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ALKALOID AND CYCLOHEXYLETHANOL DERIVATIVES FROM THE LEAVES OF *ARDISIA SILVESTRIS* PIT.

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Summary

Alkaloid and Cyclohexylethanol Derivatives from the Leaves of *Ardisia silvestris* Pit.

One alkaloid and four cyclohexylethanol derivatives, 2-methylpyridin-3-ol (**1**), rengyol (**2**), isorengyol (**3**), *trans*-1-[2-(acetyloxy) ethyl]-cyclohexane-1,4-diol (**4**), and cleroindicin B (**5**) were isolated from the ethanol extract of the leaves of *Ardisia silvestris* Pit. Their structures were identified by spectroscopic analysis and comparisons with previous reports. All compounds were reported for the first time from genus *Ardisia*.

Keywords: *Ardisia silvestris, 2-methylpyridin-3-ol, Cyclohexylethanol, Rengyol, Isorengyol, Cleroindicin B.*

1. Introduction

Ardisia silvestris Pit. (Primulaceae) (Vietnamese name: Khôi tía) is widely distributed in the northern and central provinces of Vietnam. This medicinal plant is also found in China and Laos [1]. The leaves of *A. silvestris* have been used in folk medicine for treating stomach pain by using alone or in combination with some other herbs such as *Taraxacum officinale*, *Croton tonkinensis*, and *Abrus precatorius* [2].

The ethanol extract of *A. silvestris* showed anti-bacterial activity on *Helicobacter pylori* (ATCC 51932, OX.20, OX.68 and OX.93) with MICs from 1.04 to 1.94 mg/mL [3] and on *Escherichia coli* and *Samonella* sp. at the concentration of 100 µg/mL [4]. In addition, the ethyl acetate and water extracts of *A. silvestris* showed the antioxidant activity in scavenging DPPH radical assay with IC_{50} values of 30.51 µg/mL and 31.87 µg/mL, respectively [4].

A previous phytochemical study reported two resorcinol derivatives, 2-methyl-5-(*Z*-nonadec-14-enyl)-resorcinol and 5-(*Z*-nonadec-14-enyl) resorcinol from the methanol extract of the leaves of *A. silvestris* [5]. This study reports the isolation and elucidation of one alkaloid and four cyclohexylethanol derivatives from the leaves of *A. silvestris*.

2. Materials and methods

2.1. General procedures

NMR spectra were measured by ASCEND™ 400 FT-NMR spectrometer (BRUKER) using tetramethylsilane as the internal standard. Chemical shift values were expressed in δ (*ppm*). Mass spectra were acquired on LCMS-IT-TOF™ (SHIMADZU) and UPLC-ACQUITY QDa system. The column chromatography was performed with *silica gel* (40 - 63 µm) (Merck, Germany) and Sephadex LH-20 (GE Healthcare Life). Thin layer chromatography (TLC) was conducted on *silica gel* 60 F_{254} plates (Merck, Germany). Fractions were monitored by TLC and spots were detected by spraying with the vanillin - sulfuric reagent, followed by heating. All solvents were of analytical grade.

2.2. Plant material

The leaves of *A. silvestris* were collected from Bach Ma National Park, Thua Thien Hue Province (February 2020) and authenticated by Dr. Tran Thi Van Anh. A voucher specimen of plant (No KT-022020) was deposited at Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City.

Extraction and isolation

The dried leaves powder of *A. silvestris* (3.7 kg) was percolated with 96% ethanol (90 L) at room temperature. Ethanol was removed under reduced pressure to give a crude extract (650 g). The crude extract was suspended in water (1 L) and then partitioned with *n*-hexane (1 L × 4 times), chloroform (1 L × 4 times), and ethyl acetate (1 L × 12 times). The organic solvents were evaporated *in vacuo* to yield *n*-hexane (123.8 g), chloroform (78.5 g), and ethyl acetate (84.3 g) extracts.

The ethyl acetate extract (45.0 g) was subjected to *silica gel* column chromatography (Φ 6.5 × 50 cm) using gradient solvent system of chloroform - methanol (10:0 to 5:5, v/v) to obtain 23 fractions (C.1 - C.23). The fraction C.12 (2.0 g) was separated on a *silica gel* column chromatography eluting with chloroform - acetone (9:1 to 7:3, v/v) to give 14 fractions (C.12.1 - C.12.14). The fraction C.12.5 (125 mg) was further separated on a Sephadex LH-20 column using methanol as mobile phase and then was purified on a *silica gel* column using chloroform - methanol (95:5, v/v) to afford compounds **4** (10 mg) and **5** (17 mg). The fraction C.18 (3.0 g) was chromatographed on *silica gel* column with gradient elution of chloroform - acetone (6:4 to 4:6, v/v) to obtain 14 fractions (C.18.1 - C.18.14). The fractions C.18.5 (30 mg), C.18.7 (130 mg) and C.18.9 (330 mg) were purified on a Sephadex LH-20 column eluting by methanol to yield compounds **1** (22 mg), **3** (21 mg), and **2** (23 mg), respectively.

2-Methylpyridin-3-ol (**1**): colorless needles; ESI-MS (*m/z*) 110.3 $[M+H]^+$, 108.4 $[M-H]^-$ (C_6H_7NO); ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 7.14 (1H, *dd*, J = 8.0; 1.6 Hz, H-4), 7.08 (1H, *dd*, J = 8.0; 4.8 Hz, H-5), 7.85 (1H, *dd*, J = 4.8; 1.6 Hz, H-6), and 2.40 (3H, *s*, 2-CH_3); ^{13}C-NMR (CD_3OD, 100 MHz) δ_C: 147.6 (C-2), 153.8 (C-3), 123.1 (C-4), 123.6 (C-5), 139.5 (C-6), and 18.4 (2-CH_3).

Rengyol (**2**): colorless oil; ESI-MS (*m/z*) 183.4 [M+Na] ($C_8H_{16}O_3$); ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 1.72 (2H, *m*, H-2/6), 1.40 (2H, *m*, H-2/6), 1.67 (2H, *m*, H-3/5), 1.63 (2H, *m*, H-3/5), 3.52 (1H, *m*, H-4), 1.68 (2H, *t*, J = 7.2 Hz, H-7), and 3.73 (2H, *t*, J = 7.2 Hz, H-8); ^{13}C-NMR (CD_3OD, 100 MHz), see **Table 1**.

Isorengyol (**3**): colorless oil; ESI-MS (*m/z*) 183.3 $[M+Na]^+$ ($C_8H_{16}O_3$); ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 1.79 (2H, *m*, H-2/6), 1.47 (2H, *m*, H-2/6), 1.86 (2H, *m*, H-3/5), 1.48 (2H, *m*, H-3/5), 3.80 (1H, *m*, H-4), 1.79 (2H, *t*, J = 7.2 Hz, H-7), 3.77 (2H, *t*, J = 7.2 Hz, H-8); ^{13}C-NMR (CD_3OD, 100 MHz), see **Table 1**.

trans-1-[2-(acetyloxy) ethyl]-cyclohexane-1,4-diol (**4**): colorless oil; ESI-MS (*m/z*) 225.5 $[M+Na]^+$ ($C_{10}H_{14}O_4$); ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 1.78 (2H, *m*, H-2/6), 1.47 (2H, *m*, H-2/6), 1.88 (2H, *m*, H-3/5), 1.49 (2H, *m*, H-3/5), 3.81 (1H, *dt*, J = 6.8; 3.2 Hz, H-4), 1.86 (2H, *t*, J = 7.2 Hz, H-7), 4.24 (2H, *t*, J = 7.2 Hz, H-8), and 2.04 (3H, *s*, H-10); ^{13}C-NMR (CD_3OD, 100 MHz), see **Table 1**.

Cleroindicin B (**5**): colorless oil; HR-ESI-MS (*m/z*) 157.0866 $[M-H]^-$ ($C_8H_{14}O_3$) (calcd. for 158.0943); ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 2.00 (2H, *m*, H-2/6), 1.83 (2H, *m*, H-2/6), 2.68 (2H, *m*, H-3/5), 2.18 (2H, *m*, H-3/5), 1.81 (2H, *t*, J = 6.8 Hz, H-7), and 3.79 (2H, *t*, J = 6.8 Hz, H-8); ^{13}C-NMR (CD_3OD, 100 MHz), see **Table 1**.

Table 1. ^{13}C-NMR (CD_3OD, 100 MHz) chemical shifts (*ppm*) of compounds **2 - 5**, and reference compounds

Position	2	Rengyol *	3	Isorengyol *	4	5
1	70.8	70.8	71.9	72.0	71.2	70.5
2/6	36.1	36.0	34.2	34.2	34.2	37.8
3/5	31.1	31.3	30.7	30.7	30.6	37.7
4	70.8	70.8	68.5	68.5	68.3	214.8
7	45.6	45.6	43.2	43.0	40.2	44.6
8	59.2	59.2	59.0	59.0	62.0	59.1
9					173.1	
10					21.0	

* data from reference [7].

3. Results and discussion

The ethanol extract from the leaves of *A. silvestris* was chromatographed that led to the isolation of one alkaloid and four cyclohexylethanol derivatives (**1 - 5**) (**Fig. 1**). The chemical structures of the isolated compounds were elucidated by the analyses of their MS and NMR data as well as comparison with those in the reported literatures.

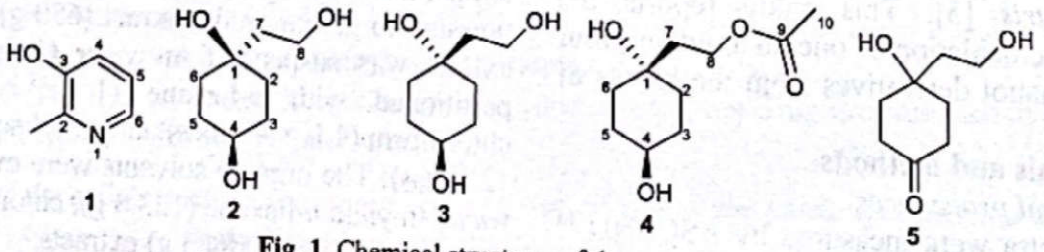

Fig. 1. Chemical structures of the compounds **1 - 5**

Compound **1** was isolated as colorless needles. The mass spectrum of **1** exhibited pseudo-ion peaks at *m/z* 110.3 $[M+H]^+$ and 108.4 $[M-H]^-$. The ^{1}H-NMR spectrum of compound **1** showed three aromatic protons at δ_H 7.85 (1H, *dd, J* = 4.8; 1.6 Hz), 7.14 (1H, *dd, J* = 8.0; 1.6 Hz) and 7.08 (1H, *dd, J* = 8.0; 4.8 Hz) and one methyl group at 2.40 (3H, s). The ^{13}C-NMR and HSQC spectra exhibited signals of 6 carbons including one aromatic carbon linked to hydroxy group (δ_C 153.8); two aromatic carbons connected with a nitrogen (δ_C 147.6 and 139.5); two aromatic carbons (δ_C 123.6 and 123.1) and one methyl group (δ_C 18.4). The analysis of NMR spectra suggested the structure of compound **1** was a pyridine derivative. The doublet of doublet splitting patterns of three aromatic protons were characteristic for 2,3-disubstituted pyridine ring. In addition, the HMBC correlations from the protons at δ_H 2.40 to C-2 (δ_C 147.6) and C-3 (δ_C 153.8) supported the presence of a methyl group at C-2 and a hydroxy group at C-3 on the pyridine ring. Based on NMR data analysis and comparing with data in the literature [6], compound **1** was identified as 2 methylpyridin-3-ol.

Compound **2** was obtained as a colorless oil. The ^{1}H-NMR and HSQC spectra of compound **2** revealed proton signals of one hydroxymethine at δ_H 3.52 (1H, *m*); one hydroxymethylene at δ_H 3.73 (2H, *t, J* = 7.2 Hz); five methylene at δ_H 1.68 (2H, *t, J* = 7.2 Hz), δ_H 1.72, 1.67, 1.63, and 1.40 (each, 2H, *m*). The ^{13}C-NMR and HSQC spectra exhibited signals of 8 carbons including one non-protonated carbon linked to the hydroxy group (δ_C 70.8); one hydroxymethine carbon (δ_C 70.8), one hydroxymethylene carbon (δ_C 59.2) and five methylene carbons (δ_C 45.9, 36.1 x 2 and 31.1 x 2). The structure of **2** was suggested as a cyclohexane ring with substituents at C-1 and C-4. The HMBC correlations from H-2/6 (δ_H 1.72 and 1.40) and H-3/H-5 (δ_H 1.67 and 1.63) to C-1/C-4 (δ_C 70.8) and the COSY correlation between H-3/5 (δ_H 1.67 and 1.63) and H-4 (δ_H 3.52) determined the location of the hydroxy group at C-4. Additionally, the HMBC correlations from methylene protons at δ_H 1.68 (2H) to C-1 (δ_C 70.8) and C-2/6 (δ_C 36.1) and from hydroxymethylene protons at δ_H 3.73 (2H) to C-1 (δ_C 70.8) proved that the 2-hydroxyethyl moiety at C-1 of the cyclohexane ring. Comparison with data in the literature [7], compound **2** was identified as *cis*-1-(2-hydroxyethyl)cyclohexane-1,4-diol also known as rengyol.

Compound **3** was isolated as a colorless oil. The NMR spectral data of compound **3** were very similar with those of compound **2**, suggested that compound **3** had the same structure as 1-(2-hydroxyethyl) cyclohexane-1,4-diol. The cyclohexane ring with two substituents at C-1 and C-4 has the symmetrical characteristic. This structure has the geometrical isomers based on the relative placement of substituents across a ring. The chemical shifts of C-1 (δ_C 71.9) and C-4 (δ_C 68.5) of compound **3** are different with those of compound **2** (δ_C 70.8, for C-1 and δ_C 70.8 for C-4). By comparison with published data [7] the structure of **3** was confirmed as *trans* relationship between the two hydroxyl groups in the 1 and 4 positions, while compound **2** was the *cis* isomer. The structure of compound **3** was identified as isorengyol.

Compound **4** was obtained as a colorless oil. The ^{1}H-NMR spectra of compound **4** showed one hydroxymethine at δ_H 3.81 (1H, *dt, J* = 6.8; 3.2 Hz); one hydroxymethylene at δ_H 4.24 (2H, *t, J* = 7.2 Hz); a methyl group at δ_H 2.04 (3H, *s*); five methylene at δ_H 1.86 (2H, *t, J* = 7.2 Hz), δ_H 1.88, 1.78, 1.49, and 1.47 (each, 2H, *m*). The ^{13}C-NMR and HSQC spectra showed signals of 10 carbon consisting of one carbonyl carbon (δ_C 173.1); one non-protonated carbon (δ_C 71.2); one hydroxymethine carbon (δ_C 68.3); one oxygenated methylene carbon (δ_C 62.0), five methylene carbons (δ_C 40.2, 34.2 x2, and 30.6 x2) and a methyl carbon (δ_C 21.0). The structure of **4** was estimated as a cyclohexylethanol derivative which was similar to the structure of **3**. In addition, the HMBC correlations from the hydroxymethylene protons (δ_H 4.24) and the methyl group (δ_H 2.04) to the carbonyl carbon (δ_C 173.1) indicated the position of the acetoxy group at C-8. Therefore compound **4** was indicated to be *trans*-1-[2-(acetyloxy) ethyl]-cyclohexane-1,4-diol [8].

Compound **5** was obtained as a colorless oil. The HR-ESI-MS of **5** exhibited an ion peak at *m/z* 157.0866 $[M-H]^-$, corresponding to the molecular formula of $C_8H_{14}O_3$. The ^{1}H-NMR spectrum of compound **5** showed one hydroxymethylene at δ_H 3.79 (2H, *t, J* = 6.8 Hz); five methylene at δ_H 1.81 (2H, *t, J* = 6.8 Hz), δ_H 2.68, 2.18, 2.00 and 1.83 (each, 2H, *m*). The ^{13}C-NMR and HSQC spectra indicated signals of 8 carbons including one carbonyl carbon (δ_C 214.8), one non-protonated carbon (δ_C 70.5), one oxygenated methylene carbon (δ_C 59.1), five methylene carbons (δ_C 44.6, 37.8 x 2 and 37.7 x 2). Compound **5** was also a cyclohexylethanol derivative similar to compounds

2 and **3**, except for the replacement of hydroxy group at C-4 by oxo group. The detailed analysis of 2D-NMR spectral data led to determine the structure of compound **5** as cleroindicin B, that was confirmed by comparison with published literature [9].

According to our best knowledge, all compound **1** - **5** were firstly isolated from the genus *Ardisia*. 2-Methylpyridin-3-ol was reported from the root of *Astragalus mongholicus* [10] or the leaves of *Calotropis gigantea* [11].

Cyclohexylethanol derivatives exhibited diversity biological activities including anti-cancer agents and antiulcer properties [12]. Therefore, these structures have been the focus of several synthetic efforts [13].

4. Conclusion

One alkaloid and four cyclohexylethanol derivatives were isolated from the ethanol extract of the leaves of *A. silvestris*. Their chemical structures were identified as 2-methylpyridin-3-ol (**1**), rengyol (**2**), isorengyol (**3**), *trans*-1-[2-(acetyloxy) ethyl]-cyclohexane-1,4-diol (**4**), and cleroindicin B (**5**). All of them were reported from genus *Ardisia* for the first time. This study provides further knowledge about the chemical constituents of this medicinal plant.

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PHENOLIC COMPOUNDS FROM α-GLUCOSIDASE INHIBITORY ACTIVE FRACTIONS OF *HOMONOIA RIPARIA* LOUR.

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Summary

Phenolic Compounds from α-Glucosidase Inhibitory Active Fractions of *Homonoia riparia* Lour.

The ethyl acetate (EtOAc) fraction from an ethanol *extract* of *Homonoia riparia* leaves (Euphorbiaceae) exhibited *in vitro* inhibitory activity on α-glucosidase, one of the key enzymes that are the therapeutic target for the treatment of diabetes mellitus. An intensive phytochemical study of the EtOAc fraction led to the isolation of quercetin (**1**), myricetin (**2**), ethyl gallate (**3**), gallic acid (**4**) and myricitrin (**5**). Their structures were elucidated by spectroscopic methods (including MS, 1D, and 2D-NMR) and by comparison with published data. These compounds, for the first time, have been isolated from *H. riparia* leaves collected in Quang Ngai Province, Vietnam.

Keywords: *Homonoia riparia* Lour., *NMR, α-Glucosidase inhibitor, Plant extracts, Phenolic.*