Acetylated Triterpene Saponins from the Thai Medicinal Plant, *Sapindus emarginatus*

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From the pericarps of *Sapindus emarginatus* (Sapindaceae), three new acetylated triterpene saponins were isolated together with hederagenin and five known triterpene saponins, as well as one known sweet acyclic sesquiterpene glycoside, mukurozioside IIb. The structures of new compounds were elucidated as hederagenin 3-*O*-(2-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, 23-*O*-acetyl-hederagenin 3-*O*-(4-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and oleanolic acid 3-*O*-(4-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside by chemical and spectroscopic data.

Key words *Sapindus emarginatus*; Sapindaceae; triterpenoidal saponin; acetylated saponin; mukurozioside IIb

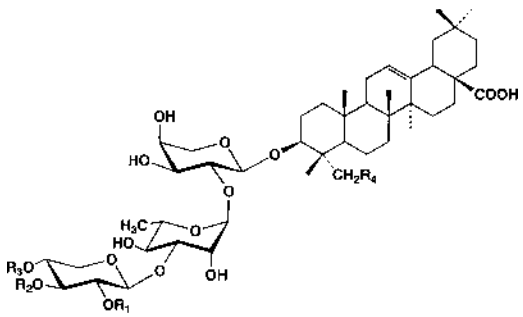
Sapindus emarginatus (Sapindaceae, Thai name: Makham-dee-khwaai) is a tall tree distributed in South and South-east Asia. In Thai traditional medicine, the pericarps are used as an antipruritic, as well as natural surfactant. In a preliminary study, antifertility has also been reported.[1] The present study deals with the isolation and structural elucidations of three new acetylated triterpene saponins (**4**, **7**, **9**) together with seven known compounds (**1**—**3**, **5**, **6**, **8**, **10**) from the pericarps of this plant.

Results and Discussion

The methanolic extract of pericarps of *S. emarginatus* was subjected to a column of a highly porous copolymer of styrene and divinylbenzene, and eluted with H_2O, MeOH and Me_2CO, successively. The portion eluted with MeOH was repeatedly chromatographed on columns of silica gel and RP-18 as well as HPLC to afford 10 compounds.

Compounds **1** and **2** have been assigned as hederagenin and hederagenin 3-*O*-α-L-arabinopyranoside,[2] respectively. The ^{1}H- and ^{13}C-NMR spectral data of compounds **3** and **5** were coincident with those of hederagenin 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (sapindoside B) and hederagenin 3-*O*-(4-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (mukurozi-saponin E_1) respectively, isolated from *Sapindus mukurossi*[3] as well as *S. delavayi.*[4] Compound **6** was elucidated as a diacetylated saponin of **3**, hederagenin 3-*O*-(3,4-di-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, previously reported from *Gliricidia sepium.*[5] The data of compound **8** were superimposable with those of oleanolic acid 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (prosapogenin CP_3) previously isolated from *Clematis chinensis.*[6] Compound **10** was elucidated as mukurozioside IIb by comparison of NMR spectral data,[7] and was previously isolated from *S. mukurossi.* This compound was ascribed as the first sweet acyclic sesquiterpene glycoside by Chung *et al.*[8]

Compound **4** has the molecular formula, $C_{48}H_{76}O_{17}$, determined by HR-FAB mass spectrometry. Inspection of the ^{13}C-NMR spectral data revealed the presence of three sugar moieties (anomeric carbons at δ 104.5, 104.5, 101.1) along with 30 carbon signals for an aglycone which was identified as hederagenin.[3] In addition, a portion signal at δ 2.00 (3H, s) in the ^{1}H-NMR spectrum as well as the carbon signals at δ 170.5 and 21.2 indicated the presence of an acetyl group. The chemical shifts of C-3 (δ 81.3) and C-28 (δ 180.2) suggested that **4** is a monodesmoside with a glycosyl linkage at C-3. Alkaline hydrolysis of **4** gave **3** which was identified by TLC and spectral data. The ^{1}H-NMR spectrum showed the signals at δ 5.05 (1H, d, J=6.6 Hz), δ 5.31 (1H, d, J=7.8 Hz)



	R_1	R_2	R_3	R_4
3	H	H	H	OH
4	Ac	H	H	OH
5	H	H	Ac	OH
6	H	Ac	Ac	OH
7	H	H	Ac	OAc
8	H	H	H	H
9	H	H	Ac	H

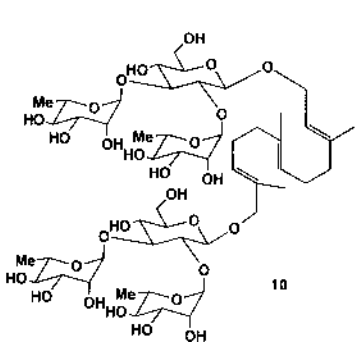


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Table 1. ^{1}H-NMR Spectral Data of Compounds **4**, **7** and **9** (400 MHz, C_5D_5N)

H	4	7	9
H-3	4.27[a)]	3.93, dd (11.7, 3.9)	3.20[a)]
H-12	5.45, br s	5.45, br s	5.45, br s
H-18	3.26, dd (13.7, 3.7)	3.28, dd (13.7, 3.7)	3.20, dd (11.2, 3.4)
H-23	4.27[a)] 3.90, d (10.7)	4.55[a)]	1.29, s
H-24	1.09, s	1.11, s	1.09, s
H-25	0.92, s	0.87, s	0.82, s
H-26	1.00, s	0.99, s	0.96, s
H-27	1.22, s	1.29, s	1.28, s
H-29	0.91, s	0.92, s	0.94, s
H-30	0.98, s	0.99, s	0.99, s
H-Ac	2.00, s	2.05, s 1.91, s	1.92, s
H-1 Ara(p)	5.05, d (6.6)	4.90, d (6.6)	4.85, d (6.1)
H-1 Rham	6.26, br s	6.32, br s	6.20, br s
H-1 Xyl	5.31, d (7.8)	5.50, d (7.8)	5.33, d (8.1)

J (Hz) in parentheses. *a*) Overlapped signals.

Table 2. ^{13}C-NMR Spectral Data of the Aglycone Moiety of Compounds **4**, **7** and **9** (100 MHz, C_5D_5N)

C	4	7	9
1	39.0	38.7	38.9
2	26.3	26.2	26.6
3	81.3	82.3	88.7
4	43.6	42.4	39.5
5	47.8	48.6	56.0
6	18.1	18.4	18.5
7	33.2	33.2	33.2
8	39.7	39.7	39.7
9	48.1	48.3	48.0
10	36.9	36.9	37.0
11	23.8	23.7	23.7
12	122.6	122.5	122.5
13	144.8	144.8	144.6
14	42.1	42.1	42.1
15	28.3	28.2	28.3
16	23.7	23.6	23.7
17	46.6	46.7	46.6
18	42.0	42.0	41.9
19	46.4	46.4	46.4
20	30.9	30.9	30.9
21	34.2	34.2	34.2
22	32.9	33.0	33.2
23	64.1	66.0	28.2
24	14.0	13.5	17.1
25	16.0	15.9	15.5
26	17.4	17.4	17.3
27	26.1	25.9	26.1
28	180.2	180.2	180.2
29	33.2	33.2	33.3
30	23.7	23.7	23.7

Table 3. ^{13}C-NMR Spectral Data of the Sugar and Acyl Moieties of Compounds **4**, **7** and **9** (100 MHz, C_5D_5N)

C	4	7	9
Ara(p)-1	104.5	105.2	105.1
2	75.5	75.3	75.7
3	75.3	75.0	74.8
4	69.5	69.5	69.2
5	66.0	66.3	65.5
Rham-1	101.1	101.5	101.5
2	71.6	71.7	71.8
3	82.1	83.4	82.6
4	72.1	72.9	72.9
5	70.0	69.6	69.6
6	18.4	18.4	18.4
Xyl-1	104.5	107.2	107.0
2	76.2	76.0	75.5
3	74.9	74.9	74.4
4	71.1	73.0	72.8
5	67.1	63.4	63.4
$CH_3\underline{C}O$	170.5	170.7 170.6	170.5
$\underline{C}H_3CO$	21.2	20.9 20.8	20.8

and δ 6.26 (1H, br s) for anomeric protons of α-arabinopyranosyl, β-xylopyranosyl and α-rhamnopyranosyl units, respectively. Negative FAB-MS of **4** exhibited a significant fragment ion at *m*/*z* 749 $[M-\text{pentose(Ac)}]^-$, indicating that the acetyl group is located on the terminal xylopyranosyl unit. Comparison of the ^{13}C-NMR spectral data of **4** with those of **3** revealed the downfield shift of C-2 of the xylopyranosyl unit (+1.2 ppm), and an upfield shift for C-1 and C-3 (−2.9, −3.4 ppm), respectively, while the other carbon signals remained almost unchanged. On the basis of this evidence, the acetyl group is located at C-2 of the xylopyranosyl unit. Consequently, the structure of **4** was elucidated as hederagenin 3-*O*-(2-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

The molecular formula of compound **7** was determined as $C_{50}H_{78}O_{18}$ by HR-FAB mass spectrometry. The ^{1}H- and ^{13}C-NMR spectra of **7** were very similar to those of **3**. However, the signals for two acetyl groups were observed in the ^{1}H-NMR spectrum at δ 2.05 (3H, s) and δ 1.91 (3H, s), as well as the signals at δ 170.7, 170.6, 20.9 and 20.8 from the ^{13}C-NMR spectrum. Comparison of the ^{13}C-NMR spectral data of **7** with those of **5** showed the signals due to the same sugar moiety, indicating the presence of one acetyl group at C-4 of the xylopyranosyl unit in **7**. The other acetyl group was assigned to attach at C-23 (δ 66.0), since the chemical shifts of C-23, -4, -3, -5 and -24 changed by +1.9, −1.2, +1.0, +0.8 and −0.5 ppm, respectively. The HMBC spectrum provided further confirmation of this acetyl group from the correlation between H-23 (δ 4.55) and δ 170.7 of an acetyl group. Additionally, negative FAB-MS displayed a fragment ion at *m*/*z* 791 $[M-\text{pentose(Ac)}]^-$. On the basis of this forgoing data, the structure of **7** was elucidated as 23-*O*-acetyl-hederagenin 3-*O*-(4-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Compound **9** has the molecular formula, $C_{48}H_{76}O_{16}$, established by HR-FAB mass spectrometry. ^{13}C-NMR spectral data revealed that **9** contains the same sugar moiety as **5** with a different aglycone. The absence of the methylene signal at δ 64.1 (C-23) in addition to the downfield shift of C-3 from 81.3 in **5** to 88.7 in **9** indicated that this aglycone is oleanolic acid. Alkaline hydrolysis of **9** gave **8**. Moreover, negative FAB-MS exhibited a quasi-molecular ion peak at *m*/*z* 907 $[M-H]^-$ together with an other peak at *m*/*z* 733 $[M-\text{pentose(Ac)}]^-$. Consequently, the structure of **9** was assigned as oleanolic acid 3-*O*-(4-*O*-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

The water solubilities of compound **3** and related saponins

which have been reported from *Sapindus mukurossi*, caused enhancement of the absorption of sodium ampicillin from rat intestine and rectum.[3] Further investigations of the isolated compounds are in progress.

Experimental

NMR spectra were recorded in C_5D_5N or 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 Diol-120A (8.0×300 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rate was 3 ml/min. 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.

Plant Material The fruit of *Sapindus emarginatus* Wall. was purchased from a traditional medicine market in Bangkok, Thailand, in October 2000. The identification of the plant was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. A voucher sample is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Extraction and Isolation The pericarps (1.6 kg) of *S. emarginatus* were extracted with hot MeOH. After removal of the solvent by evaporation, the dried residue (660 g) was subjected to a column of highly porous copolymer of styrene and divinylbenzene and eluted with H_2O, MeOH and Me_2CO, successively. The portion eluted with MeOH (90 g from 389 g) was subjected to a column of silica gel using a gradient system [(CH_2Cl_2–MeOH (9 : 1 to 1 : 1)] affording eight fractions. Fraction 1 (900 mg) was repeatedly chromatographed on a column of silica gel [CH_2Cl_2–MeOH (19 : 1)] to provide compounds **1** (66 mg) and **6** (288 mg). Fraction 2 (4.9 g) was subjected to a column of RP-18 [70—100% MeOH], then purified by prep. HPLC-Diol 120A [MeCN] to give compounds **2** (12 mg), **5** (2.1 g), **7** (45 mg) and **9** (57 mg). Fraction 3 (4.0 g) was separated on a RP-18 column [70—100% MeOH] to give compounds **4** (54 mg) and **8** (115 mg). Fraction 4 (13.7 g) was chromatographed on a column of RP-18 [70—100% MeOH] to provide compound **3** (3.6 g). Finally, fraction 7 (21.8 g) was chromatographed on a column of RP-18 [60—100% MeOH] to provide compound **10** (6.2 g).

Hederagenin 3-*O*-(2-*O*-Acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (4) White amorphous powder. $[\alpha]_D^{22}$ +5.9° (*c*=3.7, MeOH); ^{1}H-NMR: Table 1; ^{13}C-NMR: Tables 2 and 3; negative HR-FAB-MS, *m/z*: 923.5028 $[M-H]^-$ ($C_{48}H_{75}O_{17}$ requires 923.5003).

23-*O*-Acetyl-hederagenin 3-*O*-(4-*O*-Acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (7) White amorphous powder. $[\alpha]_D^{22}$ −10.4° (*c*=0.7, MeOH); ^{1}H-NMR: Table 1; ^{13}C-NMR: Tables 2 and 3; negative HR-FAB-MS, *m/z*: 965.5118 $[M-H]^-$ ($C_{50}H_{77}O_{18}$ requires 965.5109).

Oleanolic Acid 3-*O*-(4-*O*-Acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (9) White amorphous powder. $[\alpha]_D^{22}$ −20.2° (*c*=3.8, MeOH); ^{1}H-NMR: Table 1; ^{13}C-NMR: Tables 2 and 3; negative HR-FAB-MS, *m/z*: 907.5090 $[M-H]^-$ ($C_{48}H_{75}O_{16}$ requires 907.5054).

Alkaline Hydrolysis of Compounds 4, 7 and 9 Compounds **4** (20 mg) and **7** (18 mg) were refluxed with 2% KOH/MeOH for 30 min. The reaction mixtures were neutralized with Amberlite MB3 resin and concentrated to dryness, affording **3** (8 mg from **4**, and 5 mg from **7**), whose structure were identified by TLC and spectral analysis. By the same method, **9** (20 mg) provided **8** (7 mg).

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