

## A New Iridoid Glucoside from the Stems of *Myoporum bontioides* (Sieb. et Zucc.) A. Gray

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**Abstract:** A phytochemical investigation of the stems of *Myoporum bontioides* (Sieb. et Zucc.) A. Gray, a semi-mangrove plant distributed along coastlines of north-eastern Vietnam and some Asian countries led to the isolation of a new iridoid glucoside (**1**), named myobontioside E, together with fourteen known compounds (**2-15**). Their structures were elucidated by means of HR ESI-MS, 1D and 2D NMR spectroscopy as well as comparison with the data reported in the literature. The cytotoxic effects on 8505C, MKN7, HT29, and T24 cell lines were assessed using SRB assay. Only iridoid **2** exhibited weak cytotoxicity against all tested cell lines with IC<sub>50</sub> values ranging from 60.19 to 69.14 μM.

**Keywords:** *Myoporum bontioides*; Myoporaceae; iridoid glucoside; dammarane saponin; cytotoxicity. © 2021 ACG Publications. All rights reserved.

### 1. Introduction

*Myoporum*, a genus of the family Myoporaceae, comprises approximately 32 species that occur throughout the Pacific region and Indian Ocean on Mauritius and Rodriguez islands [1]. *Myoporum bontioides* (Sieb. et Zucc.) A. Gray, a small shrubby semi-mangrove tree belonging to the family Myoporaceae, is mainly distributed along coastlines of north-eastern Vietnam, south China, Taiwan and Japan, and it has long been used to treat dermatitis, pulpitis and sciatica [2-3]. Previous chemical investigations mainly focused on the leaves of *M. bontioides* and revealed the presence of major constituents including sesquiterpenes [4-5], iridoids [6], flavonoids and phenylethanoids [7-10]. Some of these compounds have activities against bacteria [5, 10], plant pathogenic fungi [8, 11], and cancer [7]. In a previous paper, we reported the isolation and structural characterization of three flavonoids, along with the chemical composition of essential oil from the leaves of *M. bontioides* [12]. As a continuation of our investigation on this plant, we have isolated and structurally elucidated a new iridoid glucoside (**1**), together with fourteen known compounds including iridoids and iridoid glucosides (**2-7**), *O*-coumaric acid glucosides (**8-9**), aliphatic alcohol glycosides (**10-11**), a lignan glucoside (**12**), and dammarane saponins (**13-15**) from the *n*-BuOH soluble fraction of the methanol extract of *M. bontioides* stems. The cytotoxicity of iridoids and iridoid glucosides (**1-7**) against four human cancer cell lines 8505C, MKN7, HT29, and T24 was also reported.

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## 2. Materials and Methods

### 2.1. General Experimental Procedures

Optical rotations were measured with a JASCO P-2000 polarimeter (Hachioji, Japan) using MeOH as the solvent. NMR experiments were conducted on a Bruker Avance 500 MHz spectrometer with chemical shifts given in ppm ( $\delta$ ). HR-ESI-MS were obtained on a X500 QTOF mass spectrometer system (MA, USA). Column chromatography (CC) was performed using silica gel 60 (0.04-0.063 mm, Merck), C18 reversed-phase (RP) silica gel (150  $\mu$ m, YMC), Diaion HP 20 (Mitsubishi chemical Co.), and Sephadex LH-20 (25-100  $\mu$ m, Sigma-Aldrich). Thin-layer chromatography was performed using precoated silica gel 60 F254 and RP-18 F254S plates (0.25 mm, Merck), and compounds were detected by UV fluorescence at 254nm or spraying with 1% vanillin-H<sub>2</sub>SO<sub>4</sub> in MeOH, followed by heating at 100 °C for 1-2 min.

### 2.2. Plant Material

The stems of *Myoporum bontioides* (Sieb. et Zucc.) A. Gray were collected in May, 2020 from coastal areas of Thai Binh province in northern Vietnam. The plant material was identified by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher sample (HUST.N05) was deposited at the Department of Organic Chemistry, School of Chemical Engineering, Hanoi University of Science and Technology (HUST), Vietnam.

### 2.3. Extraction and Isolation

The air-dried stems (2.0 kg) of *M. bontioides* were extracted three times with MeOH (3  $\times$  7 L) at 45 °C for 1 h under sonication. The three extracts were combined and evaporated under reduced pressure to obtain a residue (335 g). This crude extract was suspended in H<sub>2</sub>O (2 L) and successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH to afford *n*-hexane (27 g), EtOAc (46 g), and *n*-BuOH (120 g) residues, after removal of the solvents.

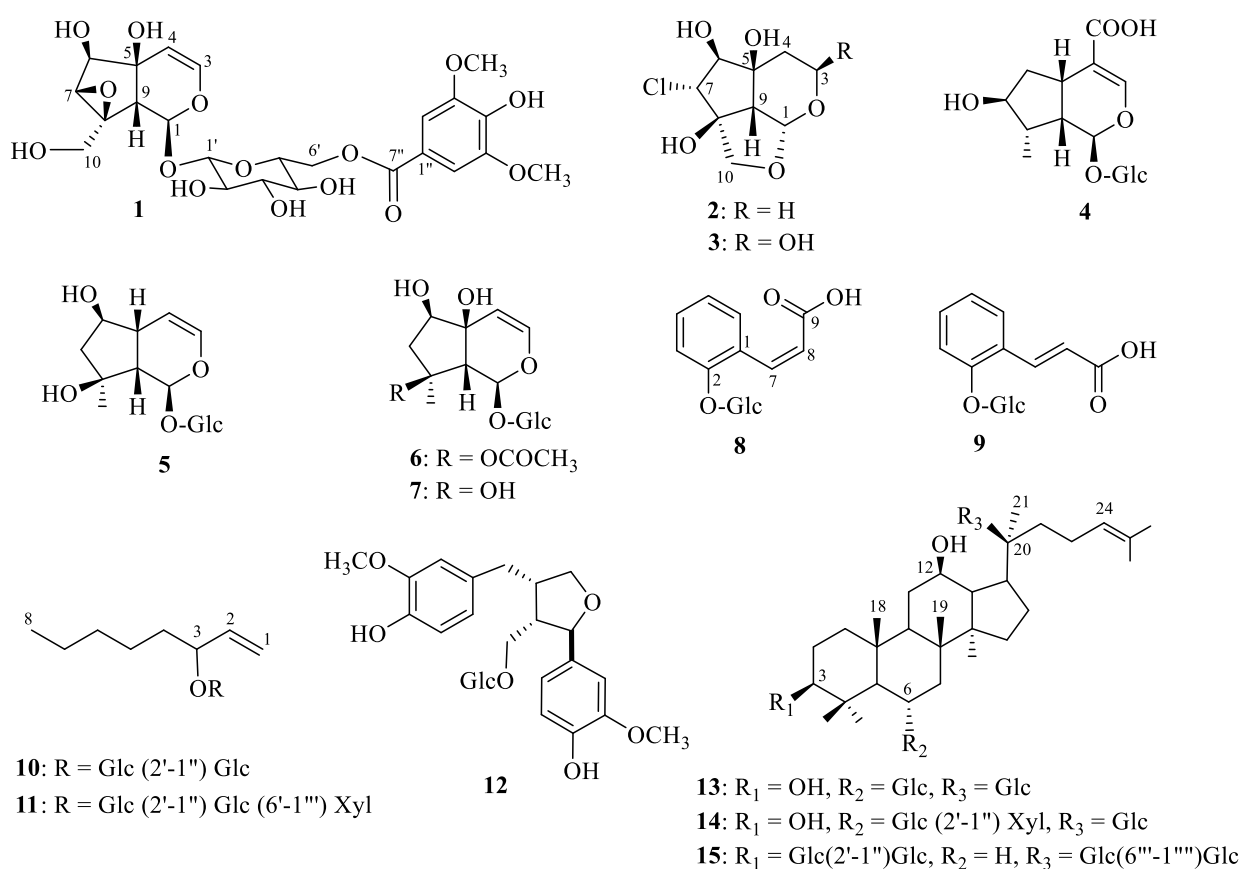
The *n*-BuOH soluble fraction (120 g) was subjected to Diaion HP20 CC, eluting with water and increasing concentration of MeOH in water (20, 40, 60, 80, 100%, v/v) to afford five fractions *Fr.1-Fr.5*. Fraction *Fr.1* (20% MeOH, 12.8 g) was chromatographed on an RP-C<sub>18</sub> silica gel column, eluting with MeOH/H<sub>2</sub>O (1:3, 1:1, v/v) to give four subfractions *Fr.1.1-Fr.1.4*. Subfraction *Fr.1.2* (0.4 g) was purified by silica gel CC with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9:1, v/v) and then by Sephadex LH-20 CC with MeOH to obtain compound **4** (6.8 mg). *Fr.1.3* (4.8 g) was fractionated on a silica gel CC, eluting with a CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10:1 to 1:1, v/v) gradient to afford eight subfractions *Fr.1.3.1-Fr.1.3.8*. Subfraction *Fr.1.3.1* was purified by Sephadex LH-20 CC with MeOH and then by silica gel CC with CH<sub>2</sub>Cl<sub>2</sub>/acetone (5:1, v/v) to give compounds **2** (5.1 mg) and **3** (25.4 mg). *Fr.1.3.6*, *Fr.1.3.7* and *Fr.1.3.8* were purified by RP-C<sub>18</sub> silica gel CC, eluting with MeOH/H<sub>2</sub>O (1:2, v/v) to yield compounds **5** (32.8 mg), **7** (17 mg) and **8** (24.2 mg), respectively. Compound **6** (25 mg) was isolated from *Fr.1.4* (1.6 g) by RP-C<sub>18</sub> CC, eluting with MeOH/H<sub>2</sub>O (1:3, v/v). Fraction *Fr.2* (40% MeOH, 8.3 g) was fractionated on an RP-C<sub>18</sub> silica gel CC, eluting with MeOH/H<sub>2</sub>O (3:1, 1:1, v/v) to provide three subfractions *Fr.2.1-Fr.2.3*. Subfraction *Fr.2.1* (0.37 g) was purified by Sephadex LH-20 CC with MeOH to give compound **9** (72 mg). *Fr.2.2* (1.1 g) was purified by Sephadex LH-20 CC with MeOH and then by RP-C<sub>18</sub> silica gel CC with MeOH/H<sub>2</sub>O (1:1, v/v) to yield compound **11** (21 mg). Compound **10** (40.5 mg) was obtained from the *Fr.2.3* by RP-C<sub>18</sub> silica gel CC, eluting with acetone/H<sub>2</sub>O (2:1, v/v). Fraction *Fr.3* (60% MeOH, 7.4 g) was subjected to silica gel CC, eluting with a CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10:1 to 1:1, v/v) gradient to afford twelve subfractions *Fr.3.1-Fr.3.12*. Subfractions *Fr.3.5* and *Fr.3.7* were purified by RP-C<sub>18</sub> silica gel CC, eluting with acetone/H<sub>2</sub>O (1:2, v/v) to yield compounds **14** (27 mg) and **12** (17 mg). *Fr.3.10* was subjected to Sephadex LH-20 CC with MeOH, followed by RP-C<sub>18</sub> silica gel CC with MeOH/H<sub>2</sub>O (1:2, v/v) to afford compounds **1** (8 mg) and **13**

(29.7 mg). *Fr.3.11* was purified by Sephadex LH-20 CC with MeOH and then by RP-C<sub>18</sub> silica gel CC with MeOH/H<sub>2</sub>O (2:3, v/v) to yield **15** (3.5 mg).

*Myobontioside E (1)*: White powder.  $[\alpha]_D^{25} = -49.3$  (*c* 0.1, MeOH); HR ESI-MS: *m/z* 603.1560 [*M* + HCOOH - H]<sup>-</sup> (calcd. for C<sub>25</sub>H<sub>31</sub>O<sub>17</sub>, 603.15667); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) data are given in Table 1.

#### 2.4. Acid hydrolysis and sugar identification

Compound **1** (2.0 mg) was refluxed with 2 N methanolic HCl (5 mL) for 2 h. After neutralization with 1 N sodium carbonate solution, the reaction mixture was extracted with CHCl<sub>3</sub> to remove the aglycone. In the aqueous layer, D-glucose was identified by the optical rotation and TLC comparison with an authentic sample [*R*<sub>f</sub> 0.17, EtOAc-MeOH-H<sub>2</sub>O (4:1:0.1, v/v),  $[\alpha]_D^{25} +45.7$ ].



**Figure 1.** Chemical structures of compounds **1-15**

#### 2.5. Cytotoxic Assay

The sulforhodamine B (SRB) method [13] was used to evaluate cytotoxic activity of seven iridoids **1-7** against four human cancer cell lines as 8505C (thyroid carcinoma), MKN7 (gastric carcinoma), HT29 (colorectal adenocarcinoma), and T24 (urinary bladder carcinoma) following the previously described protocol [14]. All tumor cell lines were supplied by Professor J. M. Pezzuto (Long-Island University, US) and Professor Jeanette Maier (Milan University, Italia). Ellipticine was used as a positive control.

### 3. Results and Discussion

#### 3.1. Structure Elucidation

The *n*-BuOH soluble fraction was subjected to multiple chromatographic separations to afford fifteen compounds (**1-15**, Figure 1).

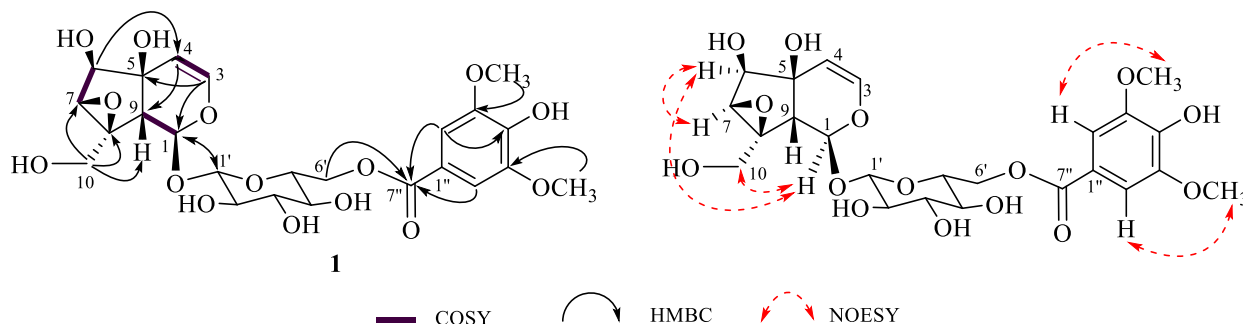
Myobontioside E (**1**) was isolated as a white powder. Its molecular formula was determined as C<sub>24</sub>H<sub>30</sub>O<sub>15</sub> by the HR ESI-MS at *m/z* 603.1560 [M + FA - H]<sup>-</sup> (calcd. 603.15667) and NMR spectroscopic data (Table 1). The <sup>1</sup>H NMR spectrum of **1** showed signals of two symmetrical aromatic protons at δ<sub>H</sub> 7.35 (2H, *s*), *cis*-olefinic protons at δ<sub>H</sub> 6.35 and 4.92 (both 1H, *d*, *J* = 6.0 Hz), an acetal proton at δ<sub>H</sub> 5.13 (1H, *d*, *J* = 9.0 Hz), two oxygenated methylene groups at δ<sub>H</sub> 4.60 (2H, *m*), 4.10 and 3.54 (both 1H, *d*, *J* = 13.0 Hz), an anomeric proton at δ<sub>H</sub> 4.78 (1H, *d*, *J* = 8.0 Hz), along with other sugar proton signals at δ<sub>H</sub> 3.30 - 3.64, two symmetrical methoxy groups at δ<sub>H</sub> 3.92 (6H, *s*), and one non-desielded methine proton δ<sub>H</sub> 2.55 (1H, *d*, *J* = 9.0 Hz). The <sup>13</sup>C NMR spectrum exhibited signal for a carboxyl carbon at δ<sub>C</sub> 167.9, four carbon signals for a symmetrical aromatic ring, a carbon signal for two methoxy groups at δ<sub>C</sub> 57.0, nine carbon signals for C<sub>9</sub>-type iridoid aglycone, and six carbon signals for a glucosyl moiety, which included an anomeric carbon signal at δ<sub>C</sub> 99.8. The <sup>1</sup>H and <sup>13</sup>C NMR signals of **1** (Table S1) were in good agreement with those of myobontioside B, a known iridoid glucoside isolated from the same species of Japan [6], except that the signals of the ferulic acid in myobontioside B replaced by signals of a syringic acid. The syringyl moiety was linked by an ester bond to the hydroxyl group of C-6' (δ<sub>C</sub> 64.4, δ<sub>H</sub> 4.60), which was confirmed by the HMBC correlation from H<sub>2</sub>-6' to C-7'' (δ<sub>C</sub> 167.9). Noted that the HMBC spectrum also showed the correlations from two methoxy groups (δ<sub>H</sub> 3.92) to C-3'', 5'' (δ<sub>C</sub> 149.0), from H-2'', 6'' (δ<sub>H</sub> 7.35) to the carboxyl carbon C-7'' (δ<sub>C</sub> 167.9). In addition, the NOESY spectrum of **1** exhibited correlation between δ<sub>H</sub> 3.92 (OCH<sub>3</sub>) and δ<sub>H</sub> 7.35 (H-2'', 6''), indicating the syringyl moiety. The relative configuration of **1** was confirmed according to structure of myobontioside B and based on *J* values and NOESY spectroscopic analysis. The NOE correlations of H-6 (δ<sub>H</sub> 3.81) with H-7 (δ<sub>H</sub> 3.45), and also of H-6 with H-1 (δ<sub>H</sub> 5.13) indicated that H-1, H-6 and H-7 were on the same side and designated as α-oriented (Figure 2). The small coupling constant (*J*<sub>6,7</sub> = 1.0 Hz) between H-6 and H-7 also supported this conformation. The large coupling constant (*J*<sub>1,9</sub> = 9.0 Hz) between H-1 and H-9 suggested that they were present in *trans*-diaxial relationship and positioning H-9 in β-orientation [6, 15]. Based on this evidence, the compound **1** was determined to be a new compound as macfadyenoside-6'-syringic acid ester.

**Table 1.** <sup>1</sup>H (at 500 MHz) and <sup>13</sup>C (at 125 MHz) NMR data for compound **1** in CD<sub>3</sub>OD

Position	δ <sub>C</sub>	δ <sub>H</sub> , <i>J</i> in Hz	Position	δ <sub>C</sub>	δ <sub>H</sub> , <i>J</i> in Hz
1	95.5 (CH)	5.13 (1H, <i>d</i> , <i>J</i> = 9.0)	<b>Glc-1'</b>	99.8 (CH)	4.78 (1H, <i>d</i> , <i>J</i> = 8.0)
2	-	-	2'	74.7 (CH)	3.30 (1H, <i>dd</i> , <i>J</i> = 8.0, 9.0)
3	142.6 (CH)	6.35 (1H, <i>d</i> , <i>J</i> = 6.0)	3'	77.5 (CH)	3.45 (1H, <i>m</i> )
4	108.0 (CH)	4.92 (1H, <i>d</i> , <i>J</i> = 6.0)	4'	71.8 (CH)	3.45 (1H, <i>m</i> )
5	74.4 (C)	-	5'	76.0 (CH)	3.64 (1H, <i>m</i> )
6	78.7 (CH)	3.81 (1H, <i>d</i> , <i>J</i> = 1.0)	6'	64.4 (CH <sub>2</sub> )	4.60 (2H, <i>m</i> )
7	63.2 (CH)	3.45 (1H, <i>m</i> )	<b>Acyl-1''</b>	121.3 (C)	-
8	66.5 (C)	-	2'', 6''	108.4 (CH)	7.35 (2H, <i>s</i> )
9	50.9 (CH)	2.55 (1H, <i>d</i> , <i>J</i> = 9.0)	3'', 5''	149.0 (C)	-
10	61.5 (CH <sub>2</sub> )	4.10 (1H, <i>d</i> , <i>J</i> = 13.0)	4''	142.6 (C)	-
		3.54 (1H, <i>d</i> , <i>J</i> = 13.0)	7''	167.9 (C)	-
			3'',5''-OCH <sub>3</sub>	57.0 (CH <sub>3</sub> )	3.92 (6H, <i>s</i> )

The structures of fourteen isolates (**2-15**) were identified as myopochlorin (**2**) [6], 3-hydroxymyopochlorin (**3**) [16], 8-*epi*-loganic acid (**4**) [17], ajugol (**5**) [18], 8-*O*-acetylharpagide (**6**) [19], harpagide (**7**) [20], *cis*-melilotoside (**8**) and *trans*-melilotoside (**9**) [21-22], octane-1-en-3-ol-3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**10**) [23], ebracteatoside B (**11**) [24], lariciresinol-9-

*O*- $\beta$ -D-glucopyranoside (**12**) [25], ginsenoside Rg1 (**13**) [26], notoginsenoside-R1 (**14**) [27], and ginsenoside Rb1 (**15**) [28] by detailed analysis of their 1D- and 2D-NMR spectroscopic data in comparison with those reported in the literature. This is the first report on the isolation of aliphatic alcohol glycosides (**10-11**) and dammarane saponins (**13-15**) from this genus [3].



**Figure 2.**  $^1\text{H}$ - $^1\text{H}$  COSY and the selected HMBC, NOESY correlations of compound **1**

### 3.2. Cytotoxicity Activity

Iridoids and iridoid glucosides **1-7** were evaluated *in vitro* for their cytotoxicity against the 8505C, MKN7, HT29, and T24 human cancer cell lines using SRB method with ellipticine as the positive control [13-14]. As the obtained results, only myopochlorin (**2**) exhibited weak cytotoxicity against all the tested cancer cell lines with the  $\text{IC}_{50}$  values of  $60.19 \pm 1.75$ ,  $64.25 \pm 1.41$ ,  $67.65 \pm 1.32$ , and  $69.14 \pm 1.37$   $\mu\text{M}$ , respectively. The other compounds were inactive in this test ( $\text{IC}_{50} > 100$   $\mu\text{M}$ ).

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### Supporting Information

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