Minor Iridoids from the Roots of *Valeriana wallichii*

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Four new iridoids, valeriotetrates B and C (**1** and **2**), 8-methylvalepotriate (**3**), and 1,5-dihydroxy-3,8-epoxyvalechlorine A (**4**), together with three known iridoids, were isolated from the roots of *Valeriana wallichii*. The structures of the new compounds were elucidated by analysis of 1D and 2D NMR and HRESIMS data. Compound **4** is an unusual iridoid bearing a C-10 chloro group and an oxo bridge connecting C-3 and C-8, resulting in a rigid skeleton.

The genus *Valeriana* of the Valerianaceae family comprises approximately 250 species and is widely distributed throughout the world.[1] Phytochemical studies have indicated that their plant parts contain iridoids, sesquiterpenoids, lignans, and alkaloids with various pharmacological properties such as sedative, cytotoxic, antitumor, antioxidant, and vasorelaxant activities.[2,3] *V. wallichii* DC., an annual herb with fragrant roots and rhizomes that is endemic to southwestern China, is well known in Traditional Chinese Medicine for its sedative, hypnotic, and antiviral activities.[1] Some valepotriates such as valtrate and didrovaltrate are effective cytotoxic agents against HTC hepatoma cells.[4] In addition, didrovaltrate can also induce noticeable remissions of KREBS II ascetic tumors. A recent report has also indicated the anti-HIV activity of valtrate as a new rev-transport inhibitor.[5]

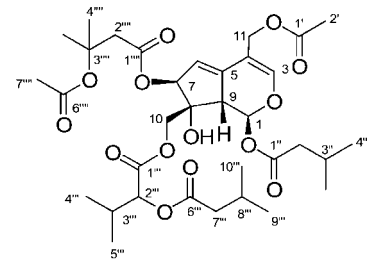
Previous phytochemical studies on this plant have revealed the presence of sesquiterpenoids, valerianandids A−C, essential oils, and iridoids.[6–8] Our study reports four new and three known iridoids isolated from the roots of *V. wallichii.* The known compounds were identified as valtrate,[9–11] didrovaltrate,[9–12] and acetovaltrate[9–12] by comparison of their physical data with reported values.

Valeriotetrate B (**1**) was isolated as a colorless oil. The molecular formula of compound **1** was determined to be $C_{34}H_{50}O_{14}$ by HRESIMS. The ^{13}C NMR and DEPT spectra showed a total of 34 signals, consistent with the carbon count. The signals were assigned as 10 methyls, five methylenes, nine methines, and 10 quaternary carbons. Comparison of the NMR data (Table 1) with those of valtrate hydrine showed that **1** had a similar skeleton.[7]

The isovalerate group in compound **1** was established by ^{13}C NMR and DEPT spectra (Table 1). The location of the isovalerate group at C-1 was confirmed by the HMBC correlation from H-1 (δ_H 6.22) to C-1″ (δ_C 170.7). The two sharp methyl singlets at δ_H 2.03 (H-2′) and 1.95(H-7‴″) were consistent with the methyl protons of acetate residues. The ^{13}C NMR signal at δ_C 170.8 was assigned to the carbonyl carbon of one of the two acetate residues on the basis of the HMBC correlation between H-2′ (δ 2.03) and C-1′ (δ_C 170.8). This carbonyl is bonded to C-11 as assessed from long-range correlations from H-11 (δ 4.71, 4.62) to C-1′ (δ_C 170.8).

The ^{13}C NMR and ^{1}H NMR spectra also indicated the presence of one isovaleroyloxyisovaleryl moiety in compound **1** (Table 1).[8] The HMBC correlations from H-10 (δ_H 4.70, 4.28) to the ester carbonyl carbon of the isovaleroyloxyisovaleryl C-1‴ (δ_C 169.6) supported a structure in which the isovaleroyloxyisovaleryl group is attached to C-10.

Additionally, the ^{13}C NMR, DEPT, and HMBC spectra showed the presence of an acetoxy-isovalerate group in **1**.[7,9] This partial structure was established by the analysis of the ^{13}C NMR and DEPT data (Table 1). Further HMBC correlations from H-7‴″ (δ_H 1.95) to C-6‴″ (δ_C 170.3) and from H-2‴″ (δ_H 2.98, 2.80) to C-1‴″ (δ_C 168.9), C-3‴″ (δ_C 79.2), C-4‴″ (δ_C 26.4), and C-5‴″ (δ_C 26.5) established the connectivity between these carbons.



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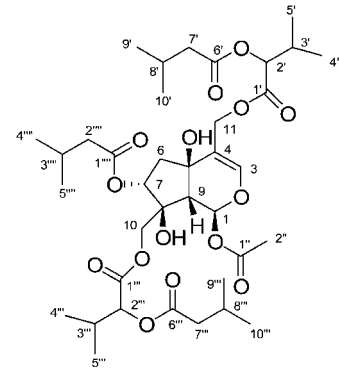

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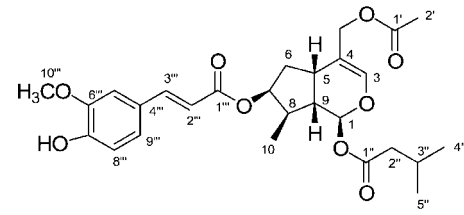

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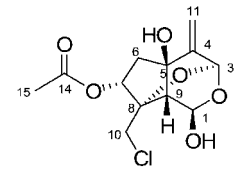

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Table 1. ^{13}C and ^{1}H NMR Data of **1**−**3** in $CDCl_3$

	valeriotetrate B (**1**)		valeriotetrate C (**2**)		8-methylvalepotriate (**3**)	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	92.5 d	6.22 (1H, d, 10.0)	89.0 d	6.57 (1H, d, 1.4)	91.0 d	6.02 (1H, d, 3.7)
3	148.2 d	6.68 (1H, s)	145.0 d	6.61 (1H, s)	139.9 d	6.34 (1H, s)
4	108.6 s		112.5 s		113.4 s	
5	139.1 s		69.6 s		32.2 d	2.93 (1H, m)
6	117.3 d	5.75 (1H, dd, 2.6, 2.4)	40.5 t	2.60 (1H, dd, 13.2, 6.2), 2.11 (1H, m)	37.3 t	1.98, 2.10 (each 1H, m)
7	83.2 d	5.38 (1H, d, 2.6)	79.8 d	4.93 (1H, dd, 6.2, 2.4)	76.7 d	5.33 (1H, td, 5.2, 2.2)
8	80.0 s		79.1 s		39.2 d	2.10 (1H, m)
9	48.3 d	2.89 (1H, dd, 10, 2.4)	53.3 d	2.58 (1H, d, 1.4)	45.8 d	2.19 (1H, m)
10	65.5 t	4.70, 4.28 (each 1H, AB, 11.4)	67.3 t	4.27, 4.23 (each 1H, AB, 11.4)	12.9 q	1.11 (3H, d, 6.7)
11	60.8 t	4.71, 4.62 (each 1H, AB, 12.4)	61.9 t	4.91, 4.69 (each 1H, AB, 12.7)	64.0 t	4.57, 4.42 (each 1H, AB, 12.1)
1′	170.8[a−i] s		169.9[f] s		170.9 s	
2′	20.9 q	2.03 (3H, s)	76.7[g] d	4.80 (1H, d, 4.8)	20.9 q	2.07 (3H, s)
3′			30.0[b] d	2.21 (1H, m)		
4′			17.2[e] q	1.01 (3H, d, 6.4)		
5′			18.9[i] q	0.99 (3H, d, 6.4)		
6′			173.2[h] s			
7′			42.9[a−i] t	2.26 (2H, m)		
8′			25.7[c] d	2.09 (1H, m)		
9′			22.4[d] q	0.96 (3H, overlapped)[a−i]		
10′			22.4[d] q	0.97 (3H, overlapped)[a−i]		
1″	170.7[a−i] s		170.7 s		171.8 s	
2″	42.9 t	2.31 (2H, m)	20.7 q	2.09 (3H, s)	43.4 t	2.14 (2H, m)
3″	25.5 d	2.15 (1H, m)			25.6 d	2.11 (1H, m)
4″	22.3 q	0.97 (3H, d, 6.4)[a−i]			22.3 q	0.99 (3H, d, 6.7)[a−i]
5″	22.3 q	0.96 (3H, d, 6.4)[a−i]			22.3 q	0.98 (3H, d, 6.7)[a−i]
1‴	169.6 s		169.6[f] s		166.7 s	
2‴	76.8 d	4.72 (1H, d, 4.2)	76.6[g] d	4.79 (1H, d, 4.8)	115.4 d	6.28 (1H, d, 15.8)
3‴	30.0 d	2.24 (1H, m)	29.9[b] d	2.21 (1H, m)	145.0 d	7.61 (1H, d, 15.8)
4‴	18.6 q	1.00 (3H, d, 6.4)	17.3[e] q	1.00 (3H, overlapped)	126.9 s	
5‴	17.1 q	0.99 (3H, d, 6.4)	18.6[i] q	0.99 (3H, overlapped)	109.4 d	7.03 (1H, d, 1.7)
6‴	173.7 q		173.0[h] s		146.8 s	
7‴	43.0 t	2.34 (2H, m)	43.0[a−i] t	2.28 (2H, m)	148.0 s	
8‴	25.6 d	2.09 (1H, m)	25.6[c] d	2.09 (1H, m)	114.7 d	6.92 (1H, d, 8.3)
9‴	22.3 q	1.02 (3H, d, 6.4)[a−i]	22.3[d] q	0.96 (3H, overlapped)[a−i]	123.0 d	7.08 (1H, dd, 8.3, 1.7)
10‴	22.3 q	0.98 (3H, d, 6.4)[a−i]	22.3[d] q	0.97 (3H, overlapped)[a−i]	55.9 q	3.93 (3H, s)
1⁗	168.9 s		171.2[h] s			
2⁗	44.1 t	2.98, 2.80 (each 1H, AB, 14.2)	43.1[a−i] t	2.29 (2H,m)		
3⁗	79.2 s		25.7[c] d	2.08 (1H, m)		
4⁗	26.4[b] q	1.51 (3H, s)[b]	22.3[d] q	0.98 (3H, overlapped)[a−i]		
5⁗	26.5[b] q	1.50 (3H, s)[b]	22.3[d] q	0.99 (3H, overlapped)[a−i]		
6⁗	170.3 s					
7⁗	22.2 q	1.95 (3H, s)				

[a−i] Assignments may be interchanged in each column.

Table 2. ^{13}C and ^{1}H NMR Data of **4** in $CDCl_3$

position	δ_C	δ_H	position	δ_C	δ_H
1	90.5 d	5.60 (1H, d, 3.2)	8	82.5 s	
3	94.1 d	5.30 (1H, s)	9	46.6 d	2.64 (1H, d, 3.2)
4	151.6 s		10	45.5 t	3.78, 3.73, (each 1H, AB, 11.5)
5	77.4 s		11	108.3 t	5.38, 5.12 (each 1H, s)
6	46.5 t	2.59 (1H, dd, 14.3, 7.3), 2.03 (1H, dd, 14.3, 2.6)	14	169.6 s	
7	74.6 d	4.95 (1H, dd, 2.6, 7.3)	15	21.0 q	2.08 (3H, s)

The chemical shift of C-7 (δ_C 83.2) in a valtrate hydrin-type skeleton indicated the presence of a C−O bond, confirming the attachment of the acetoxy-isovalerate group to C-7. The relative configuration of **1** was confirmed by comparison of NMR data with those of known compounds.[7,15] Therefore, compound **1** was identified as valeriotetrate B.

Valeriotetrate C (**2**) was obtained as colorless needles. The molecular formula of **2** was determined to be $C_{37}H_{58}O_{15}$ by HRESIMS. ^{13}C NMR and DEPT spectra data showed the presence of 11 methyls, six methylenes, 11 methines, and nine quaternary carbons. Comparison of the NMR data (Table 1) with those of valeriotetrate A showed that compound **2** had a similar skeleton.[8] The 1D and 2D NMR data of **2** also showed the presence of an acetate residue at C-1 and an isovalerate group at C-7. These data and the correlations from H-2′ (δ_H 4.80) to C-6′ (δ_C 173.2) and C-1′ (δ 169.9) and from H-2‴ (δ_H 4.79) to C-6‴ (δ_C 173.0) and C-1‴ (δ 169.6) in the HMBC established the presence of two isovaleroyloxyisovaleryl groups in **2**.[8] HMBC correlations from H-10 (δ 4.27, 4.23) to C-1‴ (δ 169.6) and from H-11 (δ 4.91, 4.69) to C-1′ (δ 169.9) suggested the presence of two isovaleroyloxyisovaleryl groups at C-10 and C-11, respectively. The relative configuration of **2** was confirmed by the NOESY experiment. On the basis of comparison of NMR data of **2** with those reported for valepotriates,[7,8] H-9 and 5-OH had β-orientations. The NOE between H-1 and H-10, H-9, and H-7 indicated the α-orientation of H-1 and β-orientations of 8-OH and H-7. Therefore, compound **2** was established to be valeriotetrate C.

8-Methylvalepotriate (**3**) was obtained as a yellow oil. The molecular formula of **3** was determined to be $C_{27}H_{34}O_9$ by HRESIMS. The ^{13}C NMR and DEPT showed the presence of five methyls, three methylenes, 12 methines, and seven quaternary carbons. Comparison of the NMR data (Table 1) with those of

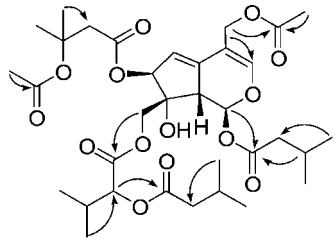

Figure 1. Key HMBC correlations for **1**.

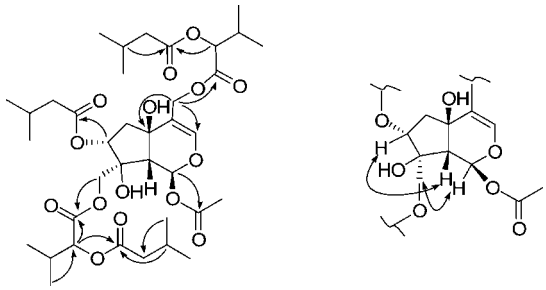

Figure 2. Key HMBC and NOESY correlations for **2**.

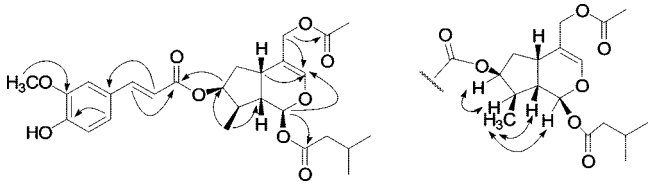

Figure 3. Key HMBC and NOESY correlations for **3**.

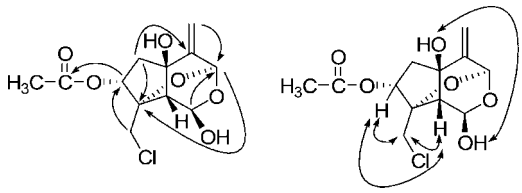

Figure 4. Key HMBC and NOESY correlations for **4**.

valeriotetrate A[8] and didrovaltrate[9] supported the presence of an iridoid skeleton in **3**.

The presence of a 1,2,4-trisubstituted phenyl ring[13] was confirmed by ^{1}H and ^{13}C NMR data (Table 1). The spectra showed the presence of a double bond conjugated at C-4‴, an $-OCH_3$ at C-6‴, and a hydroxy group at C-7‴. An ester carbonyl carbon at δ_C 166.7 supported an α,β-unsaturated ester carbonyl carbon. The HMBC correlations from H-3‴(δ_H 7.61) and H-2‴ (δ_H 6.28) to C-1‴ (δ_C 166.7) further confirmed this structural feature. The 1D and 2D NMR spectra also showed an acetate group at C-11, an isovalerate group at C-1, and the ester carbonyl carbon at C-7. The relative configuration of **3** was determined by NOESY experiment. By comparison of NMR data with those of valepotriates,[7,8,16] H-9 and H-5 were deduced as having β-orientations. The information from molecular modeling and the NOE between H-1 and H-10, H-10 and H-9, and H-7 and H-8 indicated that H-1 and H-7 were α-oriented and 10-CH_3 was β-oriented.[17] Thus, compound **3** was determined to be 8-methylvalepotriate.

1,5-Dihydroxy-3,8-epoxyvalechlorine A (**4**) was obtained as a colorless oil. The molecular formula of **4** was determined to be $C_{12}H_{15}ClO_6$ by HRESIMS. The IR spectrum showed the presence of OH (3436 cm^{-1}), ester C=O (1736 cm^{-1}), and C=C groups (1628 cm^{-1}).

The ^{13}C NMR and DEPT data (Table 2) showed the presence of one methyl, three methylenes, four methines, and four quaternary carbons. The 1D NMR data together with HSQC and HMBC revealed the presence of an acetate group, an acetal group, and an exocyclic olefinic bond in compound **4**. The HMBC correlation from H-7 (δ_H 4.95) to C-14 (δ_C 169.6) suggested that the acetate group was attached to C-7. On the basis of the downfield shift of C-8 (δ_C 82.5) and HMBC correlation from H-3 (δ_H 5.30) to C-8, C-3 (δ_C 94.1) was showed to be linked to C-8 via an oxo bridge. The methylene carbon at δ_C 45.5 was assigned to C-10, bonded to a Cl atom,[14,15] and the HMBC correlation from H-10 (δ_H 3.78) to C-8 confirmed that C-10 was linked to C-8. The HMBC data also showed the following correlations: from H-6 to C-7, C-8, C-5, and C-4, H-1 to C-5 and C-3, H-9 to C-5 and C-4, both H-3 and H-7 to C-5, and H-7 to C-9. Herein a cyclopentanopyran fused ring skeleton was shown to be present in **4**. The relative configuration of this compound was confirmed by a combination of NOESY experiment and molecular modeling with a rigid oxo-bridge skeleton. According to molecular modeling of this compound, the oxo bridge from C-3 to C-8 could only be α-oriented, and the 5-OH and H-9 could only be β-oriented. The NOE between 1-OH and 5-OH, H-10 and H-9, and H-10 and H-7 indicated that 1-OH, H-7, and H-9 were all present in the β-orientation. Therefore, **4** was identified as 1,5-dihydroxy-3,8-epoxyvalechlorine A.

Experimental Section

General Experimental Procedures. Melting points were measured on an XRC-1 apparatus and are uncorrected. Optical rotations were recorded on a PE-341 polarimeter. NMR spectra were recorded on a Bruker AV-600 MHz spectrometer using TMS as an internal standard. A Bruker BioTOF Q spectrometer was used to record HRESIMS, and a Finnigan LCQ^{DECA} to record ESIMS. Sephadex LH-20 (Pharmacia), Si gel (200−300 mesh, Qingdao Marine Chemical Group Co.), and ODS (Cosmosil 75 C_{18}-OPN, Nacalai Tesque) were employed in column chromatography.

Plant Material. The plant was collected from Anshun, Guizhou Province, People's Republic of China, in April 2006, and identified as *V. wallichii* DC. by Prof. Yu-Ying Ma. A voucher specimen (2006-408) was deposited at the Herbarium of Chengdu Institute of Biology, Chinese Academy of Sciences.

Extraction and Isolation. Dried root powder of *V. wallichii* (8 kg) was extracted with EtOH at room temperature to give an extract (2 kg) after removal of solvent. The EtOH extract was suspended in H_2O (3 L) and then partitioned successively with petroleum ether (3 × 2 L) and EtOAc (5 × 2 L). A part of the EtOAc extract (92 g) was subjected to Si gel column chromatography eluted with petroleum ether−EtOAc (from 20:1 to 1:1) to give six fractions, A−F, and afforded valtrate (1.2 g) and didrovaltrate (5 mg). Fraction C was purified over a Sephadex LH-20 column eluted with MeOH to furnish acevaltrate (25 mg). Fraction D (4.85 g) was chromatographed over a Sephadex LH-20 column, using MeOH as solvent, to give four fractions, Da−Dd. Subfraction Db (3.83 g) was chromatographed over an RP-18 column eluted with a MeOH−H_2O gradient system (53−100%) to give six subfractions, Db1−Db6. Subfraction Db6 was further separated on an RP-18 column eluted with a solvent of decreasing polarity (MeOH−H_2O, 50% to 100%) to afford compounds **1** (10 mg) and **2** (100 mg). Fraction E (3.6 g) was separated into two subfractions, Ea and Eb, over an RP-18 column eluted with a MeOH−H_2O system (50−100%). Fraction Eb (3.0 g) was chromatographed on Sephadex LH-20 eluted with MeOH to afford compound **3** (50 mg). Fraction F (3.10 g) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to yield six subfractions, Fa−Ff. Compound **4** (30 mg) was obtained from Ff by repeated column chromatography on RP-18 eluted with MeOH−H_2O (15−100%) and on Sephadex LH-20 eluted with MeOH.

Valeriotetrate B (1): colorless oil; $[\alpha]^{20}_D$ +190.2 (0.1, MeOH); ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 705; HRESIMS *m/z* 705.3097 (calcd for $C_{34}H_{50}O_{14}Na$ $[M + Na]^+$ *m/z* 705.3093).

Valeriotetrate C (2): colorless needles; mp 54−55 °C; $[\alpha]^{20}_D$ −63.8 (0.1, MeOH); IR (KBr) ν_{max} 3460, 1742, 1659, 1247, 1186 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 765; HRESIMS *m/z* 765.3652 (calcd for $C_{37}H_{58}O_{15}Na$ $[M + Na]^+$ *m/z* 765.3673).

8-Methylvalepotriate (3): yellow oil; $[\alpha]^{20}_D$ −50.0 (0.1, MeOH); IR (KBr) ν_{max} 3436, 1740, 1627, 1599, 1516, 1458, 1270, 1157 cm^{-1};

^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 525; HRESIMS *m/z* 525.2087 (calcd for $C_{27}H_{34}O_9Na$ [M + Na]$^+$ *m/z* 525.2095).

1,5-Dihydroxy-3,8-epoxyvalechlorine A (4): colorless oil; $[\alpha]^{20}_D$ +86.2 (0.1, MeOH); IR (KBr) ν_{max} 3436, 2920, 1736, 1628, 1247, 1108, 963 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 313.0436 (100%) (calcd for $C_{12}H_{15}ClO_6Na$ [M + Na]$^+$ *m/z* 313.0449) and isotope peak *m/z* 315.0425 (29%) (calcd for $C_{12}H_{15}ClO_6Na$ [M + Na]$^+$ *m/z* 315.0425).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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