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Phenolic glycosides from *Markhamia stipulata*

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Abstract

From the leaves and branches of *Markhamia stipulata*, five verbascoside derivatives (markhamiosides A–E), and one hydroquinone derivative (markhamioside F) were isolated together with 13 known compounds. Their structural elucidation was based on analyses of chemical and spectroscopic data.

Keywords: *Markhamia stipulata*; Bignoniaceae; Phenylpropanoid glycoside; Phenylethanoid glycoside; Hydroquinone derivative; Markhamiosides A–F

1. Introduction

As part of an on a going study on Thai Bignoniaceous plants (Kanchanapoom et al., 2001, 2002), we investigated the constituents of *Markhamia stipulata* Seem. ex K. Schum. (Thai name: Khae-Hua-Mu) collected from the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. *M. stipulata* is a tree distributed in South and South-east Asia. In Thai traditional medicine, the leaves and barks are externally used for treating skin diseases as well as being internally used for analgesic effect. In preliminary investigations of this plant, dehydro-α-lapachone, lapachol, dehydro-iso-α-lapachone, β-sitosterol, β-lapachone, tectol, paulownin and palmitone have been isolated from the alcoholic extract of stem-heartwood (Joshi et al., 1978). The present study deals with the isolation and structural determination of 19 compounds. Five were new verbascoside derivatives (**3**, **9**–**12**), one was a new hydroquinone derivative (**13**) along with 13 known compounds from the leaves and branches of this plant.

2. Results and discussion

The methanolic extract of the leaves and branches of *M. stipulata* was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer resin of styrene and divinylbenzene, using H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH was repeatedly applied to columns of silica gel, RP-18, or prep. HPLC to afford 19 compounds (**1**–**19**). Thirteen were identified as known compounds; phenethyl-*O*-β-glucopyranosyl-(1→2)-*O*-β-glucopyranoside (**1**) (Ono et al., 1999), decaffeoylverbascoside (**2**) (Karasawa et al., 1986), verbascoside (**4**), isoverbascoside (**5**) (Miyase et al., 1982), 2″-*O*-apiosylverbascoside (**6**) (Kanchanapoom et al., 2001), luteoside A (**7**), luteoside B (**8**) (Kernan et al., 1998), khaephuoside B (**14**) (Kanchanapoom et al., in press), sequinoside K (**15**) (Zhong et al., 1999), (6*S*,9*R*)-roseoside (**16**) (Otsuka et al., 1995), rengyoside B (**17**) (Seya et al., 1989), ajugol (**18**) (Nishimura et al., 1989), (+)-lyoniresinol 3α-*O*-β-glucopyranoside (**19**) (Achenbach et al., 1992) by comparison of physical data with literature values and from spectroscopic evidence.

The molecular formula of compound **3** was determined as $C_{25}H_{38}O_{16}$ by HR–FAB mass spectrometry. The ^{1}H NMR spectrum showed the presence of ABX systems [δ 6.64 (*d*, $J=1.7$ Hz), δ 6.63 (*d*, $J=8.1$ Hz) and δ 6.50 (*dd*, $J=8.1$, 1.7 Hz)] for 3,4-dihydroxy-β-phenylethoxyl moiety together with three anomeric protons, δ 4.31 (*d*, $J=7.8$ Hz) for β-glucose, δ 4.94 (*d*, $J=1.3$ Hz) for α-rhamnose and δ 5.16 (*d*, $J=1.5$ Hz) for β-apiose. Acid hydrolysis of **3** afforded apiose, rhamnose and glucose, identified by TLC. The ^{1}H and ^{13}C NMR spectra were very similar to those of decaffeoylverbascoside (**2**) except for the additional signals of an apiofuranosyl unit. Comparison of the ^{13}C NMR spectral data of **3**

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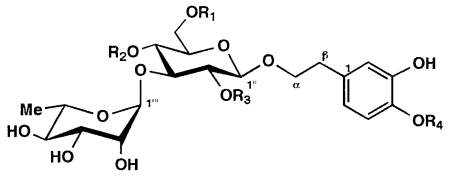
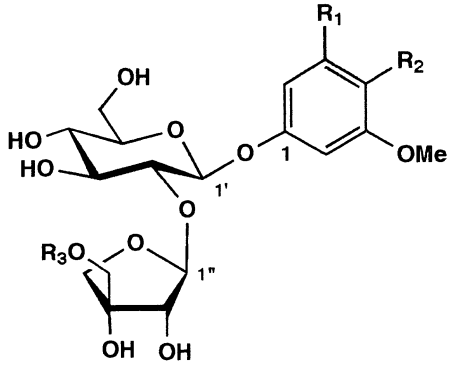
with those of **2**, the chemical shifts of C-1″, C-2″ and C-3″ were changed by −1.2, +3.8 and +1.8 ppm, respectively, indicating that the apiofuranosyl residue is located at C-2″. Furthermore, the HMBC spectrum provided significant correlations between H-1″ (δ 4.31) and CH_2-α (δ 71.9); H-1‴ (δ 4.94) and C-3″ (δ 86.3) as well as between H-1⁗ (δ 5.16) and C-2″ (δ 79.4). Consequently, compound **3** was established as 3,4-dihydroxy-β-phenylethoxy-*O*-[β-apiofuranosyl-(1⁗→2″)-α-rhamnopyranosyl-(1‴→3″)-*O*-β-glucopyranoside], named markhamioside A.

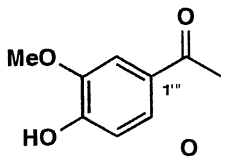
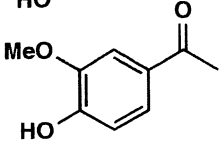
The molecular formula of compound **9** was determined as $C_{36}H_{48}O_{19}$ by HR-FAB mass spectrometry. The ^1H and ^{13}C NMR spectral data were closely related to those of luteoside B (**8**) except for additional resonances for two methoxy groups (δ 3.70 and δ 3.81) in the ^1H NMR spectrum, and at δ 56.4 and δ 56.4 in the ^{13}C NMR spectrum). The chemical shifts of an ester moiety were in agreement with those of a feruloyl moiety, as well as the carbon signals of an aglycone part were superimposable with those of a 3-hydroxy-4-methoxy-β-phenylethoxyl moiety (Miyase et al., 1991). Acid hydrolysis of **9** provided apiose, rhamnose and glucose, identified by TLC. The complete set of assignments were confirmed by using COSY, HSQC and difference NOE experiments. On irradiation of the signal at δ 3.70 (3-OMe), the intensity of H-5 (δ 6.62, *d*, *J* = 8.0 Hz) was enhanced, and irradiation of the signal at δ 3.81 (4′-OMe) caused an increase in the NOE enhancement at H-2′ (δ 7.09, *d*, *J* = 1.9 Hz). Upon irradiation of the anomeric signals of β-apiofuranosyl (δ 5.15, *d*, *J* = 1.9 Hz) and α-rhamnopyranosyl units, NOE enhancements were observed for the H-2″ (δ 3.42, *dd*, *J* = 8.8, 7.8 Hz) and H-3″ (δ 3.57, *dd*, *J* = 9.0, 8.8 Hz) of the glucopyranosyl unit, respectively. On the basis of this spectral data, the structure of compound **9** was elucidated as 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-[β-apiofuranosyl-(1⁗→2″)-α-rhamnopyranosyl-(1‴→3″)-6″-*O*-feruloyl-β-glucopyranoside], named markhamioside B.

The molecular formula of compound **10** was determined as $C_{34}H_{44}O_{19}$ by HR–FAB mass spectrometry. The ^1H and ^{13}C NMR spectral data indicated that compound **10** is a phenylpropanoid with three sugar moieties. The chemical shifts of compound **10** were almost the same as those of **8**. However, the signals for an α-arabinopyranosyl unit at δ 104.4, 72.8, 74.3, 69.3 and 66.5 (Lahloub et al., 1986) were observed from the ^{13}C NMR spectrum instead of the signals for a β-apiofuranosyl unit, suggesting that the α-arabinopyranosyl unit is attached to C-2″. Acid hydrolysis of **10** gave arabinose, rhamnose and glucose (identified by TLC). Assignment of all signals was based on the results of COSY, HSQC and HMBC. COSY and HSQC were used to determine the sugar protons. The HMBC spectrum provided further confirmation from the correlations between H-1⁗ (δ 4.47, *d*, *J* = 6.9 Hz) and C-2″



	R_1	R_2	R_3	R_4
2	H	H	H	H
3	H	H	apiosyl	H
4	H	caffeoyl	H	H
5	caffeoyl	H	H	H
6	H	caffeoyl	apiosyl	H
7	Ac	caffeoyl	apiosyl	H
8	caffeoyl	H	apiosyl	H
9	feruloyl	H	apiosyl	Me
10	caffeoyl	H	arabinosyl	H
11	Ac	caffeoyl	arabinosyl	H
12	Ac	caffeoyl	galactosyl	H



	R_1	R_2	R_3
13	H	OH	H
14	OMe	OMe	
15	H	OH	

(δ 81.4) as well as H-1‴ (δ 5.07, *d*, *J* = 1.3 Hz) and C-3″. Therefore, compound **10** was 3,4-dihydroxy-β-phenylethoxy-*O*-[α-arabinopyranosyl-(1‴→2″)-α-rhamnopyranosyl-(1‴→3″)-6″-*O*-caffeoyl-β-glucopyranoside], named markhamioside C.

The molecular formula of compound **11** was determined as $C_{36}H_{46}O_{20}$ by HR–FAB mass spectrometry. Inspection of the ^{1}H NMR spectrum revealed the presence of two sets of ABX systems [δ 6.72 (*d*, *J* = 2.0 Hz), δ 6.69 (*d*, *J* = 8.1 Hz) and δ 6.56 (*dd*, *J* = 8.1, 2.0 Hz) for the 3,4-dihydroxy-β-phenylethoxyl moiety; and δ 7.05 (*d*, *J* = 2.0 Hz), δ 6.78 (*d*, *J* = 8.1 Hz) and δ 6.94 (*dd*, *J* = 8.1, 2.0 Hz) for the caffeoyl moiety], two *trans*-olefinic protons [δ 6.26 and 7.57 (each *d*, *J* = 15.6 Hz)], a singlet signal of acetyl group (δ 2.00), together with three anomeric protons at δ 4.55 (*d*, *J* = 7.8 Hz) for β-glucose, δ 5.17 (*d*, *J* = 1.5 Hz) for α-rhamnose and δ 4.50 (*d*, *J* = 7.0 Hz) for α-arabinose. Acid hydrolysis of **11** afforded arabinose, rhamnose and glucose, identified by TLC. The chemical shifts of compound **11** were almost the same as those of **7**, except for signals of α-arabinose instead of β-apiose. The assignments were confirmed by HOHAHA, HSQC, HMBC experiments, in which long-range correlations were observed from the HMBC spectrum between H-4″ (δ 5.03, *dd*, *J* = 9.5, 9.3 Hz) and a carbonyl carbon of caffeoyl moiety (δ 168.0), C-5″ (δ 72.9), C-3″ (δ 81.1) ; H-6″ (δ 4.14, *dd*, *J* = 12.0, 5.0 Hz) and a carbonyl carbon of an acetyl group (δ 172.6); H-1″ (δ 4.55, *d*, *J* = 7.8 Hz) and CH_2-α (δ 72.1); H-1‴ (δ 5.17, *d*, *J* = 1.5 Hz) and C-3″ (δ 81.1) as well as between H-1⁗ (δ 4.50, *d*, *J* = 7.0 Hz) and C-2″ (δ 82.5). Moreover, the difference NOE experiments provided further support by irradiation of the anomeric signals at δ 4.50 of arabinose and δ 5.17 of rhamnose, which resulted in NOE enhancement for the H-2″ (δ 3.68, *dd*, *J* = 8.1, 7.8 Hz) and H-3″ (δ 3.96, *dd*, *J* = 9.3, 8.1 Hz) signals, respectively. Accordingly, the structure of compound **11** was identified as 3,4-dihydroxy-β-phenylethoxy-*O*-[α-arabinopyranosyl-(1⁗→2″)-α-rhamnopyranosyl-(1‴→3″)-4-*O*-caffeoyl-6-*O*-acetyl-β-glucopyranoside], named markhamioside D.

The molecular formula of compound **12** was deduced as $C_{37}H_{48}O_{21}$ by HR–FAB mass spectrometry. The ^{1}H and ^{13}C NMR spectral data were almost the same as those of **7**. However, the signals at δ 103.8, 73.1, 75.2, 70.4, 76.5 and 62.5 for β-galactopyranosyl unit (Sasaki et al., 1989) were observed instead of the signals for a β-apiofuranosyl unit, suggesting that the β-galactopyranosyl unit is located at C-2″. Acid hydrolysis of **12** afforded galactose, rhamnose and glucose, identified by TLC. The assignments were supported by COSY, HSQC, HMBC and difference NOE experiments. In the HMBC spectrum, significant correlations were found between H-4″ (δ 5.00, *dd*, *J* = 9.5, 9.3 Hz) and a carbonyl carbon of a caffeoyl moiety (δ 168.0), C-5″ (δ 72.9), C-3″ (δ 81.0); between H-6″ (δ 4.11, *dd*, *J* = 12.2, 5.1 Hz) and a cabonyl carbon of an acetyl group (δ 172.5); between H-1″ (δ 4.57, *d*, *J* = 7.8 Hz) and CH_2-α (δ 72.0); between H-1‴ (δ 5.14, *d*, *J* = 1.5 Hz) and C-3″ (δ 81.0) as well as between H-1⁗ (δ 4.53, *d*, *J* = 7.8 Hz) and C-2″ (δ 82.1). Furthermore, irradiation of the anomeric signals at δ 4.53 of galactose and δ 5.14 of rhamnose caused NOE enhancements of the signal at δ 3.48 (H-2″) and δ 3.95 (H-3″), respectively. Therefore, the structure of compound **12** was established as 3,4-dihydroxy-β-phenylethoxy-*O*-[β-galactopyranosyl-(1⁗→2″)-α-rhamnopyranosyl-(1‴→3″)-4-*O*-caffeoyl-6-*O*-acetyl-β-glucopyranoside], named markhamioside E.

The molecular formula of compound **13** was determined as $C_{18}H_{26}O_{12}$ by HR-FAB mass spectrometry. The ^{1}H NMR spectrum revealed the presence of a set of ABX systems at δ 6.67 (1H, *d*, *J* = 2.7 Hz, H-2), δ 6.59 (1H, *d*, *J* = 8.8 Hz, H-5) and δ 6.46 (1H, *dd*, *J* = 8.8, 2.7 Hz, H-6); a methoxy signal at δ 3.73 (3H, *s*, MeO-3) as well as two anomeric protons at δ 4.69 (*d*, *J* = 7.8 Hz) for β-glucopyranosyl and δ 5.36 (*d*, *J* = 1.5 Hz) for β-apifuranosyl units. The ^{13}C NMR spectral data were similar to those of sequinoside K (**15**), except that the signals for an acyl ester were not observed. Also, the chemical shifts of C-5″ and C-3″ were changed by-1.8 and +1.5 ppm, respectively. Consequently, the structure of compound **13** was identified as deacyl ester of sequinoside K (**15**), named markhamioside F.

The pharmalogical activities of the isolated compounds have not been investigated. However, the pharmacological activities of verbascoside derivatives have been reviewed by Cometa et al. (1993) and Jimenez and Riguera (1994). Some have antifungal, antibacterial and analgesic activities, in agreement with the traditional uses of the plants in Thailand.

3. Experimental

3.1. General

NMR spectra 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. 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 refractive 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) 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) EtOAc– MeOH–H_2O (6:4:1), (IV) 40–70% aq.

Table 1
^{1}H NMR Spectral data of compounds **3**, **9**–**12** (400 MHz, CD_3OD)

H	**3**	**9**	**10**	**11**	**12**	**13**
Aglycone						
2	6.64 (1H, *d*, *J* = 1.7 Hz)	6.64 (1H, *d*, *J* = 2.0 Hz)	6.68 (1H, *d*, *J* = 1.3 Hz)	6.72 (1H, *d*, *J* = 2.0 Hz)	6.71 (1H, *d*, *J* = 2.0 Hz)	6.67 (1H, *d*, *J* = 2.7 Hz)
5	6.63 (1H, *d*, *J* = 8.1 Hz)	6.62 (1H, *d*, *J* = 8.0 Hz)	6.59 (1H, *d*, *J* = 8.1 Hz)	6.69 (1H, *d*, *J* = 8.1 Hz)	6.64 (1H, *d*, *J* = 8.1 Hz)	6.59 (1H, *d*, *J* = 8.8 Hz)
6	6.50 (1H, *dd*, *J* = 8.1, 1.7 Hz)	6.56 (1H, *dd*, *J* = 8.0, 2.0 Hz)	6.48 (1H, *dd*, *J* = 8.1, 1.3 Hz)	6.56 (1H, *dd*, *J* = 8.1, 2.0 Hz)	6.54 (1H, *dd*, *J* = 8.1, 2.0 Hz)	6.46 (1H, *dd*, *J* = 8.8, 2.7 Hz)
α	3.93 (1H, *m*)	3.94 (1H, *m*)	3.93 (1H, *m*)	3.98 (1H, m)3.66 (1H, m)	3.95 (1H, *m*)3.64 (1H, *m*)	–
	3.62 (1H, *m*)	3.66 (1H, *m*)	3.68 (1H, *m*)	3.66 (1H, *m*)	3.64 (1H, *m*)	–
β	2.72 (2H, *t*, *J* = 7.3 Hz)	2.75 (2H, *t*, *J* = 7.5 Hz)	2.71 (2H, *m*)	2.76 (2H, *m*)	2.73 (2H, *m*)	–
3-OMe	–	3.70 (3H, *s*)	–	–	–	3.73 (3H, *s*)
Ester moiety						
2′		7.09 (1H, *d*, *J* = 1.9 Hz)	6.99 (1H, *d*, *J* = 1.5 Hz)	7.05 (1H, *d*, *J* = 2.0 Hz)	7.00 (1H, *d*, *J* = 2.0 Hz)	–
5′		6.74 (1H, *d*, *J* = 8.1 Hz)	6.72 (1H, *d*, *J* = 8.1 Hz)	6.78 (1H, *d*, *J* = 8.1 Hz)	6.73 (1H, *d*, *J* = 8.3 Hz)	
6′		6.96 (1H, *dd*, *J* = 8.1, 1.9 Hz)	6.84 (1H, *dd*, *J* = 8.1, 1.5 Hz)	6.94 (1H, *dd*, *J* = 8.1, 2.0 Hz)	6.90 (1H, *dd*, *J* = 8.3, 2.0 Hz)	
α′		6.32 (1H, *d*, *J* = 15.9 Hz)	6.23 (1H, *d*, *J* = 15.9 Hz))	6.26 (1H, *d*, *J* = 15.6 Hz)	6.21 (1H, *d*, *J* = 15.9 Hz)	
β′		7.57 (1H, *d*, *J* = 15.9 Hz)	7.51 (1H, *d*, *J* = 15.9 Hz)	7.57 (1H, *d*, *J* = 15.6 Hz)	7.53 (1H, *d*, *J* = 15.9 Hz)	
4′-OMe		3.81 (3H, *s*)	–	–	–	
Glucose						
1″	4.31 (1H, *d*, *J* = 7.8 Hz)	4.36 (1H, *d*, *J* = 7.8 Hz)	4.42 (1H, *d*, *J* = 7.8 Hz)	4.55 (1H, *d*, *J* = 7.8 Hz)	4.57 (1H, *d*, *J* = 7.8 Hz)	4.69 (1H, *d*, *J* = 7.8 Hz)
2″	3.39 (1H, *dd*, *J* = 9.3, 7.8 Hz)	3.42 (1H, *dd*, *J* = 8.8, 7.8 Hz)	3.54 (1H, *dd*, *J* = 8.3, 7.8 Hz)	3.68 (1H, *dd*, *J* = 9.0, 7.8 Hz)	3.48 (1H, *dd*, *J* = 9.0, 7.8 Hz)	[a]
3″	3.53 (1H, *dd*, *J* = 9.3, 8.0 Hz)	3.57 (1H, *dd*, *J* = 9.0, 8.8 Hz)	3.64 (1H, *dd*, *J* = 9.3, 8.3 Hz)	3.96 (1H, *dd*, *J* = 9.3, 8.1 Hz)	3.95 (1H, *dd*, *J* = 9.3, 9.0 Hz)	[a]
4″	3.94[a]	3.90[a]	3.92 (1H, *dd*, *J* = 9.3, 9.3 Hz)	5.03 (1H, *dd*, *J* = 9.5, 9.3 Hz)	5.00 (1H, *dd*, *J* = 9.5, 9.3 Hz)	[a]
5″	3.23 (1H, *m*)	3.48 (1H, *m*)	3.48[a]	3.58 (1H, *m*)	3.68 (1H, *m*)	[a]
6″	3.81 (1H, *brd*, *J* = 12.2 Hz)	4.45 (1H, *dd*, *J* = 12.0, 2.0 Hz)	4.47[a]	4.14 (1H, *dd*, *J* = 12.0, 5.0 Hz)	4.11 (1H, *dd*, *J* = 12.2, 5.1 Hz)	3.79 (1H, *dd*, *J* = 12.2, 2.0 Hz)
	3.63 (1H, *dd*, *J* = 12.2, 4.9 Hz)	4.31 (1H, *dd*, *J* = 12.0, 6.1 Hz)	4.29 (1H, *dd*, *J* = 12.0, 6.1 Hz)	4.05[a]	4.00 (1H, *dd*, *J* = 12.2, 2.6 Hz)	3.57 (1H, *dd*, *J* = 12.2, 5.4 Hz)
Rhamnose						
1‴	4.94 (1H, *d*, *J* = 1.3 Hz)	4.96 (1H, *d*, *J* = 1.5 Hz)	5.07 (1H, *d*, *J* = 1.3 Hz)	5.17 (1H, *d*, *J* = 1.5 Hz)	5.14 (1H, *d*, *J* = 1.5 Hz)	–
2‴	3.96 (1H, *dd*, *J* = 3.4, 1.3 Hz)	3.94 (1H, *m*)	4.01 (1H, *dd*, *J* = 3.4, 1.5 Hz)	4.04[a]	4.03 (1H, *dd*, *J* = 3.5, 1.5 Hz)	–
3‴	3.62[a]	3.62[a]	3.60[a]	3.57[a]	3.53 (1H, *dd*, *J* = 9.6, 3.5 Hz)	–
4‴	3.36[a]	3.30[a]	3.31[a]	3.30[a]	3.29[a]	–
5‴	3.96[a]	3.98[a]	3.98[a]	3.52[a]	3.40 (1H, *dd*, *J* = 9.8, 6.1 Hz)	–
6‴	1.21 (3H, *d*, *J* = 6.1 Hz)	1.21 (3H, *d*, *J* = 6.1 Hz)	1.21 (3H, *d*, *J* = 6.1 Hz)	1.10 (3H, *d*, *J* = 6.1 Hz)	1.05 (3H, *d*, *J* = 6.1 Hz)	–
2″-O-Sugar	*Apiose*	*Apiose*	*Arabinose*	*Arabinose*	*Galactose*	*Apiose*
1⁗	5.16 (1H, *d*, *J* = 1.5 Hz)	5.15 (1H, *d*, *J* = 1.9 Hz)	4.47 (1H, *d*, *J* = 6.9 Hz)	4.50 (1H, *d*, *J* = 7.0 Hz)	4.53 (1H, *d*, *J* = 7.8 Hz)	5.36 (1H, *d*, *J* = 1.5 Hz)
2⁗	3.87 (1H, *d*, *J* = 1.5 Hz)	3.86 (1H, *d*, *J* = 1.9 Hz)	3.56[a]	3.56[a]	3.57[a]	3.86 (1H, *d*, *J* = 1.5 Hz)
3⁗	–	–	3.48[a]	3.52[a]	3.47 (1H, *dd*, *J* = 7.8, 4.1 Hz)	–
4⁗	3.91 (1H, *d*, *J* = 9.8 Hz)	3.93 (1H, *d*, *J* = 9.8 Hz)	3.76 (1H, *m*)	3.77 (1H, *m*)	3.54[a]	4.01 (1H, *d*, *J* = 9.5 Hz)
	3.67 (1H, *d*, *J* = 9.8 Hz)	3.66 (1H, *d*, *J* = 9.8 Hz)	–	–	–	3.69 (1H, *d*, *J* = 9.5 Hz)
5⁗	3.54 (2H, *s*)	3.52 (2H, *s*)	3.81 (1H, *dd*, *J* = 12.2, 3.4 Hz)	3.82[a]	3.21 (1H, 11.9, 6.8 Hz)	3.49 (2H, *s*)
	–	–	3.25 (1H, *dd*, *J* = 12.2, 1.5 Hz)	3.20 (1H, *d*, *J* = 12.0, 1.5 Hz)	–	–
6⁗	–	–	–	–	3.70[a]	–
	–	–	–	–	3.62[a]	–
Ac	–	–	–	2.0 (3H, *s*)	1.97 (3H, *s*)	–

[a] Signal pattern unclear due to overlapping.

MeOH, (V) 40% aq. MeOH, (VI) 45% aq. MeOH, (VII) 20–70% aq. MeOH, (VIII) 5% aq. MeCN, (IX) 8% aq. MeCN, (X) 15% aq. MeCN, (XI) 45% aq. MeCN, (XII) 10% aq. MeCN, (XIII) 85% aq. MeCN), (XIV) 20% aq. MeCN, (XV) 25% aq. MeCN and (XVI) 28% aq. MeCN. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. Plant material

The leaves and branches of *Markhamia stipulata* Seem. ex K. Schum were collected in April 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 (KKU-0022) 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 (1.7 kg) of *M. stipulata* were extracted with hot MeOH. After removal of the solvent by evaporation, the residue (117.0 g) was defat-

Table 2
^{13}C NMR Spectral data for compounds **2**–**12** (100 MHz, CD_3OD)

Carbon no.	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aglycone											
1	131.5	131.5	131.5	131.4	131.6	131.4	131.4	132.8	131.7	131.8	131.8
2	117.1	117.1	117.1	117.0	117.2	117.1	117.1	117.0	117.4	117.4	117.5
3	144.6	144.4	144.6	144.5	144.6	144.4	144.5	147.5	144.6	144.7	144.7
4	146.0	145.9	146.1	146.0	146.1	145.8	146.0	147.3	146.0	146.0	146.0
5	116.3	116.3	116.3	116.3	116.3	116.3	116.4	112.9	116.4	116.4	116.4
6	121.2	121.3	121.3	121.3	121.3	121.2	121.3	121.1	121.4	121.5	121.6
α	72.3	71.9	72.2	72.4	72.2	72.2	72.2	72.1	72.2	72.1	72.0
β	36.5	36.5	36.5	36.5	36.5	36.4	36.6	36.7	36.7	36.7	36.7
4-OMe	–	–	–	–	–	–	–	56.4	–	–	–
Ester moiety											
1′			127.7	127.6	127.7	127.5	127.6	127.7	127.7	127.6	127.7
2′			115.2	115.1	115.2	115.3	115.1	111.7	115.1	115.3	115.3
3′			149.7	149.5	149.7	149.5	149.5	149.4	149.5	149.7	149.7
4′			146.8	146.6	146.8	146.5	146.7	150.6	146.7	146.8	146.8
5′			116.5	116.5	116.5	116.5	116.6	116.5	116.6	116.6	116.5
6′			123.2	123.1	123.2	123.1	123.2	124.3	123.1	123.2	123.2
α′			114.7	114.8	114.9	114.7	114.8	115.3	114.8	114.7	114.8
β′			148.0	147.2	147.9	147.8	147.2	147.1	147.2	148.0	148.0
C=O			168.3	169.1	168.3	167.9	169.1	169.0	169.1	168.0	168.0
3′-OMe			–	–	–	–	–	56.4	–	–	–
Glc											
1″	104.1	102.9	104.2	104.2	103.1	102.9	103.1	103.2	103.0	103.0	102.8
2″	75.6	79.4	76.2	75.2	81.0	80.6	79.6	79.7	81.4	82.5	82.1
3″	84.5	86.3	81.6	83.9	82.0	81.7	85.6	85.8	85.3	81.1	81.0
4″	70.1	70.3	70.4	70.0	70.8	70.6	70.5	70.7	70.5	70.6	70.6
5″	77.7	77.5	76.0	75.5	76.0	72.7	75.1	75.2	75.0	72.9	72.9
6″	62.6	62.5	62.4	64.6	62.4	63.8	64.5	64.6	64.6	64.0	64.0
Rham											
1‴	102.7	103.3	103.0	102.6	103.5	103.3	103.3	103.4	103.1	103.1	103.1
2‴	72.2	72.1	72.3	72.2	72.3	72.1	72.2	72.3	72.2	72.1	72.0
3‴	72.1	72.0	72.0	72.2	71.9	71.7	72.1	72.2	72.1	71.9	72.0
4‴	73.9	73.6	73.8	73.9	73.8	73.5	73.7	73.8	73.9	73.8	73.8
5‴	70.2	70.5	70.6	70.3	70.9	70.5	70.5	70.5	70.5	70.6	70.6
6‴	17.9	17.8	18.4	17.8	18.4	18.3	17.8	17.8	17.9	18.4	18.4
2″-O-Sugar		*Api*			*Api*	*Api*	*Api*	*Api*	*Ara*	*Ara*	*Gal*
1⁗		110.9			111.2	111.0	111.0	111.0	104.4	104.1	103.8
2⁗		78.4			78.5	78.4	78.4	78.5	72.8	72.8	73.1
3⁗		80.5			80.4	80.3	80.5	80.5	74.3	74.5	75.2
4⁗		75.1			75.1	75.0	75.1	75.2	69.3	69.5	70.4
5⁗		65.7			65.5	65.4	65.7	65.7	66.5	66.8	76.5
6⁗											62.5
$\underline{C}OCH_3$						172.5				172.6	172.5
$CO\underline{C}H_3$						20.7				20.7	20.7

ted 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 (39.0 g) was subjected to a column of silica gel (systems I, II and III, respectively) affording six fractions. Fraction 2 (3.8 g) was applied to a column of RP-18 using system IV to provide seven fractions. Fraction 2-1 was purified by prep. HPLC (system V) to provide compounds **4** (277 mg) and **15** (110 mg). Fraction 2-3 was purified by prep. HPLC (system VI) to give compounds **5** (190 mg) and **14** (61 mg). Fraction 3 (11.0 g) was subjected to a column of RP-18 (system VII) to afford 13 fractions, together with compound **7** (3.2 g) from fraction 3-8. Fraction 3-1 was purified by prep. HPLC-ODS (systemVIII) to provide compound 13 (4 mg). Fraction 3-3 was purified by prep. HPLC-ODS (system IX) to afford compound **2** (47 mg). Fraction 3-5 was purified by prep. HPLC-ODS (system X) to give compounds **16** (133 mg) and **19** (16 mg). Fraction 3-5 was similarly purified by prep. HPLC-ODS (system VI) to afford compounds **6** (31 mg) and **8** (125 mg). Fraction 3-12 was purified by prep. HPLC–ODS (system XVI) to give compound 9 (18 mg). Fraction 4 (6.2 g) was re-applied for a RP-18 column (system VII) to afford nine fractions. Fraction 4-1 was purified by prep. HPLC-ODS (system IX) to provide compounds **17** (93 mg) and **18** (45 mg). Fraction 4-3 was further purified by prep. HPLC–ODS (system XII) and HPLC-Diol (system XIII) to give compound **3** (141 mg). Fraction 4-6 was similarly purified by prep. HPLC-ODS (system XIV) to afford compounds **1** (27 mg) and **10** (43 mg). Finally, fraction 4-7 was purified by prep. HPLC-ODS (system XV) to provide compounds **11** (143 mg) and 12 (39 mg).

3.4. Markhamioside A (*3*)

Amorphous powder, $[\alpha]_D^{31}-74.8^\circ$ (MeOH, *c* 3.23); for ^1H NMR and ^{13}C NMR of (CD_3OD) spectra, see Tables 1 and 2. Negative HR–FAB–MS, *m/z*: 593.2075 $[M-H]^-$ ($C_{25}H_{37}O_{16}$ requires 593.2081).

3.5. Markhamioside B (*9*)

Amorphous powder, $[\alpha]_D^{31}-46.5^\circ$ (MeOH, *c* 0.75); for ^1H NMR and ^{13}C NMR (CD_3OD) spectra see Tables 1 and 2. Negative HR–FAB–MS, *m/z*: 783.2704 $[M-H]^-$ ($C_{36}H_{47}O_{19}$ requires 783.2711).

3.6. Markhamioside C (*10*)

Amorphous powder, $[\alpha]_D^{31}+61.1^\circ$ (MeOH, *c* 3.48); for ^1H NMR and ^{13}C NMR (CD_3OD) spectra, see Tables 1 and 2. Negative HR–FAB–MS, *m/z*: 755.2413 $[M-H]^-$ ($C_{34}H_{43}O_{19}$ requires 755.2398).

3.7. Markhamioside D (*11*)

Amorphous powder, $[\alpha]_D^{31}-57.6^\circ$ (MeOH, *c* 2.54); for ^1H NMR and ^{13}C NMR (CD_3OD) spectra, see Tables 1 and 2. Negative HR–FAB–MS, *m/z*: 797.2501 $[M-H]^-$ ($C_{36}H_{45}O_{20}$ requires 797.2504).

3.8. Markhamioside E (*12*)

Amorphous powder, $[\alpha]_D^{31}-43.8^\circ$ (MeOH, *c* 1.71); for ^1H NMR and ^{13}C NMR (CD_3OD) spectra, see Tables 1 and 2. Negative HR–FAB–MS, *m/z*: 827.2615 $[M-H]^-$ ($C_{37}H_{47}O_{21}$ requires 827.2609).

3.9. Acid hydrolysis of markhamiosides A–E (*3*, *9*–*12*)

Compound **3** (ca 5 mg) was dissolved in 0.2 N H_2SO_4 (5 ml) and heated at 95 °C for 1 h. After cooling, the reaction mixture was extracted with Et_2O. 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). Rhamnose (R_f 0.52), apiose (R_f 0.48) and glucose (R_f 0.32) wew detected by comparison with authentic samples. By the same method, (i) compound **9** yielded rhamnose R_f 0.52),

Table 3
^{13}C NMR spectral data compounds **13**, **14** and **15** (100 MHz, CD_3OD)

C	13	14	15
1	152.8	134.2	152.4
2	103.7	95.4	103.1
3	149.3	154.6	149.2
4	142.9	155.4	142.7
5	116.1	154.6	116.0
6	109.8	95.4	109.4
1′	102.5	101.1	101.8
2′	78.8	78.8	78.8
3′	78.1	78.3	78.5
4′	71.6	71.7	71.7
5′	78.8	78.8	78.8
6′	62.6	62.6	62.6
1′	110.8	110.6	110.5
2′	78.1	78.2	78.0
3′	80.7	79.1	79.2
4′	75.5	75.2	75.4
5′	66.1	67.4	67.9
1‴		122.1	122.3
2‴		113.8	113.8
3‴		152.8	152.9
4‴		148.6	148.7
5‴		125.2	125.3
6‴		115.8	115.9
C=O		167.6	167.8
MeO-3	56.4	56.4	56.3
MeO-4		61.2	
MeO-5		56.4	
MeO-3‴		56.5	56.3

apiose (R_f 0.48) and glucose (R_f 0.32); (ii) compounds **10** and **11** yielded rhamnose (R_f 0.52), arabinose (R_f 0.41) and glucose (R_f 0.32); (iii) compound **12** yielded rhamnose (R_f 0.54), glucose (R_f 0.31) and galactose (R_f 0.27) with solvent system *n*-BuOH–C_5H_5N–H_2O (6:4:3).

3.10. Markhamioside F (*13*)

Amorphous powder, $[\alpha]_D^{31}-110.7^\circ$ (MeOH, *c* 0.28); for ^1H NMR and ^{13}C NMR (CD_3OD) spectra, see Tables 1 and 3. Negative HR–FAB–MS, *m/z*: 433.1319 $[M-H]^-$ ($C_{18}H_{25}O_{12}$ requires 433.1346).

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