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NITRILE GLUCOSIDE, FLAVONOL GLUCOSIDE AND POLYPHENOLIC ACIDS FROM *EHRETIA DENTATA* COURCH.

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SUMMARY

Chemical investigation of the leaves of Ehretia dentata Couch. growing in Hoa Binh, Vietnam led to the isolation and structural elucidation of a nitril glucoside, ehretioside A1, the flavonol glucoside astragalin, as well as rosmarinic acid and methyl rosmarinat. These structures were determined on the basis of MS and NMR spectroscopic data.

Key words: *Ehretia dentata; boraginaceae; nitrile glucoside; cyanoglucoside; astragalin; rosmarinic acid.*

I - INTRODUCTION

Ehretia dentata (local name Cùm rụm răng or Cườm cườm) belongs to the family Boraginaceae, is a small trees growing wild in Vietnam [1]. Its stem bark and leaves are useful in the therapy of certain inflammatory processes [2]. Some *Ehretia* species contain cyanoglucosides and phenolic acids. Chemical constituents of *E. dentata* have not yet been studied. In our search for biological active compounds from Vietnamese plants, we now report the isolation and structural determination of nitrile glucoside, ehretioside A1; flavonol glucoside, astragalin; rosmarinic acid and methyl rosmarinate from the leaves of *E. dentata*. These structures were elucidated by MS, including HR-MS, ^{1}H- and ^{13}C-NMR techniques.

II - EXPERIMENTAL

1. General

Optical rotation $[\alpha]_D$: Digital Polarimeter Jasco DIP 1000. EIMS: ADM 402, 70 eV, Finigan TSQ 700. HR-ESI-MS: BRUKER BIOAPEX 70e Fourier transform. NMR: BRUKER Avance 500 spectrometer at 499.8 MHz (^{1}H) and 125 MHz (^{13}C, ^{13}C-DEPT). Chemical shifts were referenced to internal TMS (δ = 0, ^{1}H) and CD_3OD (δ = 49.0, ^{13}C) or pyridine-d_5 (δ = 75.3, ^{13}C). CC: Silica gel 60, 0.06 - 0.2 mm (Merck) for the first column, silica gel 60, 40 - 63 μm (Merck) for the following columns. TLC: Silica gel 60 F-254 (Merck).

2. Plant material

The leaves of *E. dentata* were collected in Hoa Binh province, in North of Vietnam, in 2005. The species was identified by Dr. Vu Xuan Phuong, Institute of Ecology and Natural Resources, VAST, Hanoi, Vietnam. A voucher specimen is deposited in the Herbarium at the same Institute.

3. Extraction and isolation

The dried and powdered leaves of *E. dentata* (600 g) were extracted with 90% aq. EtOH at room temperature. EtOH was evaporated *in*

vacuo at 45°C and the aq. solution (45 g) was partitioned with *n*-hexane followed by EtOAc and *n*-BuOH. The organic solvents were evaporated *in vacuo* to afford 25.5, 2.5 and 18.6 g of *n*-hexane, EtOAc and *n*-BuOH extracts, respectively. The n-BuOH extract (18 g) was chromatographed over silica gel with gradient EtOAc-MeOH → EtOAc-MeOH-H_2O (60 : 10 → 60 : 10 : 1) to give 10 fractions (F-1→F-10).

a) (-)-Ehretioside A1 (1)

Compound **1** was purified from fraction 6 by silica gel CC with $CHCl_3$-EtOAc-MeOH (80:20:1), white powder from MeOH, yield 70 mg (0.0116%); HR-ESI-MS (*m/z*): 452.15211 (calc. 452.15272, $C_{19}H_{27}NO_{10}Na$). $[\alpha]_D^{19}$ –343° (*c* 0.5, MeOH); Lit. [2]: $[\alpha]_D$ + 39° (*c* 1.0, pyridine); IR ν_{max}^{KBr} (cm^{-1}): 3480 (OH), 3366, 3202, 2917, 2234 (C≡N), 1689, 1644 (C=O), 1428, 1379, 1237, 1164, 1072, 1019; ^{1}H- and ^{13}C-NMR data (see table 1).

b) Astragalin (kaempferol-3-O-β-D-glucoside, (2)

Astragalin was isolated from F-1 + F-2, yield 56 mg (0.0933%). Powder from MeOH/EtOAc; ESI-MS (*m/z*): 471.5 $[M+Na]^+$ ($C_{21}H_{20}O_{11}Na$), 446.9 $[M-H]^-$.

c) Rosmarinic acid (3)

The crude compound **3** was isolated from F-3 and further purified by chromatography on sephadex LH-20 (MeOH) and then silica gel with EtOAc-MeOH (60:10). Powder from MeOH/EtOAc, yield 30 mg (0.005%); $[\alpha]^{20}_D$ +71° (*c* 1.0, MeOH) (Lit. [7]: $[\alpha]_D^{22}$ +56°] for R (+)-form); IR ν_{max}^{KBr} (cm^{-1}): 3427 (OH), 1681 (C=O), 1600, 1523, 1379, 1268, 1181, 1115, 815, 560; ESI-MS (*m/z*): 383 $[M+Na]^+$ (90), 361 $[M+H]^+$ (13), 163 (12).

d) Methyl rosmarinate (4)

Compound **4** was isolated from F-3 and further purified by chromatography on silica gel with EtOAc-MeOH (60:10). White powder from MeOH-EtOAc, yield 27 mg (0.0045%); $[\alpha]^{20}_D$ +33° (*c* 1.0, MeOH); IR ν_{max}^{KBr} (cm^{-1}): 3395 (OH), 2960, 2852, 1697 (C=O), 1600, 1521, 1447, 1279, 1162, 1017; ESI-MS (*m/z*): 397 $[M+Na]^+$, 375 $[M+H]^+$ ($C_{19}H_{18}O_8$), 302 (24), 163 (12).

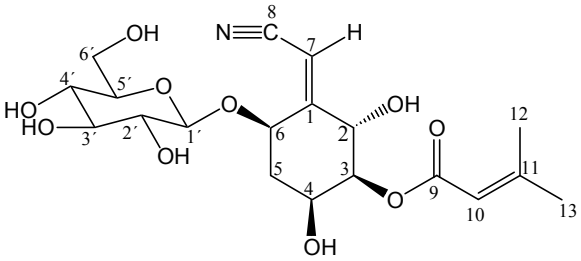
III - RESULTS AND DISCUSSION

The residue of an ethanol extract of the leaves of *E. dentata* was partitioned with *n*-hexane, ethyl acetate and *n*-butanol, successively. The EtOAc and *n*-butanol extracts, after evaporation of the solvents, were subjected to column chromatography and then recrystallization to give compounds **1 - 4** and other triterpenes.

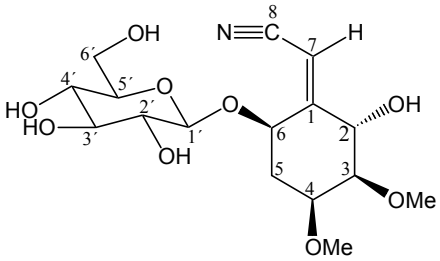
The HR-ESI-MS of compound **1** gave the $[M+Na]^+$ peak at *m/z* 452.15211 corresponding to the molecular formula $C_{19}H_{27}NO_{10}Na$ (M = 429). The IR spectrum showed absorption at ν*3480 (OH), 2234 (CN) and 1689 cm^{-1} (CO). The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a sugar moiety and was identified as β-D-glucopyranose by anomeric signals at δ_H5.07 (d, J = 7.8 Hz, H-1′) and δ_C102.27 (C-1′). Its characteristic signals in the ^{1}H- and ^{13}C-NMR spectra (table 1) and the fragment in ESI-MS spectrum (*m/z* 268, $[M+H-Glu]^+$) indicated the molecular formula $C_{13}H_{17}NO_5$ for the aglycone. The ^{1}H-NMR spectrum displayed an olefinic proton (H-10) at δ5.85 (t, J = 1.2 Hz) coupling with protons of two methyl groups at δ1.96 and 2.02 (each 3H, d, J = 1.1 Hz, in CD_3OD), indicating a senecioic acid ester moiety [2, 3]. This was further confirmed by the signals at δ116.61 and 157.01 for C-10 and C-11, respectively in the ^{13}C-NMR spectrum. Compound **1** was significant for the confirmation of the position of the senecioic acid moiety. The signal of the proton at C-3 (δ_H 4.24) was significant downfield-shifted ($\Delta\delta \approx$ 1.0 ppm) compared with the corresponding proton of **1a** [2].

NMR spectra of **1** indicated the presence of cyanomethylene group by methine signals [δ_H6.28 (d, J = 1.8 Hz), δ_C95.68] and a

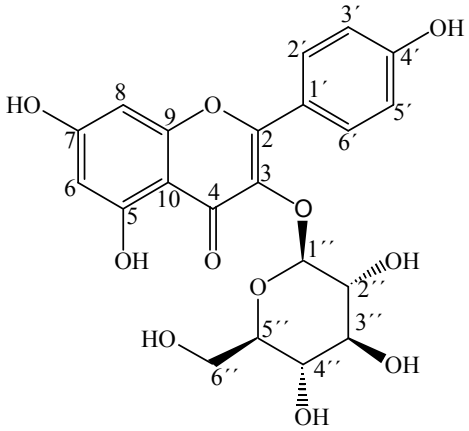
quaternary carbon (δ_C 166.03). Comparison the ^{13}C-NMR data of **1** with those of simmondsin (**1a**) showed a significant differences: 17.6 ppm downfield shift at C-3, where the senecioyl moiety is attached, 7.1 ppm upfield shift at C-2 and 8.4 ppm downfield shift at C-4 for **1** (table 1). This results confirmed the position of the senecioic acid ester linkage at C-3. The sugar moiety is attached to C-6 of the aglycone was determined by comparison with reported data of simmondsin (**1a**) [3]. The absolute configuration of **1a** was determined as (1Z)-(2*S*,3*R*,4*S*,6*R*)-1-cyanomethylene-2-hydroxy-3,4-dimethoxycyclohexyl β-D-glucopyranoside by total synthesis [3]. The ^{1}H-, ^{13}C-NMR spectral data of **1** were in good agreement with those of ehretioside A1. The optical rotation of **1** was found to be $[\alpha]^{19}_D$ - 345^O (*c* 0.5, MeOH). Thus, compound **1** was established as (-)-ehretioside A1. The enantiomeric (+)-ehretioside A1 (lit. [2]: $[\alpha]_D^{20}$ +39^O, MeOH) has been isolated for the first time from *E. philippinensis*. Cyanoglucoside showed significant anti-bacterial activity, inhibition of feeding in rat and other unique biological activity [2, 3].



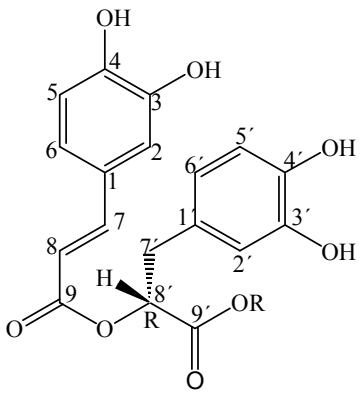
1: Ehretioside A1



1a: Simmondsin



2: Astragalin



3: R = H Rosmarinic acid
4: R = Me Methyl rosmarinate

Compound **2** was isolated as yellow solid in a yield of 0.0933% from the ethyl acetate extract using column chromatography on silica gel. The ESI-MS spectrum of **2** gave a peak at *m/z* 471 $[M+Na]^+$, combination with ^{1}H- and ^{13}C-NMR spectra leading to the molecular formula $C_{21}H_{20}O_{11}$. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a β-D-glucopyranose linked to a flavonol [anomeric proton and carbon at δ_H4.83 (d, J ≈ 7.5 Hz); δ_C104.13; CH x 6 and Cq x 9 (C=O x 1)]. The aglycon is substuted with 5,7-dihydroxy groups (δ_H 6.29 and 6.43 (each 1H, d, J ≈ 2 Hz). One substituent in ring B was identified as a hydroxy group by

Table 1: ^{13}C- and ^{1}H-NMR spectral data of simmondsin (**1a**), ehretioside A1 and compound **1** [125/500 MHz, δ (ppm), J (Hz)]

	1a CD_3OD [2]	ehretioside A1 pyridin-d_5 [2]		**1**, pyridin-d_5		**1**, CD_3OD	
C	δ_C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	166.4	165.0		165.05		165.82	
2	70.7	67.9	5.68 dd (10, 2)	67.98	5.71 d (9.7)	77.87	4.65 dd (3.2, 9.9)
3	86.3	79.0	5.18 dd (10, 3)	78.96	5.22 dd (9.9, 3.1)	68.73	4.24 dd (3.3, 8.2)
4	76.7	67.7	4.66 ddd (3, 3,3)	67.73	4.68 s br	68.38	3.85 dd (12.0, 2.4)
5	32.0	34.3	2.69 dt (15, 3) 1.82 dt (15, 3)	34.3	2.69 dt (15.3, 2.8) 1.88 dd (15.3, 3.0)	35.36	2.45 dt (15.3, 3.7) 1.91 dt (15.3, 3.7)
6	76.5	76.0	5.52 t (3)	75.98	5.57 d (1.0)	77.60	4.91 dd (9.9; 1.8)
7	95.2	95.6	6.25 d (2)	95.68	6.28 d (1.8)	95.88	5.78 d (2.0)
8	117.6	116.9		117.01		117.28	
9	58.5	166.0		166.03		167.38	
10	58.2	116.6	5.56 sept. (5)	116.61	5.56 d (5.3)	116.85	5.85 t (1.2)
11		156.9		157.01		159.06	
12		26.9	1.56 d (1.3)	26.90	1.55 s	27.43	1.96 d (1.1)
13		20.0	2.07 d (1.3)	20.02	2.07 s	20.42	2.20 d (1.1)
1′	104.1	102.7	5.03 d (7.8)	102.77	5.07 d (7.8)	104.46	4.45 d (7.8)
2′	74.7	75.0	3.97 dd (7.8, 8.8)	75.07	3.94 d (2.6)	74.81	3.86 dd (12.0, 2.4)
3′	78.1	78.4	4.21 dd (8.8, 8.3)	78.40	4.22 dd (8.3, 8.6)	78.16	3.27 dd (8.0, 8.9)
4′	71.4	71.4	4.21 dd (8.3, 9.3)	71.48	4.22 dd (8.3, 8.6)	71.30	3.41 d (8.8)
5′	78.1	78.5	3.91 ddd (9.3, 2.4, 5.2)	78.59	3.94 d (2.6)	78.55	3.37 dd (8.8, 9.3)
6′	62.7	62.7	4.44 dd (11.0, 2.4) 4.28 dd (12, 5)	62.74	4.48 d (11.3) 4.29 s br	62.45	3.85 d (12.0, 2.4) 3.72 dd (12.0, 5.0)

the high chemical shift of connected carbon (δ_C161.57, C-4′). Comparison of MS and NMR spectra with those of reported data [4] confirmed the structure of **2** as 4′,5,7-trihydroxyflavone-3-O-β-D-glucopyranoside (astragalin, kaempferol-3-O-β-D-glucopyranoside). Astragalin was found for the first time from *Astragalus* species and showed strong antibacterial and anticandidal activity [4].

The molecular formula ($C_{18}H_{16}O_8$) of **3** was established on the basis of ESI-MS spectrum (*m/z* 383 $[M+Na]^+$, 361 $[M+H]^+$). The ^{13}C-NMR and DEPT spectra showed signals for 18 carbons, including one carbonyl, and 12 aromatic carbons.

The structure of **3** was confirmed as 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid 2-O-(3,4-dihydroxyphenyl-2*E*-cinnamoyl) (rosmarinic acid) by comparison of ^{1}H- and ^{13}C-NMR spectral data with the literature [5]. Rosmarinic acid was isolated for the first time from *Rosmarinus officinalis*, and its novel butyl ester was found in *Isodon oresbius* [6].

Compound **4** was obtained as white amorphous powder and its spectra data closely resembled to those of **3**. The molecular formula of **4** was established as $C_{19}H_{18}O_8$ by peaks at *m/z* 397 $[M+Na]^+$ and 375 $[M+H]^+$, with one methyl group more than **3**. This was confirmed by signals δ_H 3.72 and δ_C 52.69 of methyl ester group in NMR spectra. The structure of **4** was elucidated as 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid 2-O-(3,4-dihydroxyphenyl-2*E*-cinnamoyl) methyl ester (methyl rosmarinate). Rosmarinic acid and its methyl ester were isolated from *E. philippinensis* and showed strong anti-histamine release, anti-HIV, antibacterial, antifungal effects, plant-growth inhibitory and anti-inflammatory activity [2].

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REFERENCES

1. Pham Hoang Ho. An Illustrated Flora of Vietnam, Youth publishing House, Vol. II, P. 804 (2000).
2. Lourder R. Simpol, Hideaki Otsuka, Kasuhiro Ohtani, Ryoji Kasai, Kauo Yamasaki. Phytochemistry, 36 (1), 91 - 95 (1994).
3. N. Chi Da, K. Yamada, S. Ogawa. J. Chem. Soc. Perkin Trans., 1, 1131 - 1137 (1992).
4. Nobutoshi Tanaka, Toshiko Satake, Akira Takanashi, Michiko Mochizuko, Takao Murakani, Yashuhisa Saiki, Zin-Zhen Yang, Chiu-Ming Chen. Chem. Pharm. Bull., 30, 3640 - 3646 (1982).
5. A. J. M. Jassen, A. J. H. Klunder, B. Zwanenburg. Tetrahedron, 47, 7645 - 7662 (1991).
6. H. Huang, Q. R. Chao, R. X. Tan, H. D. Sun, D. C. Wang. J. Zhao S. X., 1999. Planta Medica, 65, 92 - 93 (1999).