

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

Part 2. Compounds isolated from the *n*-butanol extract

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Abstract

Four phenol glycosides together with cannabicide B were isolated from the *n*-butanol extract of the aerial part of *Chirita drakei* Burt 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. Compounds **4** and **5** were obtained for the first time from the genera *Chirita*.

Keywords. *Chirita drakei*, phenylethanoid glycoside, phenol glucoside, α,β -unsaturated γ -lactone C-glucoside.

1. INTRODUCTION

In a previous article [1] we have reported the isolation and structure determination of compounds with the medium polarity from *n*-hexane and ethyl acetate extracts of aerial parts of *Chirita drakei* Burt plant collected in the islands, limestone slopes in the Ha Long Bay, Quang Ninh province. This paper reports continuously the isolation and structure elucidation of the more polar components from *n*-butanol extract, including three phenylethanoid glycosides (**1-3**), one phenol glucoside (**4**) and an α,β -unsaturated γ -lactone C-glucoside (Cannabicide B) (**5**).

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 ¹H) and solvent signal (for ¹³C). 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. H₂SO₄ and heating at 110 °C.

2.2. Plant material

The aerial parts of *C. 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, Vietnam National Museum of Nature, VAST, Hanoi, Vietnam.

2.3. Extraction and isolation

The dried powdered aerial parts of *C. drakei* (1.7 kg) were extracted exhaustively with MeOH:H₂O (9:1) at room temperature. The methanol extract was concentrated under vacuum and then aq. 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*-BuOH extract (55 g) was chromatographed on a silica gel column, eluted with a gradient solvent system of CH₂Cl₂:MeOH (from 100:0 to 0:100), to give 5 fractions (F1–F5). The fraction F4 (11 g) was subjected to silica gel column, eluting with a gradient system of EtOAc:MeOH:H₂O (from 5:0.5:0.1 to 2.5:0.5:0.1),

followed by RP-18 (MeOH:H₂O, 2:3) and Sephadex LH-20 (MeOH) to yield compounds **1** (20 mg), **2** (10 mg), **4** (10 mg) and **5** (10 mg). The fraction F5 (7 g) was purified over column silica gel, eluting with a gradient system of CH₂Cl₂:MeOH:H₂O (from 4:1:0.1 to 2:1:0.1) followed by RP-18 (acetone:H₂O, 3:7) and Sephadex LH-20 (MeOH) to yield **3** (10 mg).

3. RESULTS AND DISCUSSION

The structures of five compounds isolated from *n*-BuOH extract of *C. drakei* were identified as 2-(3,4-dihydroxyphenyl)ethyl-β-D-glucopyranoside (**1**), desrhamnosyl isoacteoside (**2**), chiritoside C (**3**), brachyanin D (**4**) and cannabioside B (**5**) by the analysis of their 1D, 2D-NMR and ESI-MS spectra and comparison with published data.

Compound **1**, obtained as amorphous pale yellow powder. It has the pseudo-molecular ion peak at *m/z* 315 [M-H]⁻ in the negative ESI-MS spectrum, according to the molecular formula C₁₄H₂₀O₈. Its ¹H and ¹³C-NMR spectra revealed the signals of a 1,3,4-trisubstituted phenylethyl alcohol: three aromatic protons at δ_H 6.71 (d, *J* = 1.5 Hz); 6.69 (d, *J* = 8.5 Hz); 6.57 (dd, *J* = 1.5 & 8.5 Hz), oxymethylene protons at δ_H 3.72 (m), 4.05 (m), methylene protons at δ_H 2.80 (m) and one β-glucopyranose unit: anomeric signals at δ_H 4.31 (d, *J* = 8.0 Hz) and δ_C 104.35. The spectroscopic data of **1** were identical with those of 2-(3,4-dihydroxyphenyl)ethyl-β-D-glucopyranoside in the literature [2].

Compound **2**, obtained as a pale yellow powder. The molecular formula of **2** was determined to be C₂₃H₂₆O₁₁, based on the positive ESI-MS (*m/z* 501 [M+Na]⁺) and NMR data. The ¹H and ¹³C-NMR spectra of **2** were very similar to those of **1** but appeared additionally the signals of a *trans*-caffeoyl moiety with an ABX spin system at δ_H 7.05 (d, *J* = 1.5 Hz), 6.78 (d, *J* = 8.0 Hz), 6.91 (dd, *J* = 1.5 & 8.0 Hz); a *trans* double bond at δ_H 6.30 (d, *J* = 15.7), 7.58 (d, *J* = 15.7 Hz) and a carbonyl carbon at δ_C 169.29. The connection of the caffeoyl moiety in **2** was determined at C-6' of β-glucopyranose by the downfield shifted signals of CH₂-6' compared with those of **1** (Δδ_C = 1.92 and Δδ_H = 0.65). Consequently, the structure of **2** was determined to be desrhamnosyl isoacteoside by comparison with reported data [3].

Compound **3**, pale yellow powder, showed pseudo-molecular ion peak at *m/z* 639 [M-H]⁻ according to C₂₉H₃₆O₁₆. Its NMR data indicated that **3** is also a phenylethanoid glycoside with phenylethyl alcohol, caffeoyl and two glucopyranose

units. The two anomeric protons at δ_H 4.88 (d, *J* = 7.5 Hz) and 4.35 (d, *J* = 8.0 Hz) confirmed this suggestion. The connection of the second β-glucopyranosyl unit at C-4' of caffeoyl group was identified when compared with spectroscopic data of chiritoside C in [4]. Chiritoside C was one of the first three representatives possessed β-glucopyranosyl moiety at C-4 of caffeoyl group, isolated from *Chirita sinensis* since 1994.

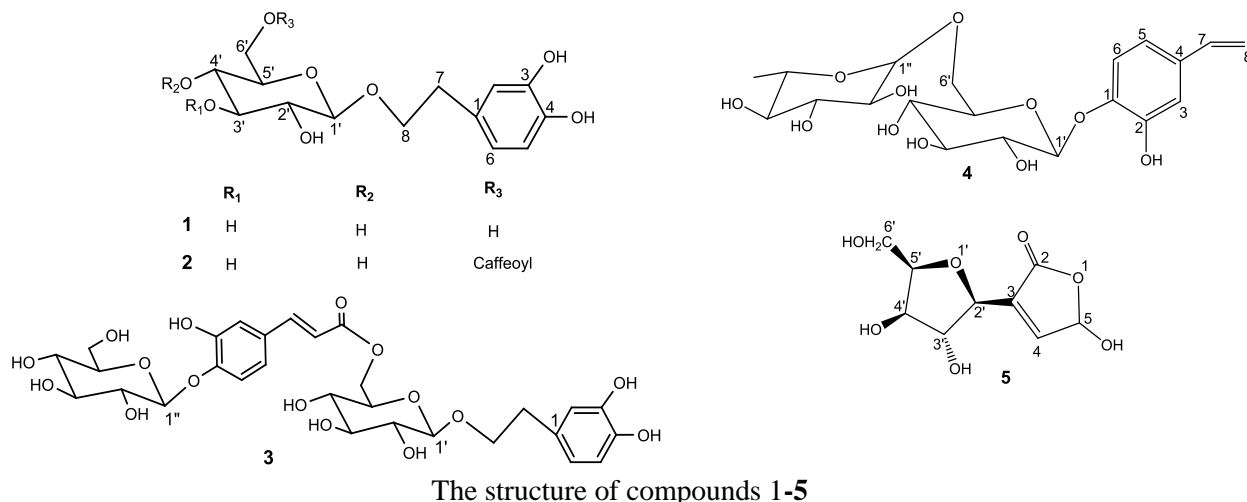
Compound **4** was obtained as a colourless oil. Its molecular formula was determined to be C₂₀H₂₈O₁₁ based on the NMR and positive ESI-MS data (*m/z* 467 [M+Na]⁺). Its ¹H-NMR spectrum showed three aromatic ABX protons at δ_H 6.97 (d, *J* = 2.0 Hz); 6.89 (dd, *J* = 2.0 & 8.5 Hz) and 7.13 (d, *J* = 8.5 Hz) and three protons of a terminal double bond at δ_H 6.63 (dd, *J* = 11.0 & 17.5 Hz), 5.65 (d, *J* = 17.5 Hz) and 5.13 (d, *J* = 11.0 Hz). Two sugar units of **4** were showed to be β-glucopyranose and α-rhamnopyranose by revealing of the anomeric signals at δ_H 4.75 (d, *J* = 6.5 Hz), δ_C 104.12 and δ_H 4.76 (br s), δ_C 102.25, respectively in the NMR spectra. The linkage of the α-rhamnose to the β-glucose is the same as rutinose, indicating by the HMBC correlation of H-1'' (δ_H 4.76) to C-6' (δ_C 67.97). The position of rutinose and ethenyl moieties was confirmed by HMBC correlations between H-1' (δ_H 4.75) and C-1 (δ_C 146.59), H-7 (δ_H 6.63) and C-4 (δ_C 135.01), C-5 (δ_C 119.52) and C-3 (δ_C 114.37). The above mentioned analysis led to conclude that **4** is 2-hydroxy-4-ethenylphenyl-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside (brachyanin D) [5]. This compound was isolated for the first time from *Stauntonia brachyanthera* Hand-Mazz in 2015.

Compound **5** obtained as a colorless liquid and gave the pseudo-molecular ion peak at *m/z* 234 [M+2H]⁺ in the positive ESI-MS spectrum, according to the molecular formula C₉H₁₂O₇. Its NMR data confirmed the presence of one alkenyl moiety signal at (δ_H 8.02)/δ_C 153.03, 142.73); an oxy-methylen group at δ_H 3.86 (dd, *J* = 3.0 & 12.0 Hz), 3.75 (dd, *J* = 2.5 & 12.0 Hz)/δ_C 62.29); five oxy-methine group at δ_H 5.71 (d, *J* = 7.5 Hz)/δ_C 102.65; δ_H 5.91 (d, *J* = 4.5 Hz)/δ_C 90.76; δ_H 4.20 (t, *J* = 5.0 Hz)/δ_C 75.73; δ_H 4.16 (t, *J* = 5.0 Hz)/δ_C 71.31 and δ_H 4.02 (m)/δ_C 86.38 together with one lactone group at δ_C 166.19. The linkages of two rings were established by HMBC correlations of H-4 (δ_H 8.02) to C-2 (δ_C 166.19), C-5 (δ_C 102.65) and C-2' (δ_C 90.76). The relative stereochemistry of **5** was determined by NOESY correlations between H-4'/H-5', H-5'/H-2' indicating that H-2', H-4' and H-5' are on the same side of the molecule.

Consequently, the structure of **5** was concluded to be cannabicide B by comparison with reported data [6]. It was isolated the first time from *Senecio cannabifolius* and showed antibacterial activities

against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.

The ^1H and ^{13}C -NMR data of compounds **1-5** were given in tables 1 and 2.



The structure of compounds **1-5**

Table 1: ^1H -NMR spectroscopic data for compounds **1-5** (500 MHz, CD_3OD)

Position	1	2	3	4	5
2	6.71 d (1.5)	6.69 d (2.0)	6.69 d (2.0)	-	-
3	-	-	-	6.97 d (2.0)	-
4	-	-	-	-	8.02 d (8.0)
5	6.69 d (8.5)	6.65 d (8.0)	6.65 d (8.0)	6.89 dd (2.0 & 8.5)	5.71 d (7.5)
6	6.57 dd (1.5 & 8.5)	6.56 dd (2.0 & 8.0)	6.55 dd (2.0 & 8.0)	7.13 d (8.5)	-
7	2.80 m	2.81 t (6.7)	2.81 t (7.5)	6.63 dd (11.0 & 17.5)	-
8	3.72 m 4.05 m	3.74 m 3.99 m	3.74 m 3.96 m	5.65 d (17.5) 5.13 d (11.0)	-
Glucose-1					
1'	4.31 d (8.0)	4.35 d (8.0)	4.35 d (8.0)	4.75 d (6.5)	-
2'	3.20 t (8.0)	3.24 t (8.0)	3.24 t (8.0)	3.38 m	5.91 d (4.5)
3'	3.28-3.39 m	3.38 m	3.32-3.99 m	3.47 m	4.20 t (5.0)
4'				3.73 m	4.16 t (5.0)
5'				3.57 m	4.02 m
6'	3.68 dd (5.0 & 12.0) 3.88 dd (1.5 & 12.0)	4.36 dd (5.5 & 11.5) 4.51 dd (2.0 & 11.5)	4.34 dd (5.5 & 11.5) 4.55 dd (2.0 & 11.5)	4.06 dd (1.5 & 11.0) 3.64 dd (6.5 & 11.0)	3.86 dd (3.0 & 12.0) 3.75 dd (2.5 & 12.0)
Caffeoyl					
2	-	7.05 d (1.5)	7.11 d (2.0)	-	-
5	-	6.78 d (8.0)	7.20 d (8.5)	-	-
6	-	6.91 dd (1.5 & 8.0)	6.94 dd (2.0 & 8.5)	-	-
β	-	6.30 d (15.7)	-	-	-
γ	-	7.58 d (15.7)	-	-	-

Glucose-2				Rhamnose	
1''	-	-	4.88 d (7.5)	4.76 br s	-
2''	-	-	3.32-3.99 m	3.38 m	-
3''	-	-		3.89 m	-
4''	-	-		3.50 m	-
5''	-	-		3.69 dd (6.5 & 9.5)	-
6''	-	-		1.25 d (6.5)	-

Table 2: ^{13}C -NMR spectroscopic data for compounds **1-5** (125 MHz, CD_3OD)

No	1	2	3	4	5
1	131.55 C	131.42 C	131.34 C	146.59 C	-
2	117.13 CH	117.12 CH	117.08 CH	148.31 C	166.19 C
3	146.11 C	146.06 C	146.16 C	114.37 CH	153.03 C
4	144.64 C	144.57 C	144.67 C	135.01 C	142.73 CH
5	116.33 CH	116.42 CH	116.38 CH	119.52 CH	102.65 CH
6	121.27 CH	121.30 CH	121.24 CH	118.55 CH	-
7	36.55 CH_2	36.62 CH_2	36.74 CH_2	137.65 CH	-
8	72.09 CH_2	72.38 CH_2	72.45 CH_2	112.84 CH_2	-
Glucose-1					
1'	104.35 CH	104.47 CH	104.57 CH	104.12 CH	-
2'	75.11 CH	75.01 CH	75.08 CH	74.06 CH	90.76 CH
3'	77.92 CH	77.87 CH	77.97 CH	77.63 CH	75.73 CH
4'	71.62 CH	71.70 CH	71.85 CH	72.43 CH	71.31 CH
5'	78.07 CH	75.36 CH	75.42 CH	77.10 CH	86.38 CH
6'	62.72 CH_2	64.64 CH_2	64.81 CH_2	67.97 CH_2	62.29 CH_2
Caffeoyl					
1	-	127.65 C	130.99 C	-	-
2	-	115.10 CH	115.80 CH	-	-
3	-	146.73 C	148.49 C	-	-
4	-	149.59 C	148.92 C	-	-
5	-	116.58 CH	118.11 CH	-	-
6	-	123.20 CH	122.45 CH	-	-
α	-	169.29 C	168.69 C	-	-
β	-	114.80 CH	117.08 CH	-	-
γ	-	147.29 CH	146.37 CH	-	-
Glucose-2				Rhamnose	
1''	-	-	103.45 CH	102.25 CH	-
2''	-	-	74.81 CH	71.60 CH	-
3''	-	-	77.56 CH	72.19 CH	-
4''	-	-	71.35 CH	74.88 CH	-
5''	-	-	78.36 CH	69.89 CH	-
6''	-	-	62.47 CH_2	17.95 CH_3	-

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