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New phragmalin-type limonoids from *Swietenia macrophylla* King

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ABSTRACT

The fruit of *Swietenia macrophylla* is commonly known as "sky fruit". The fruit, which contains flavonoids and saponins, is processed commercially into a wide range of health foods and healthcare products. The fruit concentrate is used traditionally to improve blood circulation and impart a healthy skin. In this paper, we describe the isolation and structure elucidation of three new phragmalin *ortho* esters, named swietephragmin H-J (**1**–**3**), and a new polyhydroxylated phragmalin, named swietemacrophine (**4**), from the leaves of *S. macrophylla*. The structures of the compounds were elucidated by spectroscopic methods, including HRESIMS, ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra. This is the first report of phragmalin *ortho* esters and a polyhydroxylated phragmalin from this plant.

1. Introduction

Swietenia macrophylla King (Meliaceae), also known as Big-leaf mahogany, is a tropical timber tree that can grow to a height of 40–60 m. The tree is native to the tropical regions of the Americas, including Southern Mexico, Central America and Bolivia. This tree produces a fruit commonly called "sky fruit" because the latter seems to hang upwards from the tree. Sky fruit has been processed commercially to a wide range of health foods and healthcare products. The fruit concentrate, in particular, has been used traditionally to improve blood circulation and skin condition. Previous phytochemical investigations have revealed the presence of limonoids such as swietenine, swietenolide, 8, 30-epoxyswietenine acetate, swietenine acetate, swietenolide diacetate, swietenolide tiglate, augustineolide and 3β, 6-dihydroxydihydrocarapin in the seeds (Chakravarty & Chatterjee, 1955; Chakravarty, Chatterjee, & Krishnagar, 1957; Chan, Tang, & Toh, 1976; Connolly, Henderson, McCrindle, Overton, & Bhacca, 1964; Connolly & Labbe, 1980; Ghosh, Chakrabartty, & Chatterjee, 1960; Mootoo et al., 1999). Some of these limonoids have been shown to exhibit antimalarial (Jean, Njikam, Johnson, Leonardo, & Pascal, 2000) and insect antifeedant activities ((Nsiama et al., 2008). The leaves have been reported to yield essential oils which contain himachalene, germacrene D, germacrene A, cadina-1,4-diene, hexadecanoic acid, and ethyl hexadecanoate (Marisi et al., 2003). In the course of our study to find new natural products from Malaysian medicinal plants, we isolated four new phragmalin-type limonoids, named swietephragmin H-J (**1**–**3**) and swietemacrophine (**4**) from the dichloromethane extract of the leaves of this plant. The structures of **1**–**4** were elucidated by spectroscopic methods (Fig. 1).

2. Materials and methods

2.1. General

NMR spectra were recorded in $CDCl_3$ using a JOEL ECA-400 spectrometer [^{1}H (400 MHz) and ^{13}C (100 MHz)], with TMS as the internal standard. HRESIMS spectra were recorded using a Micro TOF-Q mass spectrometer in positive-ion mode. IR spectra were recorded by a Perkin–Elmer System 2000 FT-IR spectrometer. UV spectra were recorded on a U-2000 Hitachi UV–Visible spectrophotometer. Optical rotations were measured using a JASCO DIP 370 digital polarimeter. TLC was carried out on Merck 60 GF_{254} silica gel plates (absorbent thickness 0.25 mm). Column chromatography was performed using silica gel (Merck, 230–400 mesh ASTM). All solvents used were of analytical grade and were distilled before use.

2.2. Plant material

Leaves of *S. macrophylla* were collected from the campus of Universiti Sains Malaysia, Penang, Malaysia, in January 2006, and the species was identified by staff of the School of Biological Sciences of the university where a voucher specimen (code: 10942) has been deposited.

2.3. Extraction and isolation

The air-dried and powdered leaves of *S. macrophylla* (1 kg) were extracted successively with *n*-hexane, CH_2Cl_2 and MeOH (each

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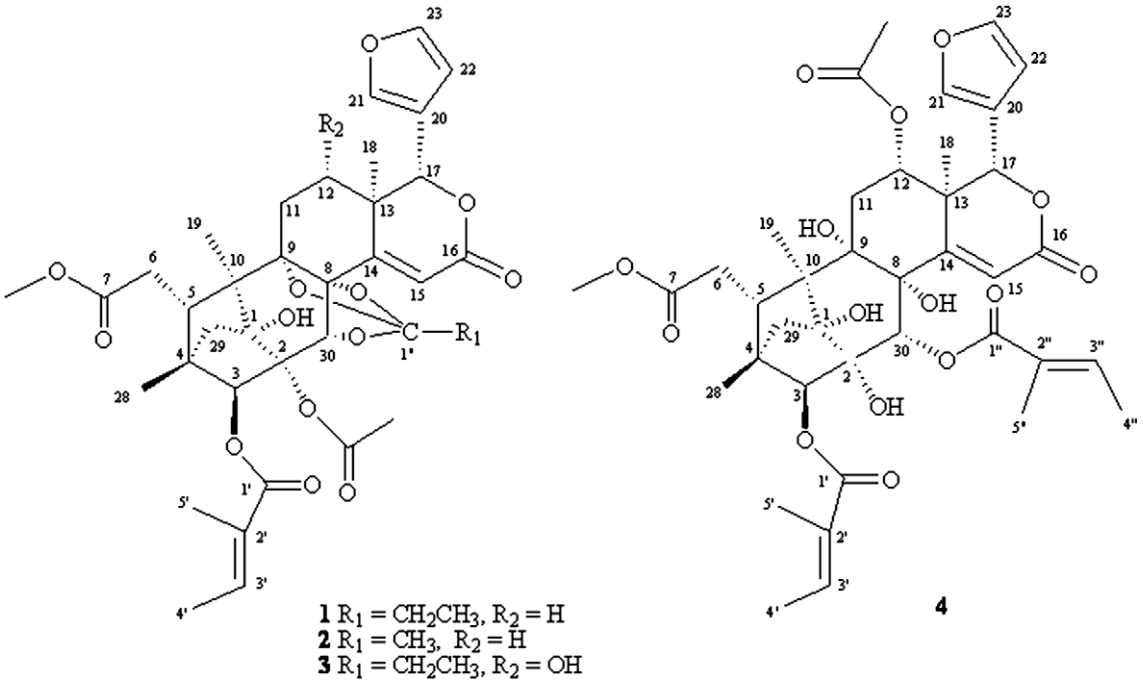

Fig. 1. Structures of compounds **1–4**.

Table 1
^{1}H NMR data of 1–4 (400 MHz, $CDCL_3$).

No.	1	2	3	4
	δ_H; mult; J(Hz)	δ_H; mult; J(Hz)	δ_H; mult; J(Hz)	δ_H; mult; J(Hz)
3	5.33 (1H, s)	5.32 (1H, s)	5.31 (1H, s)	4.60 (1H, s)
4				
5	2.48 (1H, d, 11.0)	2.47 (1H, d, 11.4)	2.28 (1H, d, 11.4)	2.42 (1H, d, 4.8)
6a	2.36 (1H, d, 11.0)	2.35 (1H, d, 11.4)	2.37 (1H, d, 11.4)	2.45 (lH, br d, 18.4)
6b	2.39 (1H, br s)	2.38 (1H, br s)	2.39 (1H, brs)	3.32 (lH, m)
11α	1.87 (lH, m)	1.92 (lH, m)	1.92 (1H, m)	1.91 (lH, m)
11β	2.14 (1H, dt, 15.2, 3.6)	2.13 (1H, dt, 15.1, 3.6)	2.32 (lH, m)	2.09 (1H, m)
12α	1.18 (1H, dt, 14.4, 3.6)	1.16 (1H, dt, 14.6, 3.6)	–	–
12β	1.55 (1H, d, 14.4)	1.57 (1H, m)	3.91 (1H, d, 13.8)	4.76 (1H, dd, 13.7, 4.6)
15	6.33 (lH, s)	6.33 (1H, s)	6.35 (1H, s)	6.13 (lH, s)
17	5.72 (1H, s)	5.71 (1H, s)	5.83 (1H, s)	5.82 (1H, s)
I8	1.36 (3H, s)	1.40 (3H, s)	1.44 (3H, s)	1.56 (3H, s)
19	1.30 (3H, s)	1.29 (3H,s)	1.33 (3H, s)	1.41 (3H, s)
21	7.49 (1H, br s)	7.48 (1H, br s)	7.67 (1H, brs)	7.45(lH, br s)
22	6.47 (1H, br s)	6.45 (1H, br s)	6.62 (1H, br s)	6.54 (lH, br s)
23	7.42 (1H, br s)	7.41 (lH, br s)	7.53 (1H, br s)	7.37 (lH, br s)
28	0.73 (3H, s)	0.72 (3H, s)	0.71 (3H, s)	0.86 (3H, s)
29a	1.73(1H, d, 11.4)	1.72 (1H, d, 11.5)	1.73 (1H, d, 11.4)	1.72 (lH, d, 11.0)
29b	1.95 (1H, d, 11.4)	1.94 (1H, d, 11.5)	1.96 (1H, d, 11.4)	1.98 (1H, d, 11.0)
30	5.46 (1H, s)	5.45 (1H, s)	5.43 (1H, s)	5.78 (lH, s)
OMe	3.68 (3H, s)	3.67 (3H, s)	3.70 (3H, s)	3.76 (3H, s)
2-OAc	2.19 (3H, s)	2.18 (3H, s)	2.19 (3H, s)	–
12-OAc	–	–	–	1.52 (3H, s)
1-OH	3.47 (lH, s)	3.48 (1H, s)	3.41 (1H, s)	3.89 (1H, s)
2-OH	–	–	–	3.57 (lH, s)
8-OH	–	–	–	4.15 (1H, s)
9-OH	–	–	–	4.72 (1H, s)
12-OH	–	–	1.93 (1H, s)	–
3-O-tigloyl				
3′	6.62 (1H, qq, 6.9, 1.4)	6.62 (1H, qq, 6.8, 1.4)	6.57 (1H, qq, 6.8, 1.4)	6.85 (lH, qq, 6.9, 1.4)
4′	1.71 (3H, br d, 6.9)	1.70 (3H, br d, 6.8)	1.68 (3H, br d, 6.8)	1.81 (3H, br d, 6.9)
5′	1.87 (3 H, br s)	1.86 (3H, br s)	1.85 (3H, br s)	1.87 (3H, s)
30-O-tigloyl				
3″	–	–	–	7.04 (lH, qq, 6.9, 1.4)
4″	–	–	–	1.75 (3H, br d, 6.9)
5″	–	–	–	1.93 (3H, s)
Orthoester				
2″	1.94 (2H, q, 7.6)	1.67 (3H, s)	1.94(2H, q, 7.8)	–
3″	1.03 (3H, t, 7.6)	–	1.03 (3H, t, 7.8)	–

solvent 5 L × 5 days) at room temperature. The CH_2Cl_2 extract was concentrated under reduced pressure at 40 °C to afford a dark greenish residue (18.2 g) which was fractionated by a silica gel column, eluting with *n*-hexane-CH_2Cl_2-EtOAc (1:0:0–0:0:1, v/v) to afford 22 fractions (A–V). Fraction G (1.06 g, dark green liquid, eluted with *n*-hexane-CH_2Cl_2 1:1 v/v) was further fractionated with a silica gel column, eluting with *n*-hexane-EtOAc (1:0–0:1, v/v) to give 11 sub-fractions (G1–G11). Sub-fractions G5 (80 mg) and G6 (90 mg) were purified by preparative TLC, with a *n*-hexane-EtOAc (2:1, v/v) solvent system to give swietephragmin H (**1**) (29 mg, 0.00066% w/w of fresh leaves) and swietephragmin I (**2**) (32 mg, 0.00073% w/w of fresh leaves), respectively. Fraction P (0.69 g), a brownish solid which eluted with CH_2Cl_2-EtOAc (1:1, v/v), was further fractionated with a silica gel column, eluting with *n*-hexane-EtOAc (1:0–0:1, v/v) to yield five sub-fractions (P1–P5). Sub-fraction P2 (0.35 g), which eluted with *n*-hexane-EtOAc (8:2–7:3, v/v), was separated by preparative TLC, using *n*-hexane-EtOAc (3:2, v/v) to afford another five fractions (P2F1–P2F5). Fractions P2F2 (70 mg) and P2F5 (60 mg) were purified by preparative TLC, utilising $CHCl_3$-EtOAc (9:1, v/v) as the solvent system to give swietemacrophine (**4**) (35 mg, 0.00080% w/w of the fresh leaves) and swietephragmin J (**3**) (12 mg, 0.00027% w/w of the fresh leaves), respectively.

Swietephragmin H (**1**): pale yellow oil; $[\alpha]_D^{28}$ +32.8 (*c* 0.042, MeOH); HRESIMS *m/z* 719.2669 (calculated for $C_{37}H_{44}O_{13}Na$ 719.2674); IR (ZnSe) ν_{max} 3547, 2971, 1726, 1241, 1027 and 872 cm^{-1}; UV (MeOH) λ_{max} (log ε) 240 nm (4.07). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 2.

Swietephragmin I (**2**): colourless oil; $[\alpha]_D^2$8 +38.3 (*c* 0.025, MeOH); HRESIMS *m/z* 705.2539 (calculated for $C_{37}H_{44}O_{13}Na$ 705.2518); IR (ZnSe) ν_{max} 3574, 2954,1725, 1236, 1025 and 865 cm^{-1}; UV (MeOH) λ_{max} (log ε) 240 nm (4.13). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 2.

Swietephragmin J (**3**): colourless oil; $[\alpha]_D^2$8 0.0 (too small to be measured); HRESIMS *m/z* 735.2638 (calculated for $C_{37}H_{44}O_{13}Na$ 735.2623); IR (ZnSe) ν_{max} 3453, 2927, 2856, 1732, 1262, 1048 and 875 cm^{-1}; UV (MeOH) λ_{max} (log ε) 240 nm (4.07). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 2.

Swietemacrophine (**4**): yellow gum; $[\alpha]_D^2$8 −29.7° (*c* 0.015, $CHCl_3$); HRESIMS *m/z* 779.2922 (calculated for $C_{39}H_{48}O_{15}Na$ 779.2885); IR (ZnSe) υ_{max} 3434, 2927, 2860, 1723, 1261, 1025 and 875 cm^{-1}; UV (MeOH) λ_{max} 240 nm (log ε 4.17). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 2.

3. Results and discussion

3.1. Structure elucidation of the new phragmalin-type limonoids

Compound **1** was isolated as pale yellow oil. The overall structure of **1** was elucidated by spectroscopic analyses, including high resolution mass spectrometry and NMR spectroscopy (^1H NMR, ^{13}C NMR, DEPT experiments, ^1H–^1H-COSY, HMQC, HMBC and NOESY). The positive-ion mode HRESIMS of 1 showed a pseudo-molecular ion peak $[M + Na]^+$ at *m/z* 719.2669 (calculated for $C_{37}H_{44}O_{13}Na$: 719.2674) which was consistent with the molecular formula of $C_{37}H_{44}O_{13}$. This formula was in agreement with the ^1H NMR (Table 1) and ^{13}C NMR data (Table 2). The molecular formula suggested that 1 had 16 degrees of unsaturation, with eight of these due to C=O esters and C=C, and the other eight due to eight rings. The IR spectrum showed absorption bands at 3547, 1726 and 872 cm^{-1}, indicating the presence of hydroxyl, ester carbonyl and furanyl groups, respectively. The UV absorption at 240 nm also indicated the presence of an α,β-unsaturated ester group.

The ^{13}C NMR and DEPT experiments showed that **1** possessed eight methyl, five methylene, nine methine and 15 quaternary carbons. Analysis of ^1H and ^{13}C NMR spectra, in association with HMQC and HMBC correlations, enabled the recognition of a trisubstituted double bond [δ_H 6.33 (1H, s); δ_C 123.3 (d), 151.6 (s)], three oxymethines [δ_H 5.33 (1H, s), δ_C 84.8 (d); δ_H 5.46 (1H, s), δ_C 73.9 (d); and δ_H 5.72 (1H, s), δ_C 79.8 (d)], three tertiary methyls [δ_H 1.30 (3H, s), δ_C 15.6 (q); δ_H 1.36 (3H, s), δ_C 19.4 (q); and δ_H 0.73 (3H, s), δ_C 14.0 (q)], an acetyl group [δ_H 2.19 (3H, s); δ_C 21.9 (q), 169.9 (s)], a tigloyl group [δ_H 6.62 (1H, qq, *J* = 6.9, 1.4 Hz), 1.71 (3H, br d, *J* = 6.9 Hz) and 1.87 (3H, br s); δ_C 136.0 (d), 13.7 (q),13.0 (q), 167.7 (s), 130.7 (s)] and a methoxycarbonyl group [δ_H 3.68 (3H, s); δ_C 52.2 (q), 173.7 (s)].

The ^1H and ^{13}C NMR spectroscopic data of **1** were characteristic of those of a phragmalin limonoid (Anne & David, 1983). The appearance of a low-field one-proton singlet at δ_H 5.72, assignable to H-17, and the coupling of the methylene protons at δ_H 2.36 (1 H, d, *J* = 11.0 Hz) and 2.39 (1 H, br s) attached to C-6 with the H-5 proton at δ_H 2.48 (1 H, d, *J* = 11.0 Hz), were evidence that **1** was a ring B, D-*seco* limonoid. Further, the absence of signals due to two

Table 2
^{13}C NMR data of 1–4 (100 MHz, $CDCl_3$).

No.	δc; mult[a]			
	1	2	3	4
1	84.7 (s)	84.6 (s)	84.5 (s)	83.9 (s)
2	83.9 (s)	84.1 (s)	83.7 (s)	76.3 (s)
3	84.8 (d)	84.7 (d)	84.8 (d)	88.8 (d)
4	44.8 (s)	44.8 (s)	44.8 (s)	43.0 (s)
5	39.7 (d)	39.7 (d)	40.1 (d)	38.7 (d)
6	33.8 (t)	33.8 (t)	33.8 (t)	33.4 (t)
7	173.7 (s)	173.7 (s)	174.2 (s)	174.6 (s)
8	84.0 (s)	83.9 (s)	83.9 (s)	72.9 (s)
9	86.0 (s)	86.3 (s)	86.0 (s)	78.4 (s)
10	48.2 (s)	48.1 (s)	48.3 (s)	48.3 (s)
11	26.4 (t)	26.4 (t)	34.6 (t)	31.9 (t)
12	29.5 (t)	29.5 (t)	67.2 (d)	68.6 (d)
13	37.9 (s)	37.9 (s)	44.7 (s)	43.3 (s)
14	151.6 (s)	151.5 *(d)*	151.1 (s)	160.1 (s)
16	123.3 (d)	123.4(d)	124.7 (d)	121.3(d)
16	163.3(s)	163.2 (s)	162.8 (s)	163.4(s)
17	79.8 (d)	79.8 (d)	78.3 (d)	77.8 (d)
18	19.4 (q)	19.5 (q)	13.1 (q)	16.9 (q)
19	15.6 (q)	15.5 (q)	15.6 (q)	13.8 (q)
20	119.S(s)	119.6 (s)	121.5 (s)	121.6 (s)
21	141.9 (d)	141.8 (d)	142.3 (d)	142.1 (d)
22	110.1 (d)	110.1 (d)	109.8 (d)	110.6(d)
23	143.1 (d)	143.1 (d)	145.0(d)	142.8 (d)
28	14.0 (q)	14.0 (q)	14.0 (q)	14.7 (q)
29	39.2 (t)	39.2 (t)	39.5 (t)	39.8 (t)
30	73.9 (d)	73.9 (d)	74.1 (d)	70.4 (d)
OMe	52.2 (q)	52.2 (q)	52.3(q)	52.2 (q)
2-OAc	169.9(s)	169.9 (s)	170.1 (s)	–
	21.9 (q)	21.9 (q)	22.0 (q)	–
12-OAc	–	–	–	170.8 (s)
	–	–	–	20.0 (q)
3-O-tigloyl				
1'	167.7(s)	167.6 (s)	167.6 (s)	168.1 (s)
2'	130.7(s)	130.7 (s)	131.1 (s)	128.0 (s)
3'	136.0 (d)	136.0 (ct)	135.6 (d)	139.1 (d)
4'	13.7 (q)	13.7 (q)	13.7 (q)	14.7 (q)
5′	13.0 (q)	13.0 (q)	12.8 (q)	12.4 (q)
30-O-tigloyl				
1″	–	–	–	168.9 (s)
2″	–	–	–	129.5 (s)
3″	–	–	–	141.2 (d)
4″	–	–	–	14.7 (q)
5″	–	–	–	12.4 (q)
Orthoester				
1″	121.4 (s)	119.8 (s)	121.8 (s)	–
2″	23.3 (t)	16.6 (q)	23.2 (t)	–
3″	7.9 (q)		7.9 (q)	–

[a] ^{13}C multiplicities were determined by DEPT experiments.

tertiary methyls at 4β (C-29) and 8β (C-30) in the basic limonoid skeleton, and the presence of the two proton signals at δ_H 1.73 and 1.95 (each 1H, d, J = 11.4 Hz), which were characteristic of the 29-methylene group, strongly suggested that **1** had a tricyclo[3.3.1 ^{2,10}.1 ^{1,4}]decane ring system.

All naturally occurring limonoids contain a furan ring attached to the D-ring, at C-17 (Amit & Shailendra, 2006). In 1, the signature monosubstituted furan moiety had characteristic chemical shifts [δ_H 6.47 (1H, br s, H-22), 7.42 (1H, br s, H-23), 7.49 (1H, br s, H-21); δ_C 110.0 (d) (C-22), 119.8 (s) (C-20), 143.1 (d) (C-23) and 141.9 (d) (C-21)]. The methine proton at δ_H 6.33 (1H, s, H-15) showed HMBC correlation (Fig. 2) to C-14 [δ_C 151.6 (s)], and also to a carbonyl carbon at δ_C 163.3 (s) (C-16), whilst the methyl protons at δ_H 1.36 (3H, s) assigned to C-18 [δ_C 19.4 (q)] showed HMBC correlations to C-12 [δ_C 29.5 (t)], C-13 [δ_C 37.9 (s)] and C-14. Further, two geminal protons at δ_H 1.87 (1H, m) and 2.14 (1H, dt, J = 15.2, 3.6 Hz) which showed HMBC correlation to C-13 were assigned to C-11 [δ_C 26.4 (t)]. These correlations, together the methine proton at δ_H 5.72 (1H, d) assigned to C-17 [δ_C 79.8 (d)] which correlated to C-12, C-14, and the furanyl carbons C-21 and C-22 through HMBC, characterised the C and D rings of **1**.

The two geminal protons at δ_H 1.73 and 1.95, which were assigned to C-29 [δ_C 39.2 (t)] showed HMBC correlations to C-2 [δ_C 83.9 (s)], C-5 [δ_C 39.7 (d)] and C-10 [δ_C 48.2 (s)], whilst the methyl protons at δ_H 1.30 (3H, s) which showed HMBC correlations to C-5 and C-9 [δ_C 86.0 (s)] was assigned to H_3-19. Further, an oxymethine proton at δ_H 5.33 (1H, s) which showed HMBC correlations to C-2, C-28 [δ_C 14.0 (q)], C-29, C-30 [δ_C 73.9 (d)] and tigloyl carbonyl carbon C-1′ [δ_C 167.7 (s)] was assigned to C-3 [δ_C 84.8 (d)]. The proton signal at δ_H 3.47 (1H, s), which was determined to be that of a hydroxyl proton, showed no HMQC correlation but was correlated to C-1 [δ_C 84.7 (s)] in HMBC, thus confirming the position of the hydroxyl group at C-1. The NOESY (Fig. 3) correlations between the acetyl protons at δ_H 2.19 (3H, s) and two oxymethine protons, H-3 and H-30 [δ_H 5.46 (1H, s)], placed the acetyl group at the C-2 position.

A typical orthoester carbon signal was observed at δ_C 121.4 (s) (C-1″) (Saad, Iwagawa, Doe, & Nakatani, 2003), indicating that **1** is a phragmalin-type limonoid with an orthoester group. HMBC correlations between an ethyl group containing C-2″ [δ_C 23.3 (t), δ_H 1.94 (2H, q, J = 7.6 Hz)] and C-3″ [δ_C 7.9 (q), δ_H 1.03 (3H, t, J = 7.6 Hz)] and C-1″ suggested the presence of an orthopropionate group in the molecule. The chemical shifts of C-8 [δ_C 84.0 (s)], C-9 and C-30 suggested their linkages to oxygen atoms. These oxygenated carbons were positioned similarly to those in swietephragmins A–G (Abdelgaleil, Doe, Morimoto, & Nakatani, 2006). The presence of the 1-OH group, and the existence of the trisubstituted C_{14}–C_{15} double bond, further supported the assignment of the orthoester group to the 8,9,30-position. The correlations above characterised the tricyclo[3.3.1^{2,10}.1^{1,4}] decane ring system in **1**.

The relative stereochemistry of **1** was determined by analysing the correlations observed in the NOESY spectrum as shown in a computer generated 3D drawing (Fig. 3). Limonoids are stereochemically homogenous compounds since they have a prototypical structure that either contains or is derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton (Amit & Shailendra, 2006). The H-17 configuration is found to be exclusively β in all known phragmalins (Cui et al., 2005; Wu et al., 2004). For compound **1**, there were NOESY correlations between H-12β/H-5/H_3-28, H-17/H-5/H-12β and H-15/H-30, indicating these protons were β-oriented. Thus, the furanyl group was in the α-configuration. The NOESY correlations between H_3-18/H-12α, 1-OH/H_3-19 and H-3/2-OAc suggested that H-3, H_3-18, H_3-19 and 2-OAc were in the α-orientation.

Compound **2** was obtained as a colourless oil. The positive-ion mode HRESIMS of **2** showed a pseudo-molecular ion peak $[M + Na]^+$ at m/z 705.2539 (calculated for $C_{36}H_{42}O_{13}Na$ 705.2518), suggesting a molecular formula of $C_{36}H_{42}O_{13}$. The IR spectrum showed absorption bands at 3574, 1725 and 865 cm^{-1}, indicating the presence of hydroxyl, ester carbonyl and furanyl groups, respectively. The UV spectrum showed an absorption at 240 nm similar to that of **1**. The NMR spectroscopic data of **2** (Tables 1 and 2) were also very similar to those of compound **1**, with the only difference observed in the orthoester group, where the orthoester carbon, C-1" [δ_C 119.8 (s)] was attached to a methyl group [δ_H 1.67 (3H, s), δ_C 16.6 (q) (C-2″)] in **2** rather than to an ethyl group

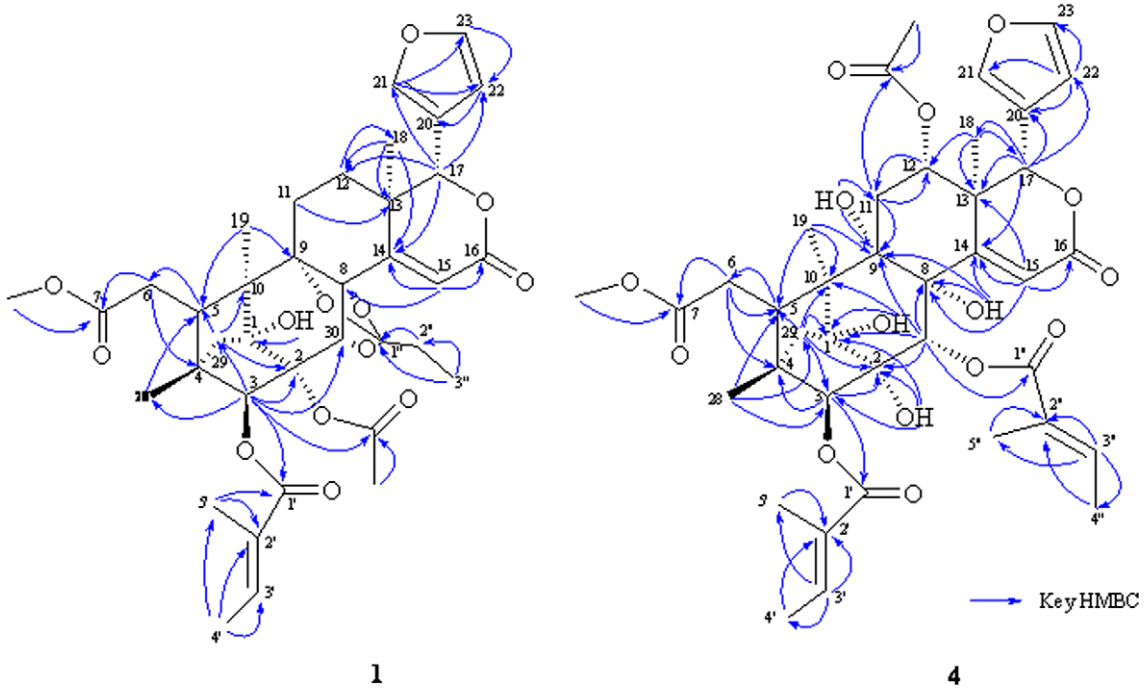

Fig. 2. Selected HMBC correlations of compounds **1** and **4**.

as in **1**. The similarity in NMR spectroscopic data between **2** and **1** also suggested similar stereochemistry in the core skeletons of **2** and **1**, with H-5, H-12$_\beta$, H-17 and H$_3$-28 in the β-orientation, and H-3, H$_3$-18, H$_3$-19, 1-OH, 2-OAc and the furanyl group in the α-orientation.

Compound **3** was isolated as a colourless oil. The HRESIMS of **3** showed a pseudo-molecular ion peak $[M + Na]^+$ at *m/z* 735.2638 (calculated for $C_{37}H_{44}O_{14}Na$ 735.2623), suggesting a molecular formula of $C_{37}H_{44}O_{14}$. The formula was consistent with the 1D NMR data (Tables 1 and 2). The IR spectrum showed absorption bands at 3453 cm^{-1} (hydroxyl group), 1732 cm^{-1} (ester carbonyl group) and 875 cm^{-1} (furanyl group). The α,β-unsaturated ester group gave an UV absorption at 240 nm. Comparison of the spectroscopic data for **3** with those of **1** showed similarities, suggesting that they are structurally similar, with the major difference being the location of a hydroxyl group at C-12 [δ_C 67.2 (d)] in 3 in place of a hydrogen atom as in 1. The HMBC correlation of 12-OH [δ_H 1.93 (1H, s)] with C-12 further confirmed this assignment. In addition, the NOESY correlation between H-12 [δ_H 3.91 (1H, br d, J = 13.8 Hz)]/H-11$_\beta$/H-17 showed that the 12-OH was α-oriented.

Compound **4** appeared as a yellow gum. The positive-ion mode HRESIMS of **4** showed a pseudo-molecular ion peak $[M + Na]^+$ at *m/z* 779.2922 (calculated for $C_{39}H_{48}O_{15}Na$ 779.2885), establishing that **4** had a molecular formula $C_{39}H_{48}O_{15}$ which was consistent with the ^{1}H and ^{13}C NMR data. The molecular formula, in association with ^{1}H and ^{13}C NMR spectral data, suggested that **4** had 16 degrees of unsaturation, ten of which came from carbon–carbon double bonds and five from CO (as esters). Therefore, the molecule is hexacyclic.

The IR spectrum showed absorption bands at 3434, 1723 and 875 cm^{-1}, indicating the presence of hydroxyl, ester carbonyl and furanyl groups, respectively. The UV absorption at 240 nm also indicated the presence of an α,β-unsaturated ester group. DEPT experiments revealed that **4** had nine methyl, three methylene, eleven methine (six olefinic) and sixteen quaternary carbons (including five carbonyls). Analysis of 1D and 2D NMR spectra enabled the recognition of a trisubstituted double bond [δ_H 6.13 (1H, s); δ_C 121.3 (d), 160.1 (s)], four hydroxyl groups [δ_H 3.57 (1H, s), 3.89 (1H, s), 4.15 (1H, s) and 4.72 (1H, s)], a methoxycarbonyl [δ_H 3.76 (3H, s); δ_C 52.2 (q), 174.6 (s)], an acetyl group [δ_H 1.52 (3H, s); δ_C 20.0 (q) and 170.8 (s)], two tigloyl groups [δ_H 6.85 (1H, qq, J = 6.9 Hz, 1.4 Hz), 1.81 (3H, br d, J = 6.9 Hz), 1.87 (3H, br s), δ_C 139.1 (d), 14.7 (q), 12.4 (q), 168.1 (s), 128.0 (s); δ_H 7.04 (1H, qq, J = 6.9 Hz, 1.4 Hz), 1.75 (3H, br d, J = 6.9 Hz) and 1.93 (3H, br s), δ_C 141.2 (d), 14.7 (q), 12.4 (q), 168.9 (s) and 129.5 (s)], and a β-furyl ring [δ_H 6.54 (1H, br s), 7.37 (1H, br s) and 7.45 (1H, br s); δ_C 110.6 (d), 142.8 (d), 142.1 (d) and 121.6 (s)]. The above spectroscopic data of 4 were characteristic of those of a phragmalin limonoid (Anne & David, 1983). The characteristic geminal proton resonances at δ_H 1.72 (1H, d, J = 11.0) and 1.98 (1H, d, J = 11.0) which were assignable to C-29, suggested that 4 also had a 4,29,1-bridge and thus a tricyclo[3.3.1^{2,10}.1^{1,4}]decane ring system as those found in **1**–**3**.

The ^{1}H and ^{13}C NMR spectral data due to the tricyclo[3.3.1^{2,10}.1^{1,4}]decane ring system of **4** showed a strong similarity to those of tabulalin, a phragmalin limonoid previously isolated from *Chukrasia tabularis* (Nakatani et al., 2004). Indeed, the chemical shifts of C-1 [δ_C 83.9 (s)], C-2 [δ_C 76.3 (s)], C-8 [δ_C 72.9 (s)] and C-9 [δ_C 78.4 (s)] were almost the same of those of the corresponding carbons (δ_C 83.5, 76.2, 72.3 and 78.5) of tabulalin, suggesting that the four hydroxyl groups in **4** were located at C-1, C-2. C-8 and C-9, like those in tabulalin. An oxygenated methine proton at δ_H 5.78 (1 H, s) attached to the carbon at δ_C 70.4 (d) which showed HMBC correlations (Fig. 2) to C-1, C-2, C-8, C-9 and a tigloyl carbonyl carbon at δ_C 168.9 (C-1″) was assigned to H-30. Another oxygenated methine proton at δ_H 4.60 (1 H, s) showing HMBC correlations to C-2, C-4 (δ_C 43.0, s) and a tigloyl carbonyl carbon at δ_C 168.1 (C-1′) was assigned to H-3. The two geminal protons at δ_H 1.72 and 1.98 showed HMBC correlations to C-1, C-2, C-3, C-5 and C-10, thus confirming their assignments to H$_2$-29. The methyl protons at δ_H 1.41 (3H, s) which showed HMBC correlations to C-10, C-5 and C-9, were assigned to H$_3$-19, whilst one other methyl proton signal at δ_H 0.86 (3H, s) was attributed to H$_3$-28, based on HMBC correlations of this signal to C-3, C-5 and C-29. The above correlations thus enabled the characterisation of the tricyclo[3.3.1^{2,10}.1^{1,4}]decane ring system of **4**.

An olefinic proton at δ_H 6.13 (1H, s) attached to the carbon at δ_C 121.3 (d) was assigned to H-15 on account of its HMBC correlations

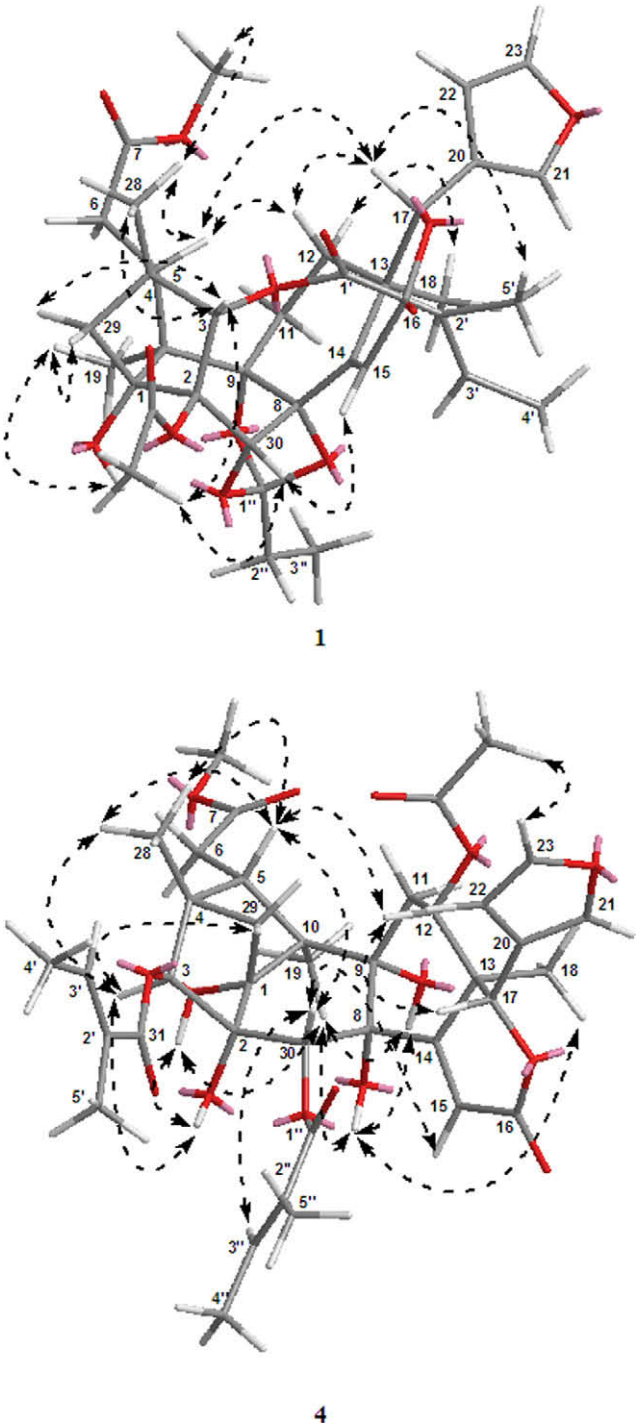

Fig. 3. Selected NOESY correlations of compounds **1** and **4**.

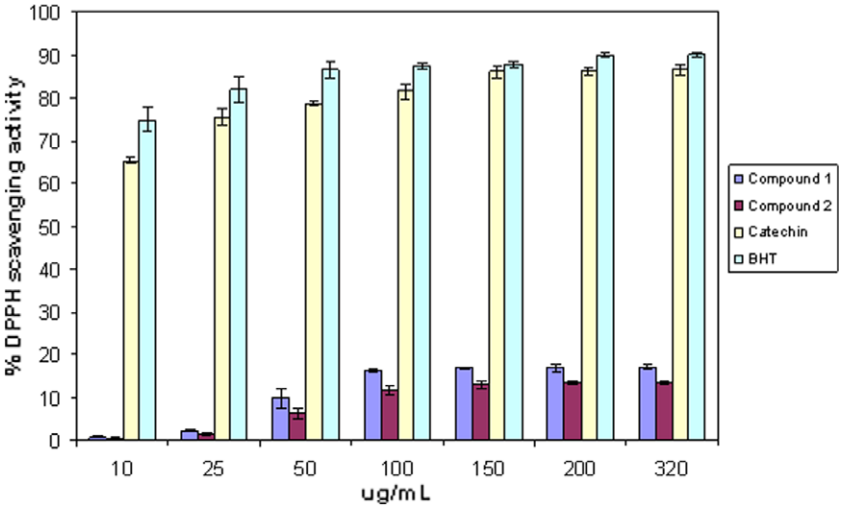

Fig. 4. % DPPH· scavenging activity of compounds **1** and **2**.

to the carbonyl carbon at δ_C 163.4 (s) (C-16), another olefinic carbon at δ_C 160.1 (s) (C-14), C-8 and a quaternary carbon at δ_C 43.3 (s) (C-13), whilst the low-field singlet at δ_H 5.82 (H-17) showed long-range C–H correlations with C-13, C-14, C-18 [δ_C 16.9 (q)] and the furan carbons C-20 [δ_C 121.6 (s)] and C-22 [δ_C 110.6 (d)]. Further, the methyl protons at δ_H 1.56 (H_3-18) showed HMBC correlations to C-13, C-12 [δ_C 68.6 (d)] and C-17 [δ_C 77.8 (d)], whilst the methylene protons at δ_H 1.91 and 2.09 were assignable to H_2-11 based on HMBC correlations to C-9, C-12 and an acetyl carbonyl carbon at δ_C 170.8 (s). The above correlations enabled the C and D rings of 4 to be characterised.

The stereochemistry of **4** was determined by NOESY correlations (Fig. 3). The NOESY correlations between H-17/H-12/H-5 and H_3-28/H-5 established that H-17, H-12, H-5 and H_3-28 were in the β-orientation, whilst correlations between H-3/2-OH, 2-OH/1-OH/H_3-19, H_3-19/8-OH/9-OH and 8-OH/H_3-18 indicated that H-3, 2-OH, 1-OH, H_3-19, 8-OH, 9-OH and H_3-18 were in the α-orientation.

3.2. Significance of phragmalin-type limonoids in genus Swietenia

Until recently, most of the described phragmalin *ortho* esters were found to possess the orthoester group at the 8,9,14- or the 1,8,9- positions (Coombees, Mulholland, & Randrianavelojosia, 2003; Hay et al., 2007; Naidoo, Mulholland, Randrianavelojosia, & Coombes, 2003; Saad et al., 2003). Phragmalins with an orthoester group at the 8,9,30-position, however, have been found exclusively in two species, namely, *Swietenia mahogany* (Abdelgaleil et al., 2006) and *Xylocarpus granatum* (Cui et al., 2005; Wu et al., 2004, 2005). With the isolation of swietephragmins H-J (**1**–**3**) from *S. macrophylla,* this species is the third known to produce this kind of compound. Amongst the 8,9,30-phragmalin *ortho* esters previously reported from *S. mahogany*, C-12 in the C-ring has never been found to be oxygenated, whereas the same carbon is an oxymethine carbon in the similar compounds isolated from *X. granatum*. However, in the present study, one of the 8,9,30-phragmalin *ortho* esters isolated from *S. macrophylla*, swietephragmin J (**3**), has a hydroxylayed C-12, thus demonstrating the structural variation possible in this type of natural products in the genus *Swietenia*. This observation may be of chemotaxonomic significance in the identification of the two *Swietenia* species (Wu, Xiao, & Li, 2006).

It is also interesting to note that phragmalins with an 8, 9-dihydroxy group are very uncommon amongst plants of the Meliaceae family, being previously reported only from *C. tabularis* ((Nakatani et al., 2004) and *X. granatum* (Zhou, Cheng, Wu, & Zou, 2006) from which five of this type of limonoid were isolated. These compounds are considered the precursors of phragmalin *ortho* esters from the biosynthetic viewpoint (Wu et al., 2004). As far as we know, **4** is the first 8, 9-dihydroxyphragmalin found in the genus *Swietenia*, and the sixth found in nature.

3.3. DPPH free radical scavenging activity

Compounds **1** and **2** isolated from *S. macrophylla* were evaluated at different concentrations for their ability to act as antioxidants to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) radicals (Kukic et al., 2008). Briefly, 4.0 mL of a methanolic solution of each sample was mixed with 0.5 mL of DPPH· solution and kept in the dark for 30 min, after which the absorbance at 517 nm was measured against a blank solution consisting of 4.0 mL of MeOH and 0.5 mL DPPH· solution. The DPPH· scavenging activity was calculated using the equation:

$$\%\ \text{DPPH}^{\cdot}\ \text{scavenging activity} = (A_o - A_s/A_o) \times 100$$

where A_o is the absorbance of the blank solution, and A_s is the absorbance of the test sample. Catechin and butylated hydroxytoluene (BHT) were used as positive controls. The mean values were obtained from triplicate experiments with three replications.

The results (Fig. 4) showed that **1** and **2** possessed low scavenging activities (17.12 ± 0.49% and 13.43 ± 0.28%, respectively) at the highest concentration (320 μg/mL) tested. Owing to the low scavenging activity of **1** and **2**, the IC_{50} value (defined as the concentration of test material required to scavenge 50% of free radicals) of each of these compounds cannot be determined in the current study. The observed low antioxidant activity of **1** and **2** might be due to their chemical structures which lack H-atom donating ability and electron delocalised potential (Gary, 2007), two factors which are of vital importance in determining the free radical scavenging ability of an antioxidant.

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