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Isoflavone glycosides from *Derris scandens*

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Abstract

Five isoflavone glycosides, named derriscandenosides A–E (**1**–**5**), were isolated from the stems of *Derris scandens*, together with ten known compounds comprising one isoflavone, two benzoic acid derivatives, three glucosyl isoflavones and four rhamnosyl-(1→6)-glucosyl isoflavones. The structures of the glycosides were assigned on the basis of spectroscopic data, especially of the acetate derivatives. Three known rhamnosyl-(1→6)-glucosyl isoflavones isolated from a crude fraction were retested for hypotensive activity with varying results.

Keywords: *Derris scandens*; Leguminosae; Isoflavone glycosides; Isoflavone glucosides; Rhamnosyl-(1→6)-glucosyl isoflavones; Hypotensive activity

1. Introduction

Derris scandens, locally called ''Thao-wan–priang'', is a spreading, climbing shrub and is widely distributed throughout Thailand. Its dried stems are used for the treatment of muscle ache and pain as well as arthritis symptoms (Tiangburanatham, 1996). It has been reported that a *n*-butanol extract of an aqueous extract of the stems had hypotensive activity in rats (Jansakul et al., 1997). In our search for hypotensive constituents from the stems of *D. scandens*, we have isolated one new glucosyl isoflavone, named derriscandenoside A (**1**), and four new rhamnosyl-(1→6)-glucosyl isoflavones, named derriscandenosides B–E (**2**–**5**). Previous chemical investigation of the stems afforded a number of isoflavones (Sekine et al., 1999) along with two isoflavone glucosides: derriscanosides A (**6**) and B (**7**) (Dianpeng et al., 1999). We have also isolated **7** together with 7,8-dihydroxy-4′-methoxyisoflavone (Shukla and Misra, 1981), 4-hydroxy-3-methoxybenzoic acid and 4-hydroxy-3,5-dimethoxybezoic acid (Rukachaisirikul et al., 1998), three glucosyl isoflavones: formononetin 7-*O*-β-glucopyranoside (**8**) (Cui et al., 1993), 8-hydroxy-4′,7-dimethoxyisoflavone 8-*O*-β-glucopyranoside (**9**) (Fujita et al., 1982) and 7-hydroxy-4′,8-dimethoxyisoflavone 7-*O*-β-glucopyranoside (**10**) (Mitrocotsa et al., 1999) and three rhamnosyl-(1→6)-glucosyl isoflavones: diadzein 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (**11**) (Markham and Mabry, 1968), formononetin 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (**12**) (Parthasarathy et al., 1976) and genistein 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (**13**) (Guang et al., 1998).

Attempted purification of fractions containing derriscandenosides A–E (**1**–**5**) as major components was unsuccessful and therefore most of the isoflavone glycosides were isolated and identified as their corresponding acetate derivatives, except for compounds **7**, **9**, **12** and **13**. Their structures were elucidated using 1D and 2D NMR spectroscopic data. The ^{1}H chemical shifts were assigned using ^{1}H NMR and ^{1}H–^{1}H COSY data while the ^{13}C chemical shifts were assigned using ^{13}C NMR, 2D HMQC and 2D-HMBC data. Monitoring of crude glycoside fractions by NMR spectroscopy did not indicate the presence of acetoxyl groups; it is therefore unlikely that partially acetylated derivatives were present as natural products.

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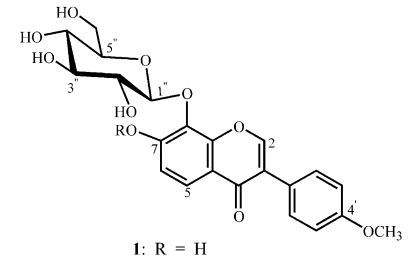
2. Results and discussion

Derriscandenoside A (**1**) tetraacetate was obtained as a colourless viscous gum. It has the quasimolecular formula $C_{30}H_{31}O_{14}$ consistent with its $[M+H]^+$ at m/z 615.1712 in the HR–FABMS. The UV spectrum with absorption bands at 211, 254 and 306 nm suggested an isoflavone skeleton. The IR spectrum showed absorption bands for a hydroxyl group (3443 cm^{-1}), acetyl carbonyl groups (1755 cm^{-1}) and an isoflavone carbonyl group (1630 cm^{-1}). The ^1H NMR spectrum (Table 1) displayed a characteristic *singlet* at δ_H 7.85 for H-2 of an isoflavone, two *doublets* with *ortho* coupling constant of 9.0 Hz at δ_H 8.05 and 7.05 for H-5 and H-6, respectively, and a pair of *doublets* of a *p*-disubstituted benzene ring at δ_H 7.47 and 6.98 with a spacing of 9.0 Hz. These results implied that **1** had a 4′,7,8-trioxygenated isoflavone framework. The methoxyl group at δ_H 3.84 was assigned to C-4′ in B-ring as irradiation of the aromatic protons (H-3′, H-5′) at δ_H 6.98 enhanced the signals of the methoxyl group and H-2′ and H-6′ in the NOED spectrum. This was further confirmed by the HMBC correlation (Table 3) between the methoxy proton at δ_H 3.84 and C-4′ (δ_C 159.9). In addition, proton signals ascribable to the glucose unit (Table 1) were observed together with methyl protons of four acetyl groups (δ_H 2.20, 2.12, 2.07 and 2.06), indicating the presence of glucose as tetraacetate derivative. The anomeric proton of the glucose residue at δ_H 4.99 with a large coupling constant of 8.1 Hz implied that the glucose moiety must have a β-glucopyranose form (Özden et al., 1998). A cross peak in the HMBC spectrum between H-1″ (δ_H 4.99) of the glucose unit with C-8 (δ_C 131.1) of the isoflavone moiety indicated that the glucose residue was attached to the 8-hydroxyl of the isoflavone moiety. Therefore, **1** was characterized as 7,8-dihydroxy-4′-methoxyisoflavone 8-*O*-β-glucopyranoside, a new isoflavone glucoside.

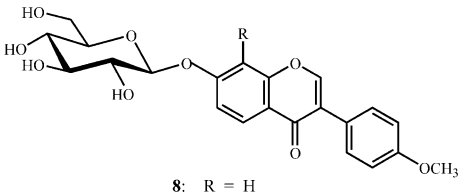
The heptaacetate of derriscandenoside B (**2**) was obtained as a colourless viscous gum. It showed in the HR–FABMS a quasimolecular ion $[M+H]^+$ at m/z 887.2637 in agreement with the molecular formula $C_{42}H_{47}O_{21}$. The UV spectrum with absorption bands at 213, 257 and 306 nm was typical for an isoflavone. The IR spectrum displayed absorption bands for acetate carbonyl groups (1732 cm^{-1}) and an isoflavone carbonyl group (1659 cm^{-1}). The isoflavone unit of **2** appeared to be 4′,7,8-trioxygenated, the same as **1**, as indicated by the ^1H NMR signals at δ_H 7.90 (*s*, H-2), 8.20 (*d*, $J=9.0$ Hz, H-5), 7.12 (*d*, $J=9.0$ Hz, H-6), 7.46 (*d*, $J=8.5$ Hz, H-2′,6′) and 6.96 (*d*, $J=8.5$ Hz, H-3′,5′). The additional methoxyl group at δ_H 3.84 was assigned to C-4′ in B-ring due to enhancement of the aromatic protons H-3′ and H-5′ (δ_H 6.96) after irradiation of this methoxy proton. In the HMBC spectrum, a cross peak between the methoxyl proton (δ_H 3.84) and C-4′ (δ_C 159.7) also supported the assigned position for the methoxyl group. In addition, ^1H NMR signals (Table 1) belonging to two sugar residues and seven acetyl groups

Table 1
^1H NMR spectral data for acetate derivatives of compounds **1, 2, 3, 4** and **5**

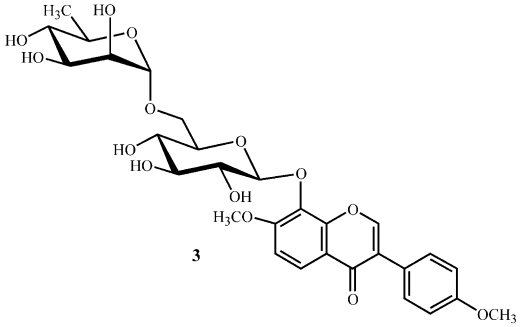
Position	Tetraacetate of **1**	Heptaacetate of **2**	Hexaacetate of **3**	Hexaacetate of **4**	Hexaacetate of **5**
2	7.85 (*s*)	7.90 (*s*)	7.99 (*s*)	7.97 (*s*)	7.95 (*s*)
5	8.05 (*d*, 9.0)	8.20 (*d*, 9.0)	8.06 (*d*, 9.1)	7.65 (*s*)	
6	7.05 (*d*, 9.0)	7.12 (*d*, 9.0)	7.04 (*d*, 9.1)		
8				7.20 (*s*)	6.71 (*s*)
2′,6′	7.47 (*d*, 9.0)	7.46 (*d*, 8.5)	7.52 (*d*, 9.1)	7.51 (*d*, 9.3)	7.48 (*d*, 9.0)
3′,5′	6.98 (*d*, 9.0)	6.96 (*d*, 8.5)	6.98 (*d*, 9.1)	6.98 (*d*, 9.3)	6.99 (*d*, 9.0)
Glc-1″	4.99 (*d*, 8.1)	5.21 (*d*, 7.5)	5.22 (*d*, 6.8)	5.13 (*d*, 7.6)	5.12 (*d*, 7.5)
2″	5.40 (*dd*, 9.8, 8.1)	5.35–5.31 (*m*)	5.36 (*dd*, 9.2, 6.8)	5.35–5.32 (*m*)	5.36 (*t*, 9.0)
3″	5.33 (*t*, 9.8)	5.34–5.30 (*m*)	5.31 (*t*, 9.2)	5.35–5.32 (*m*)	5.33 (*t*, 9.0)
4″	5.20 (*t*, 9.8)	5.09 (*t*, 10.0)	5.15 (*t*, 9.2)	5.09 (*t*, 9.9)	5.07 (*t*, 9.0)
5″	3.82 (*ddd*, 9.8, 5.6, 2.8)	3.93 (*ddd*, 10.0, 6.5, 2.5)	3.75 (*ddd*, 9.2, 6.8, 2.4)	3.89 (*ddd*, 9.9, 7.0, 2.5)	3.93–3.86 (*m*)
6″	4.30 (*dd*, 12.6, 5.6)	3.79 (*dd*, 11.5, 2.5)	3.62 (*dd*, 12.0, 2.4)	3.77 (*dd*, 11.5, 2.5)	3.76 (*dd*, 12.0, 3.0)
	4.20 (*dd*, 12.6, 2.8)	3.67 (*dd*, 11.5, 6.5)	3.69 (*dd*, 12.0, 6.8)	3.65 (*dd*, 11.5, 7.0)	3.63 (*dd*, 12.0, 9.0)
Rha-1‴		4.74 (*d*, 1.5)	4.56 (*d*, 1.6)	4.74 (*d*, 1.7)	4.76 (*d*, 1.5)
2‴		5.24 (*dd*, 3.5, 1.5)	5.06 (*dd*, 3.2, 1.6)	5.31 (*dd*, 3.4, 1.7)	5.34 (*dd*, 3.0, 1.5)
3‴		5.30 (*dd*, 10.0, 3.5)	5.12 (*dd*, 9.2, 3.2)	5.26 (*dd*, 9.9, 3.4)	5.27 (*dd*, 9.0, 3.0)
4‴		5.06 (*t*, 10.0)	4.98 (*t*, 9.2)	5.04 (*t*, 9.9)	5.05 (*t*, 9.0)
5‴		3.86 (*dq*, 10.0, 6.5)	3.70 (*dq*, 9.2, 6.3)	3.83 (*dq*, 9.9, 5.9)	3.86–3.81 (*m*)
6‴		1.17 (*d*, 6.5)	1.11 (*d*, 6.3)	1.19 (*d*, 5.9)	1.20 (*d*, 6.4)
5-OH					12.90 (*s*)
6-OMe				3.92 (*s*)	3.83 (*s*)
7-OMe			3.99 (*s*)		
4′-OMe	3.84 (*s*)	3.84 (*s*)	3.84 (*s*)	3.84 (*s*)	3.84 (*s*)
CH_3CO	2.20, 2.12, 2.07, 2.06	2.40, 2.09, 2.08, 2.07, 2.06, 2.04, 2.01	2.11, 2.08, 2.06, 1.99 (6H), 1.94	2.09, 2.08, 2.07, 2.05, 2.04, 1.96	2.10, 2.08, 2.07, 2.05, 2.04, 1.96



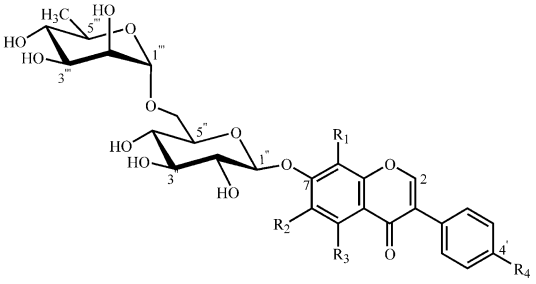
1: R = H
9: R = CH_3



8: R = H
10: R = OCH_3



3



2: R_1 = OH, R_2 = R_3 = H, R_4 = OCH_3
4: R_1 = R_3 = H, R_2 = R_4 = OCH_3
5: R_1 = H, R_2 = R_4 = OCH_3, R_3 = OH
6: R_1 = R_2 = R_3 = H, R_4 = OCH_3
7: R_1 = R_4 = OCH_3, R_2 = R_3 = H
11: R_1 = R_2 = R_3 = H, R_4 = OH
12: R_1 = R_2 = R_3 = H, R_4 = OCH_3
13: R_1 = R_2 = H, R_3 = R_4 = OH

were observed. According to the chemical shift and splitting patterns of the sugar protons, the two sugar residues were determined as glucose and rhamnose. Two one-proton *doublets* at δ_H 5.21 (J = 7.5 Hz) and δ_H 4.74 (J = 1.5 Hz) were attributed to H-1″ of the glucose unit and H-1‴ of the rhamnose unit, respectively. The large coupling constant (J = 7.5 Hz) of H-1″ and the small coupling constant (J = 1.5 Hz) of H-1‴ established the presence of the glucose and rhamnose units in β-glucopyranose and α-rhamnopyranose forms (Agrawal, 1992). The HMBC spectrum (Table 3) exhibited a correlation between H-1‴ (δ_H 4.74) of the rhamnose unit and C-6″ (δ_C 66.4) of the glucose unit, indicating that C-1‴ of rhamnose was attached to C-6″ of glucose through a (1→6)-glycosidic bond. Furthermore, a cross peak between H-1″ (δ_H 5.21) of glucose and C-7 (δ_C 151.9) established the attachment of the glucose unit to the 7-hydroxyl of the isoflavone aglycone. The presence of seven acetate carbonyl carbons apart from the carbonyl carbon of the isoflavone suggested that **2** contained one hydroxyl substituent on the isoflavone skeleton apart from six hydroxyl groups of two sugar units. Other protons of the sugar residues were assigned by analysis of the ^{1}H–^{1}H COSY spectrum using the anomeric protons as starting points. Complete ^{1}H and ^{13}C assignments were reported in Tables 1 and 2, as a result of DEPT, HMQC and HMBC experiments. These data permitted the identification of **2** as 7,8-dihydroxy-4′-methoxyisoflavone 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside, a new isoflavone glycoside.

The hexaacetate of derriscandenoside C (**3**) was obtained as a colourless viscous gum. It showed the quasimolecular formula $C_{41}H_{47}O_{20}$ from its $[M+H]^+$ at m/z 859.2664 in the HR–FABMS. This compound also exhibited, in the UV spectrum, characteristic absorption bands of an isoflavonoid at 212, 253 and 306 nm. IR absorption bands were found at 1748 cm^{-1} (acetate carbonyl group) and 1645 cm^{-1} (isoflavone carbonyl group). The ^{1}H NMR spectrum (Table 1) suggested the presence of a 4′,7,8-trioxygenated isoflavone moiety, with a sharp *singlet* at δ_H 7.99 (H-2), two *doublets* with *ortho* coupling constant (J = 9.1 Hz) at δ_H 8.06 (H-5) and 7.04 (H-6), an AA′XX′ system with two *doublets* with spacing of 9.1 Hz at δ_H 7.52 (H-2′,6′) and 6.98 (H-3′,5′) and two methoxyl groups at δ_H 3.84 and 3.99. HMBC data established the attachment of the methoxyl groups at C-7 of A-ring and C-4′ of B-ring as the downfield methoxy proton (δ_H 3.99) showed a cross peak with C-7 (δ_c 156.1) while the other methoxy proton (δ_H 3.84) had a correlation with C-4′ (δ_c 159.9). This conclusion was supported by cross peaks between H-6 (δ_H 7.04) and the downfield methoxyl group and between H-3′, H-5′ (δ_H 6.98) and the other methoxy group in the NOESY spectrum. Comparison of its ^{1}H NMR spectrum with that of **2** indicated that **3** also contained β-glucopyranosyl and α-rhamnopyranosyl residues with anomeric protons at δ_H 5.22 (*d*, J = 6.8 Hz) and δ_H 4.56 (*d*, J = 1.6 Hz) which were attributed to H-1″ of the glucose unit and H-1‴ of the rhamnose unit, respectively. The presence of an α-rhamnosyl residue was confirmed by a methyl *doublet* at δ_H 1.11 (J = 6.3 Hz). The HMBC correlation (Table 3) between H-1‴ (δ_H 4.56) of the rhamnose unit and C-6″ (δ_C 66.5) of the glucose unit revealed that the rhamnose unit formed a (1→6) glycosidic

Table 2
^{13}C NMR spectral data for acetate derivatives of compounds **1, 2, 3, 4** and **5**

Position	Tetraacetate of **1**	Heptaacetate of **2**	Hexaacetate of **3**	Hexaacetate of **4**	Hexaacetate of **5**
2	151.3	152.2	152.7	152.9	153.7
3	125.3	125.1	124.7	124.3	122.9
4	175.6	175.3	176.0	175.8	181.4
4a	118.7	120.1	119.4	120.7	108.4
5	124.3	123.9	123.4	106.6	154.3
6	115.3	112.0	110.3	148.8	133.7
7	154.4	151.9	156.1	151.0	155.7
8	131.1	152.0	132.4	107.2	95.4
8a	150.2	149.6	150.8	151.5	152.7
1′	123.5	125.0	124.2	124.7	123.0
2′,6′	130.2	130.1	130.5	130.3	130.1
3′,5′	114.1	114.0	114.2	114.2	114.2
4′	159.9	159.7	159.9	159.8	159.8
Glc-1″	103.4	98.0	101.2	100.0	99.5
2″	71.0	70.5	72.1	71.2	70.7
3″	72.1	72.3	72.9	72.6	72.4
4″	67.9	68.7	69.2	69.3	68.9
5″	72.7	73.8	74.4	73.9	73.7
6″	61.3	66.4	66.5	66.8	66.5
Rha-1‴		97.0	97.9	98.4	98.3
2‴		69.4	69.6	69.6	69.2
3‴		68.8	69.3	69.4	69.2
4‴		70.8	70.9	71.1	70.7
5‴		66.8	66.9	66.9	66.7
6‴		17.3	17.5	17.6	17.3
6-OMe				56.6	61.1
7-OMe			56.8		
4′-OMe	55.4	55.3	55.6	55.6	55.4
CH_3CO	20.6, 20.7	20.8, 20.7, 20.6, 20.5, 20.2	21.0, 20.9, 20.9 (2C), 20.8	21.0, 20.9, 20.8	20.8, 20.6
$COCH_3$	170.6, 170.1, 169.3, 169.2	170.1, 170.0, 169.99, 169.9, 169.5, 169.4, 168.1	170.5, 170.3, 170.2, 170.1, 169.9, 169.6	170.4, 170.1, 170.0, 169.9, 169.7 169.5	170.2 169.9, 169.8 169.5, 169.3

linkage with the glucose unit, C-1″ of which was connected to the 8-hydroxyl of the aglycone due to a correlation between the H-1″ (δ_H 5.22) of the glucose unit with C-8 (δ_C 132.4) of the aglycone. Therefore, **3** was identified as 8-hydroxy-4′,7-dimethoxyisoflavone 8-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside, a new isoflavone glycoside.

Derriscandenoside D (**4**) hexaacetate was obtained as a colourless viscous gum. It showed in the HR–FABMS a quasimolecular ion $[M+H]^+$ at *m/z* 859.2650 in agreement with the molecular formula $C_{41}H_{47}O_{20}$. The UV spectrum with absorption bands at 209, 260 and 322 nm suggested an isoflavone skeleton. The IR spectrum exhibited absorption bands for acetyl carbonyl groups (1750 cm^{-1}) and an isoflavone carbonyl group (1634 cm^{-1}). The ^1H NMR spectrum (Table 1) suggested that the isoflavone had a 4′,6,7-trioxygenated skeleton which showed the resonances at δ_H 7.97 (*s*, H-2), 7.65 (*s*, H-5), 7.20 (*s*, H-8), 7.51 (*d*, *J*=9.3 Hz, H-2′,6′) and 6.98 (*d*, *J*=9.3 Hz, H-3′,5′). Two methoxy signals were observed at δ_H 3.92 and 3.84. The location of the methoxyl groups at C-6 and C-4′ was determined by HMBC data between the methoxy protons (δ_H 3.92 and 3.84) and C-6 (δ_C 148.8) and C-4′ (δ_C 159.8) of the isoflavone, respectively. In addition, NOESY data gave support as the aromatic proton (H-5, δ_H 7.65) correlated with the methoxy proton at δ_H 3.92 while the aromatic protons (H-3′, H-5′, δ_H 6.98) correlated with the methoxy proton at δ_H 3.84. These data suggested that the isoflavone moiety had an afromosin skeleton. Two anomeric protons (δ_H 5.13, *d*, *J*=7.6 Hz, H-1″ and δ_H 4.74, *d*, *J*=1.7 Hz, H-1‴) together with proton signals of sugar units (Table 1) suggested the presence of β-glucopyranosyl and α-rhamnopyranosyl units. Comparison of the ^{13}C chemical shift of C-6″ of the glucose residue (Table 2) with those of **2** and **3** indicated that rhamnose was linked to the 6-hydroxyl of glucose unit. This (1→6)-glycosidic linkage was confirmed by a HMBC correlation (Table 3) between H-1‴ (δ_H 4.74) of rhamnose and C-6″ (δ_C 66.8) of glucose. The attachment of the disaccharide to the 7-hydroxyl of the aglycone was deduced from a correlation observed between the anomeric proton, H-1″ (δ_H 5.13), of the glucose moiety and C-7 (δ_C 151.0) of the aglycone in the HMBC spectrum. The location of the sugars was further supported by a cross peak between the methoxy proton at C-6 (δ_H

3.92) and H-1″ of glucose in the NOESY spectrum. The presence of six acetyl methyl signals in the ^{1}H NMR spectrum indicated that all hydroxyl groups of the glucose and rhamnose units were protected as acetate esters. Therefore, **4** was identified as afromosin 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside, a new isoflavone glycoside.

Derriscandenoside E (**5**) hexaacetate was obtained as a colourless viscous gum. It showed a quasimolecular formula $C_{41}H_{47}O_{21}$ from its $[M+H]^+$ at *m*/*z* 875.2597 in the HR–FABMS. This compound exhibited characteristic UV absorption bands of an isoflavone at 212, 265 and 330 nm. IR absorption bands were found at 1756 cm^{-1} (acetate carbonyl group) and 1659 cm^{-1} (isoflavone carbonyl group). The ^{1}H NMR spectrum (Table 2) revealed the presence of a chelated hydroxyl group (δ_H 12.90 ppm, *s*, 5-OH) and a sharp *singlet* at δ_H 7.95 assigned to H-2 of an isoflavonoid nucleus. It exhibited two *singlets* at δ_H 3.84 and 3.83 for two methoxyl groups and a one proton *singlet* at δ_H 6.71 for H-8. It also displayed an AA′XX′ pattern, typical of a 4′-substituted B-ring, with two *doublets* with spacing of 9.0 Hz at δ_H 7.48 (H-2′ and H-6′) and 6.99 (H-3′ and H-5′). The location of the methoxyl groups at C-4′ and C-6 was established by HMBC data (Table 3) which showed correlations between the methoxyl groups at δ_H 3.84 and 3.83 and C-4′ (δ_C 159.8) and C-6 (δ_C 133.7), respectively. Furthermore, a correlation between the chelated hydroxy proton (5-OH) and C-6 confirmed the attachment of the methoxyl group at C-6. These data indicated that **5** had an isoflavone structure of the irisolidone type. Two anomeric proton signals at δ_H 5.12 (J = 7.5 Hz) and δ_H 4.76 (J = 1.5 Hz) were attributed to H-1″ and H-1‴ of the β-glucose and α-rhamnose units, respectively. The presence of an α-rhamnose unit was confirmed by a methyl *doublet* at δ_H 1.20 (J = 6.4 Hz). Moreover, the C-6″ carbon signal of the glucose unit in the ^{13}C NMR spectrum (Table 2) was observed at δ_C 66.5 which suggested the attachment of the rhamnose unit at the 6″-hydroxyl of the glucose unit. The HMBC spectrum revealed a correlation between H-1‴ (δ_H 4.76) of the rhamnose unit and C-6″ (δ_C 66.5) of the glucose unit as well as a correlation between H-1″ (δ_H 5.12) of the glucose unit and C-7 (δ_C 155.7) of the aglycone. These results confirmed the (1→6)-glycosidic linkage between the rhamnose residue and the glucose unit, C-1″ of which was attached to the 7-hydroxyl of the aglycone. The presence of six acetyl methyl groups at δ_H 2.10, 2.08, 2.07, 2.05, 2.04 and 1.96 suggested that all of hydroxyl groups of the rhamnose and glucose units were acetylated while the chelated 5-hydroxyl group of the aglycone was intact. Therefore, **5** was characterized as irisolidone 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside, a new isoflavone glycoside.

Compounds **7**, **12** and **13**, isolated from a crude hypotensive fraction, were examined for hypotensive activity in the in vivo preparation. On intravenous injection at the dose 4 mg/kg of animal weight, **7** caused a small decrease in mean arterial blood pressure (26.7 mmHg) and heart rate (10 beats/min) of anesthetized rats while **13** caused a slight increase (5 mmHg) in mean arterial blood pressure with no change in heart rate; **12** had no

Table 3
HMBC correlation data of acetate derivatives of compounds **1, 2, 3, 4** and **5**

Positon -H	Tetraacetate of **1**	Heptaacetate of **2**	Hexaacetate of **3**	Hexaacetate of **4**	Hexaacetate of **5**
2	C-3, C-4, C-8a, C-1′	C-3, C-4, C-8a	C-4, C-7, C-8a	C-4, C-8a, C-1′	C-3, C-4, C-8a
5	C-4, C-7, C-8, C-8a	C-4, C-7, C-8a	C-4, C-7, C-8a	C-4, C-4a, C-6, C-8a	
6	C-4a, C-7, C-8, C-8a	C-8	C-4a, C-7, C-8		
7				C-4, C-4a, C-6, C-8a	
8					C-4, C-4a, C-6, C-7, C-8a
2′,6′	C-3, C-2′, 6′,C-4′	C-3, C-2′, 6′,C-4′	C-3, C-2′, 6′,C-4′	C-3, C-2′, 6′,C-4′	C-1′, C-2′,6′, C-4′
3′,5′	C-1′, C-3′,5′, C-4′	C-1′, C-3′,5′, C-4′	C-1′, C-2′,6′, C-4′	C-1′, C-3′,5′, C-4′	C-3, C-3′, 5′,C-4′
Glc-1″	C-8	C-7	C-8, C-3″,C-5″,C-6″	C-7	C-7, C-3″
2″	C-3″	C-3″	C-2″,C-3″,C-4″	C-2″	C-3″,C-4″
3″	C-2″,C-4″	C-3″	C-2″,C-4″,C-5″	C-2″	C-2″,C-4″
4″	C-3″,C-5″,C-6″	C-3″	C-4″,C-5″	C-3″	C-3″,C-5″
5″				C-4″	C-1″,C-4″
6″	C-5″	C-1‴	C-5″	C-5″,C-1‴	C-4″,C-6″
Rha-1‴		C-6″,C-3‴	C-6″,C-3‴,C-5‴	C-6″,C-3‴	C-6″,C-3‴
2‴			C-3‴,C-4‴	C-3‴,C-4‴	C-3‴,C-4‴
3‴			C-3‴,C-4‴	C-4‴	C-2‴,C-4‴,C-5‴
4‴			C-2‴,C-3‴,C-5‴	C-3‴,C-5‴	C-2‴,C-3‴,C-5‴
5‴			C-1‴	C-4‴	C-3‴,C-4‴
6‴		C-4‴,C-5‴	C-4‴,C-5‴	C-4‴,C-5‴	C-4‴,C-5‴
5-OH					C-5, C-6, C-4a
6-OMe				C-6	C-6
7-OMe			C-7		
4′-OMe	C-4′	C-4′	C-4′	C-4′	C-4′

effect on blood pressure or heart rate. Further work is required to explain the activity of the crude fraction.

3. Experimental

3.1. General

IR spectra were recorded using a FTS165 FT-IR spectrometer. UV absorption spectra were recorded using a UV-160A spectrophotometer (SHIMADZU). NMR spectra were recorded on either a Bruker AVANCE 400 (400 MHz) spectrometer or Varian UNITY INOVA (500 MHz) spectrometer using $CDCl_3$ as solvent unless otherwise stated. FAB MS and HRMS data were determined on a VG ZAB 2SEQ mass spectrometer. Optical rotation was measured with sodium D line (590 nm) on an AUTOPOL^R II automatic polarimeter. For TLC Merck (catalogue no. 105715) precoated silica gel plates were used. Acetylation reaction was performed using a sample (20 mg) and acetic anhydride (0.5 ml) in the presence of pyridine (0.2 ml). The reaction mixture was stirred at room temperature overnight. Ice water was added and the mixture was then extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness in vacuo to yield a crude mixture.

3.2. Plant material

Stems of *Derris scandens* were collected in Phang-nga Province, Thailand. Proper identification was made by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University, and a specimen deposited at the Prince of Songkla University Herbarium.

3.3. Extraction and isolation

Stems of *D. scandens* were chopped into small pieces and air dried. Dried stems (≈10 kg) were simmered in hot water twice for a 3 hour period. The two extracts were combined and simmered at 50 °C to reduce the volume to 50%. The cooled liquid was extracted with water-saturated *n*-butanol. The *n*-butanol phase was evaporated to dryness under reduced pressure to give 170 g of a yellow powder. The crude extract (122.5 g) was fractionated by CC over silica gel, eluted with $CHCl_3$, $CHCl_3$–MeOH gradient and finally with pure MeOH to afford 7 frs. Fr. 4 (1.9 g) was further subjected to CC over reversed-phase silica gel (RpC-18), eluted with 30% MeOH–H_2O and gradually enriched with MeOH up to pure MeOH to yield 11 subfrs. Subfr. 4 (115.2 mg) contained 4-hydroxy-3-methoxybenzoic acid as a white solid. Subfr. 5 (59.1 mg) was separated on TLC, using 60% EtOAc-petroleum ether (3 runs) as a mobile phase to afford 4-hydroxy-3-methoxybenzoic acid (16.1 mg) and 4-hydroxy-3,5-dimethoxybenzoic acid (8.2 mg) as a white solid. Subfr. 8 (241.7 mg) was submitted to CC over silica gel, eluted with 10% MeOH–$CHCl_3$ and gradually enriched with MeOH up to pure MeOH to yield 6 subfrs. The third fraction (85.6 mg) was further purified on TLC, using 15% MeOH–$CHCl_3$ (3 runs) as a mobile phase to afford 3 bands. Bands 2 (6.6 mg) and 3 (13 mg) were further subjected to acetylation reaction, followed by purification on TLC using 45% EtOAc–petroleum ether (3 runs) as a mobile phase to yield a white solid, the tetracetate of **10** (3.5 mg) and a colourless viscous gum, the tetraacetate of **8** (4.3 mg). The fourth fraction (86.5 mg) was subjected to TLC using 15% MeOH-$CHCl_3$ (4 runs) as a mobile phase to afford a white solid (12.5 mg) which contained **1** as a major component. Upon acetylation and subsequent purification of this mixture on TLC using 50% EtOAc–petroleum ether (2 runs) as a mobile phase yielded the tetraacetate of **1** as a colourless viscous gum (8.5 mg). Subfrs. 9 was further subject to CC over silica gel, eluted with 10% MeOH–$CHCl_3$, $CHCl_3$–MeOH gradient and finally with pure MeOH to afford 6 subfrs. The second and the fourth fractions afforded **9** (15 mg) and **7** (61 mg), respectively, as white solid. The fifth fraction (58 mg) was applied to a silica gel column, eluted with 15% MeOH–$CHCl_3$ to afford 4 subfrs. The third subfr. (18 mg) was further purified on TLC using 15% MeOH–$CHCl_3$ (8 runs) as a mobile phase to yield **12** as a white solid (6.5 mg). Subfr. 10 was acetylated and then subjected to silica gel column chromatography, eluted with 40% EtOAc–petroleum ether, gradually enriched with EtOAc, followed by MeOH–EtOAc gradient and finally with 10% MeOH–EtOAc to yield 7 subfrs. The first fraction (8 mg) was further purified on TLC using 30% EtOAc–petroleum ether (8 runs) as a mobile phase to afford the diacetate of 7,8-dihydroxy-4′-methoxyisoflavone as a colourless viscous gum (2.5 mg). Fr. 5 (2.09 g) was separated by CC over reversed-phase silica gel (RpC-18), eluted with 30% MeOH–H_2O up to pure MeOH to yield 12 subfrs. Subfr. 3 (30 mg) was separated on reversed-phase TLC (RpC-18) using 33% MeOH–H_2O as a mobile phase to yield 3 bands. The second band (8.5 mg), which contained **5** as a major component, was acetylated and subsequently purified on TLC using 8% acetone-$CHCl_3$ (4 runs) as a mobile phase to afford the hexaacetate of **5** as a colourless viscous gum (6.3 mg). Subfr. 6 (23.0 mg), which contained **4** as a major component, was subjected to acetylation reaction and then further purified on TLC using 50% EtOAc-petroleum ether (3 runs) as a mobile phase to afford the hexaacetate of **4** (17.5 mg) as a colourless viscous gum. Subfr. 9 (50.5 mg) was further separated by CC over

silica gel, eluted with 14% MeOH–$CHCl_3$ to afford 2 subfrs. The second fraction (13.0 mg), which contained **3** as a major component, was acetylated and further purified on TLC using 50% EtOAc–petroleum ether (4 runs) to yield the hexaacetate of **3** as a colourless viscous gum (7.8 mg). Fr. 7 (903.1 mg) was separated by CC over reverse-phase silica gel (RpC-18), eluted with 50% MeOH–H_2O to yield 3 subfrs. Subfr. 2 (226 mg) was further separated by radial chromatography (silica-gel plate), eluted with $CHCl_3$, $CHCl_3$–MeOH gradient and finally with pure MeOH to yield 8 subfrs. The fourth fraction (55 mg) was further recrystallized from a mixture of MeOH–$CHCl_3$ to afford **13** as a white solid while the fifth fraction (64 mg) was acetylated and subsequently purified on TLC using 45% EtOAc–petroleum ether (7 runs) as a mobile phase to yield the heptaacetate of **11** as a colourless viscous gum (4.3 mg). Subfr. 3 (330 mg) was purified by CC over silica gel, eluted with 14% MeOH–CH_2Cl_2 to yield 4 subfrs. The third fraction (98.9 mg), which contained **2** as a major component, was subjected to acetylation reaction and subsequently chromatographed on TLC using 1% MeOH–$CHCl_3$ (7 runs) as a mobile phase to afford the heptaacetate of **2** as a colourless viscous gum (69 mg).

3.4. Tetraacetate of 7,8-dihydroxy-4′-methoxyisoflavone 8-O-β-glucopyranoside (*1*)

Colorless viscous gum; $[\alpha]_D^{29}$ -55.56° (*c* 0.36, $CHCl_3$); UV: λ_{max} $CHCl_3$ nm: 211, 254, 306; IR ν_{max} (neat) cm^{-1}: 3443, 2920, 2850, 1755, 1630, 1609; ^1H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HR–FABMS $[M+H]^+ = m/z$ 615.1712 (calc. for $C_{30}H_{31}O_{14}$, 615.1714); FABMS (rel. int.) 615 (M + H, 25), 552 (25), 281 (75), 207 (100).

The tetraacetate of **1** was obtained from a glycoside mixture containing **1** as a major component: ^1H NMR (500 MHz, CD_3OD) δ 8.22 (*s*, H-2), 7.89 (*d*, *J* = 8.5 Hz, H-5), 7.47 (*d*, *J* = 8.5 Hz, H-2′,6′), 7.04 (*d*, *J* = 8.5 Hz, H-6), 6.99 (*d*, *J* = 8.5 Hz, H-3′,5′), 4.90 (*d*, *J* = 8.1 Hz, H-1″), 3.83 (*s,* OMe), 3.82–3.35 (*m,* glucose protons).

3.5. Heptaacetate of 7,8-dihydroxy-4′-methoxyisoflavone 7-O-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (*2*)

Colorless viscous gum; $[\alpha]_D^{29}$ −51.47° (*c* 1.36, $CHCl_3$); UV: λ_{max} $CHCl_3$ nm: 213, 233 (sh), 257, 306; IR ν_{max} (neat) cm^{-1}: 2940, 1732, 1659, 1613, 1573; ^1H NMR (500 MHz) see Table 1; ^{13}C NMR (125 MHz) see Table 2; HR–FABMS $[M+H]^+ = m/z$ 887.2591 (calc. for $C_{42}H_{47}O_{21}$, 887.2610); FABMS (rel. int.) 887 (M + H, 100), 663 (41) 327 (86), 273 (68).

The heptaacetate of **2** was obtained from a glycoside mixture containing **2** as a major component: ^1H NMR (500 MHz, CD_3OD) δ 8.27 (*s*, H-2), 7.70 (*d*, *J* = 9.5 Hz, H-5), 7.50 (*d*, *J* = 9.0 Hz, H-2′,6′), 7.35 (*d*, *J* = 9.5 Hz, H-6), 7.00 (*d*, *J* = 9.0 Hz, H-3′,5′), 4.99 (*d*, *J* = 7.5 Hz, H-1″), 4.72 (*d*, *J* = 1.5 Hz, H-1‴), 4.05–3.36 (*m*, glucose and rhamnose protons), 3.83 (*s*, OMe), 1.20 (*d*, *J* = 6.5 Hz, H-6‴).

3.6. Hexaacetate of 8-hydroxy-4′,7-dimethoxyisoflavone 8-O-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (*3*)

Colorless viscous gum; $[\alpha]_D^{29}$ −40.54° (*c* 0.74, $CHCl_3$); UV: λ_{max} $CHCl_3$ nm: 212, 253, 306; IR ν_{max} (neat) cm^{-1}: 2939, 2848, 1748, 1645, 1609, 1567; ^1H NMR (500 MHz) see Table 1; ^{13}C NMR (125 MHz) see Table 2; HR–FABMS $[M+H]^+ = m/z$ 859.2664 (calc. for $C_{41}H_{47}O_{20}$, 859.2661); FABMS (rel. int.) 859 (M + H, 100), 577 (85), 551 (68), 299 (100), 273 (48).

The hexaacetate of **3** was obtained from a glycoside mixture containing **3** as a major component: ^1H NMR (500 MHz, CD_3OD) δ 8.25 (*s*, H-2), 8.00 (*d*, *J* = 9.0 Hz, H-5), 7.50 (*d*, *J* = 8.5 Hz, H-2′,6′), 7.27 (*d*, *J* = 9.0 Hz, H-6), 6.99 (*d*, *J* = 8.5 Hz, H-3′,5′), 5.07 (*d*, *J* = 7.5 Hz, H-1″), 4.56 (*d*, *J* = 1.5 Hz, H-1‴), 4.03 (*s*, OMe), 3.82 (*s*, OMe), 3.84–3.37 (*m*, glucose and rhamnose protons), 1.06 (*d*, *J* = 6.5 Hz, H-6‴).

3.7. Hexaacetate of afromosin 7-O-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (*4*)

Colorless viscous gum; $[\alpha]_D^{29}$ −68.18° (*c* 0.44, $CHCl_3$); UV: λ_{max} $CHCl_3$ nm: 209, 226 (*sh*), 260, 322; IR ν_{max} (neat) cm^{-1}: 2938, 1750, 1634, 1602, 1579; ^1H NMR (500 MHz) see Table 1; ^{13}C NMR (125 MHz) see Table 2; HR–FABMS $[M+H]^+ = m/z$ 859.2650 (calc. for $C_{41}H_{47}O_{20}$, 859.2661); FABMS (rel. int.) 859 (M + H, 25), 299 (100).

The hexaacetate of **4** was obtained from a glycoside mixture containing **4** as a major component: ^1H NMR (500 MHz, CD_3OD) δ 8.31 (*s*, H-2), 7.63 (*s*, H-5), 7.50 (*d*, *J* = 9.0 Hz, H-2′,6′), 7.36 (*s,* H-8), 6.99 (*d*, *J* = 8.5 Hz, H-3′,5′), 5.05 (*d*, *J* = 7.5 Hz, H-1″), 4.65 (*d*, *J* = 1.5 Hz, H-1‴), 3.97 (*s*, OMe), 3.82 (*s*, OMe), 3.84–3.37 (*m*, glucose and rhamnose protons), 1.22 (*d*, *J* = 6.5 Hz, H-6‴).

3.8. Hexaacetate of irisolidone 7-O-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (*5*)

Colorless viscous gum; $[\alpha]_D^{29}$ −90.90° (*c* 0.11, $CHCl_3$); UV: λ_{max} $CHCl_3$ nm: 212, 265, 330; IR ν_{max} (neat) cm^{-1}: 3430, 2923, 1756, 1659, 1613, 1591; ^1H NMR (400 MHz) see Table 1; ^{13}C NMR (100 MHz) see Table 2; HR–FABMS $[M+H]^+ = m/z$ 875.2597 (calc. for $C_{41}H_{47}O_{21}$, 875.2610); FABMS (rel. int.) 875 (M + H, 17), 315 (100), 281 (70).

The hexaacetate of **5** was obtained from subfr. 3 separated from fr. 5: ^1H NMR (500 MHz, CD_3OD) δ

8.25–8.18 (*singlets*, H-2), 7.90–6.60 (other isoflavone protons), 5.10–5.00 (*doublets*, $J = 7.0$–8.0 Hz, H-1″), 4.75–4.65 (*doublets*, $J = 1.5$ Hz, H-1‴), 4.00–3.78 (*singlets*, OMe), 4.10–3.36 (*m*, glucose and rhamnose protons), 1.24–1.06 (*doublets*, $J = 6.0$–7.0 Hz, H-6‴).

3.9. Examination of hypotensive activity

Female Wistar rats in esterus were anesthetized with Nembutal (50 mg/kg). A polyethylene catheter was cannulated through the right common carotid artery and connected to a pressure transducer and polygraph for monitoring blood pressure and heart rate. The animal was then equilibrated for 40 min. The sample was dissolved in saline or 10% DMSO in saline in a concentration of 10 mg/ml and injected through the left jugular vien at the dose of 4 mg/kg of animal weight. The pronounced hypotensive and negative chronotropic activities of each sample were defined when it caused a decrease in mean arterial blood pressure of more than 75 mmHg and a decrease in heart rate more than 60 beats/min and these activities were prolonged for at least one min.

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