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TWO NEW VALEPOTRIATES FROM THE ROOTS OF *VALERIANA SISYMBRIIFOLIA*

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ABSTRACT

A dicholoromethane extract of the roots of *Valeriana sisymbriifolia* Vahl. afforded a new valepotriate, 1-α-aceisovaltrate and a new valepotriatehydrine, acetoxydeisovaleroxy-1-α-acetoxy-isovaleroxy isovaltratehydrine together with a known compound, valtrate. Structural assignments of the compounds were based on spectroscopic methods (UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR).

Keywords: *Valeriana sisymbriifolia*, Valepotriate, 1-α-Aceisovaltrate, Iranian valerian

INTRODUCTION

Valeriana sisymbriifolia is one of the seven species of the Valeriana genus widely distributed in northern and central regions of Iran. It has been used therapeutically for more than ten centuries. The valepotriates and terpenoids of the essential oil of this genus have been partly associated with the pharmacological activities such as sedative, hypnotic and antispasmodic (1). A number of valepotriates have been previously isolated from the roots of some Valeriana species (2-6). In this paper the isolation and identification of the constituents of the dichloromethane extract of *Valeriana sisymbriifolia* including two new valepotriates by various chromatographic and spectroscopic methods is described.

MATERIALS AND METHODS

Apparatus

IR spectra were taken in KBr and CCl_4 and recorded on a 1420 Perkin-Elmer instrument. The UV spectra were taken in methanol on a Secomam-100 spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra of $CDCl_3$ or C_6D_6 solutions were determined on a Varian 400 Unity plus NMR instrument using TMS as internal standard. Mass spectra were recorded by direct inlet on a Keratos Concept 25 spectrometer. TLC scanner was Shimadzu model CS-9000.

Plant material

Valeriana sisymbriifolia was collected in Darrehbid of Khansar, Iran in September 1997 and was identified by Mr. M. Noroozi from the Forest Research Institute of Isfahan. A voucher specimen (No. 8875) has been deposited at the herbarium of the institute.

Extraction and isolation

The dried rhizomes and roots of *Valeriana sisymbrifolia* (65 g) was extracted with dichloromethane (650 mL). The extract was dissolved in acetone (200 mL) and stored at 4 °C for one hour. The mixture was filtered; the filtrate was dried and redissolved in toluene (4 mL). The toluene solution was chromatographed over an alumina column (200 g alumina) employing gradient elution using petroleum ether (40-60 °C) and ethyl methyl ketone from a ratio of 98:2 to a ratio of 80:20.

Totally 140 fractions of 10 ml were collected. Combined fractions were chromatographed over silica-gel GF 254 plates employing two different eluent systems, toluene:ethyl acetate (78:22) and hexane:ethyl methyl ketone (80:20). The plates after drying were spotted with two different reagent systems, either HCl-acetic acid and placed in an oven at 120 °C for 10 min. or using DNPH and placed in an oven at 105 °C for 5-10 min. The spots were then visualized under 256-nm UV lamp to check for the existence of diene, monoene, hydroxymonoene and diene hydrine valepotriates. The fractions of the three spots corresponded to compounds **1**, **2** and **3** with R_f of 0.72, 0.59 and 0.32 respectively (Fig.1) were separately collected and dried to give pale yellow oils. A TLC of the dichloromethane extract followed by scanning at 256 nm indicated that compounds **1**,**2** and **3** corresponded to 62.25%, 26.24% and 2.57% of the

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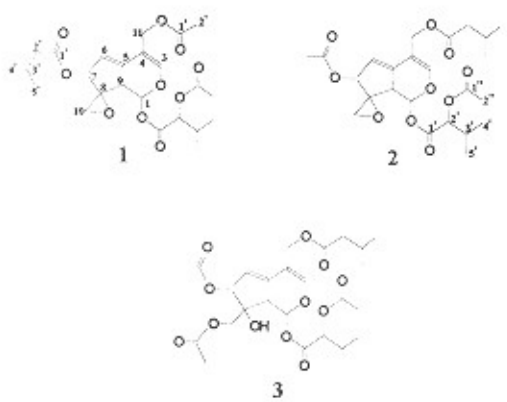
total valepotriates respectively (Fig.2). Subsequent spectroscopic analysis identified **1** as a valepotriate previously reported in other genera (7) and **2** and **3** as new ones that have not been reported previously. All the three compounds show maximum UV absorbance at 256 nm characteristic of dieno type valepotriates.

Valtrate (**1**): Yellow oil, UV (MeOH): λ_{max}=256 nm; FT-IR (CCl_4): ν_{max}= 2980, 2960, 2900, 1775, 1750, 1650, 1620, 1480, 1380, 1300, 1240, 1155, 1105, 1030, 980 cm^{-1}. EIMS: m/z (%), 422 (7) $[M]^+$, 363 (6) $[M\text{-}AcO]^+$, 321 (7) $[M\text{-}Iso]^+$, 261 (7), 236 (20), 219 (100), 206 (10), 191 (18), 177 (70), 164 (17), 148 (71), 97 (13), 85 (100), 57 (15). FABMS: m/z (%), 423 (2) $[M+1]^+$, 422 (3) $[M]^+$, 363 (3) $[M\text{-}AcO]^+$, 337 (2), 321 (19) $[M\text{-}Iso]^+$, 304 (4), 261 (8), 235 (9), 219 (100), 203 (9), 191 (21), 177 (40), 159 (17), 149 (55), 131 (31). ^{1}H-NMR ($CDCl_3$): δ= 0.94 (6H, d, J= 6.4 Hz), 0.98 (6H, d, J= 6.4 Hz), 1.25 (1H, m), 2.05 (3H, s), 2.17 (2H, d, J= 6Hz), 2.20 (2H, d, J= 5.2 Hz), 2.89, 3.02 (2H, ABq, J= 5 Hz), 3.42 (1H, dd, J= 2.8, 10 Hz), 4.63, 4.76 (2H, ABq, J= 12.5 Hz), 5.36 (1H, d, J= 2.8 Hz), 5.86 (1H, t, J= 2.8 Hz), 5.98 (1H, d, J= 10 Hz), 6.7 (1H, s). ^{13}C-NMR and DEPT (Table 1).



Scheme 1. Chemical structure of compounds **1-3**.

1-α-Aceisovaltrate (**2**): Yellow oil, UV (MeOH): λ_{max}= 256 nm; FT-IR (CCl_4): ν_{max}= 2880, 2860, 2800, 1740, 1700, 1600, 1570, 1430, 1400, 1335, 1250, 1195, 1130, 1110, 1080, 1070, 1015, 985, 940 cm^{-1}. EIMS: m/z (%)= 480 (3) $[M]^+$, 463 (14), 438 (7), 421 (19) $[M\text{-}AcO]^+$, 396 (22), 379 (40) $[M\text{-}Iso]^+$, 363 (13), 339 (22), 321 (31) $[M\text{-}(1\text{-}\alpha\text{-}OacIso)]^+$, 236 (5), 219 (100) $[321\text{-}IsOH]^+$, 191 (13), 175 (12), 143 (24), 115 (17), 85 (4). ^{1}H-NMR (C_6D_6): δ= 0.817 (3H, d, J= 6.4 Hz), 0.839 (3H, d, J= 6.8 Hz), 0.89 (3H, d, J= 6.4 Hz), 0.979 (3H, d, J= 6.8 Hz), 1.32 (1H, m), 1.603 (3H, s), 1.674 (3H, s), 1.993 (2H, d, J= 6.8 Hz), 2.094 (1H, m), 2.793, 2.919 (2H, ABq, J= 4.8 Hz), 3.40 (1H, dd, J= 2.8, 10 Hz), 4.31, 4.44 (2H, ABq, J= 0.8, 12.4 Hz), 5.048 (1H, d, J= 3.6 Hz), 5.563 (1H, d, J= 3 Hz), 5.777 (1H, t, J= 3Hz), 6.164 (1H, s), 6.243 (1H, d, J= 10 Hz). ^{13}C-NMR and DEPT (Table 1).

Acetoxydeisovaleroxy-(1-α-acetoxyisovaleroxy isovaltratehydrine) (**3**): Yellow oil, UV (MeOH): λ_{max}= 256 nm; FT-IR (CCl_4): ν_{max}= 3500, 2960, 2930, 2880, 1740, 1645, 1620, 1470, 1375, 1235, 1160, 1120, 1100, 1040, 980, 960 cm^{-1}. EIMS: m/z (%)= 540 (5) $[M]^+$, 522 (9) $[M\text{-}H_2O]^+$, 497 (4) $[M\text{-}Ac]^+$, 482 (47), 481 (94) $[M\text{-}OAc]^+$, 480 (17) $[M\text{-}HOAc]^+$, 441 (24), 439 (100) $[M\text{-}OIso]^+$, 438 (20) $[M\text{-}HOIso]^+$, 421 (64) $[M\text{-}(H_2O+OIso)]^+$, 381 (8) $[M\text{-}1\text{-}\alpha\text{-}OAcIso]^+$, 380 (20) $[M\text{-}1\text{-}\alpha\text{-}HOAcIso]^+$ 362 (21), 340 (13), 321 (18) $[M\text{-}(2\ OAc + OIso)]^+$ 279 (60) $[M\text{-}(OIso +1\text{-}\alpha\text{-}OAcIso)]^+$, 278 (37), 218 (5). ^{1}H-NMR (C_6D_6): δ= 0.8095 (3H, d, J= 6 Hz), 0.8245 (3H, d, J= 6 Hz), 0.9315 (3H, d, J= 6.8 Hz), 1.0525 (3H, d, J= 6.8 Hz), 1.605 (3H, s), 1.711 (3H, s), 1.87 (3H, s), 2.012 (2H, d), 2.035 (1H, m), 2.328 (1H, m), 2.610 (1H, brs), 2.848 (1H, dd, J= 2.8, 10 Hz), 4.3585, 4.4575 (2H, ABq, J= 12.4 Hz), 4.3955, 4.446 (2H, ABq, J= 11.4 Hz), 5.0875 (1H, d, J= 4.4 Hz), 5.6797 (1H, d, J= 2.8 Hz), 5.760 (1H, t, J= 2.4 Hz), 6.224 (1H, s), 6.5945 (1H, d, J= 10 Hz). ^{13}C-NMR and DEPT (Table 1).

RESULTS AND DISCUSSION

The plant extract, after column chromatography and repeated preparative TLC, afforded 11 compounds of which the major components were **1**,**2** and **3**. Compound **1** was obtained as yellow oil having a greenish blue chromatographic spot. Its R_f value, the UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR spectra were almost the same as those of Valtrate (7). Compound **2**, obtained as yellow oil had a R_f value in the range of that of Acevaltrates. The absorption at 256 nm in the UV spectrum shows that this iridoid contained the conjugated valtrate chromophore. This was supported by the stretching bond for the C=C bond at 1570 and 1600 cm^{-1}. The IR spectrum also showed the characteristic bonds for ester groups (1700-1740 cm^{-1}). In the EI mass spectra the presence of molecular ion at m/z 480 and the peaks at m/z 421 $[M\text{-}OAc]^+$ and m/z 379 $[M\text{-}Iso]^+$ and m/z 321 $[M\text{-}1\text{-}\alpha\text{-}AcIso]^+$ and the ions at m/z 43, 85 and 143 suggest that the molecule contained acetyl, isovaleryl and 1-α-acetoxy-isovaleryl moieties. The ^{1}H-NMR spectrum exhibited a couple of doublets at δ 0.89 and 0.81

ppm for two methyl groups of isovaleryl similar to that of isovaltrates and two singlets at δ 1.603 and 1.674 ppm for two methyl groups belonging to acetyl groups. A doublet at δ 5.048 with an integration of one proton instead of two protons indicated the presence of an α-moiety at isovaleryl group at C-1. Two singlets at δ 1.603 and 1.674 for two methyl groups belonging to acetyl groups revealed that the α-moiety on C-1 isovaleryl and the ester on C-7 are both acetoxy groups. The ^{13}C-NMR spectra (Table 1) displayed four carbonyl carbons rather than three supporting the presence of an extra acetoxy group. Compound **3**, obtained as yellow oil, displayed the IR absorption at 3500, 1740, 1645 and 1620 cm^{-1} indicating the presence of hydroxy, carbonyl and conjugated double bonds in the molecule, respectively. The presence of conjugated double bonds is supported by the UV absorption at 256 nm. The EIMS spectra showed the molecular ion at 540. The ions at m/z 522 [M-H_2O]$^+$, 481 [M-OAc]$^+$, 439 [M-OIso]$^+$ and 381 [M-1-α-AcIso]$^+$ indicated the presence of hydroxy, acetyl, isovaleryl and 1-α-acetoxyisovaleryl functionality in **3**. The ^{1}H-NMR spectra showed the shift of the chemical shift of C-10 protons to lower field in comparison to valtrates indicating the cleavage of the epoxy ring. This is confirmed by the broad 1H proton at δ 2.61 for the hydroxy group. The rest of the peaks for the C-1 and C-11 esters were similar to **2**. The ^{13}C-NMR spectra showed a change in the chemical shifts of C-8, C-9 and C-10 to lower fields (δ 80.1, 48.7 and 65.8 respectively) in comparison to valtrates and similar to valepotriatehydrines (8). The five peaks for carbonyl carbons are in accordance to the proposed structure for **3**.

Table 1. The ^{13}C-NMR data and DEPT for compounds **1-3**

C	1	2	3	DEPT
1	92.6	93.6	93.5	CH
3	148.5	148.3	148.1	CH
4	108.4	108.9	109.2	C
5	141.0	140.9	139.4	C
6	118.65	119.3	117.9	CH
7	83.1	83.5	83.2	CH
8	64.2	64.4	80.1	C
9	43.1	43.3	48.7	CH
10	47.9	47.7	65.8	CH_2
11	60.8	60.4	60.6	CH_2
7-Acetyl				
1'		170.0	170.0	C
2'		20.4	20.37	CH_3
10/11-Acetyl				
1'	170.6		170.4	C
2'	20.8		20.25	CH_3
1-Isovaleryl				
1'	170.1			C
2'	43.09			CH_2
3'	25.6			CH
4'	22.2			CH_3
5'	22.2			CH_3
7/11- Isovaleryl				
1'	172.3	172.0	171.5	C
2'	43.37	43.4	43.38	CH_2
3'	25.8	25.9	25.8	CH
4'	22.3	22.3	22.3	CH_3
5'	22.3	22.3	22.2	CH_3
1-α-AceIsovaleryl				
1'		167.5	167.9	C
2'		76.2	76.6	CH
3'		30.2	30.4	CH
4'		18.8	18.7	CH_3
5'		16.7	16.9	CH_3
1"		170.0	170.2	C
2"		20.0	20.1	CH_3

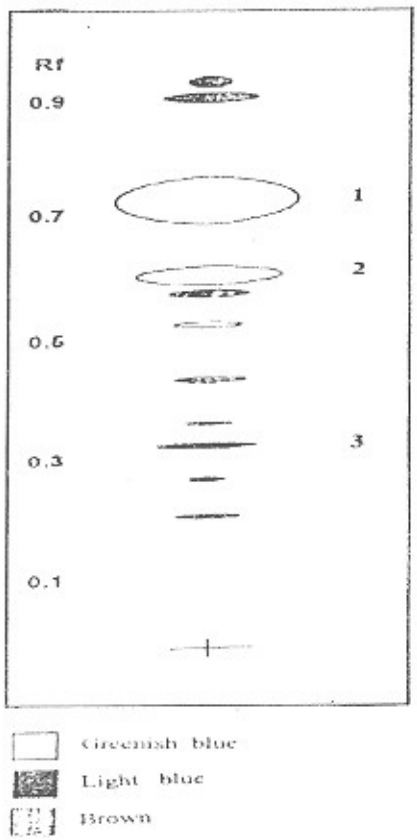


Fig 1. TLC of the methanolic extract of the roots of *Valeriana sisymbriifolia*.
1.2.3 represent compounds **1**, **2** and **3** respectively

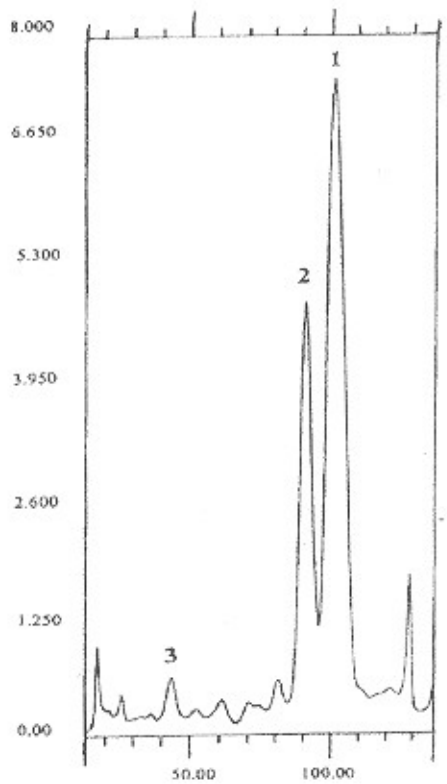


Fig 2. TLC scanning of the methanolic extract of the roots of *Valeriana sisymbriifolia*.
1= compound **1** (62.25%), 2= compound **2** (26.24%), 3= compound **3** (2.57%)

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