Revision of the Structures of 1,5-Dihydroxy-3,8-epoxyvalechlorine, Volvaltrate B, and Valeriotetrate C from *Valeriana jatamansi* and *V. officinalis*

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The structures of 1,5-dihydroxy-3,8-epoxyvalechlorine (**1a**) and volvaltrate B (**6a**), two new chlorinated iridoids isolated from *Valeriana jatamansi* and *V. officinalis*, respectively, were originally assigned on the basis of spectroscopic methods. Reinvestigation using X-ray analysis and chemical transformation revealed that the original assignment of H-7 in **1a** and *OH*-8 in **6a** should be inverted and that the structures should be revised to **1** and **6**, respectively. Correspondingly, the structure of valeriotetrate C (**7a**) should be revised to **7**. Volvaltrate B (**6**) showed cytotoxic activity against the lung adenocarcinoma (A549), metastatic prostate cancer (PC-3M), colon cancer (HCT-8), and hepatoma (Bel7402) cell lines, with IC_{50} values of 8.5, 2.0, 3.2, and 6.1 μM, respectively.

Our recent investigation of *Valeriana jatamansi*,[1,2] a plant from the family Valerianaceae, resulted in the isolation of a number of valepotriates and iridoids, including four chlorinated iridoids, 1,5-dihydroxy-3,8-epoxyvalechlorine (**1a**), volvaltrate B (**6a**), and a new pair of C-1 epimers (**2** and **3**). 1,5-Dihydroxy-3,8-epoxyvalechlorine (**1a**) and volvaltrate B (**6a**), both containing a chlorine at C-10, were presented in separate papers as being new iridoids recently isolated from *V. jatamansi* and *V. officinalis*, respectively, and their relative configurations were proposed by NOESY, ROESY, and molecular modeling data.[3,4] Our reinvestigation using X-ray analysis and chemical transformation revealed that the original assignment of H-7 in **1a** and *OH*-8 in **6a** should be inverted and that their structures should be revised to **1** and **6**, respectively. Also, valeriotetrate C (**7a**), a new valepotriate recently isolated from *V. jatamansi*, was found to be identical to valeriotetrate A (**7b**), and their structures should be revised to **7**.[3,5]

Compound **1** was obtained as a colorless oil with the molecular formula $C_{12}H_{15}ClO_6$ as determined by HRESIMS. Comparison with the literature data showed that compound **1** was identical to 1,5-dihydroxy-3,8-epoxy valechlorine (**1a**) recently isolated from the same plant without reporting the absolute configuration.[3] Although our reisolation of **1a** provided an opportunity to determine its absolute configuration, repeated attempts to secure crystals of **1a** for single-crystal X-ray analysis were unsuccessful. However, a pair of C-1 epimers (**2** and **3**) (extraction artifacts) were obtained from the same fraction (Tables 1 and 2 and Experimental Section). Crystals of **3** suitable for single-crystal X-ray analysis were obtained from a MeOH solution. The subsequent X-ray diffraction experiment demonstrated that the absolute configuration of **3** was as depicted in Figure 1. The ORTEP diagram showed that the substituents at C-1 and C-7 in **3** were cofacial. This was different from the opposite orientation of the substituents at C-1 and C-7 assigned for **1a**.[3] Analysis of the NOESY spectra of **1** and **3** indicated the presence of NOE correlations between H-7 and H-6α and between H-9 and both H-6β and H_2-10 for both compounds (Figure 2) and between H-1 and H_2-10 for **1** but not for **3**. These NOE correlations demonstrated that **1** and **3** possessed the identical configuration at C-7 but different ones at C-1. Therefore, the previous assignment of the acetoxy group at C-7 in **1a** was incorrect, and it was revised as depicted for **1**. The MM2-opimized structures for **1** and **3** were consistent with the above NOE correlations deduced from the respective NOESY spectra (Figure 2), in full support of the above conclusion. Consequently, the structures of **1**−**3** have now been assigned as (1*S*,3*R*,5*R*,7*S*,8*R*,9*S*)-1,5-dihydroxy-3,8-epoxyvalechlorine, (1*S*,3*R*,5*R*,7*S*,8*R*,9*S*)-3,8-epoxy-1-*O*-ethyl-5-hydroxyvalechlorine, and (1*R*,3*R*,5*R*,7*S*,8*R*,9*S*)-3,8-epoxy-1-*O*-ethyl-5-hydroxyvalechlorine, respectively.

Compound **6** was obtained as a colorless oil, which analyzed for the molecular formula $C_{27}H_{41}ClO_{11}$ on the basis of HRESIMS. This substance gives 1D and 2D NMR data identical to those reported for volvaltrate B (**6a**) (Supporting Information, Figure S32−S37).[5] However, the relative configuration at C-8 in **6a** was different from those of the related valepotriates.[1,6−11] This prompted us to re-examine its configuration. Analysis of the literature revealed that derivatives of **1**−**3**, possessing the 2,9-dioxatricyclo[4,3,1,$0^{3,7}$]decane skeleton and oxo-bridge between C-3 and C-8, could be readily transformed from dihydrovaltrate hydrin-type iridoids under strong acidic conditions.[10−14] Having a large amount of **6a** in hand (1.25 g) and the absolute configuration assignment of **1**−**3**, the chemical transformation was carried out to establish the absolute configuration of the iridoid nucleus in **6a**. Treatment of compound **6a** with *p*-toluenesulfonic acid in MeOH successfully converted **6a** into **4** and **5** (Scheme 1). The NOESY spectra of **1**, **4**, and **6a** revealed that the three compounds had completely identical NOE correlations of H-1 with H-9 and H_2-10. This indicated that **6a** and **4** had the same configuration as **1**. Meanwhile, the α-orientation of *OH*-8 in **6a** was evidenced by NOE correlations between H-7 and H-6α and of H-9 with H-6β and H_2-10 in its NOESY spectrum (Supporting Information, Figure S37). The absolute configuration of the α-(isovaleroxy)isovaleroxy moiety at C-11 in **6a** was determined by basic hydrolysis. Basic hydrolysis of **6a** yielded α-(isovaleroxy)isovaleric acid (Experimental Section and Supporting Information, Figures S47−S49). Comparison of the optical rotation data of α-(isovaleroxy)isovaleric acid {$[\alpha]_D^{20}$ +7.8 (*c* 0.2, $CHCl_3$)} and (*R*)-α-(acetoxy)isovaleric acid {lit.[15] $[\alpha]_D^{25}$ +40.5 (*c* 1.0, $CHCl_3$)} demonstrated the *R* configuration of the α-(isovaleroxy)isovaleric acid. Accordingly, the correct structure of volvaltrate B was revised to **6**.

A question arises as to whether **2** and **3** occur naturally or whether their presence is an artifact of the extraction protocol. To answer this question, a simulated extraction protocol was then undertaken. Thirty milligrams each of **1** and **6** was dissolved in 20 mL of 95% EtOH and heated at 50 °C for 8 h, separately, and the reactions were monitored by LC-MS. Only in the reaction of **6** could a trace

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Chart 1

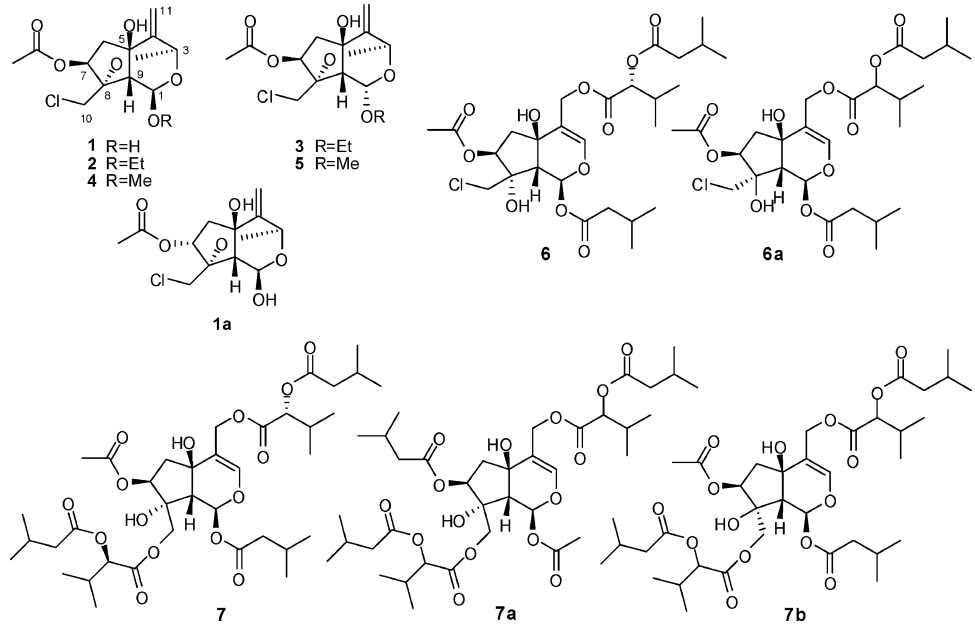


Table 1. ^{1}H NMR Data (δ) for Compounds **1**−**7**[a]

	position	**1** ($CDCl_3$)	**2** (acetone-d_6)	**3** ($CDCl_3$)	**3** (acetone-d_6)	**4** (acetone-d_6)	**5** (acetone-d_6)	**6** ($CDCl_3$)[b]	**7** ($CDCl_3$)[c]
	1	5.61 d (3.6)	5.31 d (3.0)	5.20 s	5.21 s	5.18 (3.6)	5.11 (1.2)	6.57 d (1.8)	6.57 brs
	3	5.31 s	5.30 s	5.23 s	5.22 s	5.30 s	5.22 s	6.61 s	6.61 s
	6	2.59 dd (14.4, 7.2)	2.40 dd (14.4, 7.2)	2.67 dd (14.4, 7.2)	2.51 dd (14.4, 7.2)	2.38 dd (14.4, 7.2)	2.50 dd (14.4, 7.2)	2.63 dd (13.2, 6.0)	2.59 (13.2, 6.0)
		2.02 dd (14.4,5.4)	1.93 dd (14.4, 2.4)	1.94 dd (14.4, 3.6)	1.95 dd (14.4, 3.0)	1.92 dd (14.4, 2.4)	1.94 dd (14.4, 3.0)	2.11 dd (13.2, 6.6)	2.09 (13.2, 7.2)
	7	4.95 dd (7.2, 5.4)	4.82 dd (7.2, 2.4)	4.88 dd (7.2, 3.6)	4.74 dd (7.2, 3.0)	4.82 dd (7.2, 2.4)	4.76 dd (7.2, 3.0)	4.98 dd (6.0, 6.6)	4.92 dd (6.0, 7.2)
	9	2.64 d (3.6)	2.60 d (2.4)	2.37 s	2.34 s	2.61 d (2.4)	2.35 d (1.2)	2.73 d (1.8)	2.59 brs
	10	3.79 d (11.4)	3.97 d (11.4)	4.35 d (11.4)	4.28 d (11.4)	3.96 d (11.4)	4.26 d (11.4)	3.75 d (11.4)	4.27 d (12.6)
		3.72 d (11.4)	3.81 d (11.4)	3.84 d (11.4)	3.87 d (11.4)	3.81 d (11.4)	3.86 d (11.4)	3.68 d (11.4)	4.22 d (12.6)
	11	5.38 s	5.24 s	5.30 s	5.28 s	5.23 s	5.29 s	4.92 d (12.6)	4.90 d (12.6)
		5.13 s	5.11 s	5.15 s	5.14 s	5.10 s	5.15 s	4.69 d (12.6)	4.69 d (12.6)
R_1	1		3.79 m	3.92 m	3.85 m	3.37 s	3.43 s		
			3.51 m	3.51 m	3.50 m				
	2		1.15 t (7.2)	1.28 t (7.2)	1.16 t (7.2)			2.23 m	2.20 m
	3							2.10 m	2.10 m
	4							0.98 d (6.6)	0.96 d (6.6)
	5							0.98 d (6.6)	0.97 d (6.6)
R_7	2	2.09 s	2.05 s	2.06 s	2.04 s	2.05 s	2.04 s	2.09 s	2.08 s

[a] ^{1}H NMR data (δ) were measured on a 600 MHz NMR instrument. Proton coupling constants (J) in Hz are given in parentheses. The assignments are based on DEPT, ^{1}H−^{1}H COSY, NOESY, HSQC, and HMBC experiments. [b] Data of the α-isovaleroxyisovaleroxy group at C-11 of **6**: δ 4.79 (1H, d, J = 4.8 Hz, H_{R11-2}), 2.20 (1H, m, H_{R11-3}), 1.01 (6H, d, J = 7.2 Hz, $H_{R11-4,\ 5}$), 2.28 (2H, m, H_{R11-7}), 2.10 (1H, m, H_{R11-8}), 0.98 (6H, d, J = 7.2 Hz, $H_{R11-9,\ 10}$). [c] Data of the α-isovaleroxyisovaleroxy groups at C-10 and C-11 of **7**: δ 4.80 (2H, d, J = 4.8 Hz, H_{R10-2} and H_{R11-2}), 2.26, 2.28 (each 1H, m, H_{R10-3} and H_{R11-3}), 1.01 (12H, d, J = 7.2 Hz, $H_{R10-4,\ 5}$ and $H_{R11-4,\ 5}$), 2.27 (4H, m, H_{R10-7} and H_{R11-7}), 2.10 (2H, m, H_{R10-8} and H_{R11-8}), 0.98, 0.99 (each 6H, d, J = 7.2 Hz, $H_{R10-9,\ 10}$ and $H_{R11-9,\ 10}$).

of **2** and **3** be detected. This result indicated that **2** and **3** are indeed extraction artifacts.

The NMR data of compound **7**, unambiguously assigned by its 2D NMR data analysis, were identical to those of valeriotetrate C (**7a**) and valeriotetrate A (**7b**). The latter two compounds were separately reported from the root of *V. jatamansi*, and different structure were assigned though they had the same spectroscopic data as **7** (Experimental Section and Supporting Information, Figures S38−S46).[3,5] Detailed 2D NMR data analysis of **7** revealed that it had the same planar structure as **7b**. Particularly, the HMBC spectrum of **7** showed correlations from H-1 to the carbonyl carbon of the isovaleroxy group at δ_C 170.7 and from H-7 to the carbonyl carbon of the acetoxy group at δ_C 171.2. This secured that the isovaleroxy and acetoxy groups in **7** were located at C-1 and C-7, respectively (Supporting Information, Figure S43). The NOESY spectrum of **7** displayed NOE correlations between H-7 and H-6α, of H-9 with H-6β and H_2-10, and of H-1 with H-9 and H_2-10. These NOEs revealed that the configuration of **7** was the same as that of **6**. As in **6**, the *R* configuration of the α-(isovaleroxy)isovaleroxy moiety at C-10 and C-11 in **7** was also elucidated on the basis of the basic hydrolysis. Therefore, the structure of valeriotetrate C and valeriotetrate A was revised as depicted for compound **7**.

Compounds **1**−**6** were evaluated for cytotoxicity against four human cancer cell lines, lung adenocarcinoma (A549), metastatic prostate cancer (PC-3M), colon cancer (HCT-8), and hepatoma (Bel7402), using the MTT method.[16,17] Volvaltrate B (**6**) showed weak activity against all tested cell lines, with IC_{50} values of 8.5, 2.0, 3.2, and 6.1 μM, respectively, while compounds **1**−**5** were inactive (IC_{50} > 10 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR and UV spectra were recorded on Bruker Vector-22 and Shimadzu UV-2550 UV−visible spectrophotometers, respectively. NMR spectra were obtained on a Bruker Avance 600 MHz NMR spectrometer in $CDCl_3$ or acetone-d_6 with TMS as an internal standard. ESIMS and HRESIMS were acquired on an Agilent LC/MSD Trap XCT and a Q-TOF micro mass spectrometer (Waters, Milford, MA), respectively. Column chromatography was performed using silica gel (100−200 mesh and 10−40 μm; Huiyou Silica Gel Development Co. Ltd., Yantai, People's Republic of China) and Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). Semipreparative HPLC was conducted on an ODS column (Kromasil, 5 μm, 300 × 10 mm) using a PDA UV detector at 208 nm. Preparative TLC (0.4−0.5 mm) was carried out on precoated

Table 2. ^{13}C NMR Data (δ) for Compounds **1**−**7**[a]

	position	**1**	**2**[b]	**3**	**3**[b]	**4**[b]	**5**[b]	**6**[c]	**7**[d]
	1	90.5	96.7	93.8	96.5	98.1	98.0	89.4	89.0
	3	94.1	95.2	94.8	95.1	95.3	95.2	144.7	144.9
	4	151.5	154.9	151.0	153.1	154.9	153.1	112.9	112.5
	5	77.4	78.7	77.0	78.1	78.7	78.0	70.2	69.6
	6	46.5	48.1	46.9	48.7	48.3	48.8	40.7	40.5
	7	74.6	75.3	74.2	75.5	75.2	75.5	79.7	79.7
	8	82.5	84.0	82.6	83.7	84.0	83.8	80.2	79.0
	9	46.6	48.0	49.0	50.6	48.0	50.4	54.2	53.3
	10	45.5	47.6	47.5	49.1	47.6	49.1	49.7	67.3
	11	108.4	107.9	108.2	108.8	107.9	109.0	61.9	61.9
R_1	1		64.5	64.6	65.4	55.8	56.8	170.8	170.7
	2		15.8	15.2	16.0			43.1	43.1
	3							25.7	25.7
	4							22.3	22.3
	5							22.3	22.3
R_7	1	169.7	170.4	169.5	170.3	170.4	170.3	170.7	171.2
	2	21.0	21.5	21.0	21.4	21.4	21.4	20.9	20.8

[a] ^{13}C NMR data (δ) were measured in $CDCl_3$ for **1**, **3**, **6**, and **7** at 150 MHz. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] ^{13}C NMR data (δ) were measured in acetone-d_6 for **2**, **3**, **4**, and **5** at 150 MHz. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [c] Data of the α-isovaleroxyisovaleroxy group at C-11 of **6**: δ 169.9 (C_{R11-1}), 77.0 (C_{R11-2}), 29.9 (C_{R11-3}), 18.7 (C_{R11-4}), 17.3 (C_{R11-5}), 173.3 (C_{R11-6}), 43.0 (C_{R11-7}), 25.7 (C_{R11-8}), 22.4 (C_{R11-9}), 22.3 (C_{R11-10}). [d] Data of the α-isovaleroxyisovaleroxy groups at C-10 and C-11 of **7**: δ 169.6 (C_{R10-1}), 77.0 (C_{R10-2}), 29.9 (C_{R10-3}), 18.7 (C_{R10-4}), 17.3 (C_{R10-5}), 173.1 (C_{R10-6}), 43.0 (C_{R10-7}), 25.7 (C_{R10-8}), 22.4 (C_{R10-9}), 22.3 (C_{R10-10}); 169.9 (C_{R11-1}), 77.0 (C_{R11-2}), 29.9 (C_{R11-3}), 18.7 (C_{R11-4}), 17.3 (C_{R11-5}), 173.2 (C_{R11-6}), 43.0 (C_{R11-7}), 25.6 (C_{R11-8}), 22.3 (C_{R11-9}), 22.3 (C_{R11-10}).

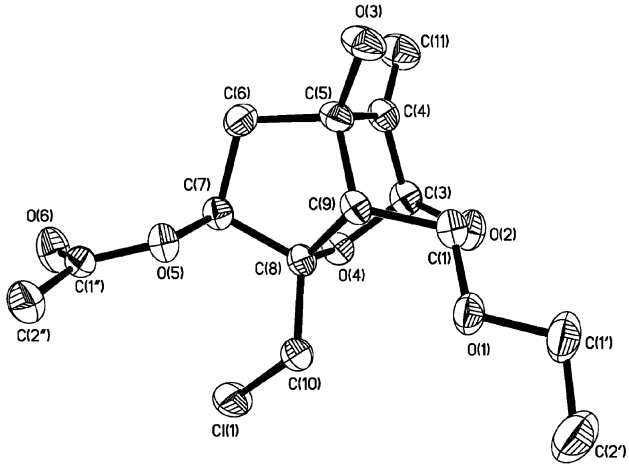


Figure 1. ORTEP drawing for compound **3** depicting its absolute configuration.

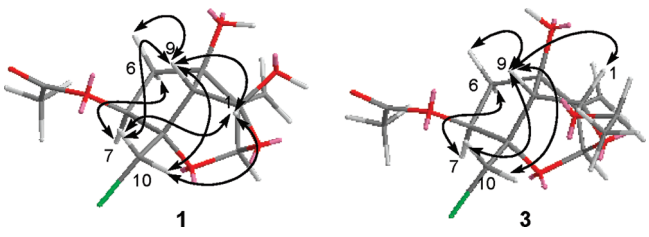
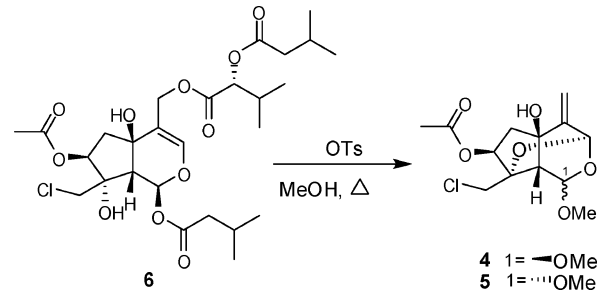


Figure 2. Key NOESY correlations for compounds **1** and **3**.

silica gel GF_{254} plates (Yantai). Zones were visualized under UV light (254 nm) or by spraying with 10% H_2SO_4 followed by heating.

Plant Material. The whole plants of *V. jatamansi* were collected in Gaopo, Guizhou Province, People's Republic of China, in July 2007, and identified by Prof. Shun-zhi He, Guiyang University of Traditional Chinese Medicine. A herbarium specimen was deposited in the School of Pharmacy, Second Military Medical University, People's Republic of China (herbarium no. 2007-08-16).

Scheme 1. Chemical Transformation of **6** into **4** and **5**



Extraction and Isolation. The air-dried whole plants of *V. jatamansi* (8.5 kg) were powdered and extracted with 11.0 L of 95% EtOH at room temperature for 3 × 48 h. The combined extracts were evaporated under reduced pressure to yield a residue. The residue was suspended in H_2O (1.5 L) and then partitioned with EtOAc (5 × 1 L). The EtOAc fraction (420 g) was chromatographed over silica gel (1500 g), eluting with increasing amounts of Me_2CO (0−100%) in petroleum ether, to afford 10 fractions (F_1−F_{10}) based on TLC analysis. Fraction F_4 (18.2 g) was chromatographed over Sephadex LH-20 eluting with petroleum ether−CH_3Cl−MeOH (5:5:1) to give five subfractions. The second subfraction was further separated by reversed-phase preparative HPLC (RP_{18}, 5 μm, 208 nm, MeOH−H_2O, 77:23) to afford **7** (20 mg). Fraction F_6 (10.8 g) was fractionated by column chromatography over Sephadex LH-20 using petroleum ether−$CHCl_3$−MeOH (5:5:1) as eluent to give two subfractions. Separation of the second subfraction by repeated chromatography over Sephadex LH-20, using $CHCl_3$−MeOH (1:1) as eluting solvent, yielded **6** (1.25 g). Fraction F_7 (25.6 g) was separated by column chromatography over Sephadex LH-20 with $CHCl_3$−MeOH (1:1) as eluent to give four subfractions. The first subfraction was further purified by preparative TLC developed with $CHCl_3$−Me_2CO (5:1) to give **1** (202.1 mg). The second subfraction was repeatedly purified by reversed-phase preparative HPLC (RP_{18}, 5 μm, 208 nm, MeCN−H_2O, 40:60) to afford **2** (15.1 mg) and **3** (5.2 mg).

(1*R*,3*R*,5*R*,7*S*,8*R*,9*S*)-1,5-Dihydroxy-3,8-epoxyvalechlorine (1): colorless oil; $[\alpha]_D^{20}$ +66.3 (*c* 0.4, MeOH); IR (KBr) ν_{max} 3438, 2923, 1736, 1627, 1247, 1108, 968 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Tables 1 and 2; positive-mode ESIMS *m/z* 315, 313 $[M + Na]^+$ (1:3); negative-mode ESIMS *m/z* 291, 289 $[M - H]^-$ (1:3); HRESIMS *m/z* 289.0483 $[M - H]^-$ (calcd for $C_{12}H_{14}ClO_6$, 289.0479).

(1*R*,3*R*,5*R*,7*S*,8*R*,9*S*)-3,8-Epoxy-1-*O*-ethyl-5-hydroxyvalechlorine (2): colorless oil; $[\alpha]_D^{20}$ +55.3 (*c* 0.9, MeOH); IR (KBr) ν_{max} 3435, 2920, 1725, 1622, 1371, 1248, 1092, 971 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Tables 1 and 2; positive-mode ESIMS *m/z* 343, 341 $[M + Na]^+$ (1:3); negative-mode ESIMS *m/z* 319, 317 $[M - H]^-$ (1:3); HRESIMS *m/z* 341.0738 $[M + Na]^+$ (calcd for $C_{14}H_{19}ClO_6Na$, 341.0768).

(1*S*,3*R*,5*R*,7*S*,8*R*,9*S*)-3,8-Epoxy-1-*O*-ethyl-5-hydroxyvalechlorine (3): colorless plate crystal (MeOH), mp 152−153 °C; $[\alpha]_D^{20}$ +75.3 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3428, 2925, 1722, 1626, 1375, 1252, 1093, 970 cm^{-1}; ^{1}H NMR ($CDCl_3$ and acetone-d_6, 600 MHz) and ^{13}C NMR ($CDCl_3$ and acetone-d_6, 150 MHz) data, see Tables 1 and 2; positive-mode ESIMS *m/z* 343, 341 $[M + Na]^+$ (1:3); negative-mode ESIMS *m/z* 319, 317 $[M - H]^-$ (1:3); HRESIMS *m/z* 341.0741 $[M + Na]^+$ (calcd for $C_{14}H_{19}ClO_6Na$, 341.0768).

Volvaltrate B (6): colorless oil; $[\alpha]_D^{20}$ −39.0 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3487, 2963, 1738, 1372, 1240, 1089, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Tables 1 and 2; positive-mode ESIMS *m/z* 601, 599 $[M + Na]^+$ (1:3); negative-mode ESIMS *m/z* 577, 575 $[M - H]^-$ (1:3); HRESIMS *m/z* 599.2250 $[M + Na]^+$ (calcd for $C_{27}H_{41}ClO_{11}Na$, 599.2235).

Compound 7: colorless oil; $[\alpha]_D^{20}$ −65.3 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Tables 1 and 2; positive-mode ESIMS *m/z* 765 $[M + Na]^+$; negative-mode ESIMS *m/z* 777 $[M + {}^{35}Cl]^-$.

X-ray Analysis of 3. Crystals of compound **3** were grown via the slow evaporation of a methanol solution. A colorless plate of appropriate dimensions was mounted on a glass fiber. All measurements were made using a Bruker SMART CCD diffractometer with Mo Kα radiation (λ = 0.71073 Å) from a normal-focus sealed tube. In total, 6790 frames were collected with the detector set in different positions of 2θ. Each

frame covered 0.20° in ω. Of the 6370 reflections collected with 2.27° $\leq 2\theta \leq$ 24.99°, 2718 were independent ($R_{int} = 0.0395$). This represents 100% of the unique data to the maximum value of 2θ. The structure was solved using the SHELXTL package. Atomic coordinates and anisotropic displacement parameters were refined for the non-hydrogen atoms. The full-matrix least-squares (on F^2) of 190 variables gave values of the conventional crystallographic residuals $R_1 = 0.0376$ ($wR_2 = 0.0882$) for 2718 observed data with $I > 2\sigma(I)$ and $R_1 = 0.0424$ ($wR_2 = 0.0905$) for all data. The goodness-of-fit was 0.989. A final difference Fourier map showed residual density between −0.242 and 0.160 e/Å^3. The absolute configuration was assigned on the basis of the absolute structure parameter, which refined to a value of 0.05(7).

Crystal data of **3**: $C_{14}H_{19}ClO_6$; fw 318.74; colorless plate; 0.20 × 0.10 × 0.08 mm; orthorhombic; space group $P2_12_12_1$; unit cell dimensions $a = 9.547(4)$ Å, $b = 12.328(5)$ Å, $c = 13.064(5)$ Å, $V = 1537.6(10)$ Å^3; $Z = 4$; $d_{calc} = 1.377$ Mg/m^3. Crystallographic data of **3** have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 722355). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/deposit or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK [fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk].

Chemical Transformation of 6 into 4 and 5. Volvaltrate B (**6**, 30 mg) was treated in 5 mL of MeOH by addition of 2.0 mg of *p*-toluenesulfonic acid. The reaction was carried out in a 10 mL flask closed with a Teflon-lined cap and kept for 30 min at 60 °C. The reaction was quenched with 10 mL of ice water and extracted with CH_2Cl_2 (3 × 15 mL) to obtain an organic layer, which was washed with H_2O and dried over Na_2SO_4. The residue from the CH_2Cl_2 extract was separated by reversed-phase preparative HPLC (RP_{18}, 5 μm, 208 nm, MeCN−H_2O, 40:60) to afford **4** (13.2 mg) and **5** (3.6 mg).

(1*R*,3*R*,5*R*,7*S*,8*R*,9*S*)-3,8-Epoxy-1-*O*-methyl-5-hydroxyvalechlorine (4): colorless oil, $[\alpha]_D^{20}$ +14.3 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3424, 2972, 1723, 1625, 1371, 1256, 1090, 970 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Tables 1 and 2; positive-mode ESIMS *m*/*z* 329, 327 $[M + Na]^+$ (1:3); negative-mode ESIMS *m*/*z* 401, 399 $[M + {}^{35}Cl]^-$ (2:3); HRESIMS *m*/*z* 327.0646 $[M + Na]^+$ (calcd for $C_{13}H_{17}ClO_6Na$, 327.0611).

(1*S*,3*R*,5*R*,7*S*,8*R*,9*S*)-3,8-Epoxy-1-*O*-methyl-5-hydroxyvalechlorine (5): colorless oil, $[\alpha]_D^{20}$ +122.0 (*c* 1.1, MeOH); IR (KBr) ν_{max} 3427, 2975, 1726, 1628, 1378, 1257, 1072, 966 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Tables 1 and 2; positive-mode ESIMS *m*/*z* 329, 327 $[M + Na]^+$ (1:3); negative-mode ESIMS *m*/*z* 401, 399 $[M + {}^{35}Cl]^-$ (2:3); HRESIMS *m*/*z* 327.0658 $[M + Na]^+$ (calcd for $C_{13}H_{17}ClO_6Na$, 327.0611).

Basic Hydrolysis of Compounds 6 and 7. To a solution of **6** (30 mg) or **7** (15 mg) in THF (2.0 mL) and H_2O (6 mL) was added NaOH (120 mg), and then the mixture was stirred at room temperature for 1 h. After neutralized with 4 N HCl, the solution was evaporated under reduced pressure to give a residue. The mixture was resolved by Sephadex LH-20 chromatography eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1) and then preparative TLC using $CHCl_3$−MeOH (20:1) as developing solvent to yield (*R*)-α-(isovaleroxy)isovaleric acid (3.0 mg from **6** and 2.3 mg from **7**): colorless oil, $[\alpha]_D^{20}$ +7.8 (*c* 0.2, $CHCl_3$; lit.[18] $[\alpha]_D^{20}$ +4.8); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.85 (1H, brs, H-2), 2.26 (1H, m, H-3), 1.01, 0.99 (each 3H, d, $J = 7.2$ Hz, H_3-4/5), 2.27 (2H, m, H_2-2′), 2.13 (1H, m, H-3′), 0.97 (6H, d, $J = 6.6$ Hz, H_3-4′/5′); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.1 (C, C-1), 76.4 (CH, C-2), 30.2 (CH, C-3), 19.0 (CH_3, C-4), 17.4 (CH_3, C-5), 173.1 (C, C-1′), 43.3 (CH_2, C-2′), 25.9 (CH, C-3′), 22.6 (CH_3, C-4′/5′); positive-mode ESIMS *m*/*z* 225 $[M + Na]^+$.

Cytotoxicity Assays. The cytotoxic activity was determined against four human cancer cell lines, A549, PC-3M, HCT-8, and Bel7402, obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were seeded in 96-well plates at a cell density of 3000 per well and were treated 24 h later with various concentrations of compounds **1**−**6**. After 24 h of incubation, MTT was added to all wells. Plates were incubated for another 24 h, and cell viability was measured by observing absorbance at 570 nm on a SpectraMax^{190} microplate reader (Molecular Devices, USA).[16,17] IC_{50} values were calculated using Microsoft Excel software. Paclitaxel was used as a positive control.

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Supporting Information Available: 1D NMR spectra of (*R*)-α-(isovaleroxy)isovaleric acid, 1D and 2D NMR spectra of compounds **1**−**7**, and X-ray crystallographic data of compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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