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WATER-SOLUBLE COMPONENTS OF *Ancistrocladus cochinchinensis*

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Abstract. Using various chromatography methods, a saponin, ginsenoside Rg1 (**1**), and three phenolic glycosides, tortoside A (**2**), phlorizin (**3**), and 4-hydroxy-2-methoxyphenyl-6-*O*-syringyl-β-D-glucopyranoside (**4**) were isolated from the water-soluble fraction of *Ancistrocladus cochinchinensis.* Their structures were elucidated by 1D- and 2D-NMR spectroscopic analyses and comparison with those reported in the literature. Compound **1** was reported from *A. cochinchinensis* for the first time.

Keywords: Ancistrocladus cochinchinensis, ginsenoside Rg1, tortoside A, phlorizin.

1. INTRODUCTION

Ancistrocladus cochinchinensis is an endemic species of Viet Nam. It has been used in folk medicines with diruric, anti-febrile, and anti-phlogistic properties [1]. They are rich in naphtylisoquinoline alkaloids (NIQs) which exhibited unique chemical structure, specific biological activities and hence recently received much interests of medicinal chemists. They have been demonstrated to exhibit excellent, specific on anti-malarial, anti-protozoal, insecticidal, antimicrobial, anti-proliferative and anti-HIV activities [2 - 7]. In this paper, we describe the isolation and structural elucidation of four compounds belonging to saponin and phenolic derivatives that were isolated from *A. cochinchinensis* leaves.

2. MATERIAL AND METHODS

2.1. Plant Material

The plant, *A. cochinchinenssis* were collected in March 2013 at Vinh Phuc province, Viet Nam and taxonomically identified by one of the authors, Prof. Ninh Khac Ban. A voucher specimen (TNSV-TQ8) was deposited at the Institute of Marine Biochemistry, VAST, Viet Nam.

2.2. General experimental procedures

NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) with TMS as the internal standard. Column chromatography (CC) was performed using silica gel (Kieselgel 60,70 - 230 mesh and 230 - 400 mesh, Merck) or RP-18 resins (30 - 50 µm, Fujisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ plates (0.25 mm, Merck).

2.3. Extraction and isolation

Air-dried leaves of *A. cochinchinensis* (3.5 kg) were powdered and ultrasonically extracted three times with methanol (each 10.0 L, 5 h). Evaporation of the solvent *in vacuo* gave a methanol extract (200.0 g). The methanol extract was suspended in distilled water and successively partitioned with dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc) to yield CH_2Cl_2 (100.0 g), EtOAc (3.5 g), and water-soluble layers. The water layer was then passed through Diaion HP-20 column chromatography, washed with distilled water and desorbed with methanol/water (25 %, 50 %, 75 % and 100 % volume of methanol, each 1.0 L, stepwise) to give two main fractions ACW1-ACW2. Fraction ACW2 (13.0 g) was chromatographed on a silica gel column, eluting with gradient of dichloromethane/methanol (from 100/1 to 0/100, v/v) to give five sub-fractions ACW2A-ACW2E. The sub-fraction ACW2C was divided into three smaller fractions, ACW3A-ACW3C, on a silica gel column, eluting with dichloromethane/methanol/water (8.0/1.0/0.1, v/v/v). Compound **2** (15.0 mg) was obtained from ACW3A fraction using a RP-18 column, eluted with methanol/water (1.0/1.4, v/v). Fraction ACW3B was chromatographed on a silica gel column, eluting with ethyl acetate/methanol (10.0/1.0, v/v) to yield compound **4** (8.0 mg).

Fraction ACW2D was chromatographed on a silica gel column, eluting with ethyl acetate/methanol/water (8.0/1.0/0.1, v/v/v) to give three fractions ACW4A-ACW4C. Compound **3** (6.0 mg) was obtained from fraction ACW4B using a silica gel column, eluted with dichloromethane/methanol/water (4.0/1.0/0.1, v/v/v). Compound **1** (8.0 mg) was obtained from fraction ACW4C using a RP-18 column, eluted with methanol/water (1.0/1.0, v/v).

Ginsenoside Rg1 (1): White powder, ESI-MS: *m/z* 801.4 $[M + H]^+$, 823.4 $[M + Na]^+$ $C_{47}H_{72}O_{14}$ (M = 800). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1.

Tortoside A (2): Amorphous powder, ESI-MS: *m/z* 603.1 $[M + Na]^+$, $C_{18}H_{36}O_{13}$ (M = 580). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 2.

Phlorizin (3): Pale yellow needles, ESI-MS: *m/z* 459.1 $[M + Na]^+$, $C_{21}H_{24}O_{10}$ (M = 436). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 2.

4-hydroxy-2-methoxyphenyl-6-*O*-syringyl-*β*-*D*-glucopyranoside (4): White powder, ESI-MS: *m/z* 505.2 $[M + Na]^+$, $C_{22}H_{25}O_{12}$ (M = 482). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 2.

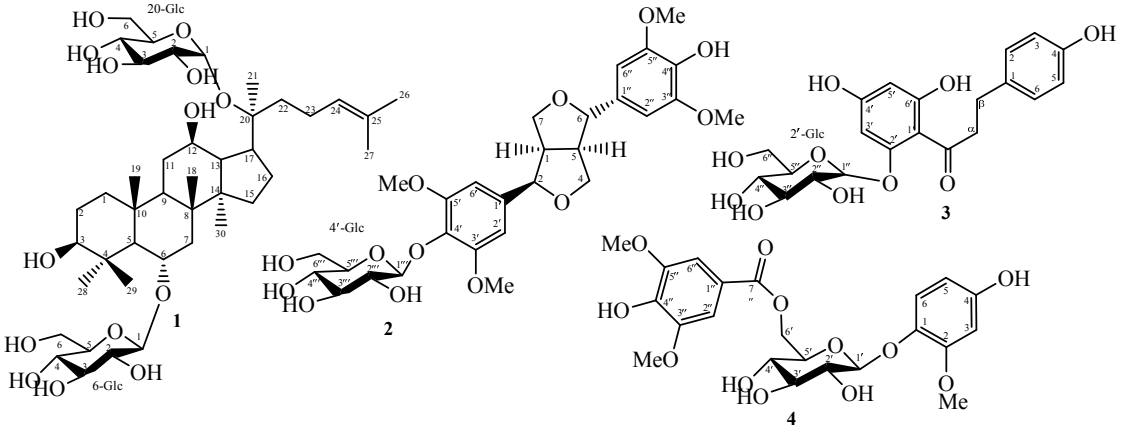

Figure 1. Chemical structures of compounds **1**-**4.**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **1** showed the presence of eight singlet methyl groups at δ_H 0.96, 1.02, 1.03, 1.12, 1.35, 1.37, 1.65, and 1.70 (each 3H, s); two anomeric protons at δ_H 4.63 (1H, d, J = 8.0 Hz), and 4.37 (1H, d, J = 7.5 Hz); and an olefin proton at 5.12 (1H, t, J = 7.0 Hz) suggesting the presence of a disaccharide triterpenoid compound. The ^{13}C-NMR and DEPT spectra of **1** exhibited 42 carbon signals, corresponding to a triterpenoid (including 8 methyl, 7 methine, 9 methylene and 6 non-protonated carbons) and two glucose moieties (including two anomeric carbons at δ_C 105.6 and 98.3; and two oxymethylene carbons at δ_C 62.9 and 62.6). The protons were assigned to respective carbons with the aid of HSQC spectrum (Table 1). On the basis of these data and by comparison with literature values, compound **1** was identified as ginsenoside Rg1 (Table 1) [8]. The structure of **1** was further confirmed based on HMBC analysis. The HMBC correlations from methyl signals at δ_H 1.02 (H-19), 1.03 (H-29) and 1.35 (H-28) to carbon C-5 (δ_C 61.8); from proton H-5 (δ_H1.15) and from anomeric proton at δ_H 4.37 (H-1′ of 6-Glc) to carbon C-6 (δ_C 80.9) confirmed the position of first glucose moiety at C-6. The position of the remaining glucose moiety at C-20 (δ_C 84.9) was demonstrated based on the HMBC correlations from anomeric proton at δ_H 4.63 (H-1″ of 20-Glc) and methyl signal at δ_H 1.37 (H-21) to carbon C-20 (δ_C 84.9). The HMBC correlations of two methyl protons at 1.65 (H-27) and 1.70 (H-26) to olefin carbons at 125.8 (C-23)/ 132.3 (C-24) confirmed the double bond were at C-23. Based on the above evidence, the chemical structure of **1** was established as ginsenoside Rg1 (Figure 1). Ginsenoside Rg1 have been reported as one of the major bioactive ingredients in *Panax ginseng* [9, 10]. To our best knowledge, compound **1** was reported from *A. cochinchinensis* for the first time.

Compound **2** was obtained as amorphous powder. The 1D, 2D-NMR spectrum showed the presence of two pairs of equivalent aromatic protons at 6.67 (2H, brs, H-2′, H-6′) and 6.73 (2H, brs, H-2″, H-6″); four aromatic methoxy groups at 3.86 (6H, s, 3′, 5″-CH_3) and 3.87 (6H, s, 3″, 5″-CH_3); one β-glucose moiety [105.3/ 4.86 (1H, d, J = 7.5 Hz), 75.7 (CH), 78.3 (CH), 71.3 (CH), 77.8 (CH) and 62.6 (CH_2)]. In addition, the signals of a bis-tetrahydrofuran ring were observed at 87.6 (CH), 87.2 (CH), 72.9 (CH_2), 72.8 (CH_2), 55.9 (CH) and 55.5 (CH). The NMR

Table 1. ^{1}H- and ^{13}C-NMR data for **1** and reference compounds.

C	δ_C	$^{\#}\delta_C$ [a]	δ_H	C	δ_C	$^{\#}\delta_C$ [a]	δ_H
1	39.5	40.2	1.75/1.08*	22	35.9	36.6	1.66/1.84 (t, 8.5)
2	27.7	27.6	1.61/1.42*	23	23.1	24.2	2.11 (m)
3	78.4	79.9	3.12 (m)	24	125.7	125.8	5.12 (t, 7.0)
4	40.2	40.5	-	25	130.8	132.3	-
5	61.2	61.8	1.15 brs	26	25.6	25.8	1.70 (s)
6	77.9	80.9	4.11 (dt, 3.0, 10.5)	27	17.6	17.8	1.65 (s)
7	44.9	45.3	2.06 (dd, 3.5, 13.0)/1.68 *	28	31.6	31.4	1.35 (s)
8	40.9	41.9	-	29	16.2	16.1	1.03 (s)
9	49.8	50.6	1.51 (dd, 2.0, 13.0)	30	17.4	17.1	0.96 (s)
10	39.5	40.4	-	1’	105.8	105.6	4.37 (d, 7.5)
11	30.1	31.0	1.87/1.62 *	2’	75.3	75.5**	3.23 (t, 8.5)
12	70.1	71.2	3.70 (dd, 5.0, 10.0)	3’	80.0	79.1*	3.38 *
13	48.9	49.5	1.77 brd, 10.5)	4’	71.6	71.9***	3.30 *
14	51.2	52.4	-	5’	80.0	78.3*	3.36 *
15	29.9	31.5	1.40/1.20 *	6’	62.9	62.9	3.84 (dd, 1.5, 12.0)/3.65 *
16	26.8	27.2	1.96/1.69 *		20-Glc		
17	51.6	53.1	2.30 (m)	1’’	98.0	98.3	4.63 (d, 8.0)
18	17.4	17.6	1.12 (s)	2’’	74.9	75.4**	3.11 (t, 8.5)
19	17.4	17.9	1.02 (s)	3’’	79.0	77.9*	3.24 *
20	83.1	84.9	-	4’’	71.3	71.7***	3.33 *
21	22.2	22.8	1.37 (s)	5’’	77.9	77.7*	3.29 *
22	35.9	36.6	1.66/1.84 (t, 8.5)	6’’	62.9	62.6	3.79 (dd, 2.0, 12.0)/3.67 *

*[a] recorded in CD_3OD, *δ_C of ginsenoside Rg1 recorded in C_5D_5N* [8]*; *, overlapped signals*

data suggested that compound **2** be a lignan glycoside. The NMR data of **2** were completely similar to those of tortoside A (Table 2) [11]. The position of glucose at C-4′ was confirmed with the aid of HMBC correlations from proton 4.73 (H-2) to carbons 139.5 (C-1′)/ 104.6 (C-2′, C-6′); and strong HMBC correlations from aromatic proton 6.67 (H-2′, H-6′) and anomeric proton 4.87 (H-1′′′) to carbon 135.6 (C-4′). From the above evidence, compound **2** was identified as tortoside A (Figure 1).

Compound **3** was obtained as pale yellow needles. The ^{1}H-NMR spectrum of **3** showed two pair of equivalent signals of a 1,4-disubstituted aromatic ring at 7.08 (2H, d, J = 8.5 Hz) and 6.70 (2H, d, J = 8.5 Hz); two other aromatic signals at 6.20 (1H, d, J = 2.0 Hz) and 5.98 (1H, d, J = 2.0 Hz); a sugar moiety with *axial* anomeric proton at 5.50 (1H, d, J = 7.0 Hz). The ^{13}C-NMR and DEPT spectra showed 21 signals including six carbons of a glucose [102.1, 74.7, 78.4, 71.1, 78.5 and 62.4], seven non-protonated carbons, six aromatic methine carbons and two methylene carbons. Its NMR data were completely similar to those of phlorizin (Table 2) [12]. The protons were assigned to corresponding carbons with the aid of HSQC spectrum (Table 2). The position of sugar moiety was confirmed by HMBC correlation between anomeric proton 5.05 (H-1’’) and carbon 162.3 (C-2′). Thus, compound **3** was identified as phlorizin (Figure 1).

Table 2. NMR data for compounds **2-4** and reference compounds.

2				**3**				**4**			
C	**$^{@}\delta_C$**	**δ_C[a]**	**δ_H**	**C**	**$^{\%}\delta_C$**	**δ_C[b]**	**δ_H**	**C**	**$^{\$}\delta_C$**	**δ_C[a]**	**δ_H**
1	55.5	55.5	3.15 (m)	1	133.9	133.9	-	1	140.3	140.8	-
2	87.6	87.6	4.73 (d, 4.5)	2, 6	130.4	130.4	7.08 (d, 8.5)	2	150.6	152.1	-
4	72.9	72.8	4.29 (m)	3, 5	116.1	116.1	6.70 (d, 8.5)	3	101.4	101.8	6.44 (d, 2.5)
5	55.7	55.9	3.15 (m)	4	156.4	156.4	-	4	153.7	155.0	-
6	87.2	87.2	4.78 (d, 4.0)	1′	106.8	106.7	-	5	107.0	107.5	6.06 (dd, 3.0, 8.5)
8	73.0	72.9	3.93 (m)	2′	162.3	162.3	-	6	118.0	120.0	6.91 (d, 8.5)
1’	139.5	139.5	-	3′	95.4	95.6	6.20 (d, 2.0)	2-OCH_3	56.2	56.5	3.79 (s)
2’, 6'	104.5	104.6	6.67 (s)	4′	167.6	167.6	-	1’	102.6	104.3	4.72 (d, 7.5)
3’, 5'	154.4	154.4	-	5′	98.3	98.4	5.98 (d, 2.0)	2’	74.1	75.0	3.50 *
4’	135.6	135.6	-	6′	166.0	166.3	-	3’	76.7	77.7	3.50 *
1’’	133.1	133.1	-	α	47.0	46.9	3.47 (t, 7.0)	4’	71.2	72.1	3.43 *
2’’, 6"	104.8	104.9	6.73 (s)	β	30.8	30.9	2.89 (t, 7.0)	5’	74.7	75.6	3.69 (ddd, 2.0, 7.5, 12.5)
3’’, 5"	149.3	149.4	-	C=O	206.5	206.5	-	6’	65.0	65.1	4.41 (dd, 7.5, 12.5) 4.69 (dd, 2.0, 12.5)
4’’	136.2	136.3	-	1″	102.0	102.1	5.05 (d, 7.0)	1’’	119.8	120.5	-
3’, 5'-CH_3	57.1	57.1	3.86 (s)	2″	74.7	74.7	3.50 *	2’’, 6"	108.1	108.5	7.32 (s)
3’’, 5" CH_3	56.8	56.8	3.87 (s)	3″	78.4	78.4	3.49 *	3’’, 5"	148.6	149.5	-
4’ -Glc				4″	71.1	71.1	3.41 (brd, 8.0)	4’’	142.8	144.9	-
1’’’	105.3	105.3	4.87 (d, 7.5)	5″	78.5	78.5	3.48 *	7’’	167.3	168.2	-
2’’’	75.7	75.7	3.51 (m)	6″	62.4	62.4	3.92 (brd,12.0) 3.73 (dd, 5.5, 12.0)	3’’, 5"-OCH_3	56.8	56.8	3.85 (s)
3’’’	78.3	78.3	3.23 (m)								
4’’’	71.4	71.3	3.44 *								
5’’’	77.8	77.8	3.44 *								
6’’’	62.6	62.6	3.79 (dd, 2.0, 12.0) 3.68 (dd, 5.0, 12.0)								

[a)]recorded in CD_3OD, $^{@}\delta_C$ of tortoside A recorded in CD_3OD [11], $^{\%}\delta_C$ of phlorizin recorded in CD_3OD [12], $^{\#}\delta_C$ of (+) 4-hydroxy-2-methoxyphenyl-6-O-syringyl-β-D-glucopyranoside recorded in acetone-d_6 [13]. *: overlapped signals.

Compound **4** was isolated as white powder. There are three protons of a trisubstituted aromatic ring at 6.98 (1H, d, J = 8.5 Hz), 6.44 (1H, d, J = 2.5 Hz) and 6.60 (1H, dd, J = 3.0, 8.5 Hz); two singlet equivalence protons 7.32 (2H, s); three aromatic methoxy groups at 3.85 (6H, s, 2 x CH_3) and 3.79 (3H, s); and an anomeric of a β-oriented sugar moiety at 4.73 (1H, d, J = 7.5 Hz) were observed. The ^{13}C-NMR and HSQC spectrum gave 22 carbons including three methoxy groups, seven non-protonate carbons, five aromatic methine carbons and three methylene carbons (Table 2). Based on its NMR data and by comparison with those of the

previous report, compound **4** was suggested as hydroxy-2-methoxyphenyl-6-*O*-syringyl-β-D-glucopyranoside [13]. The position of syringyl group at C-6′ (65.1) was assigned with the aid of HMBC correlation from oxymethylene protons 4.41 and 4.69 (H-6′) to carbonyl carbon 168.2

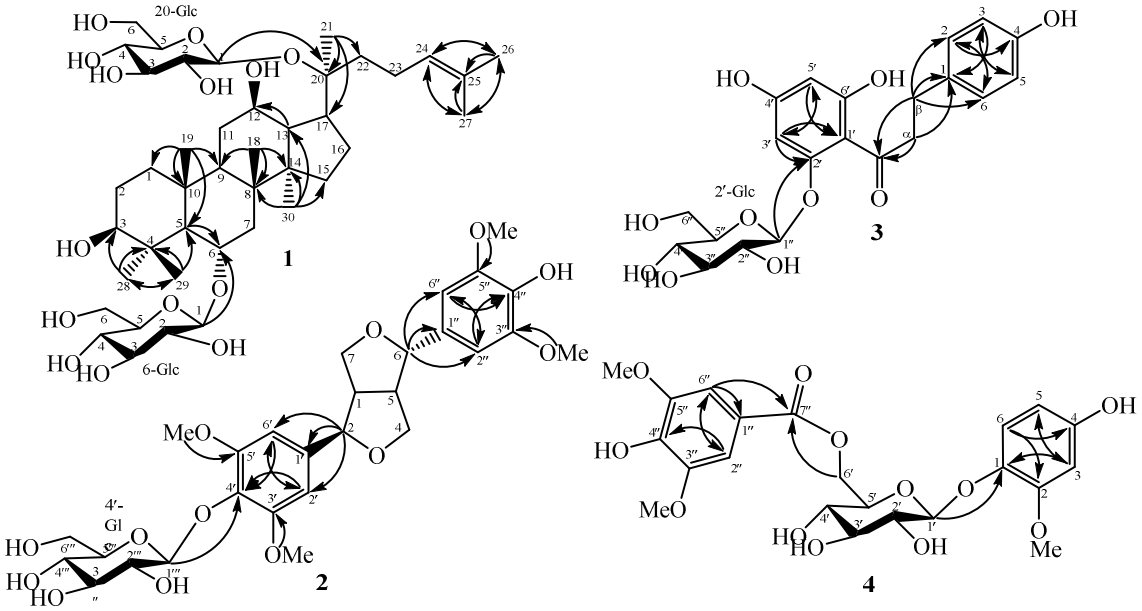

Figure 2. Key HMBC correlations of compounds **1** – **4.**

(C-7″). The strong HMBC correlations from anomeric proton 4.72 (H-1′) and aromatic proton 6.44 (H-3) to carbon 140.8 (C-1) were assigned the position of sugar moiety at C-1 of 1,3,4-trisubstituted aromatic ring. Compound **4** was identified as 4-hydroxy-2-methoxyphenyl-6-*O*-syringyl-β-D-glucopyranoside (Figure 1).

4. CONCLUSIONS

In the present study, four compounds: ginsenoside Rg1 (**1**), tortoside A (**2**), phlorizin (**3**), and 4-hydroxy-2-methoxyphenyl-6-*O*-syringyl-β-D-glucopyranoside (**4**) were isolated from the leaves of *A. cochinchinensis*. The results show that water fraction of *A. cochinchinensis* may be a source of saponins. Further study on saponin components of this genus need to be carry out.

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