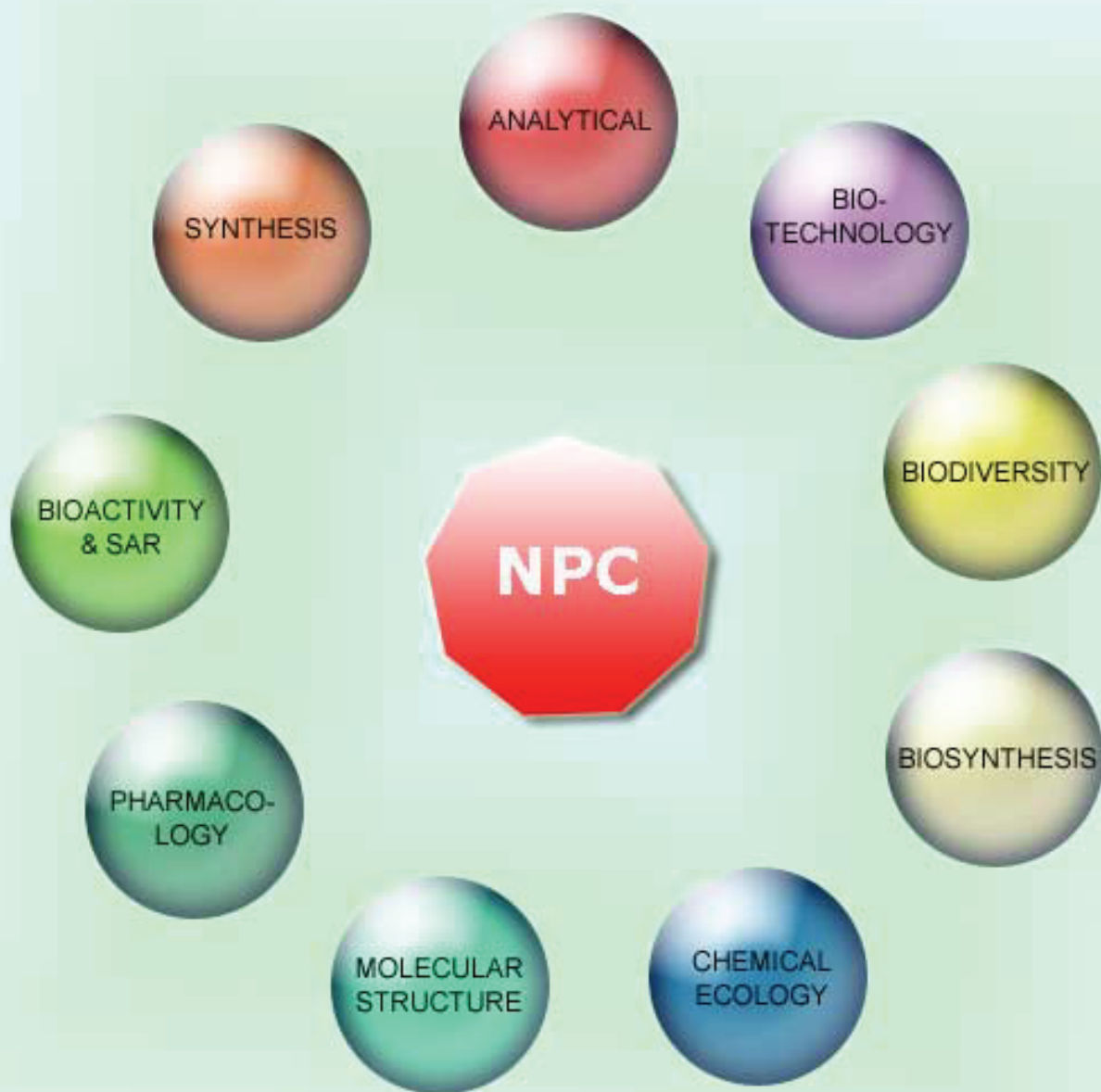


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Phenolic Components from the Aerial Parts of *Agrimonia pilosa*

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Two new resorcinol derivatives, named agrimopilosides A and B (**1** and **2**), along with two known compounds, (2*S*, 3*S*)-aromadendrin 3-*O*- β -D-glucopyranoside (**3**), (2*S*, 3*S*)-glucodistylin (**4**) were isolated from the aerial parts of *Agrimonia pilosa*. Their chemical structures were determined by mean of HRMS, NMR, CD spectra, and as well as by comparison with the reported data. At concentration of 20 μ M, compounds **1**-**4** modestly inhibited NO production in LPS-stimulated RAW264.7 cells, with the inhibitory rates in the range of 9.55-33.73%. None of them showed cytotoxicity toward HepG2, MCF-7, and SK-LU-1 human cancer cells by MTT assay.

Keywords: *Agrimonia pilosa*, Agrimopiloside A, Agrimopiloside B, Resorcinol derivative.

Agrimonia pilosa Ledeb. (Rosaceae) is widely distributed in southwest of Vietnam and has been used for treatment hemoptysis, hemorrhage, fever, diarrhea, and tuberculosis [1]. Previous phytochemical investigations of this genus confirmed the presence of flavonoids, triterpenoids, and phenolic compounds that possessed a variety of biological activities including anti-inflammatory, diabetes, anti-skin wrinkling, and whitening effects [2]. In the present study, we describe isolation and structural elucidation of two new phenolic glycosides **1** and **2** from the aerial parts of *Agrimonia pilosa*.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was deduced to be C₁₆H₂₄O₇ by HR-ESI-MS (m/z 327.1453 [M-H]⁻, calcd for C₁₆H₂₃O₇, 327.1444), in conjunction with its ¹³C-NMR data. The ¹H-NMR spectra of **1** showed three aromatic proton signals belonging to a 1,3,5-trisubstituted benzene ring at δ_H 6.45 (1H, t, $J = 2.0$ Hz), 6.41 (1H, t, $J = 2.0$ Hz), and 6.33 (1H, t, $J = 2.0$ Hz). The signal of an anomeric proton was observed at δ_H 4.86 (1H, d, $J = 7.5$ Hz); two methyl groups were at δ_H 1.20 (3H, d, $J = 7.0$ Hz) and 0.83 (3H, t, $J = 7.0$ Hz). The ¹³C-NMR spectra of **1** revealed signals of 16 carbons which were classified into three non-protonated carbons, nine methines, two methylenes, and two methyl carbons. Of these, a set of carbinol carbon signals including δ_C 102.2, 78.1, 78.1, 74.9, 71.4, and 62.5 were typically characterized of a glucose unit. Shielded carbon signals (δ_C 43.2, 32.0, 22.3, 12.6) and their corresponding proton splitting pattern [δ_H 2.49 (1H, sex), 1.58 (2H, quin), 1.20 (3H, d), 0.83 (3H, t), $J = 7.0$ Hz each] suggested the presence of a *sec*-butyl group. Remain six aromatic carbon signals containing two deshielded carbons (δ_C 160.1 and 159.3) indicated compound **1** to be a resorcinol derivative [3]. The HMBC correlations from protons H-10 (δ_H 1.20) to carbons C-1 (δ_C 151.4)/ C-7 (δ_C 43.2)/ C-8 (δ_C 32.0); from H-7 (δ_H 2.49) to C-1 (δ_C 151.4)/ C-2 (δ_C 107.9)/ C-6 (δ_C 109.2) confirmed the attachment of *sec*-butyl group to benzene ring. In addition, HMBC correlations from aromatic proton H-2 (δ_H 6.45)

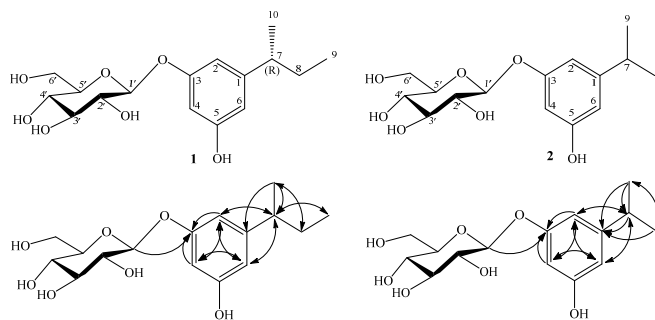


Figure 1: Chemical structures and key HMBC correlations of **1** and **2**.

and anomeric proton H-1' (δ_H 4.86) to carbon C-3 (δ_C 160.1) were demonstrated the location of glucose moiety at C-3. The absolute configuration at C-7 of **1** was assigned to be *R* based on both negative CD bands at ¹B_b and ¹L_a transitions of benzene chromophore ($\theta_{192} = -1.84$ and $\theta_{228} = -0.87$ mdeg) and in comparison with the reported literature [4]. Consequently, chemical structure of compound **1** was established and named as agrimopilosides A.

Compound **2** was isolated as a white amorphous powder. The molecular formula of **2**, C₁₅H₂₂O₇, was determined by pseudo-molecular ion peak at m/z 313.1298 [M-H]⁻ (calcd for C₁₅H₂₁O₇, 313.1287) in the HR-ESI-MS and ¹³C-NMR analysis. The ¹H- and ¹³C-NMR spectra of **2** (Table 1) were found to be similarity to those of **1**, except signals of side-chain. Three shielded carbon signals and their corresponding proton splitting pattern [δ_C/δ_H 35.5/2.79 (sep, $J = 7.0$ Hz), 24.3 and 24.2/1.21 (6H, d, $J = 7.0$ Hz)] indicated the presence of an isopropyl group in **2** instead of *sec*-butyl group in **1** [5]. Thus, compound **2** was established to be 1-isopropyl-3,5-dihydroxybenzene 3-*O*- β -D-glucopyranoside, and named as agrimopiloside B.

Table 1: ¹H- and ¹³C-NMR data for compounds 1 and 2.

Pos.	1 ^a		2 ^b	
	δ _c	δ _H (mult., J in Hz)	δ _c	δ _H (mult., J in Hz)
1	151.4	-	152.6	-
2	107.9	6.45 (t, 2.0)	107.3	6.50 (t, 2.0)
3	160.1	-	160.1	-
4	102.5	6.41 (t, 2.0)	102.5	6.41 (t, 2.0)
5	159.3	-	159.3	-
6	109.2	6.33 (t, 2.0)	108.6	6.37 (t, 2.0)
7	43.2	2.49 (sex, 7.0)	35.5	2.79 (sep, 7.0)
8	32.0	1.58 (quin, 7.0)	24.2	1.21 (d, 7.0)
9	12.6	0.83 (t, 7.0)	24.3	1.21 (d, 7.0)
10	22.3	1.20 (d, 7.0)	-	-
1'	102.2	4.86 (d, 7.5)	102.2	4.86 (d, 7.5)
2'	74.9	3.45 (m)	74.9	3.45 (m)
3'	78.1	3.43 (m)	78.1	3.43 (m)
4'	71.4	3.42 (m)	71.4	3.42 (m)
5'	78.1	3.46 (m)	78.1	3.46 (m)
6'	62.5	3.72 (dd, 5.0, 12.0)	62.5	3.72 (dd, 5.0, 12.0)
		3.91 (dd, 2.0, 12.0)		3.91 (dd, 2.0, 12.0)

Measured in ²CD₃OD, Assignments were done by HSQC and HMBC experiments.

Remaining compounds were identified as (2*S*, 3*S*)-aromadendrin 3-*O*-β-*D*-glucopyranoside (**3**) [6] and (2*S*, 3*S*)-glucodistylin (**4**) [7] on the basis of their NMR and CD spectral data which were in good agreement with those reported in the literature.

Compounds **1-4** were evaluated for their effects on NO production in the LPS-stimulated RAW 264.7 cells. The results showed that at concentration of 20 μM, all compounds modestly inhibited NO production with the inhibitory rates from 9.55% to 33.73%. The isolated compounds **1-4** were also evaluated their effects on the proliferation of HepG2, MCF-7, and SK-LU-1 human cancer cells using MTT assay. However, none of them had significant cytotoxicity against the tested cell lines.

Experimental

General: Optical rotations, Jasco DIP-370 automatic polarimeter; NMR, Bruker AM500 FT-NMR spectrometer; CD, Chirascan spectrometer; HR-ESI-MS, Agilent 6530 Accurate Mass Q-TOF LC/MS system.

Plant material: The aerial parts of *A. pilosa* were collected at Trung Khanh, Cao Bang province, Vietnam, in August, 2013. Its scientific name was identified by Dr. Pham Thanh Huyen, Institute of Ecology and Biological Resources, VAST. A voucher specimen (6695A) is deposited at the Herbarium of Military Institute of Traditional Medicine.

Extraction and isolation: The dried and powdered of aerial parts of *A. pilosa* (5.0 kg) were sonically extracted with methanol at 50 °C

for three times (10.0 L each). After removal of the solvents, methanol extract (700.0 g) was suspended with distilled water (1.5 L) and successively partitioned with dichloromethane and ethyl acetate (three times, 2.0 L each) to give corresponding soluble extracts, dichloromethane (BAPD, 320.0 g), ethyl acetate (BAPE, 80.0 g), and water layer (BAPW). The BAPE extract (80.0 g) was separated on a silica gel column chromatography, eluting with gradient of dichloromethane/ methanol (100/0, 50/1, 30/1, 20/1, 10/1, 1/1, 0/100, v/v) to give seven fractions, BAP1A - BAP1G). Fraction BAP1D (10.0 g) was continuously separated on a RP-18 column chromatography, eluting with methanol/water (1/3, v/v) to give seven smaller fractions, BAP2A - BAP2G. Fraction BAP2C (0.5 g) was chromatographed on Sephadex LH20 column, eluting with methanol/water (1/1, v/v) to give compound **4** (30 mg). Fraction BAP2D (2.0 g) was separated on a silica gel column chromatography, eluting with dichloromethane/methanol/water (4/1/0.1, v/v/v) to give three fractions, BAP3A - BAP3C. Compound **3** (15 mg) was obtained from the BAP3B fraction (0.3 g) using a RP-18 column, eluted with acetone/water (2/5, v/v). Fraction BAP2E (1.0 g) was chromatographed on a RP-18 column, eluting with acetone/water (1/2, v/v) to yield three sub-fractions, BAP4A - BAP4C. Compound **1** (7.0 mg) was obtained from the BAP4A fraction (0.1 g) using a RP-18 column, eluted with acetone/water (1/2, v/v). Fraction BAP2F was chromatographed on a RP-18 column, eluting with acetone/water (1/3, v/v) to yield four sub-fractions (BAP5A-BAP5D). Compound **2** (15.0 mg) was obtained from the BAP5B fraction (0.2 g) using a Sephadex LH-20 column, eluted with methanol/water (1/1, v/v).

Agrimopiloside A (1)

White amorphous powder.

[α]_D²⁵: +28.3 (c 0.1, MeOH).

CD (MeOH): θ₁₉₂ = -1.84 and θ₂₂₈ = -0.87 mdeg

¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (125 MHz, CD₃OD): Table 1.

HR-ESI-MS: *m/z* 327.1453 [M-H]⁻; calcd for C₁₆H₂₃O₇, 327.1444.

Agrimopiloside B (2)

White amorphous powder.

[α]_D²⁵: +41.6 (c 0.1, MeOH).

¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (125 MHz, CD₃OD): Table 1.

HR-ESI-MS: *m/z* 313.1298 [M-H]⁻; calcd for C₁₅H₂₁O₇, 313.1287.

Supplementary data: HR-ESI-MS, NMR, and CD spectra of compounds **1 - 4** can be found in the online version.

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