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Two cycloartanes-*type* triterpenoid from *Homonoia riparia* Lour

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Abstract

Using various chromatography methods, two known cycloartanes, 24-methylenecycloartane-3*β*,6*β*,7*β*,16*β*-tetraol (**1**) and riparsaponin (**2**), together with two known flavonol glycosides, quercetin 3-*O*-*β*-D-glucopyranoside (**3**) and quercetin 3-*O*-α-*L*-rhamnopyranosyl (1→6) *β*-D-glucopyranoside (**4**) were isolated from the methanol extract of the leaves of *Homonoia riparia* Lour. Their structures were determined by MS and 1D-, 2D-NMR spectra, as well as by comparison with those reported in the literature.

Keywords. *Homonoia riparia*, cycloartane, flavonol glycoside.

1. INTRODUCTION

Homonoia is a small genus of shrubs which belongs to the Euphorbiaceae family that is widespread from India to mainland China, Taiwan, and Vietnam, and found commonly at riverbanks [1]. The plant is used for the treatment of various diseases such as hepatitis, pneumonia, scald, and skin diseases [1, 2]. The phytochemical studies of *H. riparia* confirmed the presence of sterols, triterpenes, and flavonoids [3, 4]. In this paper, we report herein the isolation and structure elucidation of two cycloartane-*type* triterpenoids and two flavonol glycosides from the methanol extract of the *H. riparia* leaves.

2. MATERIAL AND METHODS

2.1. Plant materials

The leaves of *H. riparia* collected in Vinhphuc, Vietnam, in March 2016, and identified by Prof. Ninh Khac Ban, Institute of Marine Biochemistry, Vietnam Academy of Science and Technology. A voucher specimen (HR01) was deposited at Lab of Pharmaceutical Chemistry, Faculty of Chemistry, University of Science, VNU.

2.2. General experimental procedures

The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Variant AM400 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck, Whitehouse Station, NJ) or RP-18 resins (30-50 μm, Fuji silysia Chemical Ltd.), and thin layer chromatography (TLC) using precoated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried leaves of *H. riparia* (5.0 kg) were extracted with methanol (8.0 L) under sonication for 4h to yield 700.0 g extract. This extract was suspended in H_2O and successively partitioned with *n*-hexane and EtOAc to obtain the *n*-hexane (HR1, 19.8 g), EtOAc (HR2, 253.5 g), and aqueous (HR3, 430.0 g) fractions. The fraction HR2 (253.5 g) was chromatographed on a silica gel column, eluting with a gradient solvent of $CHCl_3$-MeOH (20:1→1:1, v/v) to obtain five sub-fractions, HR1A (11.4 g), HR1B (8.7 g), HR1C (3.5 g), HR1D (3.9 g), and HR1E (55.8 g). The fraction HR1D was chromatographed on a silica gel column eluting with $CHCl_3$:MeOH (20:1, v/v) to give three smaller fractions, HR1C1 (1.7 g), HR1C2 (1.0 g), and HR1C3 (0.8 g). The fraction HR1C2 was chromatographed on HPLC using J'sphere ODS H-80 (250 mm x 20 mm, 4 mm, 8 nm) column eluting with 25% aqueous acetonitrile at a flow rate of 3.0 mL/min to yield **1** (16.2 mg) and **2** (7.5 mg). The HR1E fraction was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (6:1, v/v) to yield **3** (5.6 mg), and **4** (4.8 mg).

24-Methylenecycloartane-3*β*,6*β*,7*β*,16*β*-tetraol (1): White amorphous powder; $[\alpha]_D^{25}$: +42.5 (*c* 0.1, in CD_3OD); ESI-MS *m/z*: 511 $[M+Na]^+$; $C_{31}H_{52}O_4$, M = 488.38; ^1H- and ^{13}C-NMR (CD_3OD), see table 1.

Riparsaponin (2): White amorphous powder; $[\alpha]_D^{25}$: +25.0 (*c* 0.1, in CD_3OD); ESI-MS *m/z*: 643 $[M+Na]^+$; $C_{36}H_{60}O_8$, M = 620.43; ^1H- and ^{13}C-NMR (piridine-d_5): see Table 1.

Quercetin-3-*O*-*β*-D-glucopyranoside (3): Yellow amorphous powder; ESI-MS *m/z*: 465 $[M+H]^+$; $C_{21}H_{20}O_{12}$, M = 464.09; ^1H- and ^{13}C-NMR (CD_3OD), see table 2.

Quercetin 3-*O*-α-*L*-rhamnopyranosyl (1→6)-*β*-D-glucopyranoside (4): Yellow amorphous powder; ESI-MS *m/z*: 611 $[M+H]^+$; $C_{27}H_{30}O_{16}$, M = 610.15; ^1H- and ^{13}C-NMR (CD_3OD), see table 2.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The ^1H-NMR spectrum of **1** showed signals of two olefinic protons at δ_H 4.67 (1H, s) and 4.68 (1H, s), four tertiary methyls at δ_H 1.22, 1.12, 1.07, and 1.00 (each s, 3H); three secondary methyls at δ_H 1.03 (3H, d, *J* = 6.8 Hz), 1.02 (3H, d, *J* = 6.8 Hz), and 0.95 (3H, d, *J* = 6.4 Hz); and four hydroxymethine protons at δ_H 4.32 (m), 3.97 (1H, br s), 3.41 (1H, m), and 3.14 (br d, 8.4). The two high-field doublets observed at δ_H 1.14 (1H, d, *J* = 3.5 Hz) and 0.41 (1H, d, *J* = 3.5 Hz) are characteristic of the two protons of cyclopropane ring. The ^{13}C-NMR and DEPT spectra showed the signal of 31 carbons, consisting of seven methyls, nine methylenes, nine methines, and six quaternary carbons. The analysis of NMR data suggested the structure of **1** as a cycloartane-*type* triterpene [5]. The HMBC correlations between H-24 (δ_H 4.67 and 4.68) and C-23 (δ_C 32.7)/C-24 (δ_C 158.1)/C-25 (δ_C 35.0) suggested an exocyclic olefinic methylene at C-24. H-29 (δ_H 1.07) and H-30 (δ_H 1.12) had HMBC correlations with C-3 (δ_C 79.8)/C-4 (δ_C 41.9)/C-5 (δ_C 50.4), indicating the location of two methyl groups at C-4. The HMBC correlations between H-18 (δ_H 1.22) and C-12 (δ_C 34.0)/C-13 (δ_C 46.9)/C-14 (δ_C 47.3)/C-17 (δ_C 57.0); H-28 (δ_H 1.00) and C-8 (δ_C 47.7)/C-13 (δ_C 46.9)/C-14 (δ_C 47.3)/C-15 (δ_C 52.4) confirmed two methyl groups at C-13 and C-14. H-21 (δ_H 0.95) was observed to have HMBC correlations with C-17 (δ_C 57.0)/C-20 (δ_C 31.5)/C-22 (δ_C 36.4), indicating the location of methyl group at C-20. In addition, the HMBC correlations between H-26 (δ_H 1.01), H-27 (δ_H 1.02) and C-24 (δ_C 158.1)/C-25 (δ_C 35.0) confirmed two secondary methyl groups at C-25. The HBMC correlations between H-19 (δ_H 1.14, 0.41) and carbons C-1 (δ_C 34.0)/C-5 (δ_C 50.4)/C-8 (δ_C 47.7)/C-9 (δ_C 20.0)/C-10 (δ_C 25.7) suggested the cyclopropane ring forming at C-9/C-10. Moreover, the COSY correlations between H-2 (δ_H 1.65)/H-3 (δ_H 3.14); and H-5 (δ_H 1.31), H-6 (δ_H 3.97), H-7 (δ_H 3.41), and H-8 (δ_H 2.03); and H-15 (δ_H 1.59 and 2.30)/H-16 (δ_H 4.02) as well as the HMBC correlations between H-3 (δ_H 3.14)/C-4 (δ_C 41.9) and C-29 (δ_C 25.1), H-6 (δ_H 3.97)/C-5 (δ_C 50.4) and C-7 (δ_C 74.7), H-7 (δ_H 3.41)/C-6 (δ_C 73.1) and C-8 (δ_C 47.7), and H-16 (δ_H 4.32)/C-15 (δ_C 52.4) and C-17 (δ_C 57.0) confirmed the location of four hydroxymethine groups at C-3, C-6, C-7, and C-16. The *β*-configurations of the hydroxyl groups at C-3, C-6, C-7, and C-16 were inferred from the NOE correlations between H-3 (δ_H 3.14)/H-5_{ax} (δ_H 1.31) and H_3-29 (δ_H 1.07), H-6 (δ_H 3.97)/H-7 (δ_H 3.41) and H_3-29 (δ_H 1.07), H-7 (δ_H 3.41)/H-6 (δ_H 3.97) and H_3-28 (δ_H 1.00), and H-16 (δ_H 4.02)/H-17_{ax} (δ_H 1.64) and H_3-28 (δ_H 1.00), respectively. The ^1H and ^{13}C-NMR data of **1** were identical to those of 24-methylenecycloartane-3*β*,6*β*,7*β*,16*β*-tetraol [5]. The molecular formula of **1**, $C_{31}H_{52}O_4$, was agreed with a quasi-molecular ion peak at *m/z* 511 $[M+Na]^+$ in the ESI-MS.

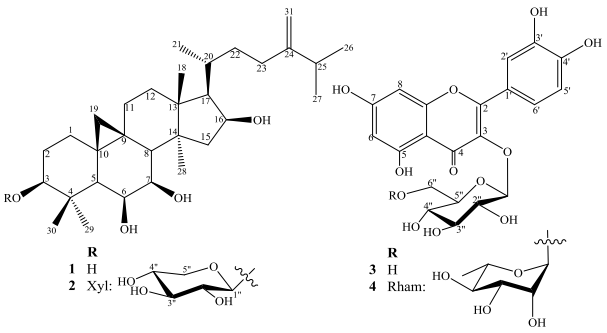

Figure 1: Chemical structures of compounds **1-4**

Compound **2** was isolated as a white amorphous powder. The ^{13}C-NMR spectrum of **2** revealed the signals of 36 carbons, of which five were assigned to a pentose residue and the remaining 31 to an aglycone. In addition, the ^1H and ^{13}C-NMR spectra of **2** were almost identical to those of **1**, except for the additional sugar moiety, indicating that **1** and **2** have the same aglycone. The carbon signals δ_C 106.8, 74.8, 77.8, 70.5, and 66.4 could further demonstrate the existence of a pentose. The sugar moiety was elucidated as D-xylopyranoside by comparison of the NMR data of **2** with those of riparsaponin [6] and found to match (table 1). The large coupling constant of protons H-1' and H-2', *J* = 6.8 Hz, indicated *β*-configuration. The sugar was

shown to be attached at C-3 aglycone by observation of HMBC correlation between the anomeric xyl H-1' (δ_H 4.83) and C-3 (δ_C 88.2). Consequently, the structure of **2** was established. The molecular formula of **2** was agreed with its ESI-MS analysis (*m/z*: 642, $[M+Na]^+$).

Compounds **3** and **4** were elucidated as flavonol glycosides in which aglycone is quercetin by analysis of ^{1}H and ^{13}C-NMR spectra. Compound **3** has one sugar unit whereas **4** has two sugar units. Comparison of 1D-NMR signals of **3** with those of published data in the literature is found to match (table 2) [7]. The ^{1}H-NMR spectrum of **4** indicated the presence of a rhamnosyl and a glucosyl moieties with characteristic signals at 4.50 (H-1''', s) and 1.10 (H-6''', d, J = 6.0 Hz) for rhamnose and the anomeric proton of *β*-glucose at 5.08 (H-1'', d, J = 7.2 Hz).

Table 1: NMR spectral data for **1** and **2** and reference compounds

C	1			2		
	δ_C [#,a]	δ_C [a,b]	δ_H [a,d] (mult., J in Hz)	δ_C [%,c]	δ_C [c,b]	δ_H [c,d] (mult., J in Hz)
1	33.9	34.0	1.51 (m)/1.70 (m)	32.6	32.6	1.60 (m)/1.82 (m)
2	31.7	30.9	1.65 (m)	29.2	29.5	2.07 (m)/2.35 (m)
3	79.0	78.8	3.14 (br d, 8.4)	88.5	88.2	3.49 (br d, 8.4)
4	41.9	41.9	-	40.8	41.2	-
5	50.4	50.9	1.31 (d, 3.0)	49.0	49.4	1.31 (br d, 3.6)
6	73.0	73.1	3.97 (br s)	71.4	71.8	4.42 (m)
7	74.1	74.7	3.41 (m)	72.7	73.1	3.80 (br d, 5.0)
8	47.8	47.7	2.03 (d, 11.6)	45.9	46.7	1.51 (br s)
9	19.9	20.0	-	24.0	24.4	-
10	25.7	25.7	-	18.7	18.9	-
11	26.8	26.9	1.05 (m)/2.04 (m)	25.4	25.6	1.10 (m)/2.28 (m)
12	33.9	33.9	1.14 (m)/1.62 (m)	32.3	32.8	1.24 (m)/1.73 (m)
13	47.0	46.9	-	45.6	46.0	-
14	47.1	47.3	-	45.4	45.8	-
15	53.3	52.4	1.59 (m)/2.30 (m)	51.7	52.1	2.18 (m)/2.85 (m)
16	72.3	73.6	4.32 (m)	70.7	71.3	4.70 (m)
17	57.1	57.0	1.64 (m)	55.4	56.0	1.82 (m)
18	19.3	19.9	1.22 (s)	19.4	19.5	1.57 (s)
19	32.9	33.0	0.41 (d, 3.5)/1.14 (d, 3.5)	31.4	31.7	0.53 (d, 3.5)/1.55 41 (d, 3.5)
20	31.3	31.5	1.84 (m)	29.8	30.2	2.28 (m)
21	18.9	18.6	0.95 (d, 6.4)	17.9	17.8	1.12 (d, 6.8)
22	36.1	36.4	1.17 (m)/1.85 (m)	34.5	35.0	1.42 (m)/2.24 (m)
23	32.8	32.7	2.00 (m)/2.15 (m)	31.4	32.8	1.50 (m)/1.74 (m)
24	157.6	158.1	-	156.2	156.5	-
25	34.5	35.0	2.26 (m)	33.1	33.4	2.32 (m)
26	22.4	22.4	1.02 (d, 6.8)	21.8	21.4	1.04 (d, 6.8)
27	22.5	22.5	1.03 (d, 6.8)	21.9	21.5	1.06 (d, 6.8)
28	20.6	20.2	1.00 (s)	19.4	19.4	1.27 (s)
29	25.7	25.1	1.07 (s)	24.1	24.1	1.55 (s)
30	16.9	16.0	1.12 (s)	16.4	16.5	1.67 (s)
31	106.7	106.6	4.67 (s)/4.68 (s)	105.8	105.7	4.82 (s)/4.89 (s)
3-*O*-Xyl						
1′				105.9	106.8	4.83 (d, 6.8)
2’				73.8	74.8	3.99 (m)
3′				76.8	77.8	4.11 (t, 8.8)
4′				69.7	70.5	4.18 (m)
5′				65.6	66.4	3.71 (m)/4.35 (m)

Measured in [a] CD_3OD, [b] 100 MHz, [c] piridine-d_5, [d] 400 MHz.
[#] δ_C of 24-methylenecycloartane-3β,6β,7β,16β-tetraol [5], [%] δ_C of riparsaponin [6].

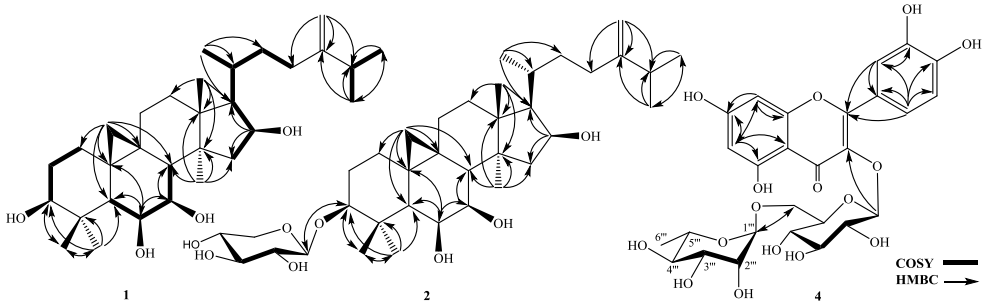

Figure 2: The key HMBC and COSY correlations of compounds **1**, **2** and **4**

Table 2: ^{1}H- and ^{13}C-NMR data for **3** and **4** and reference compounds

C	**3**			**4**		
	δ_C [$,a]	δ_C [a,b]	δ_H [a)] (mult., *J* in Hz)	δ_C [&,a]	δ_C [a,b]	δ_H [a)] (mult., *J* in Hz)
2	156.8	159.0	-	158.5	159.4	-
3	133.8	135.6	-	135.6	135.6	-
4	177.5	179.5	-	179.4	179.4	-
5	161.7	163.0	-	162.9	162.9	-
6	99.1	99.84	6.19 (s)	99.9	99.9	6.18 (s)
7	164.2	166.0	-	166.0	166.0	-
8	93.9	94.7	6.38 (s)	94.9	94.8	6.36 (s)
9	156.6	158.5	-	159.3	159.3	-
10	104.6	105.7	-	105.6	105.6	-
1'	121.6	123.1	-	123.1	123.1	-
2'	115.6	116.0	7.70 (d, 2.0)	116.0	117.7	7.65 (s)
3'	145.2	145.9	-	145.8	145.8	-
4'	148.9	149.8	-	149.8	149.8	-
5'	116.6	117.5	6.88 (d, 8.0)	117.7	116.0	6.85 (d, 8.2)
6'	120.0	123.2	7.57 (dd, 2.0, 8.0)	123.5	123.5	7.57 (d, 8.2)
1''	101.3	104.3	5.25 (d, 7.2)	104.7	104.7	5.08 (d, 7.2)
2''	74.5	75.7	3.47 (dd, 7.2, 8.2)	75.7	75.8	3.42 (m)
3''	76.9	78.1	3.41 (t, 8.2)	78.2	78.2	3.40 (m)
4''	70.4	71.2	3.21 (m)	71.3	71.4	3.25 (m)
5''	78.0	78.4	3.32 (m)	77.1	77.2	3.30 (m)
6''	61.4	62.5	3.55 (dd, 5.6, 11.4)	68.5	68.5	3.40 (dd, 5.6, 11.4)
			3.70 (dd, 2.4, 11.4)			3.78 (dd, 2.4, 11.4)
1'''				102.4	102.4	4.50 (br s)
2'''				72.0	72.1	3.62 (m)
3'''				72.2	72.2	3.52 (m)
4'''				73.9	73.9	3.25 (m)
5'''				69.7	69.7	3.41 (m)
6'''				17.9	17.9	1.10 (d, 6.0)

Measured in [a)]CD_3OD, [b)]100 MHz, [c)]400 MHz, [$)]$\delta_C$ of quercetin-3-O-β-glucopyranoside [7], [&)]δ_C of quercetin 3-O-α-L-rhamnopyranosyl (1→6) β-D-glucopyranoside [8].

The HMBC correlations between H-6" and C-1"', H-1"' and C-6", and H-1" and C-3 (δ_C 135.6) confirmed the linkage (1→6) between rhamnosyl and glucosyl moieties and the glucopyranosyl moiety connected with aglycone quercertin at C-3. Moreover, the ^{1}H and ^{13}C-NMR data of **4** were identical with those reported in the literature (table 2) [8]. Consequently, the structure of **3** and **4** was elucidated as quercetin-3-*O*-*β*-D-glucopyranoside, and quercetin 3-*O*-α-L-rhamnopyranosyl (1→6) glucopyranoside, respectively.

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