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Loi Huynh
Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy, Ho chi minh City, Vietnam.

Hung Tran
Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy, Ho chi minh City, Vietnam.

Markus Bacher
Department of Chemistry, Division of Chemistry of Renewables, UFT Research Center Tulln, University of Natural Resources and Life Sciences, A-3430 Tulln an der Donau, Austria.

Thomas Pacher
Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, Veterinaerplatz 1, A-1210 Vienna, Austria.

Correspondence:
Hung Tran
Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy, Ho chi minh City, Vietnam.

Iridoids and flavonoids from *Valeriana hardwickii* Wall

Loi Huynh, Hung Tran, Markus Bacher and Thomas Pacher

Abstract
Two iridoids - valechlorine, isovaleroxyvaltrate hydrine - were isolated by column chromatography from roots and rhizomes of *Valeriana hardwickii*. Additionally, three flavonoids - linarin, neobudofficide and rhoifolin - were also isolated by the same technique from aerial parts. Their structures were elucidated by spectral data.

Keywords: *Valeriana hardwickii*, valechlorine, isovaleroxyvaltrate hydrine, linarin, neobudofficide, rhoifolin

1. Introduction
Valeriana is the major genus of the Valerianaceae, a family comprising about 200 species distributed throughout the world and many of which are used medicinally. Valerian today is a highly respected medicinal plant listed in many pharmacopoeial monographs. Valepotriates are the iridoids which are being isolated from many *Valeriana* species. The isolation of these components led to much interest into their activity and has led to the widespread use in Europe of standardised mixture of valepotriates as a mild sedative. In addition, some flavonoids were also isolated such as luteolin, diosmetin, kaemferol, quercetin, apigenin, acacetin. Unusual compounds, linarin isovalerate and linarin were also separated [1]. Regarding *V. hardwickii*, "Nữ lang in Vietnamese", until now, a few chemical studies were reported, most of them concerning analyses of essential oils [2 ,3, 4]. About the medicinal purpose of this herb, the roots are used in Vietnam as a traditional medicine with anti-convulsant, sedative, anti-fatigue, and anti-dysmenorrheal properties. The topical uses of aerial parts are the treatment of insect bites and various skin diseases [5, 6]. This chemical study was carried out to clarify the chemical composition of this valuable plant.

2. Materials and Methods
2.1 Plant materials
Valeriana hardwickii Wall. was collected in Bidoup – Núi bà Mountains at an altitude of 1500 m above sea level, Lâm đồng province, Vietnam, in August 2011. The voucher specimen (No: 01-2011-BMDL) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy, HCM City, Vietnam.

2.2 Extraction and isolation
The air-dried powder (800 g) of rhizomes and roots of *V. hardwickii* were extracted at room temperature with CH_2Cl_2 (3 × 2 L, 24h each). The combined extract was evaporated under reduced pressure to yield the residue (58 g) which was subsequently extracted with MeOH (3 × 200 mL, 4 h each) to get MeOH crude extract (26 g) after removal of solvent. This extract was subjected to open column liquid chromatography on silica gel (250 g; Merck; 63-200 µm; 4.5 × 50 cm) using step gradient of EtOAc-Hexane (0:100, 0.3 L; 10:90, 2.2 L; 20:80, 4.0 L). Fractions of 150 mL were collected and monitored by TLC using EtOAc-Hexane (20:80) as a mobile phase and glacial acetic acid - hydrochloric acid (2:8) as a reagent. Five fractions (F_{1-5}) were collected. F_5 (612 mg) was submitted to MPLC on silica gel (40-63 µm; Merck; 460 × 35 mm) using step gradient of EtOAc in hexane (5%; 1.8 L; 10%; 1.4 L). Fractions (approx. 100 mL each) were manually collected and monitored by UV detector at 254 nm, and TLC with above conditions. Thirty five fractions (1-35) were collected. 1 (73 mg), and 2 (13 mg) were obtained from fractions 26-28, and 33, respectively.
The air-dried powder (1.8 kg) of aerial parts of *V. hardwickii* was percolated with MeOH (15 L). After removal of MeOH, the received extract (0.5 L) was added with water (1 L) and

partitioned with $CHCl_3$ (4 × 1.5 L), subsequently with EtOAc (4 × 1.2 L) and finally with BuOH (4 × 1 L) which was evaporated under reduced pressure to yield the residue (60 g). A aliquot of this residue (3.8 g) was chromatographed on Sephadex LH-20 (3 × 50 cm) using MeOH as mobile phase. Fractions of 20 mL were collected and monitored by TLC using EtOAc-MeOH-H_2O-CH_3COOH (100:17:13:1) as mobile phase. Six fractions were collected. Fraction 4 (1.0 g) was submitted to MPLC on C-18 bonded silica (Lobar® Grosse B column, 310 × 25 mm, 40-63 µm) and gradiently eluted with MeOH-H_2O (20:80, 150 mL; 40:60, 250 mL; 50:50, 500 mL; 60:40, 250 mL; 80:20, 250 mL), flow rate was about 5 mL/min. The fractions were monitored by UV detector at 254 nm. Forty-five fractions were collected. Compound 3 (64 mg), 4 (7mg) and 5 (11.5 mg) were received from fractions 42-44, 35-36 and 23-24, respectively.

2.3 Nuclear magnetic resonance (NMR) spectroscopy

All NMR spectra were recorded on a Bruker Avance II 400 (resonance frequencies 400.13 MHz for ^1H and 100.61 MHz for ^{13}C) equipped with a 5 mm observe broadband probe head (BBFO) with z–gradients at room temperature with standard Bruker pulse programs. The samples were dissolved in 0.6 ml of $CDCl_3$ (99.8 % D) or DMSO-d6 (99.8 % D). Chemical shifts are given in *ppm*, referenced to residual solvent signals ($CDCl_3$: 7.26 *ppm* for ^1H, 77.0 *ppm* for ^{13}C and DMSO: 2.49 *ppm* and 39.6 *ppm*, respectively). ^1H NMR data were collected with 32k complex data points and apodized with a Gaussian window function (lb = −0.3 Hz and gb = 0.3 Hz) prior to Fourier transformation. ^{13}C-jmod spectra with WALTZ16 ^1H decoupling were acquired using 64k data points. Signal-to-noise enhancement was achieved by multiplication of the FID with an exponential window function (lb = 1Hz). All two-dimensional experiments were performed with 1k × 256 data points, while the number of transients (2–4 scans) and the sweep widths were optimized individually. The resulting FIDs were zero-filled to a 2k × 1k data matrix and apodized with a sine function for COSY in both the $\omega_1$and ω_2 dimensions prior to Fourier transformation. Heteronuclear spectra were zero-filled only in F_1 to a 1k×512 data matrix, and apodized in both dimensions with a shifted sine function. HSQC experiments were acquired using adiabatic pulse for inversion of ^{13}C and GARP-sequence for broadband ^{13}C-decoupling, optimized for $^1J_{(CH)}$= 145 Hz.

3. Results and discussion

Compounds 1, 2 were isolated as colorless oil and colorless needle-shaped cristal in MeOH, respectively. The NMR data are shown in Table 1. Based on the spectroscopic analysis and comparison with published spectra [7, 8], compounds 1 and 2 were elucidated as valechlorine and isovaleroxyvaltrate hydrine, respectively.

Compounds 3 -5 showed characteristic UV spectra of flavonoids and in the ^1H NMR spectra the typical coupling pattern of p-substituted B-rings with methoxyl groups for compounds 3 and 4, and a hydroxyl group for compound 5, respectively. Moreover the presence of chelated 5-hydroxyl substituents was indicated by sharp singlets at d ~ 13 *ppm* for all three substances. Therefore the main structural differences of the isolated flavonoids were the attached sugar moieties on O-7. Detailed analysis of both 1D and 2D NMR data of compound 3 revealed the presence of rutinoside (a-L-rhamnopyranosyl-(1→6)-b-D-glucopyranose) as sugar unit, unequivocally confirmed by HMBC crosspeaks from Glu-H1 at d = 5.05 *ppm* to C-7 (d = 163.07 *ppm*) and from Rha-H1 (d = 4.55 *ppm*) to Glu-C6 at d = 66.20 *ppm*. The corresponding H, H-coupling constants of both anomeric protons also proved the conformation as abovementioned as a-Rha and b-Glu, respectively. Compound 5 showed similar ^1H and ^{13}C NMR spectra as 3 with the only difference that rhamnose was now 1→2 linked to glucose as shown by a crosspeak from Rha-H1 (d = 5.12 *ppm*) to Glu-C2 (d = 76.39 *ppm*) in the HMBC spectra, the stereochemistry of the anomeric centers remaining unchanged. Contrary to compounds 3 and 5 the 1D NMR spectra of compound 4 revealed the presence of two rhamnose units linked to Glu-2 and Glu-6, respectively, as demonstrated by appropriate HMBC crosspeaks. Therefore, in summary, the structures of the isolated flavonoids were elucidated as Linarin (3) (acacetin 7-*O*-rutinoside), Neobudofficide (4), and Rhoifolin (5) (apigenin-7-*O*-neohesperidoside) [9], respectively, with the corresponding NMR data listed in Table 2. The structure of compounds isolated is shown in Figure 1.

The isolated components are main compounds and firstly reported from *Valeriana hardwickii* Wall. The present of predominant valechlorine which was also isolated from *Valeriana wallichii* (syn. *Valeriana jatamansi* Jones) (Himalayan Valerian) and *Valeriana officinalis* (European Valerian) is noteworthy since a halogen incorporated into secondary metabolite is unusual amongst the flowering plant [1, 10]. The chlorinated valepotrates from *V. wallichii* have moderate cytotoxicity against lung adenocarcinoma (A 549), metastatic prostate cancer (PC-3M), colon cancer (HCT-8) and hepatoma (Bel 7402) cell lines with IC50 values of 0.89–9.76 lM [7]. Linarin was first identified in *V. wallichii* by Thies (1968) in the form of its isovaleryl ester, but its pharmacological properties were not explored. More recently, it has been demonstrated that linarin, isolated from the leaves of *Buddleia cordata*, exerts central analgesic properties and is responsible for the antipyretic activity and anti-inflammatory effects. Linarin from *V. officinalis* demonstrated its sedative and sleep-enhancing properties in mice [11]. Apigenin 7-*O*-neohesperidoside were firstly reported from *Valeriana* genus. The further chemical investigation which is comparative analysis of the essential oils of *Valeriana hardwickii* and *Valeriana officinalis* showed that thirty-two compounds are common in the root essential oil of both species with high amounts of isovaleric acid, α-pinene, camphene, bornyl acetate, and β-caryophyllene [12]. These studies revealed that *Valeriana hardwickii* has similar chemical composition with other *Valeriana* so the therapeutic uses are identical.

Table 1: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of 1 and 2 in $CDCl_3$

Position	1		2	
	δ_C *ppm*	**δ_H *ppm***	**δ_C *ppm***	**δ_H *ppm***
1	92.38	6.25 *d* (10.0)	92.52	6.25 *d* (10.0)
3	148.00	6.67 *s*	148.05	6.68 *s*
4	108.70		108.70	
5	139.30		139.06	
6	117.44	5.78 *dd* (2.9; 2.6)	117.56	5.77 *dd* (2.9; 2.5)
7	83.16	5.40 *d* (2.9)	83.13	5.47 *d* (2.9)
8	80.30		80.16	
9	49.26	2.95 *dd* (10.0; 2.6)	48.42	2.94 *dd* (10.0; 2.5)
10	48.45	4.01 *d* (11.1) 3.79 *d* (11.1)	65.47	4.39 *d* (11.5) 4.31 *d* (11.5)
11	60.87	4.70 *d* (12.4) 4.64 *d* (12.4)	60.92	4.71 *d* (12.4) 4.64 *d* (12.4)
1’	170.50		170.80	
2’	43.20	2.30 *d* (7.2)	43.17	2.32 *d* (7.2)
3’	25.72[a]	2.16 *m*	25.64	2.17 *m*
4’	22.43[b]	1.01 *d* (6.7)	22.36 [c]	0.99 *d* (6.6)
5’	22.39[b]	1.01 *d* (6.7)	22.33 [c]	0.99 *d* (6.6)
1’’	171.79		171.84	
2’’	43.50	2.22 *dd* (14.5; 7.3) 2.17 *dd* (14.5; 6.7)	43.38	2.14 *m*
3’’	25.79[a]	2.11 *m*	25.73	2.04 *m*
4’’	22.29[b]	0.96 *d* (6.6)	22.33 [c]	0.93 *d* (6.6)
5’’	22.23 [b]	0.96 *d* (6.4)	22.33 [c]	0.93 *d* (6.6)
1’’’	170.84		170.85	
2’’’	20.90	2.04 *s*	20.92	2.04 *s*
1’’’’			173.09	
2’’’’			43.03	2.23 *dd* (14.9; 7.6) 2.19 *dd* (14.9; 6.6)
3’’’’			25.64	2.07 *m*
4’’’’			22.25[c]	0.95 *d* (6.6)
5’’’’			22.25 [c]	0.95 *d* (6.6)
8-OH		2.54 *s*		2.52 *s*

[a, b, c].....exchangeable

Table 2: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of **3-5** in DMSO-*d*6

	3		4		5	
Position	δ_C	δ_H *ppm*	δ_C	δ_H *ppm*	δ_C	δ_H *ppm*
2	164.06		164.08		164.41	
3	103.92	6.93 *s*	104.02	6.94 *s*	103.27	6.85 *s*
4	182.14		182.11		182.09	
4a	105.58		105.65		105.54	
5	161.25		161.25		161.22	
6	99.77	6.44 *d* (2.2)	99.56	6.38 *d* (1.8)	99.46	6.36 *br s*
7	163.06		162.64		162.64	
8	94.89	6.78 *d* (2.2)	94.48	6.72 *d* (1.8)	94.62	6.78 *br s*
8a	157.09		157.12		157.09	
1’	122.79		122.76		121.04	
2’, 6’	128.58	8.04 *d* (9.0)	128.52	8.02 *d* (9.0)	128.70	7.92 *d* (8.8)
3’, 5’	114.83	7.14 *d* (9.0)	114.85	7.15 *d* (9.0)	116.17	6.93 *d* (8.8)
4’	162.54		162.52		161.59	
4’-OCH_3	55.68	3.85 *s*	55.66	3.85 *s*		
5-OH		12.90 *s*		12.91 *s*		12.97 *s*
Glu						
Glu-1	100.04	5.05 *d* (7.2)	97.92	5.22 *d* (7.1)	97.93	5.22 *d* (7.2)
Glu-2	73.18	3.27 *m*	76.33	3.51	76.39	3.50 *m*
Glu-3	76.36	3.29 *m*	77.09	3.49	77.13*	3.48 *m*
Glu-4	69.71	3.14 *m*	69.82	3.16	69.76	3.20 *m*
Glu-5	75.77	3.60 *m*	75.59	3.65	77.32*	3.48 *m*
Glu-6	66.20	3.85 + 3.44	66.11	3.85 + 3.43 *m*	60.59	3.70 + 3.47 *m*
^{6}Rha						
^{6}Rha-1	100.63	4.55 *d* (1.1)	100.63	4.54 *br s*		
^{6}Rha-2	70.46	3.66 *m*	70.43	3.66		
^{6}Rha-3	70.85	3.46 *m*	70.83	3.47		

^{6}Rha-4	72.17	3.14 *m*	72.14	3.14		
^{6}Rha-5	68.44	3.40 *m*	68.43	3.40		
^{6}Rha-6	17.91	1.07 *d* (6.2)	17.89	1.07 *d* (6.2)		
^{2}Rha						
^{2}Rha-1			100.57	5.12 *br s*	100.58	5.12 *br s*
^{2}Rha-2			70.13	3.68 *m*	70.51	3.69 *br s*
^{2}Rha-3			70.54	3.33 *m*	77.59	3.32 *m*
^{2}Rha-4			71.94	3.20 *m*	71.98	3.20 *m*
^{2}Rha-5			68.43	3.75 *m*	68.45	3.74 *m*
^{2}Rha-6			18.16	1.20 *d* (6.2)	18.18	1.19 *d* (6.2)

*…exchangeable

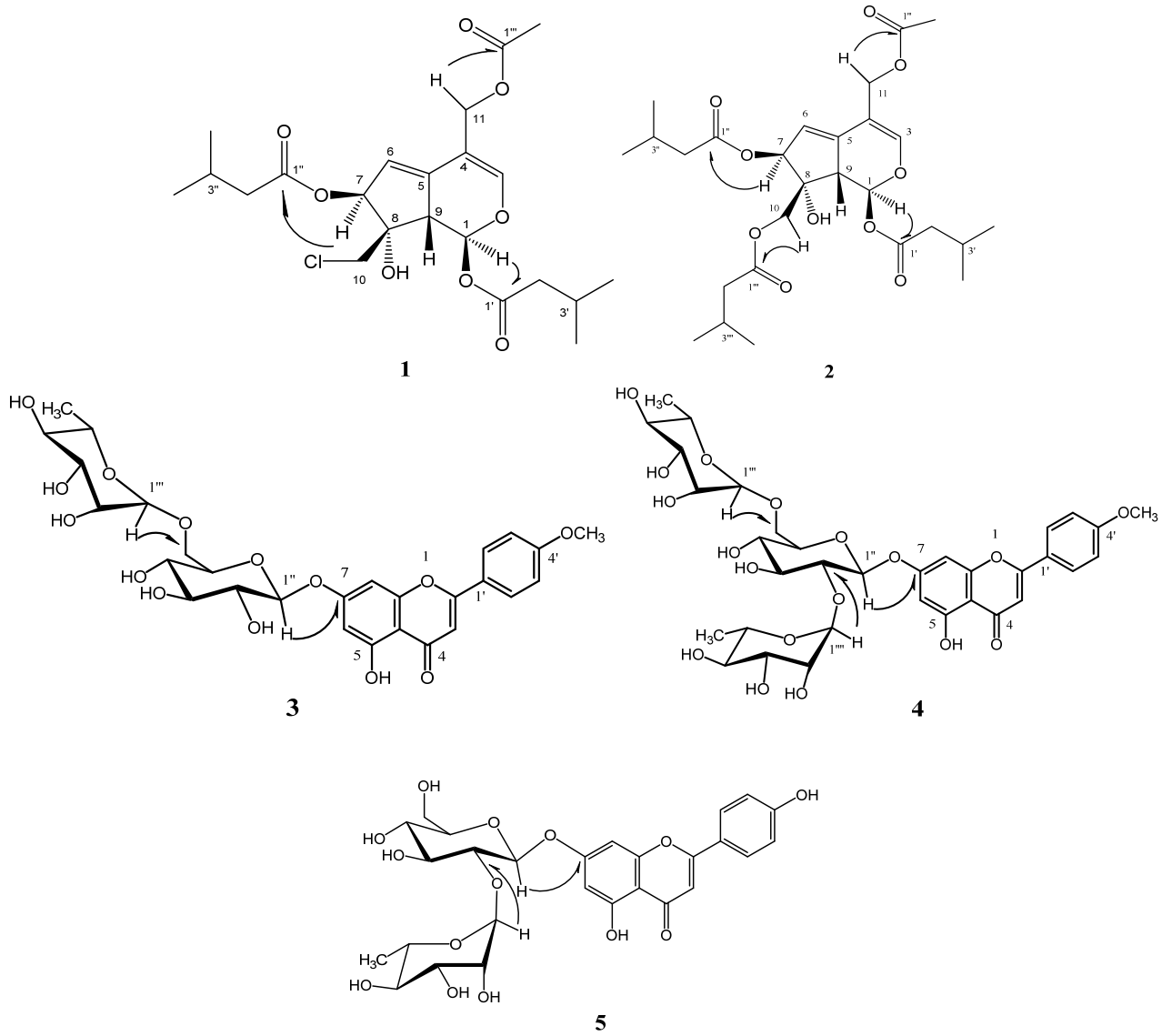

Fig 1: Chemical structure and key HMBC correlations of compounds 1-5.

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