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Iridoid glucosides from *Thunbergia laurifolia*

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Dedicated to Professor Vichiara Jirawongse on the occasion of his 84th birthday.

Abstract

Two iridoid glucosides, 8-*epi*-grandifloric acid and 3′-*O*-β-glucopyranosyl-stilbericoside, were isolated from the aerial part of *Thunbergia laurifolia* along with seven known compounds, benzyl β-glucopyranoside, benzyl β-(2′-*O*-β-glucopyranosyl) glucopyranoside, grandifloric acid, (*E*)-2-hexenyl β-glucopyranoside, hexanol β-glucopyranoside, 6-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosylapigenin. Strucural elucidation was based on the analyses of spectroscopic data.

Keywords: *Thunbergia laurifolia*; Acanthaceae; Iridoid glucoside; Grandifloric acid; 8-*epi*-Grandifloric acid; 3′-*O*-β-Glucopyranosyl-stilbericoside

1. Introduction

Thunbergia laurifloia Lindl. (Acanthaceae, Thai name: Raang-Chuet), a vine distributed in Southeast Asia, was collected in the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The leaves are used in Thai traditional medicine as an antipyretic, as well as an antidote for detoxification of poisons. In the course of our continuing study on Thai medicinal plants (Kanchanapoom et al., 2001), we report the isolation and structural determination of nine compounds (**1**–**9**), which includes two new iridoid glucosides (**4**, **5**), and seven known compounds, the benzyl alcohol glucosides (**1**, **2**), an iridoid glucoside (**3**), two aliphatic alcohol glucosides (**6**, **7**) and two flavonoid *C*-glucosides (**8**, **9**) from the aerial part of this plant. The presence of iridoids in this genus are well known (Damtoft et al., 1994a,b; Frederiksen et al., 1999; Jensen et al., 1988; Jensen and Nielsen, 1989; Ismail et al., 1996).

2. Results and discussion

From the methanolic extract of the aerial part of *T. laurifolia*, nine compounds (**1**–**9**) were isolated. Compounds **1** and **2** were identified as benzyl β-glucopyranoside (Miyase et al., 1987) and benzyl β-(2′-*O*-β-glucopyranosyl) glucopyranoside (Okamura et al., 1981), respectively. Compound **6** was elucidated as *n*-hexyl-β-glucopyranoside. The ^{1}H and ^{13}C NMR spectral data of compound **7** were coincident with those of (*E*)-2-hexenyl β-glucopyranoside (Mizutani et al., 1988). Compounds **8** and **9** have been assigned as 6-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosylapigenin (Harborne and Mabry, 1982), respectively.

Compounds **3** and **4** have the same molecular formula, $C_{15}H_{22}O_9$, based on HR-FAB MS. The ^{1}H and ^{13}C NMR spectra of compounds **3** and **4** were very similar, suggesting that the two compounds are stereoisomers. The spectra of each compound revealed the presence of a β-glucopyranosyl unit and a cyclopentanopyran ring, corresponding to a C-9 iridoid skeleton. DEPT experiments with each compound showed the presence of two methylenes, six methines and one signal for a quaternary carbon. The appearance of the methine signals at δ 140.3 (C-3) and δ 108.2 (C-4) in **3**, as well as at δ 140.7 (C-3) and δ 107.6 (C-4) in **4** suggested that the

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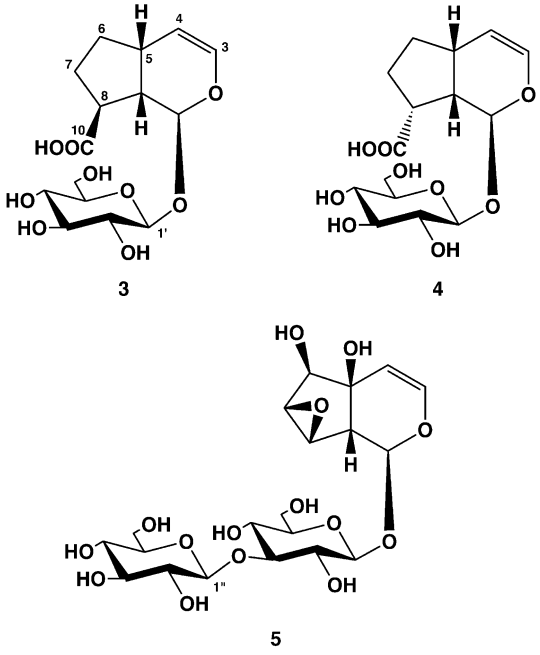
Table 1
^{13}C NMR Spectral data of compounds **3**–**5** (CD_3OD, 100 MHz)

C	3	4	5
1	96.2	95.5	96.5
3	140.3	140.7	143.1
4	108.2	107.6	108.2
5	34.4	34.6	73.6
6	33.1	33.0	79.2
7	29.8	29.0	59.4
8	46.8	45.8	56.3
9	48.4	46.4	50.7
10	185.7	179.5	
1′	99.8	99.7	100.0
2′	74.8	74.7	74.1
3′	78.2	78.1	87.4
4′	71.6	71.4	70.1
5′	77.9[a]	77.9	77.9[a]
6′	62.8	62.6	62.7
1″			105.3
2″			75.6
3″			78.1[a]
4″			71.6
5″			78.2[a]
6″			62.7

[a] May be interchanged.

C-4 position was unsubstituted (Chaudhuri et al., 1980). The quaternary signal at δ 185.7 in **3** and at δ 179.5 in **4**, together with two more oxygen atoms from the molecular formula indicated the presence of a carboxyl group in each compound; in both cases, this was assigned to C-8 due to the significant difference in the chemical shifts at C-8, C-9 and C-10 (Table 1). The complete assignments of this NMR spectroscopic data were based on COSY, HSQC and difference NOE experiments. The configuration of the carboxyl group of **3** was confirmed as β on the basis of an NOE difference experiment. Irradiation of H-1 (δ 5.28) caused an enhancement of H-8 (δ 2.67) as previously observed (Ismail et al., 1996). Compound **3** (grandifloric acid) was thus previously isolated from *Thunbergia grandiflora* (Ismail et al., 1996) but whereby some of the spectra assignments were uncertain. Additionally, the carboxyl group of **4** was concluded to be α, and the lack of an NOE between H-1 and H-8 in **4** supported this conclusion. Thus compound **4** is 8-*epi*-grandifloric acid. It should be noted that the upfield shifts of C-9 (−2.2 ppm) and C-10 (−6.2 ppm) in going from the 8β- to 8α-epimers were in agreement with those concluded for C-8 epimers of iridoids lacking oxygen substituents at C-7 and/or C-8 (Damtoft et al., 1981).

The molecular formula of compound **5** was determined as $C_{20}H_{30}O_{15}$ by HR-FAB MS. The ^{13}C NMR spectral data were very similar to those of stilbericoside (Jensen and Nielsen, 1989) except that the ^{13}C NMR signals for one more glucopyranosyl unit were observed. The additional unit was attached to C-3′ of the first glucopyranose based on an HMBC experiment, in which long-range correlations were observed between H-1″ (δ 4.58) and C-3′ (δ 87.4), as well as H-1′ (δ 4.71) and C-3′ (δ 87.4). Moreover, the chemical shifts of C-3′, C-2′ and C-4′ were changed by +9.3, −0.6 and −1.3 ppm, respectively, relative to **4**. Therefore, the structure of **5** was elucidated as 3′-*O*-β-glucopyranosyl-stilbericoside.



3. Experimental

3.1. General

NMR, MS, HPLC, and CC were performed as described (Kanchanapoom et al., 2001). The solvent systems were: (I) EtOAc–MeOH–H_2O (4:1:0.1), (II) EtOAc–MeOH–H_2O (7:3:0.3), (III) EtOAc–MeOH–H_2O (6:4:1), (IV) 15–50% MeOH, (V) 20% MeCN, (VI) 5% MeCN, (VII) 15% MeCN. The spray reagent used was 10% H_2SO_4 in 50% ethanol.

3.2. Plant material

T. laurifolia was collected in November, 1999 from the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The identification of the plant was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU-0006) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried aerial part (1.5 kg) of *T. laurifolia* was extracted with hot MeOH (40 l, with reflux for 24 h,

70 °C). After removal of the solvent in vacuo, the resulting residue (189 g) was suspended in H_2O (1 l) and defatted with Et_2O (3 l). The aqueous layer was applied to a column of highly porous copolymer of styrene and divinylbenzene and eluted with H_2O, 50% MeOH, MeOH and Me_2CO, successively. The fraction eluted with 50% MeOH (10.0 g) was applied to a column of silica gel (solvent systems I, II and III, respectively) affording six fractions. Fraction 1 (4.0 g) was separated on a RP-18 column (solvent system IV) to give six fractions. Fraction 1-2 (520 mg) was purified by prep. HPLC-ODS (solvent system V) to afford **1** (41 mg), **2** (74 mg) and **4** (10 mg). Fractions 1-3 (222 mg) and 1-5 (434 mg) were purified by using RP-18 column (solvent system VI) and prep. HPLC-ODS (solvent system VII) to give **6** (7 mg), **7** (9 mg) and **8** (47 mg). Fraction 2 (2.3 g) was applied to a RP-18 column (solvent system IV) to afford five fractions. Fraction 2-1 (877 mg) was purified by using RP-18 column (solvent system VI) and prep. HPLC-ODS (solvent system VI) to provide compounds **3** (55 mg) and **5** (17 mg). Finally, fraction 2-2 (145 mg) was purified by prep. HPLC-ODS to give **9** (20 mg).

3.4. Grandifloric acid (*3*)

Amorphous powder, $[\alpha]_D^{19}$ −115.3° (MeOH, *c* 0.98) (ref. −84°); ^{1}H NMR (CD_3OD): δ 6.17 (1H, *dd*, *J* = 6.1, 1.9 Hz, H-3), δ 5.28 (1H, *d*, *J* = 3.7 Hz, H-1), δ 4.69 (1H *dd*, *J* = 6.1, 2.4 Hz, H-4), δ 4.64 (1H, *d*, *J* = 7.8 Hz, H-1′ Glc), δ 3.87 (1H, *brd*, *J* = 11.7 Hz, H-6′ Glc), δ 3.58 (1H, *dd*, *J* = 11.7, 3.9 Hz, H-6′ Glc), δ 3.41–3.28 (3H, *m*, H-3′,4′,5′ Glc), δ 3.21 (1H, *dd*, *J* = 9.0, 7.8 Hz, H-2′ Glc), δ 2.75 (1H, *m*, H-5), δ 2.67 (1H, *m*, H-8), δ 2.50 (1H, *m*, H-9), δ 1.98 (2H, *m*, H-6,7), δ 1.81 (1H, *m*, H-7), δ 1.43 (1H, *m*, H-6); 13C NMR (CD3OD): Table 1; negative HR-FAB-MS, *m*/*z*: 345.1179 ($C_{15}H_{21}O_9$ requires 345.1185).

3.5. 8-epi-Grandifloric acid (*4*)

Amorphous powder, $[\alpha]_D^{19}$ +55.4° (MeOH, *c* 0.98); ^{1}H NMR (CD_3OD): δ 6.21 (1H, *dd*, *J* = 6.1, 2.0 Hz, H-3), δ 5.26 (1H, *d*, *J* = 3.7 Hz, H-1), δ 4.72 (1H. *dd*, *J* = 6.1, 2.0 Hz, H-4), δ 4.65 (1H, *d*, *J* = 7.8 Hz, H-1′ Glc), δ 3.87 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6′ Glc), δ 3.68 (1H, *dd*, *J* = 12.0, 4.9 Hz, H-6′ Glc), δ 3.39 (1H, *dd*, = 9.3, 8.8 Hz, H-4′ Glc) δ 3.44–3.30 (2H, *m*, H-3′,5′ Glc), δ 3.21 (1H, *dd*, *J* = 8.0, 7.8 Hz, H-2′ Glc), δ 2.80 (1H, *m*, H-8), δ 2.78 (1H, *m*, H-5), δ 2.47 (1H, *m*, H-9), δ 2.05 (1H, *m*, H-7), δ 1.96 (1H, *m*, H-6), δ 1.84 (1H, *m*, H-7), δ 1.49 (1H, *m*, H-6); ^{13}C NMR (CD_3OD): Table 1; negative HR-FAB-MS, *m*/*z*: 345.1192 ($C_{15}H_{21}O_9$ requires 345.1185).

3.6. 3′-O-β-Glucopyranosyl-stilbericoside (*5*)

Amorphous powder, $[\alpha]_D^{19}$ −105.3° (MeOH, *c* 0.69); ^{1}H NMR (CD_3OD): δ 6.37 (1H, *d*, *J* = 6.1 Hz, H-3), δ 5.21 (1H, *d*, *J* = 8.7 Hz, H-1), δ 4.92 (1H. *d*, *J* = 6.1 Hz, H-4), δ 4.71 (1H, *d*, *J* = 8.0 Hz, H-1′ Glc), δ 4.58 (1H, *d*, *J* = 7.8 Hz, H-1″ Glc), δ 4.06 (1H, *d*, *J* = 1.5 Hz, H-6), δ 3.91–3.84 (2H, *m*, H-6′,6″ Glc), δ 3.62 (1H, *d*, *J* = 2.7 Hz, H-8), δ 3.53 (1H, *m*, H-7), δ 3.47–3.25 (8H, *m*, H-2′,2″,3′,3″,4′,4″,5′,5″), δ 2.45 (1H, *d*, *J* = 8.7 Hz, H-9); ^{13}C NMR (CD_3OD): Table 1; negative HR-FAB-MS, *m*/*z*: 509.1500 ($C_{20}H_{29}O_{15}$ requires 509.1506).

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