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Lignan and phenylpropanoid glycosides from *Fernandoa adenophylla*

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Dedicated to Professor Vichiara Jirawongse on the occasion of his 83rd birthday

Abstract

From the leaves and branches of *Fernandoa adenophylla*, a lignan glycoside (fernandoside) and a phenylpropanoid glycoside (2″-*O*-β-apiosylverbascoside) were isolated together with 12 known compounds. The structural elucidations were based on analyses of physical and spectroscopic data. © 2001 Published by Elsevier Science Ltd.

Keywords: *Fernandoa adenophylla*; Bignoniaceae; Lignan glycoside; Phenylpropanoid glycoside; Fernandoside; 2″-*O*-β-Apiosylverbascoside

1. Introduction

As part of our ongoing study on Thai medicinal plants, we investigated the constituents of *Fernandoa adenophylla* Steenis (Bignoniaceae, Thai name: Khae-hang-khang) collected from the botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. *F. adenophylla* is a tree, 5–13 m high, distributed in south and south-east Asia regions. In Thai traditional medicine, the leaves are used for external treatment of skin diseases. No chemical investigation has been carried out on this genus. The present study deals with the isolation and structural elucidation of 14 compounds (**1**–**14**), including a new lignan glycoside (**8**), a new phenylpropanoid glycoside (**12**) and 12 known compounds (**1**–**7**, **9**–**11** and **13** and **14)** from this plant.

2. Results and discussion

From the methanolic extract of the leaves and branches of *F. adenophylla,* 14 compounds (**1**–**14**) were isolated. Twelve were identified as known compounds; salidroside (**1**) (Schwab and Schreier, 1988), decaffeoylverbacoside (**2**), verbascoside (**3**), isoverbascoside (**4**), leucosceptoside A (**5**) (Miyase et al., 1982), plantaginoside C (**6**) (Miyase et al., 1991), martynoside (**7**) (Sasaki et al., 1978), (6*R*, 9*S*)-oxo-α-ionol glucoside (**9**) (Pabst et al., 1992), hispidulin-7-*O*-β-glucoside (**10**) (Hase et al., 1995), (+)-lyoniresinol 3a-*O*-β-glucoside (**11**) (Achenbach et al., 1992), apigenin-7-*O*-β-glucuronide (**13**) (Hase et al., 1995) and hispidulin-7-*O*-β-glucuronide (**14**) (Subramanian and Nair, 1972) by comparison of physical data with literature values and from spectroscopic evidence.

The molecular formula of compound **8** was determined as $C_{36}H_{44}O_{16}$ by HR–FAB mass spectrometry. The ^{1}H and ^{13}C NMR spectra of **8** were very similar to those of **11**. In addition, the signals of 1,3,4-trisubstituted aromatic ring, one methoxy signal and one ester carbonyl carbon were observed. Comparison of the ^{13}C NMR spectral data of **8** with those of **11** revealed the downfield shift of C-6 (+2.2 ppm) and upfield shift of C-5 (−2.6 ppm) of the glucosyl moiety indicating that the additional unit is an ester located at C-6 of the glucosyl moiety. Moreover, the HMBC spectrum (Fig. 1) revealed the correlation between H-6 (δ 4.56 and 4.33) of the glucosyl moiety and carbonyl carbon (δ 168.0). The methoxyl group of the additional unit was assigned to position C-3″ by a difference NOE experiment in the ^{1}H NMR spectrum. Irradiation of this methoxy signal (δ 3.78) caused an NOE enhancement at H-2″ (δ 7.48, *d*, $J=2.0$ Hz). Consequently, the structure of compound **8**

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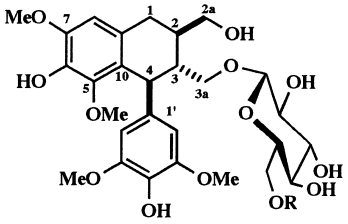
was assigned as (+)-lyoniresinol 3a-[6-(4-hydroxy-3-methoxy)-benzoyl]-*O*-β-glucopyranoside, named fernandoside.



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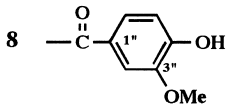
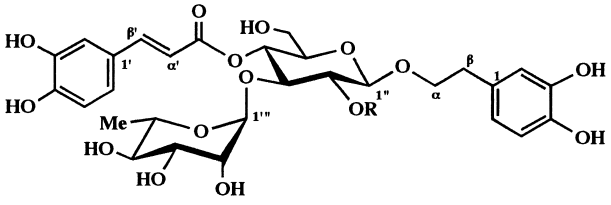


8

11 H



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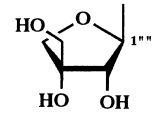
3 H



12

The molecular formula of compound **12** was determined as $C_{34}H_{44}O_{19}$ by HR–FAB mass spectrometry. Inspection of the ^{1}H NMR spectrum revealed the presence of two sets of AMX systems [δ 6.66 (*d*, J = 2.0 Hz), δ 6.63 (*d*, J = 8.1 Hz) and δ 6.52 (*dd*, J = 8.1, 2.0 Hz) for the 3,4-dihydroxy-β-phenylethoxyl moiety; and δ 7.01 (*d*, J = 2.0 Hz), δ 6.73 (*d*, J = 8.1 Hz) and δ 6.90 (*dd*, J = 8.1, 2.0 Hz) for the caffeoyl moiety], two *trans*-olefinic protons [δ 7.24 (*d*, J = 15.9 Hz) and δ 6.23 (*d*,J = 15.9 Hz)] together with three anomeric protons δ 4.41 (*d*, J = 7.8 Hz) for β-glucose, δ 5.00 (*d*, J = 1.3 Hz) for α-rhamnose and δ 5.17 (*d*, J = 2.2 Hz) for β-apiose. Acid hydrolysis with 0.2 N H_2SO_4 afforded rhamnose, apiose and glucose (identified by TLC). Comparison of the ^{13}C NMR spectral data with those of verbascoside (**3**) indicated that glucose is the core sugar with the 3,4-dihydroxy-β-phenylethoxyl moiety located at C-1″ as well as the *trans*-caffeoyl moiety linked at C-4″. The complete assignments were based on the results of COSY, HSQC and HMBC. COSY and HSQC experiments were used to determine the sugar protons (Table 2). HMBC correlations were observed between H-1″ (δ 4.41) and CH_2-

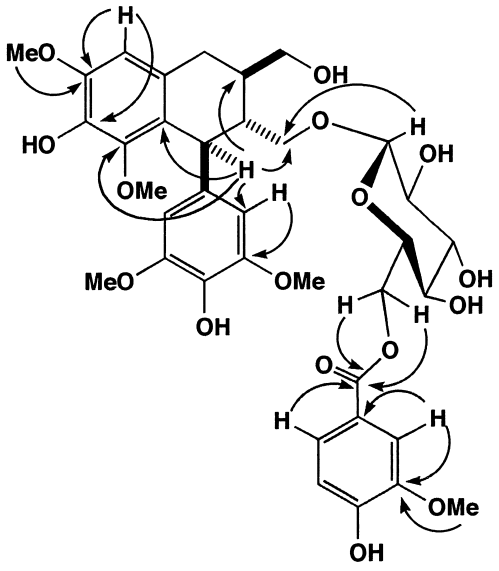


Fig. 1. The HMBC correlations of fernandoside (**8**).

α (δ 72.2); H-2″ (δ 3.47) and C-1″ and C-1‴″ H-3″ (δ 3.87) and C-2″ and C-1‴ as well as H-4″ and a carbonyl carbon (δ 168.3). Also, the reciprocal HMBC correlations were found between H-1‴″ and C-2″; H-1‴ and C-3″. On the basis of the spectral data, the structure of compound **12** was established as 3,4-dihydroxy-β-phenylethoxy - *O* - β - apiofuranosyl - (1→2) - α - rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-glucopyranoside (2″-*O*-β-apiosylverbascoside).

3. Experimental

3.1. General

NMR spectral data were recorded in CD_3OD using a JEOL JNM A-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a JEOL JMS-SX 102 spectrometer. IR were measured with a HORIBA FT 710 infrared spectrometer. UV were recorded on a JASCO V-520 spectrophotometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (20×150 mm i.d., YMC) and Diol-120 (8.0×300 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rates were 6 ml/min for ODS and 3ml/min for Diol-120. For CC, silica gel G 60 (Merck), YMC-gel ODS (50 μm, YMC), polyamide C-200 (Wako) and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) EtOAc–MeOH–H_2O (4:1:0.1), (II) EtOAc–MeOH–H_2O (7:3:0.3), (III) 20–70% MeOH, (IV) 10% MeCN, (V) 20% MeCN, (VI) 25% MeCN, (VII) MeCN, (VIII) 15% MeCN and (IX)

100–80% MeCN. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. Plant material

The leaves and branches of *Fernandoa adenophylla* Steenis were collected in May 2000 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 is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried leaves and branches (2.5 kg) of *F. adenophylla* were extracted with hot MeOH. After removal of the solvent by evaporation, the residue (240.0 g) was defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer of styrene and divinylbenzene and eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (25.0 g) was subjected to a column of silica gel (systems I and II, respectively) affording three fractions. Fraction 1 (4.8 g) was applied to a RP-18 column using system III to provide 11 fractions. Fraction 1-1 was purified by prep. HPLC–ODS (system IV) to give compounds **1** (43 mg) and **2** (120 mg). Fractions 1-5 and 1-7 were similarly purified by prep. HPLC-ODS (system V) to afford compounds **3** (1 g) and **4** (620 mg). Fractions 1-8 and 1-9 were further purified by prep. HPLC–ODS (system VI) and HPLC–Diol (system VII) to provide compound **5** (32 mg), **6** (38 mg), **7** (17 mg), **8** (24 mg), **9** (9 mg) and **10** (90 mg). Fraction 2 (8.4 g) was applied to a RP-18 column (system III) to afford 13 fractions. Fraction 2-5 was purified by prep. HPLC–ODS (system VIII) to give compound **11** (160 mg). Fractions 2-8, 2-9 and 2-10 were combined and further separated on a column of polyamide (system IX), then followed by prep. HPLC–ODS (system V) to afford compound **12** (24 mg). Fractions 3 was subjected to a RP-18 column of (system III), then

Table 1
^{13}C NMR spectral data of compounds **8** and **11** (100 MHz, CD_3OD)

Carbon no.	**8**	**11**	Carbon no.	**8**	**11**
1	33.7	33.7	Glc-1	104.8	104.7
2	40.9	40.5	2	75.1	75.1
2a	66.2	66.2	3	78.1	77.8
3	46.4	46.6	4	71.9	71.6
3a	71.8	71.5	5	75.5	78.1
4	42.8	42.7	6	65.0	62.8
5	148.9	148.5			
6	138.8	138.8	1″	122.5	
7	147.6	147.5	2″	113.6	
8	107.8	107.8	3″	152.9	
9	130.2	130.1	4″	148.7	
10	126.2	126.3	5″	116.0	
			6″	125.1	
1′	139.4	139.3			
2′	106.9	106.9	MeO-5	60.2	60.1
3′	148.9	148.9	MeO-7	56.6	56.6
4′	134.5	134.4	MeO-3′,5′	56.9	56.9
5′	148.9	148.9	MeO-3″	56.4	
6′	106.9	106.9	-COO-	168.0	

Table 2
NMR spectral data of compounds **3** and **12** (100 MHz for ^1H NMR and 400 MHz for ^{13}C NMR, CD_3OD)

No.	Carbon		Proton
	3	**12**	**12**
Aglycone			
1	131.5	131.6	
2	116.5	116.5	6.66 (*d*, *J* = 2.0 Hz)
3	144.6	144.6	
4	146.1	146.1	
5	117.1	117.2	6.63 (*d*, *J* = 8.1 Hz)
6	121.3	121.3	6.52 (*dd*, *J* = 8.1, 2.0 Hz)
α	72.2	72.2	3.96 (*m*)
			3.70 (*m*)
β	36.5	36.5	2.75 (*t*, *J* = 7.3 Hz)
Caffeoyl moiety			
1′	127.7	127.7	
2′	115.2	115.2	7.01 (*d*, *J* = 2.0 Hz)
3′	149.7	149.7	
4′	146.8	146.8	
5′	116.3	116.3	6.73 (*d*, *J* = 8.1 Hz)
6′	123.2	123.2	6.90 (*dd*, *J* = 8.1, 2.0 Hz)
α′	114.7	114.9	6.23 (*d*, *J* = 15.9 Hz)
β′	148.0	147.9	7.54 (*d*, *J* = 15.9 Hz)
C=O	168.3	168.3	
Glucose			
1″	104.2	103.1	4.41 (*d*, *J* = 7.8 Hz)
2″	76.2	81.0	3.47 (*dd*, *J* = 9.8, 8.8 Hz)
3‴	81.6	82.0	3.87 (*t*, *J* = 9.3 Hz)
4″	70.4	70.8	4.89 (*t*, *J* = 9.3 Hz)
5″	76.0	76.0	3.48–3.52[a]
6″	62.4	62.4	3.50–3.62[a]
Rhamnose			
1‴	103.0	103.5	5.00 (*d*, *J* = 1.3 Hz)
2‴	72.3	72.3	3.90–4.00[a]
3‴	72.0	71.9	3.50–3.60[a]
4‴	73.8	73.8	3.27 (*t*, *J* = 9.6)
5‴	70.6	70.9	3.50–3.60[a]
6‴	18.4	18.4	1.06 (*d*, *J* = 6.1 Hz)
Apiose			
1⁗		111.2	5.17 (*d*, *J* = 2.2 Hz)
2⁗		78.5	3.90 (*d*, *J* = 1.8 Hz)
3⁗		80.4	
4⁗		75.1	3.97 (*d*, *J* = 9.9 Hz)
			3.70 (*d*, *J* = 9.9 Hz)
5⁗		65.5	3.55 (*s*)

[a] Signal pattern unclear due to overlapping.

followed by HPLC–ODS (system IV) to provide compounds **13** (51 mg) and **14** (75 mg).

3.4. Fernandoside (**8**)

Amorphous powder, $[\alpha]_D^{26}$ +25.0° (MeOH, *c* 1.24); UV λ_{max}^{MeOH} (nm): 362, 250; IR: υ_{max}^{KBr} cm^{-1} 3430, 2938, 1698, 1701, 1610, 1515, 1427, 764; ^1H NMR (CD_3OD): δ 7.50 (1H, *dd*, *J* = 8.1, 2.0 Hz, H-6″), δ 7.48 (1H, *d*, *J* = 2.0 Hz, H-2″), δ 6.77 (1H, *d*, *J* = 8.1 Hz, H-5″), δ 6.50 (1H, *s,* H-8), δ 6.37 (2H, *s,* H-2′, 6′), δ 4.56 (1H, (*dd*, *J* = 12.0, 2.1 Hz, H-6 Glc), δ 4.33 (1H, *d*, *J* = 11.5 Hz, H-4), δ 4.31 (1H, (*dd*, *J* = 12.0, 6.3 Hz, H-6 Glc), δ 4.28 (1H, *d*, *J* = 7.8 Hz, H-1 Glc), δ 3.84 (1H, *dd*, *J* = 10.2, 9.3 H-4 Glc), δ 3.79 (3H, *s,* MeO-7), δ 3.78 (3H, *s,* MeO-3″) 3.68 (6H, *s,* MeO-3′, 5′), δ 3.58 (1H, (*dd*, *J* = 11.0, 4.4 Hz, H-2a), δ 3.52 (1H, *m*, H5 Glc), δ 3.48 (1H, *dd*, *J* = 11.0, 6.8 Hz, H-2a), δ 3.41 (1H, *dd*, *J* = 9.8, 4.2 Hz, H-3a), δ 3.37 (2H, *m,* H-3a, 3 Glc), δ 3.30 (3H, *s* MeO-5), δ 3.24 (IH, *dd*, *J* = 7.8, 7.8 Hz, H-2 Glc), δ 2.64 (1H, (*dd*, *J* = 15.1, 4.9 Hz, H-1), δ 2.53 (1H, *dd,* *J* = 15.1, 11.5 Hz, H-1), δ 2.09 (1H, *m,* H-3), δ 1.61 (1H, *m*, H-2); ^{13}C NMR (CD_3OD): Table 1; negative HR–FAB–MS, *m*/*z*: 731.2504 $[M–H]^-$ ($C_{36}H_{43}O_{16}$ requires 731.2551).

3.5. 2″-O-β-Apiosylverbascoside (**12**)

Amorphous powder, $[\alpha]_D^{26}$ −90.7° (MeOH, *c* 1.40); UV λ_{max}^{MeOH} (nm): 355, 250; IR υ_{max}^{KBr} cm^{-1}: 3400, 2936, 1698, 1604, 1518, 1447, 813; ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD): Table 2; negative HR–FAB–MS, *m*/*z*: 755.2413 $[M–H]^-$ ($C_{34}H_{43}O_{19}$ requires 755.2398).

3.6. Acid hydrolysis of 2″-O-β-apiosylverbascoside (**12**)

Compound **12** (5 mg) was dissolved in 0.2 N H_2SO_4 (5 ml) and heated at 95°C for 2 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous layer was neutralized with $NaHCO_3$, concentrated to dryness, and extracted with pyridine. The pyridine extract was then analyzed on silica gel TLC (EtOAc–MeOH–H_2O–AcOH 13:3:3:4), affording rhamnose (R_f 0.63), apiose (R_f 0.49) and glucose (R_f 0.39).

Acknowledgements

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