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Chemical constituents of *Chirita drakei* Burtt collected in Ha Long bay, Quang Ninh province, Viet Nam

Part 1. Compounds isolated from the *n*-hexane and ethyl acetate extracts

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Abstract

Two triterpenes, an anthraquinone, two lignans and a phenolic compound were isolated from the *n*-hexane and ethyl acetate extracts of the aerial part of *Chirita drakei* Burtt collected in islands, on mountain slopes of Ha Long bay, Quang Ninh province. Their structures have been elucidated by mass, NMR spectroscopy and comparison with published data. There are no report on the chemical constituents of *Chirita drakei* before our study.

Keywords. *Chirita drakei*, triterpene, anthraquinone, lignin.

1. INTRODUCTION

Ha long bay is one of seven new world's natural wonders, which contains the full value of geology, geomorphology and biodiversity. A set of different plant species were found here, including mangrove plants or species grow in coastal sand of islands, on mountain slopes, on mountain peaks or in store. Until now, 17 endemic plant species have been detected in Ha long bay, for example *Hedyotis lecomtei*, *Allophylus leviscens*, *Chirita gemella*, etc. [1]. *Chirita* is the oldest genus of the Gesneriaceae family, distributed in Malaysia, Southeast Asia and south of China. Some *Chirita* species are used in folk medicine as poultice, hemostasis agents or for treatment of hypertension,... [2]. Until now there are only few studies on chemical and biological activities of the *Chirita* species. However, these investigations revealed interesting results. For example, in the folk medicine of China, *Chirita eburnea* Hance is known as reagent to heal coughing up blood, immunodeficiency diseases. Its chemical composition contains 4 new α-dunnion derivatives, a new anthraquinone together with 7 known anthraquinones. The ethyl acetate fraction of ethanol extract together with two new compounds: (R)-7,8-dihydroxy-α-dunnion, (R)-7-methoxy-6,8-dihydroxy-α-dunnione revealed free radical scavenging activity with IC_{50} value of 5.2 µg/mL, 8.4 µM, and 3.6 µM, respectively while of ascorbate 6.8 µM [3]. *Chirita fimbrisepala* is used in folk medicine for treatment of inflammatory such as hepatitis, enteritis. One of four isolated compounds from the roots of this plant is a new flavone glycoside, named mahuangchiside [4]. Therefore, in order to contribute to the understanding, research, evaluation, protection, maintenance, and exploitation of the genetic resources of rare plant species growing in the Ha Long Bay, the chemical composition *Chirita drakei* Burtt species were investigated. This paper deals with the isolation and structural elucidation of 6 compounds from the *n*-hexane and ethyl acetate extracts of *C. drakei*'s aerial parts. All isolated compounds, except **3**, were obtained for the first time from *Chirita* genus.

2. EXPERIMENTAL

2.1. Equipments and methods

IR: Impact 410, Nicolet, Germany; ESI-MS: LC-MSD-Trap-SL, Varian, USA, NMR: Bruker Avance 500, Germany with TMS as internal reference (for ^{1}H) and solvent signal (for ^{13}C). CC used silica gel

60G, size 0.043-0.063 mm (Merck), TLC: precoated silica gel G60F254 plates (Merck), spots were detected by spraying with vanillin 1 % in conc. H_2SO_4 and heating at 110 ^oC.

2.2. Plant material

The aerial parts of *Chirita drakei* were collected in the islands of Ha Long Bay, Quang Ninh province, Vietnam in October 2013. A voucher specimen (VHH.HL 10.2013.1) is deposited in Institute of Chemistry, VAST, Hanoi, Vietnam. The scientific name was identified by Dr. Tran Thi Phuong Anh, Viet Nam National Museum of Nature, VAST, Hanoi, Vietnam.

2.3. Extraction and isolation of the compounds

The aerial parts of *C. drakei* (1.7 kg) were extracted with MeOH:H_2O (9:1) (four times) at room temperature. The methanol extract was concentrated under *vacuum* and then aqueous solution was extracted with *n*-hexane, EtOAc and *n*-BuOH, successively. The solvent was evaporated in *vacuum* to afford *n*-hexane (10.0 g), EtOAc (10.8 g) and n-BuOH (55.0 g) extracts.

The *n*-hexane extract (10.0 g) was separated by chromatography on silica gel, eluting gradient with *n*-hexane: EtOAc to furnish 10 fractions (H1-H10), which were combined according to TLC monitoring. The third fraction H3 (250 mg) was further purified over silica gel column with *n*-hexane:EtOAc (from 9:1 to 8:2) as eluting solvent to yield 7 mg of compound **1**. The tenth fraction H10 (40 mg) was chromatographed on silica gel with *n*-hexane:EtOAc (7:3) to give 11 mg compound **2**.

The EtOAc extract (10.8 g) was given on silica gel column, eluting gradient with CH_2Cl_2:MeOH (from 100:0 to 0:100) to furnish 12 fractions (E1-E12). The fifth fraction E5 (350 mg) was further purified over silica gel column with *n*-hexane:EtOAc (2:1) as eluent to yield 5 mg of compound **3** and 15 mg of **4**. The seventh fraction E7 (350 mg) was given over silica gel column, eluted with *n*-hexane:EtOAc (6:4) to furnish 11 mg of compound **5**. Compound **6** (15 mg) was obtained by repeated silica gel column chromatography of fraction E10 with CH_2Cl_2:MeOH (10:1). Besides, β-sitosterol and β-sitosterol glucoside were isolated from fraction E3 and E12, respectively, by crystallization method.

24-methylen-lanost-8-en-3β-ol (**1**)

Amorphous white powder. ESI-MS: *m/z*: 441 $[M+H]^+$, $C_{31}H_{53}O$.

^{1}H-NMR (500 MHz, CD_3OD), δ_H (ppm), *J* (Hz): 4.71, 4.66 (each br s, 2H-24), 3.24 (dd, *J* = 4.0; 11.5, H-3), 0.69, 0.81, 0.88, 0.98, 1.00 (each s, 3H, CH_3-18, 19, 28, 29, 30), 0.92 (d, *J* = 6.5, CH_3-21), 1.02, 1.03 (each d, *J* = 6.5, CH_3-26, 27).

^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 35.59 (C-1), 27.86 (C-2), 79.01 (C-3), 38.90 (C-4), 50.39 (C-5), 18.26 (C-6), 28.21 (C-7), 134.41 (C-8, C-9), 37.03 (C-10), 21.01 (C-11), 26.51 (C-12), 44.51 (C-13), 49.82 (C-14), 31.00 (C-15), 30.85 (C-16), 50.41 (C-17), 15.76 (C-18), 18.72 (C-19), 36.49 (C-20), 19.15 (C-21), 35.59 (C-22), 31.29 (C-23), 156.94 (C-24), 34.99 (C-25), 22.00 (C-26), 21.87 (C-27), 15.43 (C-28), 27.97 (C-29), 24.27 (C-30), 105.93 (C-24^1).

3α,24-dihydroxy-urs-12-ene-28-oic acid (**2**)

Amorphous white powder. ESI-MS: *m/z* 473 $[M+H]^+$, 471 $[M-H]^-$ ($C_{30}H_{48}O_4$).

^{1}H-NMR (500 MHz, CD_3OD) δ_H: 3.79 (br s, H-3), 5.26 (br s, H-12), 2.22 (d, *J* = 11.5 Hz, H-18), 3.70, 3.41 (each d, *J* = 11.5 Hz, 2H-24).

^{13}C-NMR (125 MHz, CD_3OD) δ_C: 34.38 (C-1), 71.30 (C-3), 126.91 (C-12), 139.59 (C-13), 54.39 (C-18), 66.32 (C-24), 181.63 (C-28), 17.67 (C-29), 21.57 (C-30).

Digiferruginol (**3**)

Orange-yellow powder. ESI-MS: *m/z* 253 $[M-H]^-C_{15}H_{10}O_4$. ^{1}H- and ^{13}C-NMR, see table 1.

Epoxyconiferyl alcohol (**4**)

Amorphous brown-black powder. ESI-MS: *m/z* 195 $[M-H]^-$ ($C_{10}H_{12}O_4$). ^{1}H- and ^{13}C-NMR, see table 1.

Lariciresinol (**5**)

White powder. $[\alpha]_D^{25}$ (MeOH, c 0.1) = $+32^0$. ESI-MS: *m/z* 383 $[M+Na]^+$ $C_{20}H_{24}O_6Na$. ^{1}H- and ^{13}C-NMR, see table 1.

Isolariciresinol (**6**)

White powder. $[\alpha]_D^{25}$ (MeOH, c 0.1) = $+41^0$. ESI-MS: *m/z* 359 $[M-H]^-$ $C_{20}H_{23}O_6$. ^{1}H- and ^{13}C-NMR, see table 1.

3. RESULTS AND DISCUSSION

Six compounds were isolated from *n*-hexane and ethyl acetate extracts of the aerial parts of *C. drakei* by repeated column chromatography with the appropriate solvent systems. Their structures were identified as 24-methylen-lanost-8-en-3β-ol (**1**), 3α,24-dihydroxy-urs-12-en-28-oic acid (**2**), digiferruginol (**3**), epoxyconiferyl alcohol (**4**),

lariciresinol (**5**), isolariciresinol (**6**) by the analysis of their 1D, 2D-NMR, ESI-MS spectra and compared with published data.

Compound **1** showed the pseudo molecular ion peak at *m/z* 441 $[M+H]^+$ in the positive ESI-MS spectrum, according to the molecular formula $C_{31}H_{52}O$ of this compound. Its ^{1}H-NMR spectrum revealed signals of 8 methyl groups [δ_H 0.69, 0.81, 0.88, 0.98, 1.00 (each 3H, s), 0.92 (d, *J* = 6.5 Hz), 1.02, 1.03 (each d, *J* = 6.5 Hz)], one hydroxy-methin group at δ_H 3.24 (dd, *J* = 4.0 & 11.5 Hz), one >C=CH_2 group at δ_H 4.71, 4.66 (each 1H, br s), and aliphatic protons resonanced in the range of 1.10-2.30 ppm. Besides the carbon signals, which are suitable with ^{1}H-NMR data, the ^{13}C-NMR spectrum indicated the presence of totally substituted double bond at δ_C 134.41 (x2C). These two carbons are the characteristic signals of double bond at C8-C9 in lanosten-type triterpene. Consequently, the structure of **1** was identified as 24-methylen-lanost-8-en-3β-ol from the analysis mentioned above and compared with reported data [5, 6]. It was isolated for the first time from *Neolitsea sericea* species, Lauraceae family.

Compound **2** has molecular formula of $C_{30}H_{48}O_4$ based on the NMR and positive ESI-MS data (*m/z* 473 $[M+H]^+$). The spectroscopic data suggested that **2** belong to the urs-12-en-28-oic acid series. This suggestion was confirmed by ^{1}H-NMR signals at δ_H 5.26 (br s, H-12), 2.22 (d, *J* = 11.5 Hz, H-18), 3.79 br s (H-3) together with 6 methyl groups [δ_H 0.84, 0.95, 1.05, 1.17 (each 3H, s), 0.92 (d, *J* = 6.5 Hz), 0.99 (br s)] and ^{13}C-NMR signals at δ_C 71.30 (C-3), 126.91 (C-12), 139.59 (C-13) and 181.63 (C-28). Besides, its NMR spectra showed a hydroxy-methylen group [(δ_H 3.70, 3.41 (each d, *J* = 11.5 Hz), δ_C 66.32)]. The HMBC correlations between protons at δ_H 3.70, 3.41 and carbons at δ_C 22.81 (C-23), 44.0 (C-4), 71.30 (C-3) confirmed the position of the second hydroxyl group at C-24. The α configuration of hydroxyl group at C-3 was identified by the chemical shift and multiplicity peak of hydroxyl-methine proton (3.79 ppm, brs). Thus, the structure of **2** was determined as 3α,24-dihydroxy-urs-12-en-28-oic acid by comparison with spectroscopic data in the literature [7].

Compound **3** gave the pseudo-molecular ion peak at *m/z* 253 $[M-H]^-$ in the negative ESI-MS spectrum, according to the molecular formula $C_{15}H_{10}O_4$ for this compound. Its ^{1}H- and ^{13}C-NMR of **3** showed an anthraquinone type, including six aromatic protons of two aromatic rings. The substituents in **3** were determined as two carbonyl groups at δ_C 182.30, 188.98, a hydroxyl group at δ_C 159.7 and a hydroxymethyl group at δ_H 4.87 (s, 2H); δ_C 59.13. The spectroscopic data of **3** were indentical to those of 1-hydroxy-2-hydroxymethylanthraquinone (digiferruginol) [8].

The molecular formula of **4** was deduced as $C_{10}H_{12}O_4$ from the negative ESI-MS spectrum (m/z 195 $[M-H]^-$) and NMR data. Its ^{1}H- NMR spectrum revealed a typical pattern of a 1,3,4-trisubstituted benzene ring at δ_H 6.89 (d, *J* = 2.0 Hz), 6.88 (d, *J* = 8.2 Hz) and 6.81(dd, *J* = 2.0 & 8.2 Hz), one aromatic methoxy group at δ_H 3.89 (s, 3H) and one phenolic hydroxyl group at δ_H 5.72. A hydroxy-methylene group attached to C-2' was revealed by the signals at δ_H 3.88 (dd, *J* = 3.5 & 9.0 Hz), 4.24 (dd, *J* = 6.5 & 9.0 Hz) and δ_C 71.64. In summary, the structure of **4** was elucidated as epoxyconiferyl alcohol by comparison with spectroscopic data in the literature [9].

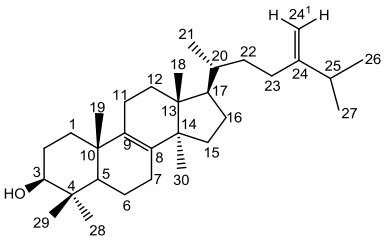
Compound **5** indicated the pseudo-molecular ion peak at *m/z* 383 $[M+Na]^+$ in the positive ESI-MS spectrum, according to the molecular formula $C_{20}H_{24}O_6$ of this compound. Analysis of the ^{1}H-, ^{13}C-NMR and DEPT spectral data indicated **5** to be a tetrahydrofuran-type lignan, showing six aromatic protons in a pair of ABX system at δ_H 6.85 (d, *J* = 2.0 Hz), 6.86 (d, *J* = 8.0 Hz), 6.80 (dd, *J* = 2.0 & 8.0 Hz) and 6.68 (2H, m), 6.83 (d, *J* = 8.0 Hz), two oxy-methylene groups at δ_H 3.73-3.78 m, 3.92 m/δ_C 60.88) and 3.74 (dd, *J* = 6.2 & 8.5 Hz), 4.04 (dd, *J* = 6.5 & 8.5 Hz)/δ_C 72.88, two phenolic hydroxyl groups at δ_H 5.65, 5.56, two methoxy groups (δ_H 3.87, 3.86/δ_C 55.92), an oxy-methine group at δ_H 4.78 (d, *J* = 7.0 Hz)/δ_C 82.83 and four aliphatic protons. Finally, **5** was established as (+) lariciresinol by comparison with spectroscopic data and $[\alpha]_D$ value in the literatures [10]. Moreover, the structure of **5** was also confirmed by 2D-NMR methods.

Compound **6** has the same molecular formula of $C_{20}H_{24}O_6$ as of **5** based on ESI-MS (*m/z* 359 $[M-H]^-$) and NMR data. Its NMR spectra were very similar to those of **5** with two exceptions. The first is the disappearance of one aromatic proton in ^{1}H-NMR of **6**. The second, an oxy-methine group in **5** was replaced by methine group at δ_H 3.82 (d, *J* = 10.0 Hz)/δ_C 48.06 in NMR spectra of **6**. Combination of the above spectroscopic analysis and comparison with spectroscopic data and $[\alpha]_D$ value in [11] led to conclusion that **6** is (+) isolariciresinol. The reassignment of signals CH-2, CH-3 and CH-5', CH-6' in the literature based on the corrections between H-2 (6.67 ppm) and C-7 (33.57 ppm), H-5 (6.21 ppm) and C-7' (48.06 ppm), H-5' (6.76 ppm) and C-1' (138.62 ppm), H-6' (6.63 ppm) and C-7'

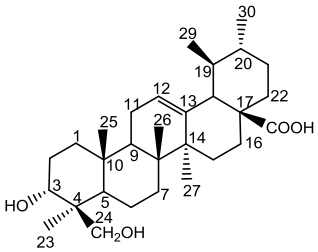
Table 1: ^{1}H- and ^{13}C-NMR (500 and 125 MHz) data of compounds **3-5** ($CDCl_3$) and **6** (CD_3OD)

C	^{1}H				^{13}C			
	3	**4**	**5**	**6**	**3**	**4**	**5**	**6**
1	-	-	-	-	159.70 C	132.88 C	134.79 C	129.03 C
2	-	6.89 d (2.0)	6.85 d (2.0)	6.67 s	137.99 C	108.66 CH	108.36 CH	112.41 CH
3	7.77 d (7.8)	-	-	-	134.09 CH	146.74 C	146.65 C	147.20 C
4	7.85 d (7.8)	-	-	-	119.34 CH	145.26 C	145.04 C	145.27 C
5	8.30 m	6.88 d (8.2)	6.86 d (8.0)	6.21 s	127.30^{+}CH	114.31 CH	114.20 CH	117.35 CH
5a	-	-	-	-	133.21*C	-	-	-
6	7.82 m	6.81 dd (2.0 & 8.2)	6.80 dd (1.5 & 8.0)	-	134.14$^{\#}$CH	118.94 CH	118.75 CH	134.17 C
7	7.82 m	-	4.78 d (7.0)	2.79 d (7.5)	134.69$^{\#}$CH	-	82.83 CH	33.57 CH_2
8	8.30 m	-	2.40 m	2.02 m	126.84^{+}CH	-	52.58 CH	40.03 CH
8a	-	-	-	-	133.73*C	-	-	-
9	-	-	3.73-3.78 m 3.92 m	3.71 m (2H)	188.98 C	-	60.88 CH_2	65.98 CH_2
9a	-	-	-	-	115.20 C	-	-	-
10	-	-	-	-	182.30 C	-	-	-
10a	-	-	-	-	131.85 C	-	-	-
1'	4.87 s	3.88 dd (3.5 & 9.0) 4.24 dd (6.5 & 9.0)	-	-	59.13 CH_2	71.64 CH_2	132.28 C	138.62 C
2'	-	3.10 m	6.68 m	6.70 d (1.5)	-	54.13 CH	111.24 CH	113.82 CH
3'	-	4.74 d (4.5)	-	-	-	85.86 CH	146.55 C	149.01 C
4'	-	-	-	-	-	-	144.01 C	145.94 C
5'	-	-	6.83 d (8.0)	6.76 d (8.0)	-	-	114.44 CH	115.99 CH
6'	-	-	6.68 m	6.63 dd (1.5 & 8.0)	-	-	121.97 CH	123.20 CH
7'	-	-	2.54 dd (10.5 & 13.0) 2.90 dd (5.0 & 13.5)	3.82 d (10.0)	-	-	33.30 CH_2	48.06 CH
8'	-	-	2.72 m	1.79 t (10.0)	-	-	42.41 CH	48.02 CH
9'	-	-	3.74 dd (6.2 & 8.5) 4.04 dd (6.5 & 8.5)	3.42 dd (4.0 & 11.0) 3.67 d (4.0)	-	-	72.88 CH_2	62.27 CH_2
OMe	-	3.89 s	3.87 s	3.79 s	-	55.94 CH_3	55.92	56.36 CH_3
OMe	-	-	3.86 s	3.82 s	-	-	55.92	56.41 CH_3
CH_2OH	2.35 brs	-	-	-	-	-	-	-
OH-1	13.01 s	-	-	-	-	-	-	-
OH-4	-	5.72 s	5.65 s	-	-	-	-	-
OH-4'	-	-	5.56 s	-	-	-	-	-

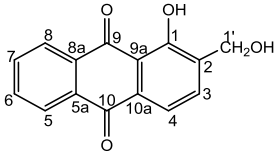
(48.06 ppm) in the HMBC spectrum. (+) lariciresinol and (+) isolariciresinol were isolated before from medicinal plant *Fagraea racemosa* [10]. The structure of compounds **1-6**.



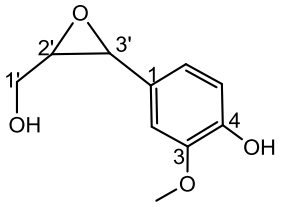
1: 24-methylen-lanost-8-en-3β-ol



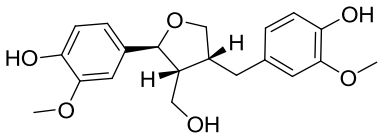
2: 3α,24-dihydroxy-urs-12-en-28-oic acid



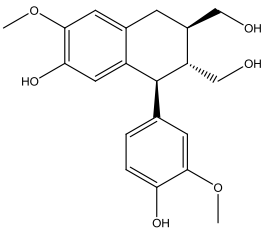
3: Digiferruginol



4: Epoxyconiferyl alcohol



5: (+)-Lariciresinol



6: (+)-Isolariciresinol

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