 28.7, 29.4, 29.7, 33.4, 37.6, 47.4, 63.2, 128.4, 129.6, 132.3, 134.9.

E-*Valerenyl isovalerate* (**7a**). EI-GC/MS *m/z* (rel. int.): 39 (11), 40 (12), 41 (52), 43 (26), 53 (10), 55 (29), 57 (57), 67 (10), 77 (15), 79 (21), 81 (17), 85 (29), 91 (34), 93 (20), 95 (14), 105 (40), 106 (11), 107 (29), 117 (15), 119 (24), 131 (37), 132 (16), 133 (16), 145 (54), 146 (15), 147 (24), 149 (12), 159 (23), 160 (52), 161 (12), 187 (100), 188 (15), 202 (59), 203 (17), 304 $[M]^+$ (0); NICI-MS: 101 (100), 201 (26), 303, $[M-1]^-$, (51); IR ν_{max}^{neat} cm^{-1}: 1740 (C=O, ester), 1183 (C–O, ester); ^1H NMR ($CDCl_3$, TMS int. standard): δ0.76 (*d*, 3H, J = 6.8 Hz), 0.96 (*d*, 6H, J = 6.6 Hz), 1.63 (*d*, 3H, J = 0.7 Hz), 1.70 (*d*, 3H, J = 1.3 Hz), 2.84 (*br s*, 1H), 3.40 (*br d*, 1H), 4.46 (*s*, CH_2-14), 5.76 [*dq*, J = 9.1 (*d*) and 1.3 (*q*) Hz]; ^{13}C NMR ($CDCl_3$, TMS int. standard): δ12.1 *q* (C-15), 13.3 *q* (C-14), 13.9 *q* (C-13), 22.4 *q* (C-4', C-5'), 24.7 *t*, 25.8 *d* (C-3'), 26.2 *t*, 28.8 *t*, 33.4 *d* (C-8/C-5), 33.5 *d* (C-5/C-8), 37.5 *t* (C-2), 43.6 *t* (C-2'), 47.5 *d* (C-9), 70.0 *t* (C-14), 128.6 *s*, 129.3 *s*, 131.0 *d* (C-11), 135.0 *s* (C-12), 172.6 *s* (C-1').

In the mixture of *E*- and *Z*-valerenyl isovalerate additional signals for *Z*-valerenyl isovalerate (**7b**) at δ4.64 (*s*) (^1H NMR) and 63.0 (^{13}C NMR) for the $-CH_2O-$ group.

Valerenyl valerate (**8**; *two isomers*). MS *m/z* (rel. int.): 39 (19), 41 (91), 43 (32), 55 (45), 57 (100), 67 (25), 69 (21), 77 (17), 79 (43), 81 (23), 82 (15), 83 (10), 85 (58), 91 (65), 93 (48), 94 (12), 95 (38), 103 (15), 104 (13), 105 (96), 106 (24), 107 (39), 108 (38), 109 (18), 117 (11), 118 (18), 119 (43), 120 (25), 123 (62), 133 (10), 134 (19), 135 (11), 145 (88), 146 (18), 147 (31), 148 (20), 160 (48), 161 (71), 162 (13), 178 (22), 187 (38), 189 (23), 202 (81), 203 (18), 204 (20), 304 $[M]^+$ (0); NICI-MS: 101 (100), 201 (15), 303 $[M-1]^-$ (41).

Valerenyl hexanoate (**9**; *two isomers*). MS *m/z* (rel. int.): 39 (12), 41 (36), 43 (48), 53 (10), 55 (29), 57 (18), 67 (15), 69 (16), 71 (20), 77 (12), 79 (15), 81 (12), 91 (20), 92 (12), 93 (11), 95 (12), 99 (15), 105 (28), 107 (11), 119 (20), 120 (15), 131 (25), 132 (10), 133 (15), 145 (48), 146 (13), 147 (24), 159 (13), 160 (38), 161 (12), 187 (100), 188 (19), 202 (69), 203 (18), 318 $[M]^+$, (0); NICI-MS: 115 (100), 201 (55), 317 $[M-1]^-$ (35).

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REFERENCES

1. Stoll, A., Seebeck, E. and Staufacher, D. (1957) *Schweizerische Apotheker-Zeitung* **95**, 115.
2. Buechi, G., Popper, T. L. and Staufacher, D. (1960) *J. Am. Chem. Soc.* **82**, 2962.
3. Smit, A. L. C. and Field, F. H. (1977) *J. Am. Chem. Soc.* **99**, 6471.
4. Bruins, A. P. (1979) *Analyt. Chem.* **51**, 967.
5. Bohlmann, F. and Lonitz, M. (1980) *Chem. Ber.* **113**, 2410.
6. Brunke, E.-J., Hammerschmidt, F.-J. and Struwe, H. (1980) *Tetrahedron Letters* **21**, 2405.
7. Snowden, R. L., Sonnay, P. and Ohloff, G. (1981) *Helv. Chim. Acta* **64**, 25.
8. Bruins, A. P. (1983) *Biomed. Mass Spectrom.* **10**, 46.
9. Baudouy, R., Sartoretti, J. and Choplin, F. (1983) *Tetrahedron* **39**, 3293.
10. Birnbaum, G. J., Findlay, J. A. and Krepinsky, J. J. (1978) *J. Org. Chem.* **43**, 272.