ISOLATION OF TWO CYCLOARTANE-TYPE SAPONINS FROM RADIX ASTRAGALI MEMBRANACEI

PHÂN LẬP HAI HỢP CHẤT CYCLOARTANE SAPONIN TỪ RỄ HOÀNG KỲ

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ABSTRACT

In this research, two cycloartane-type saponins, including 3*β*-*O*-(2′-*O*-*β*-D-glucopyranosyl-*β*-D-xylopyranosyl)-6*α*,16*β*,25-trihydroxy-(20*R*,24*S*-epoxy) cycloartane and 3*β*-*O*-(2′-*O*-acetyl-*β*-D-xylopyranosyl)-6*α*-*O*-*β*-D-glucopyranosyl-16*β*,25-dihydroxy-(20*R*,24*S*-epoxy)cycloartane were isolated from polar soluble fraction of the radix astragali membranacei. Their chemical structures were determined by analyzing ESI-MS and NMR spectra. These compounds were reported as the main constituents of *Astragalus membranaceus* and used as the references to evaluate quality of this oriental medicine.

Keywords: *Cycloartane-type saponin, astragalus membranaceus, radix astragali membranacei, astragaloside.*

TÓM TẮT

Trong nghiên cứu này, 2 hợp chất 3*β*-*O*-(2′-*O*-*β*-D-glucopyranosyl-*β*-D-xylopyranosyl)-6*α*,16*β*,25-trihydroxy-(20*R*,24*S*-epoxy)cycloartane và 3*β*-*O*-(2′-*O*-acetyl-*β*-D-xylopyranosyl)-6*α*-*O*-*β*-D-glucopyranosyl-16*β*,25-dihydroxy-(20*R*,24*S*-epoxy)cycloartane được phân lập từ phân đoạn phân cực của rễ hoàng kỳ (Radix Astragali membranacei). Cấu trúc hoá học của các hợp chất được xác định dựa trên phân tích phổ ESI-MS và phổ cộng hưởng từ hạt nhân. Các hợp chất này được xác định là những thành phần hóa học chính trong loài *Astragalus membranaceus* và được sử dụng như một trong những chất tham chiếu để đánh giá chất lượng của dược liệu Hoàng kỳ (Radix Astragali membrancacei).

Từ khóa: *Cycloartane saponin, astragalus membranaceus, rễ hoàng kỳ, astragalosit.*

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1. INTRODUCTION

Radix Astragali membranacei (Huang-qi in Traditional Chinese Medicine) is the dried root of *A. membranaceus* (Fisch) Bunge, a species of the Fabaceae (or Leguminosae) family. *A. membranaceus* is a wild growing plant. It distributed in many places in China. The roots of this plant have been used in traditional Chinese medicine for more than 2,000 years and popularly in oriental medicine of many other countries nowadays [1]. The main chemical constituents of *A. membranaceus* were reported including cycloartane-type and oleanane-type triterpenoids, and flavonoids [2]. Radix Astragali membranacei showed the potent cardiovascular protective effect [2] including protection against myocardial ischemia-reperfusion injury, myocardial injury, myocardial fibrosis, etc; and other health promoting function to reduce the efect of risk factors led to cardiovascular diseases such as protection of vascular cell [3-6], hypotension [7-9], anti-atherosclerosion [10, 11], etc.

Recently, *A. membranaceus* is being cultivated in some areas of Vietnam. However, the chemical compositions of *A. membranaceus* in Vietnam have not been deeply reported, resulting lack of reference compounds for quality control. In the aim to clarify chemical components of Radix Astragali membranacei, this research describes the isolation and the structural elucidation of two cycloartane-type saponins from the water soluble fraction of dried roots of *A. membranaceus*.

2. MATERIALS AND METHODS

2.1. General experimental procedures

NMR spectra were measured on a Bruker AVANCE NEO 600MHz spectrometer. Column chromatography was carried out by using silica gel (Kiesegel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) as stationary phase. Thin layer chromatography (TLC) was done on pre-coated silicagel 60 F254 (0.25mm, Merck). The spots were visualized under UV radiation (wavelength of 254 and 365nm) or spraying with aqueous solution of H_2SO_4 (5%) followed by heating in a hot plate.

2.2. Plant material

Radix Astragali membranacei was purchased at Ninh Hiep market of oriental medicine. A specimen (coded: RAM02) was kept at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The dried powder of Radix Astragali membranacei (1.5kg) was extracted with methanol three times (each 4L, 30

minutes) under ultrasonic condition at room temperature. After filtration, filtrate was collected and then the solvent was removed under reduced pressure to yield 94g residue. This methanolic extract was suspended in water and successively separated with n-hexane, dichloromethane, and ethyl acetate. After separation, the water layer was subjected on a diaion HP - 20 column, washed with 1L water and then eluted with methanol/water (1/4 - 1/0, v/v) to give four fractions, AMW1 - AMW4. Fraction AMW2 was separated on silica gel column, eluting with gradient dichloromethane/methanol (40/1 - 0/1, v/v) to yield five subfractions, AMW2A - AMW2E. Subfraction AMW2B was then chromatographed with silica gel column using solvent system of dichloromethane/methanol/water (4/1/0.1, v/v/v) to give four smaller subfractions, AMW2B1 - AMW2B4. Subfraction AMW2B2 was purified by an RP-18 column, eluting with acetone/water (2/1, v/v) to obtain compound **1** (125mg). Subfraction AMW2C was chromatographed by silica gel column, eluting with dichloromethane/methanol/water (5/1/0.1, v/v/v) to yield two smaller subfractions, AMW2C1 - AMW2C2. Subfraction AMW2C2 was purified by an RP - 18 column using acetone/water (2/1, v/v) as eluent to obtain compound **2** (85mg).

3*β*-*O*-(2′-*O*-*β*-D-glucopyranosyl-*β*-D-xylopyranosyl)-6*α*,16*β*,25-trihydroxy-(20*R*,24*S*-epoxy)cycloartane (**1**): white amorphous powder, $[\alpha]_D^{30}$: +26° (c, 0.1 MeOH), $C_{41}H_{68}O_{14}$, ESI-MS: m/z 807 $[M+Na]^+$, ^{1}H-NMR (600MHz, Pyridine-d_5) and ^{13}C-NMR (150MHz, Pyridine-d_5) spectral data are given in Table 1.

3*β*-*O*-(2′-*O*-acetyl-*β*-D-xylopyranosyl)-6*α*-*O*-*β*-D-glucopyranosyl-16*β*,25-dihydroxy-(20*R*,24*S*-epoxy) cycloartane (**2**): white amorphous powder, $[\alpha]_D^{30}$: +39° (c, 0.1 MeOH), $C_{43}H_{70}O_{15}$, ESI-MS: m/z 849 $[M+Na]^+$, ^{1}H-NMR (600MHz, Pyridine-d_5) and ^{13}C-NMR (150MHz, Pyridine-d_5) spectral data are given in Table 1.

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white amorphous powder. The ^{1}H-NMR spectrum of **1** contained signals of 7 methyl groups at δ_H 1.96, 1.61, 1.46, 1.45, 1.34, 1.33, 1.00ppm (each 3H, s) and the signal of 1 methylene group at up-field region [δ_H 0.58 and 0.30 (each 1H, d, J = 4.2Hz)] which were characterized for a cycloartane-type saponin. The ^{13}C-NMR spectrum of **1** revealed signals of 41 carbons. Of these, by HSQC and DEPT spectra, it can be suggested the presence of 7 non-protonated carbons, 16 methines, 11 methylenes, and 7 methyl groups. Therefore, it can be suggested that compound **1** to be a saponin contain cycloartane skeleton, which has 30 carbons of triterpene cycloartane and 11 other carbons belonging to one hexose and one pentose. The presence of two sugar units consisted of the observation of two anomeric protons [δ_H 4.92 (1H, d, J = 6.6Hz) and 5.42 (1H, d, J = 7.2Hz)] in the ^{1}H-NMR. Additionally, J values of these anomeric protons (6.6 and 7.2Hz) indicated *β*-glycosidic linkages. Signals of sugar units were then assigned by COSY spectra including correlations of H-1′ (δ_H 4.92)/ H-2′ (δ_H 4.27)/ H-3′ (δ_H 4.21)/H-4′ (δ_H 4.19)/ H-5′ (δ_H 4.32 and 3.67) and H-1″ (δ_H 5.42)/ H-2″ (δ_H 4.15)/ H-3″ (δ_H 4.30)/H-4″ (δ_H 4.33)/ H-5″ (δ_H 3.97)/ H-6″ (δ_H 4.54 and 4.47). Moreover, carbon chemical shift of sugar units [C-1′ (δ_C 106.2), C-2′ (δ_C 83.7), C-3′ (δ_C 78.4), C-4′ (δ_C 71.5), C-5′ (δ_C 67.2); and C-1″ (δ_C 106.5), C-2″ (δ_C 77.5), C-3″ (δ_C 78.5), C-4″ (δ_C 72.2), C-5″ (δ_C 78.8), C-6″ (δ_C 63.3)] suggested for assignation of a *β*-xylopyranosyl unit and a *β*-glucopyranosyl unit. Next, the HMBC correlation between H-1″ (δ_H 5.42) and C-2′ (δ_C 83.7) indicated *O*-*β*-glucopyranosyl unit link to C-2′ of *β*-xylopyranosyl unit. Meanwhile, the HMBC correlation between H-1′ (δ_H 4.92)/ H-28 (δ_H 1.96) / H-29 (δ_H 1.46) and C-3 (δ_C 89.2) indicated the *O*-glycosidic linkage between *β*-xylopyranosyl unit and C-3 of aglycone moiety. The COSY interaction from H-5 (δ_H 1.73) to H-6 (δ_H 3.75) suggested the presence of hydroxyl group at C-6. On the other hand, the value of J coupling constant (J = 9.6Hz) allowed to predict the configuration of hydroxyl group at C-6 is *alpha*-configuration. Another hydroxyl group located at C-16 was confirmed by COSY interaction between H-17 (δ_H 2.55) and H-16 (δ_H 5.04). A large *J* coupling constant value between H-17/H-16 (J = 7.2Hz), and carbon chemical shift of C-16 (δ_C 74.0) indicated for *beta*-configuration of hydroxy group at C-16 [12]. Position of the last hydroxy group was determined at C-25 by HMBC correlations from H-26 (δ_H 1.61) and H-27 (δ_H 1.33) to C-25 (δ_C 71.8). The HMBC interaction from H-24 (δ_H 3.90) to C-20 (δ_C 87.8) indicated the epoxy-bridge between C-24 and C-20. The HMBC interaction from H-21 (δ_H 1.34) to C-17 (δ_C 58.9), C-20 (δ_C 87.8) and C-22 (δ_C 35.4) also supported the linkage of C-20 and oxygen atom. Furthermore, chemical shift value of C-24 (δ_C 82.2) and C-20 (δ_C 87.8) consisted with (20*R*,24*S*)-configuration as previously described [12]. As all of NMR data analysis, compound **1** was determined to be 3*β*-*O*-(2′-*O*-*β*-D-glucopyranosyl-*β*-D-xylopyranosyl)-6*α*,16*β*,25-trihydroxy-(20*R*,24*S*-epoxy)cycloartane, a known compound having trivial name as astragaloside III. The ^{13}C-NMR spectral data of compound **1** was also well matched with those reported for astragaloside III in same NMR solvent condition (pyridine-d_5, Table 1) [12]. Astragaloside III was reported as one of the main chemical components isolated from *A. membranaceus* and can be used as a reference substance to evaluate the quality of Radix Astragali membranacei [13]. This compound was reported to exhibit various biological activities such as skin whitening in cosmetics, anti-cancer, anti-inflammatory and anti-diabetic activities [14, 15].

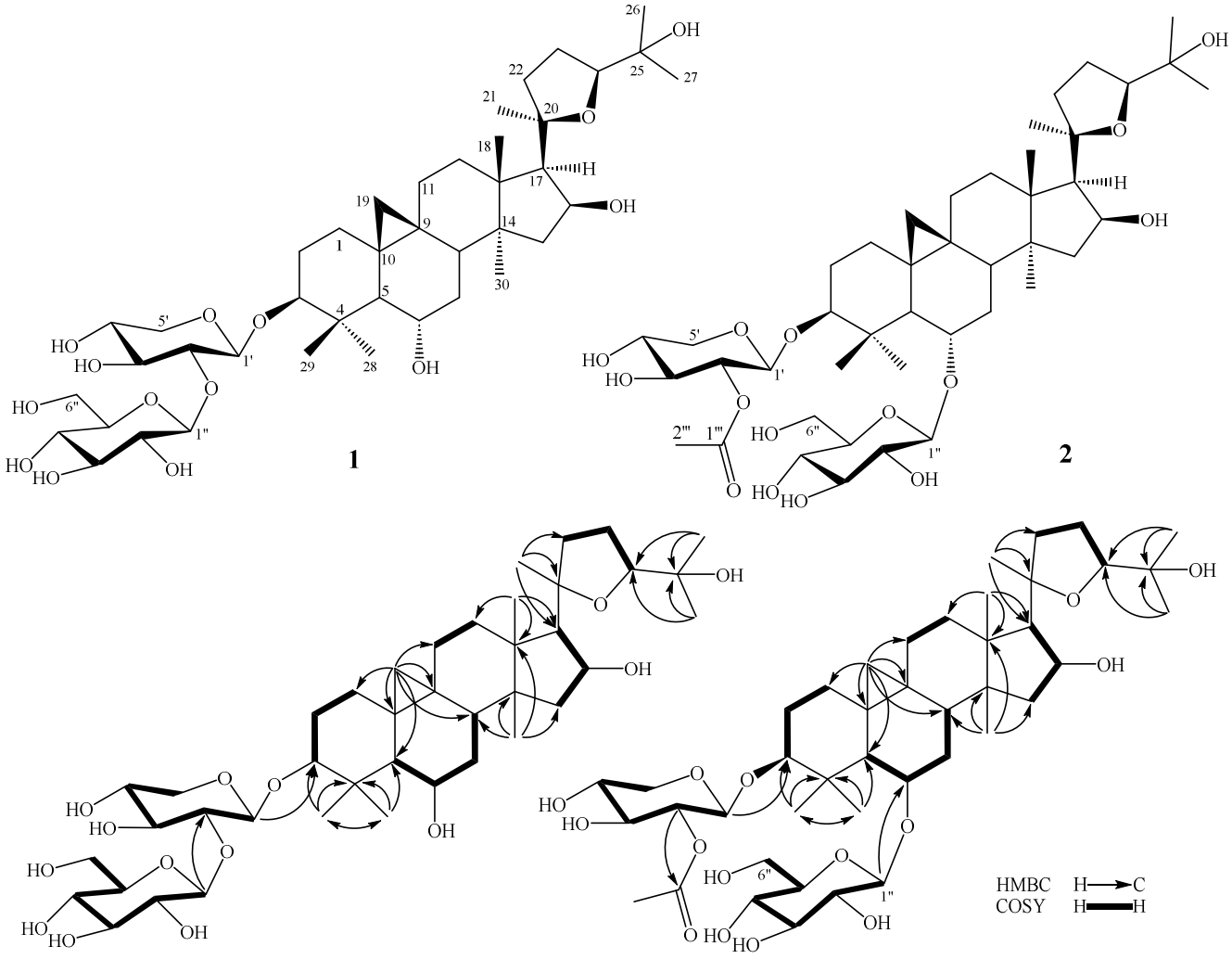

Figure1. Chemical structure and key HMBC, COSY interactions of compounds **1** and **2**

Table 1. NMR spectral data for compounds **1** and **2** in Pyridine-d_5

No	1			2		
	$^{\#}\delta_C$	$^{a}\delta_C$	$^{b}\delta_H$ (mult., *J* in Hz)	$^{\#\#}\delta_C$	$^{a}\delta_C$	$^{b}\delta_H$ (mult., *J* in Hz)
1	32.4	33.0	1.61 (m)/ 1.26 (m)	32.0	32.6	1.60 (m)/ 1.24 (m)
2	30.3	30.9	2.39 (m)/ 2.00 (m)	29.9	30.5	2.31 (m)/ 1.94 (m)
3	88.6	89.2	3.56 (dd, 4.2, 11.4)	88.9	89.4	3.42 (dd, 4.8, 11.4)
4	42.7	43.3	-	42.3	42.8	-
5	53.9	54.5	1.73 (br d, 9.6)	52.5	53.0	1.89 (br d, 9.0)
6	67.8	68.4	3.75 (ddd, 3.0, 9.6, 9.6)	79.3	79.8	3.78 (ddd, 3.6, 9.0, 9.0)
7	38.5	39.1	1.83 (m)/ 1.64 (m)	34.9	35.3	1.87 (m)/ 1.66 (m)
8	46.8	47.4	1.95 (m)	46.1	46.7	1.96 (m)
9	21.0	21.6	-	21.2	21.8	-
10	29.3	30.5	-	28.9	29.4	-
11	26.2	26.7	1.93 (m)/ 1.25 (m)	26.1	26.6	1.85 (m)/ 1.30 (m)
12	33.3	33.9	1.70 (m)/ 1.61 (m)	33.5	33.9	1.65 (m)/ 1.57 (m)
13	45.0	45.6	-	45.8	46.3	-
14	46.1	46.7	-	46.2	46.7	-
15	46.5	47.1	2.15 (m) / 1.81 (m)	45.8	45.5	2.36 (m) / 1.84 (m)
16	73.4	74.0	5.04 (q, 7.2)	73.4	73.9	5.00 (q, 7.8)
17	58.3	58.9	2.55 (d, 7.2)	58.2	58.7	2.53 (d, 7.8)
18	21.4	22.0	1.45 (s)	21.2	21.7	1.42 (s)
19	30.3	30.9	0.58 (d, 4.2) 0.30 (d, 4.2)	29.2	29.5	0.56 (d, 4.2) 0.20 (d, 4.2)
20	87.2	87.8	-	87.2	87.8	-
21	27.1	27.6	1.34 (s)	27.1	27.6	1.32 (s)
22	34.9	35.4	3.11 (m) / 1.69 (m)	35.0	35.4	3.14 (m) / 1.67 (m)
23	26.4	27.0	2.35 (m) / 2.07 (m)	26.5	27.0	2.33 (m) / 2.09 (m)
24	81.6	82.2	3.90 (dd, 5.4, 9.0)	81.7	82.2	3.90 (dd, 5.4, 9.0)
25	71.2	71.8	-	71.3	71.8	-
26	28.1	28.7	1.61 (s)	28.2	28.7	1.61 (s)
27	28.5	29.3	1.33 (s)	28.3	28.8	1.31 (s)

28	28.7	29.9	1.96 (s)	28.6	29.1	1.84 (s)
29	16.5	17.1	1.46 (s)	16.5	17.1	1.30 (s)
30	20.1	20.7	1.00 (s)	19.9	20.4	0.95 (s)
3-*O*-Xyl						
1'	105.6	106.2	4.92 (d, 6.6)	104.7	105.3	4.81 (d, 7.8)
2'	83.2	83.7	4.27 (m)	75.6	76.1	5.57 (dd, 7.8, 8.4)
3'	77.8	78.4	4.21 (m)	76.3	76.7	4.16 (dd, 8.4, 8.4)
4'	70.9	71.5	4.19 (m)	71.3	71.8	4.21 (m)
5'	66.6	67.2	4.32 (br d, 10.8) 3.67 (dd, 10.8, 10.8)	67.1	67.6	4.33 (br d, 10.8) 3.68 (dd, 10.8, 10.8)
2' -*O*-Glc						
1''	106.0	106.5	5.42 (d, 7.2)	105.2	105.8	4.94 (d, 7.8)
2''	76.9	77.5	4.15 (m)	75.6	76.1	4.06 (dd, 7.8, 8.4)
3''	78.0	78.5	4.30 (m)	79.3	79.6	4.23 (m)
4''	71.7	72.2	4.33 (m)	71.9	72.3	4.21 (m)
5''	78.2	78.8	3.97 (m)	78.2	78.7	3.93 (m)
6''	62.7	63.3	4.54 (dd, 3.0, 11.4) 4.47 (dd, 4.8, 11.4)	63.1	63.6	4.50 (br d, 10.8) 4.32 (dd, 4.8, 10.8)
2'-OAc						
1'''				21.2	21.7	2.06 (s)
2'''				170.1	170.7	-

Measured at [a]150MHz, [b]600MHz ; [#)]Prevously reported δ_C data for astragaloside III [12]; [##)]Previously reported δ_C data for astragaloside II [16]

Compound **2** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **2** also observed signals of seven singlet methyl groups (δ_H 1.84, 1.61, 1.42, 1.32, 1.31, 1.30, 0.95) and a methylene group at up-field region (δ_H 0.56 and 0.20) which characterized of a cycloartane-type triterpene. The ^{13}C-NMR of **2** revealed the signal of 43 carbons. Based on HSQC and DEPT spectrum, it can be seen as 8 non-protonated carbons, 16 methines and 11 methylenes, and 8 methyl groups. Among them, the signal of a carbonyl group at δ_C 170.7 and methyl group at δ_C 21.7 / δ_H 2.06 were assigned for an acetyl group. These NMR signals showed that compound **2** was also a cycloartane-type saponin but differed to **1** by the presence of an acetyl group and changing in chemical shift values at C-6 and C-2′. The HMBC interactions from H-1′ (δ_H 4.81), H-28 (δ_H 1.84), and H-29 (δ_H 1.30) to C-3 (δ_C 89.4) allowed to identify *O*-β-xylopyranosyl group at C-3 of cycloartane moiety. However, differ to compound **1**, the HMBC correlation between H-2′ (δ_H 5.57) and carbonyl carbon C-1‴ (δ_C 170.7) indicated acetoxy group link to C-2′ of xylopyranosyl group. On the other hand, the HMBC interaction from H-1″ (δ_H 4.94) to C-6 (δ_C 79.8) indicated *O*-β-D-glucopyranosyl group link to C-6 of aglycone moiety. The presence of *O*-β-D-glucopyranosyl group at C-6 was also supported by the down-field shifted signals of H-6 (δ_H 3.78) and C-6 (δ_C 79.8) of compound **2** in comparison with those of compound **1** ($\delta_{H\text{-}6}$ 3.75 and $\delta_{C\text{-}6}$ 68.4). Similarly, the presence of acetoxy group at C-2′ caused the change in proton ($\delta_{H\text{-}2'}$ 5.57) and carbon ($\delta_{C\text{-}2'}$ 76.1) chemical shift value at this position. Therefore, compound **2** was established as 3β-*O*-(2′-*O*-acetyl-β-D-xylopyranosyl)-6α-*O*-β-D-glucopyranosyl-16β,25-dihydroxy-(20*R*,24*S*-epoxy) cycloartane, a known compound with trivial name as astragaloside II. Its NMR data was well agreed with those reported in the literature [16]. This compound was also previously isolated as main component from *A. membrannaceus*, and can be used as a reference compound to evaluate quality of *Radix Astragali membranacei* [17]. Compound **2** was reported to have some potential bioactivities including skin whitening, anti-cancer, anti-inflammation, and anti-diabetes [18].

4. CONCLUSION

Initially studying the phytochemistry of Radix Astragali membranacei (the dried roots of *A. membrannaceus*), two cycloartane-type saponins including astragaloside III (**1**) and astragaloside II (**2**) were isolated. Their chemical structures were determined by 1D and 2D-NMR spectral analysis and comparison with literature. Both of astragaloside II and astragaloside III are major components of *A. membrannaceus* roots and hence they can be used as reference compounds for quality control of Radix Astragali membranacei.

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Khoa Công nghệ Hoá, Trường Đại học Công nghiệp Hà Nội