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Phanginin A–K, diterpenoids from the seeds of *Caesalpinia sappan* Linn.

Orapun Yodsaoue [a], Sarot Cheenpracha [a], Chatchanok Karalai [a,*], Chanita Ponglimanont [a], Suchada Chantrapromma [a], Hoong-Kun Fun [b], Akkharawit Kanjana-Opas [c]

[a] *Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*
[b] *X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia*
[c] *Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*

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Abstract

The first chemical study on the seeds of *Caesalpinia sappan* Linn. led to isolation of 11 cassane-type diterpenes, named phanginin A–K (**1**–**11**). The skeleton present in compounds **1**–**8** is rather unusual, consisting of a cassane-type diterpene with an ether bridge between C-19/C-20 in compounds **1**–**6** and C-11/C-20 in compounds **7** and **8**. Their structures were elucidated on the basis of spectroscopic techniques. In addition, the X-ray structure of phanginin A (**1**) is reported. Only phanginin I (**9**) exhibited cytotoxic effect against KB cell line with IC_{50} value of 4.4 μg/ml.

Keywords: *Caesalpinia sappan* Linn.; Leguminosae; Cassane-type diterpenes; Anticancer activity; Phanginin A–K

1. Introduction

Caesalpinia sappan Linn. (Leguminosae), known locally as "Phang" in Thai, is a shrubby tree distributed in China, India, Burma, Thailand, Indonesia and Vietnam. The heartwood of this plant has been used in folk medicine as a blood tonic, expectorant, emmenagogue, and has interesting biological activities such as antioxidation (Safitri et al., 2003; Badami et al., 2003), immunomodulation (Choi et al., 1997), antimicrobial (Lim et al., 2007), anticomplementary (Oh et al., 1998), sedative effect (Nagai et al., 1986) and vasorelaxation (Xie et al., 2000; Hu et al., 2003) properties. In the preceding papers, we isolated several cassane-type diterpenes from *Caesalpinia crista* (Cheenpracha et al., 2005, 2006). As a continuation of our study on this genus, we now report the isolation and elucidation of 11 new cassane-type diterpenoids (**1**–**11**) from the seeds of *C. sappan*. Their structures were identified on the basis of spectroscopic analysis. In addition, the structure of phanginin A (**1**) was confirmed using the X-ray diffraction technique. The biosynthetic pathway (Devon and Scott, 1972) and partial synthetic approach (Johnston and Overton, 1973) of cassane-type diterpenes were proposed to be derived from the pimarane skeleton via 1,2-methyl migration from C-13 to C-14.

2. Results and discussion

The CH_2Cl_2 extract from the seeds of *C. sappan* was subjected to repeated column chromatography over silica gel to furnish phanginin A–K (**1**–**11**). The basic skeleton of all compounds was identified to be a furanoditerpene on the basis of the UV spectra (λ_{max} 211–225 nm) (Cheenpracha et al., 2005) and a positive Ehrlich test (Kuroda et al., 2004). In addition, the IR spectrum of all of these compounds displayed a carbonyl ester (1717–1731 cm^{-1}) functionality. The ^1H NMR spectroscopic data of all compounds showed characteristics of a 1,2-disubstituted furan ring as indicated by signals between δ 6.15 and 7.35 (each

* Corresponding author. Tel.: +66 7428 8444; fax: +66 7421 2918.
E-mail addresses: chatchanok.k@psu.ac.th, chatchanok_k@yahoo.com (C. Karalai).

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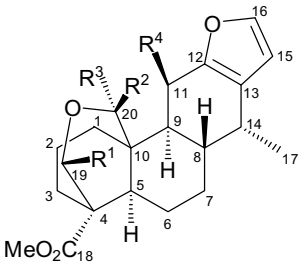
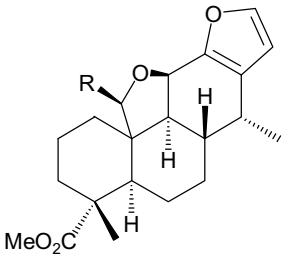
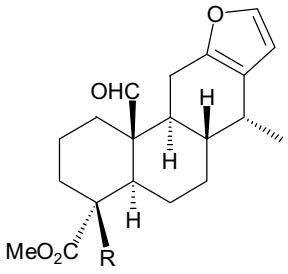
d) with coupling constants between 1.2 and 1.8 Hz. Also, the proton signals of singlets between δ 3.64 and 3.77 and doublets between δ 0.94 and 1.01 were assigned as methyl ester groups of 18-OMe and aliphatic methyl groups at C-17, respectively.





1: $R^1 = R^3 = R^4 = H$, $R^2 = OH$
2: $R^1 = OH$, $R^2 = R^3 = R^4 = H$
3: $R^1 = R^2 = R^4 = H$, $R^3 = OMe$
4: $R^1 = OMe$, $R^2 = R^3 = R^4 = H$
5: $R^1 = =O$, $R^2 = R^3 = R^4 = H$
6: $R^1 = R^2 = H$, $R^3 = R^4 = OH$

7: R = OH
8: R = H



9 : R = Me
10: R = CHO
11: R = CO_2Me

Phanginin A (**1**) was obtained as a white powder which was further recrystallized from CH_2Cl_2 to yield colorless crystals. The subsequent X-ray structure (Fig. 1) confirms a pentacyclic furano cassane-type diterpene framework with a molecular formula $C_{21}H_{28}O_5$ ($[M]^+$ *m*/*z* 360.1949 obtained from HREIMS). This structure was in agreement with the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 3). The ^{13}C NMR spectroscopic data (Table 3) displayed 21 carbons including those of an ester carbonyl carbon at δ 175.8 (C-18), and a 1,2-disubstituted furan ring moiety at δ 109.8 (C-15), 122.4 (C-13), 140.3 (C-16) and 149.5 (C-12). The ^1H NMR (Table 1) spectroscopic data displayed the presence of two AB systems. One of them was at δ 2.60 (*dd*, $J = 15.9$, 11.7 Hz, H-11α) and 2.72 (*dd*, $J = 15.9$, 5.7 Hz, H-11β), and the other as the oxymethylene protons at δ 3.69 and 4.37 (each *d*, $J = 11.7$ Hz, 2H-19), respectively. The lower field signal at δ 5.01 (*s*) was deduced to be a dioxymethine proton H-20 whose HMBC spectrum showed correlations with carbons at δ 38.6 (C-10), 45.2 (C-5) and 61.6 (C-19). The oxymethylene protons at δ 3.69 and 4.37 (2H-19) showed correlations with carbons at δ 35.5 (C-3), 45.2 (C-5) and 97.2 (C-20). These data suggested an ether bridge between C-19 and C-20. The relative stereochemistry of a pyran ring was determined on the basis of X-ray diffraction analysis and NOESY experiments whose cross-peaks of a dioxymethine proton at δ 5.01 (H-20) with protons at δ 2.02 (H-1β) and 2.72 (H-11β) suggested a β-orientation of a dioxymethine proton H-20 and in an equatorial position. From these data, **1** was concluded to be phanginin A.

Phanginin B (**2**), $[M]^+$ *m*/*z* 360.1927 ($C_{21}H_{28}O_5$) by HREIMS, exhibited a hydroxyl functionality at 3394 cm^{-1} in the IR spectrum. The ^1H (Table 1) and ^{13}C (Table 3) NMR spectroscopic data were closely related to those of **1**. The difference was in the position of the OH groups which was placed at C-20 in **1** (H-20: δ_H 5.01 (*s*), δ_C 97.2) but at C-19 in **2** (H-19: δ_H 5.33 (*s*), δ_C 94.8). The HMBC correlations of an oxymethine proton at δ 5.33 (H-19) with carbons at δ 36.9 (C-3), 44.0 (C-5), 49.1 (C-4) and 63.5 (C-20) confirmed the location of the OH functionality at C-19. The relative stereochemistry of a pyran ring was analyzed by NOESY correlations, the dioxymethine proton at δ 5.33 (H-19) showed a cross-peak with the proton at δ 1.96 (H-3β) and no cross-peak with 2H-20 suggesting equatorial orientation of H-19. Therefore, **2** was determined to be phanginin B.

Phanginin C (**3**) had a molecular formula $C_{22}H_{30}O_5$ ($[M]^+$ *m*/*z* 374.2116), based on HREIMS which was 14 mass units more than that of **1**, suggesting the addition of a Me group. The ^1H (Table 1) and ^{13}C (Table 3) NMR spectra of **3** displayed characteristics similar to those of **1**, except for the presence of an additional methoxyl group at δ_H 3.53 (*s*) in **3**. This signal correlated with a carbon resonance at δ 101.1 (C-20) in the HMBC analysis, suggesting the OMe group at C-20. In the NOESY spectrum, correlations between the dioxymethine proton at δ 5.02 (H-20) displayed a cross-peak with protons at δ 1.76 (H-6β), 3.53 (20-OMe) and 4.03 (H-19ax), indicating that this dioxymethine proton was in the axial orientation. Thus, the structure of phanginin C was concluded to be **3**.

The molecular formula of phanginin D (**4**) was found to be $C_{22}H_{30}O_5$ ($[M]^+$ *m*/*z* 374.2110), by HREIMS. The ^1H (Table 1) and ^{13}C (Table 3) NMR spectroscopic data of **4** were similar to those of **2** with an additional OMe signal at δ_H 3.35 which showed a HMBC correlation with C-19 (δ 102.6). The relative stereochemistry of a pyran ring was determined from the results of NOESY experiments and compared with phanginin B (**2**), which the dioxymethine proton at δ 4.76 (H-19) showed a cross-peak with protons at δ 1.92 (H-3β) and 3.35 (19-OMe) suggesting an equatorial orientation of H-19. Therefore, the structure of phanginin D was assigned as **4**.

Phanginin E (**5**), $C_{21}H_{26}O_5$ ($[M]^+$ at *m*/*z* 358.1744 by HREIMS), was two mass units less than that of **2**. The ^1H (Table 1) and ^{13}C (Table 3) NMR spectroscopic data resembled those of **2**, except that the signal of the dioxyme-

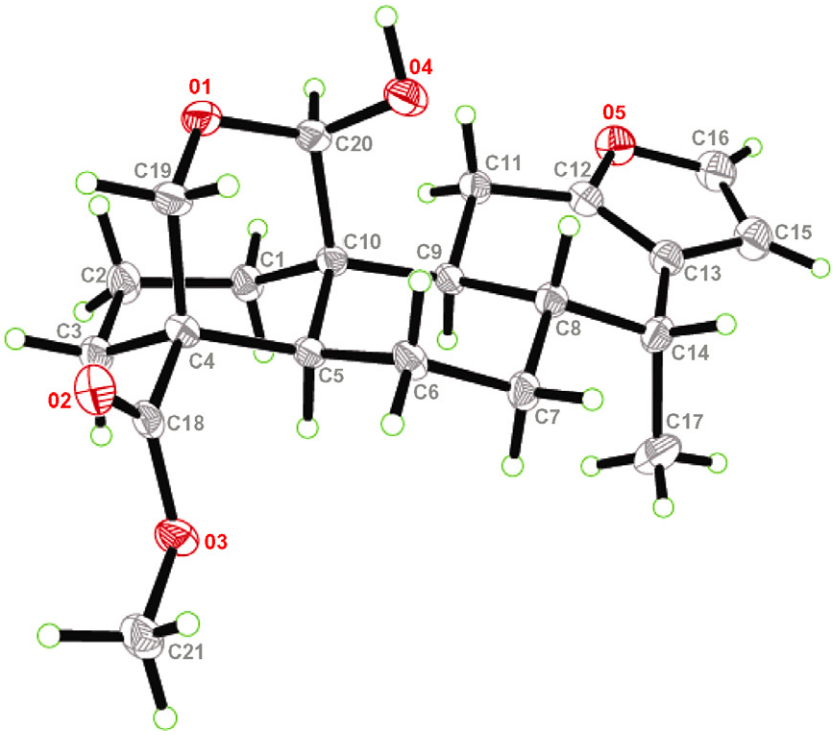


Fig. 1. ORTEP drawing of **1**.

Table 1
^{1}H NMR (300 MHz, $CDCl_3$) spectroscopic data of phanginin A–F (**1–6**) (δ in ppm, multiplicities, J in Hz)

No.	1	2	3	4	5	6
1α	1.24 *m*	1.23 *m*	1.88 *ddd* (13.8, 6.3, 1.2)	1.21 *m*	1.40 *m*	1.37 *m*
β	2.02 *m*	2.19 *m*	2.22 *m*	2.18 *m*	2.20 *m*	2.28 *m*
2	1.52 *m*, 1.62 *m*	1.64 *m*	1.22 *m*, 1.62 *m*	1.57 *m*	1.92 *m*	1.68 *m*, 2.60 *m*
3α	1.87 *m*	1.62 *m*	1.31 *m*	1.62 *m*	2.14 *m*	2.06 *m*
β	1.98 *m*	1.96 *m*	2.20 *m*	1.92 *m*		2.18 *m*
5	1.62 *m*	1.70 *m*	1.71 *m*	1.73 *dd* (12.3, 3.3)	1.73 *m*	2.18 *dd* (11.4, 6.3)
6α	1.19 *m*	1.84 *m*	1.35 *m*	1.88 *m*	1.43 *m*	1.60 *m*
β	2.23 *m*		1.76 *m*	2.07 *m*	1.62 *m*	1.70 *m*
7	1.36 *m*, 1.70 *m*	1.65 *m*	1.35 *m*, 1.73 *m*	1.33 *m*, 1.67 *m*	1.80 *m*	1.32 *m*, 1.72 *m*
8	2.38 *m*	1.62 *m*	1.70 *m*	1.73 *m*	1.90 *m*	2.07 *m*
9	1.45 *m*	1.52 *m*	1.57 *m*	1.52 *dt* (11.7, 5.7)	1.70 *m*	1.85 *dd* (12.3, 4.5)
11α	2.60 *dd* (15.9, 11.7)	2.27 *m*	2.25 *m*	2.24 *m*	2.07 *m*	5.19 *d* (4.5)
β	2.72 *dd* (15.9, 5.7)	2.72 *dd* (16.2, 5.7)	2.73 *dd* (16.2, 6.0)	2.71 *dd* (16.2, 5.7)	2.78 *dd* (16.2, 5.7)	
14	2.58 *m*	2.62 *brq* (7.2)	2.64 *brq* (6.9)	2.62 *m*	2.71 *brq* (7.2)	2.71 *qd* (7.2,4.2)
15	6.17 *brs*	6.17 *d* (1.5)	6.17 *d* (1.8)	6.17 *d* (1.8)	6.18 *d* (1.8)	6.22 *d* (1.8)
16	7.19 *brs*	7.22 *d* (1.5)	7.22 *d* (1.8)	7.21 *d* (1.8)	7.23 *d* (1.8)	7.35 *d* (1.8)
17	0.96 *d* (6.9)	0.97 *d* (7.2)	0.95 *d* (6.9)	0.96 *d* (6.9)	0.99 *d* (7.2)	0.96 *d* (7.2)
19ax	4.37 *d* (11.7)	–	4.03 *dd* (11.7,2.7)	–	–	4.22 *dd* (12.6, 3.0)
eq	3.69 *d* (11.7)	5.33 *s*	3.81 *d* (11.7)	4.76 *s*	–	4.07 *d* (12.6)
20ax	–	4.16 *dd* (11.4, 2.4)	–	4.04 *dd* (11.1, 2.4)	4.67 *dd* (11.7, 2.4)	–
eq	5.01 *s*	3.56 *d* (11.4)	5.02 *s*	3.54 *d* (11.1)	4.24 *dd* (11.7, 0.9)	5.47 *s*
18-OMe	3.65 *s*	3.64 *s*	3.69 *s*	3.70 *s*	3.77 *s*	3.68 *s*
19-OMe	–	–	–	3.35 *s*	–	–
20-OMe	–	–	3.53 *s*	–	–	–

thine proton of **2** at δ_H 5.33 (δ_C 94.8, C-19) was replaced by a carbonyl carbon at δ_C 170.5. This finding was supported by analysis of the HMBC spectrum, in which the oxymethylene protons of 2H-20 at δ 4.24 (*dd*, $J = 11.7$, 0.9 Hz) and 4.67 (*dd*, $J = 11.7$, 2.4 Hz) were correlated with carbons at δ 35.4 (C-10), 38.1 (C-1), 40.9 (C-9), 46.7 (C-5) and 170.5 (C-19). Thus, the structure of **5** was assigned as phanginin E.

The molecular weight of phanginin F (**6**), $C_{21}H_{26}O_5$ ($[M-H_2O]^+$ was assigned at m/z 358.1765 by HREIMS. The NMR spectroscopic data (Tables 1 and 3) of **6** displayed similarities with phanginin A (**1**). The ^{13}C NMR

spectrum (Table 3) exhibited a couple of oxymethine carbons at δ 71.1 and 104.5, these being assigned to C-11 and C-20, respectively. The ^{1}H NMR signal of H-11 was observed as a doublet at δ 5.19 ($J = 4.5$ Hz), whose HMBC spectrum showed correlations to carbons at δ 38.5 (C-8), 43.4 (C-9), 129.0 (C-13) and 148.0 (C-12). The relative stereochemistry of **6** was determined on the basis of coupling constants and the results of NOESY experiments. The small J values for H-9 and H-11 ($J = 4.5$ Hz) indicated that H-11 should be an equatorial proton. In addition, the oxymethine proton at δ 5.19 (H-11) showed a cross-peak with the protons at δ 1.85 (H-9α) and 2.28 (H-1β) and the dioxymethine proton at δ 5.47 (H-20) with protons at δ 1.70 (H-6β), 2.07 (H-8β) and 4.22 (H-19ax) in NOESY (Fig. 2) experiment confirming the α-orientation of H-11 and the axial position of H-20. Thus, phanginin F was characterized as **6**.

Phanginin G (**7**) had the molecular formula $C_{21}H_{28}O_5$ ([M]$^+$ m/z 360.1916), based on HREIMS. The IR absorption exhibited an hydroxyl functionality at 3395 cm^{-1}. The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) of **7** and phanginin F (**6**) were comparable, except that the methylene proton signals of 2H-19 at δ 4.22 (*dd*, $J = 12.6$, 3.0 Hz) and 4.07 (*d*, $J = 12.6$ Hz) in **6** were replaced by a singlet methyl proton at δ 1.25 (Me-19). The latter protons showed HMBC correlations with carbons at δ 37.1 (C-3), 48.1 (C-4), 48.5 (C-5) and 179.0 (C-18), suggesting its location at C-4. The ^{1}H NMR signal of a dioxymethine proton H-20 at δ 5.52 (*s*); δ_C 103.3 showed HMBC correlations with carbons at δ 36.0 (C-1), 47.9 (C-10), 48.5 (C-5), 48.7 (C-9) and 69.3 (C-11). The oxymethine proton H-11 (δ_H 4.85, *d*, $J = 4.8$ Hz; δ_C 69.3) had HMBC correlations with carbons at δ 36.2 (C-8), 48.7 (C-9), 103.3 (C-20), 128.0 (C-13) and 147.0 (C-12). These data suggested an ether bridge between C-20 and C-11 (forming a tetrahydrofuran skeleton). The small J value for H-11 ($J = 4.8$ Hz) indicated that H-11 should be an equatorial proton. In the NOESY spectrum (Fig. 3), the dioxymethine proton at δ 5.52 (H-20) correlated with the methyl protons at δ 1.25 (H-19) and protons at δ 1.70 (H-2β) and 1.92 (H-1β), and the oxymethine proton at δ 4.85 (H-11) displayed a cross-peak with protons at δ 1.79 (H-9α) and 1.92 (H-1β) confirming that protons H-20 and H-11 of a tetrahydrofuran ring were in *cis* configuration. Thus, phanginin G was concluded to be **7**.

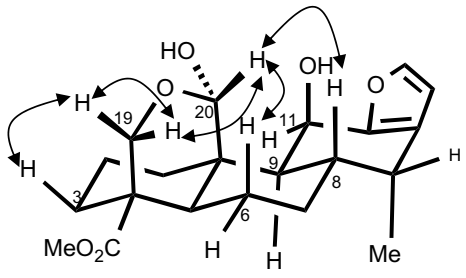


Fig. 2. Selected NOESY cross-peak for compound **6**.

Phanginin H (**8**) was found to have the molecular formula $C_{21}H_{28}O_4$ ([M]$^+$ m/z 344.1996) by HREIMS. The ^{1}H (Table 2) and ^{13}C (Table 3) NMR spectroscopic data of **8** were similar to those of **7**, except that the signal of a dioxymethine proton at δ 5.52 (*s*, H-20); δ_C 103.3 in **7** was replaced by those of oxymethylene protons at δ 3.92 (*d*, $J = 8.1$ Hz) and 3.97 (*brdd*, $J = 8.1$, 3.0 Hz). Both oxymethylene protons showed HMBC correlations with the carbons at δ 35.9 (C-1), 45.8 (C-10), 47.4 (C-5), 49.4 (C-9) and 68.7 (C-11). Therefore, phanginin H was assigned as **8**.

The molecular formula of phanginin I (**9**) was found to be $C_{21}H_{28}O_4$ [M]$^+$ m/z 344.1983, by HREIMS. The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) showed similarity with **8** and taepeenin H previously isolated from *C. crista* (Cheenpracha et al., 2005). The ^{1}H NMR spectral data exhibited a signal of an aldehydic proton at δ 10.21 (*d*, $J = 1.2$ Hz, H-20); δ_C 208.4 whose HMBC spectrum showed correlations with carbons at δ 31.9 (C-1) and 49.9 (C-5), confirming its location at C-20. The relative stereochemistry of **9** was assigned by NOESY experiments in which aldehydic proton H-20 (δ 10.21) showed a cross-peak with δ 1.04 (Me-19) and 2.06 (H-8). Thus, the structure of phanginin I was identified to be **9**.

Phanginin J (**10**) was deduced as $C_{21}H_{26}O_5$ from an exact mass measurement ([M]$^+$ m/z 358.1779) by HREIMS. The ^{1}H (Table 2) and ^{13}C (Table 3) NMR spectra of **10** were comparable with those of **9**. The difference was shown as the disappearance of a singlet at δ 1.04 (Me-19) in **9**, and the appearance of an additional aldehydic proton signal at δ 9.68 (*s*, H-19) in **10** whose HMBC spectrum showed correlations with carbons at δ 30.6 (C-3), 48.1 (C-5), 60.7 (C-4) and 173.1 (C-18), confirming the attachment of an aldehyde group at C-4. Therefore, phanginin J was assigned as **10**.

Phanginin K (**11**), [M]$^+$ m/z 388.1881 ($C_{22}H_{28}O_6$) by HREIMS, exhibited similar ^{1}H and ^{13}C NMR spectroscopic data (Tables 2 and 3) to those of **10**, except that an aldehydic proton signal at δ 9.68 in **10** was replaced by methoxyl protons at δ 3.74 (*s*). The ^{13}C NMR spectra indicated the presence of two ester carbonyl carbons at δ 171.2 (C-18) and 172.8 (C-19). The location of 19-OMe was confirmed by its HMBC correlation with C-19 (δ 172.8). In the NOESY spectrum, correlations between the methoxyl protons at δ 3.74 (OMe-19) with the aldehydic proton signal at δ 10.04 (H-20) indicated that this ester carbonyl group was β-oriented. Thus, the structure of phanginin K was deduced to be **11**.

All isolated compounds were evaluated for their cytotoxicity against the human MCF-7 (breast adenocarcinoma), HeLa (human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer) cell lines by the sulphorhodamine B (SRB) assay (Skehan et al., 1990). Phanginin I (**9**) demonstrated moderate inhibitory activity against KB cell line with IC_{50} value of 4.4 μg/ml, but was inactive against MCF-7, HeLa and HT-29 cell lines (IC_{50} values of 14.6, 19.0 and 14.0 μg/ml, respectively). The rest of the compounds were inactive.

Table 2
^{1}H NMR (300 MHz, $CDCl_3$) spectroscopic data of phanginin G–K (**7–11**) (δ in ppm, multiplicities, J in Hz)

No.	7	8	9	10	11
1α	1.22 *m*	1.22 *m*	0.90 *m*	1.07 *dd* (13.2, 3.3)	0.98 *m*
β	1.92 *m*	2.08 *m*	2.46 *brd* (12)	2.44 *m*	2.42 *m*
2	1.70 *m*	1.65 *m*	1.61 *m*, 1.80 *m*	1.69 *m*, 1.85 *m*	1.67 *m*
3	1.64 *m*	1.60 *m*, 1.86 *m*	1.59 *m*, 1.77 *m*	1.65 *m*, 2.32 *m*	1.66 *m*, 2.40 *m*
5	2.20 *brd* (12.0)	2.20 *d* (11.7, 3.0)	2.18 *dd* (12.9, 2.1)	2.18 *brd* (11.7)	2.16 *dd* (12.6, 2.4)
6	1.14 *m*, 2.10 *m*	1.29 *m*, 1.43 *m*	1.40 *m*, 2.17 *m*	1.60 *m*, 2.50 *m*	1.65 *m*, 2.61 *m*
7	1.39 *m*, 1.68 *m*	1.46 *m*, 1.71 *m*	1.69 *m*, 1.94 *m*	1.52 *m*, 1.87 *m*	1.60 *m*, 1.92 *m*
8	2.69 *m*	1.93 *m*	2.06 *m*	1.97 *m*	2.20 *m*
9	1.79 *dd* (12.0, 4.8)	1.80 *dd* (12.0, 3.9)	1.84 *m*	1.83 *m*	1.82 *dt* (11.4, 6.6)
11α	4.85 *d* (4.8)	4.83 *d* (3.9)	2.69 *dd* (17.1, 6.6)	2.77 *dd* (16.8, 6.6)	2.72 *dd* (16.8, 6.6)
β			2.18 *m*	2.31 *dd* (16.8, 10.5)	2.17 *m*
14	2.62 *m*	2.64 *qd* (7.2, 4.2)	2.65 *brq* (7.2)	2.67 *brq* (7.2)	2.69 *m*
15	6.23 *d* (1.5)	6.22 *d* (1.8)	6.15 *d* (1.8)	6.16 *d* (1.5)	6.17 *d* (1.8)
16	7.33 *d* (1.5)	7.31 *d* (1.8)	7.19 *d* (1.8)	7.19 *d* (1.5)	7.21 *d* (1.8)
17	0.94 *d* (6.6)	0.97 *d* (7.2)	1.00 *d* (7.2)	1.00 *d* (7.2)	1.01 *d* (7.2)
19	1.25 *s*	0.99 *s*	1.04 *s*	9.68 *s*	
20	5.52 *s*	3.97 *brdd* (8.1, 3.0) 3.92 *d* (8.1)	10.21 *d* (1.2)	9.98 *s*	10.04 *d* (1.2)
18-OMe	3.69 *s*	3.69 *s*	3.70 *s*	3.77 *s*	3.70 *s*
19-OMe					3.74 *s*

Table 3
^{13}C NMR (75 MHz, $CDCl_3$) spectroscopic data of phanginin A–K (**1–11**)

No.	1	2	3	4	5	6	7	8	9	10	11
1	37.8	38.4	29.0	38.4	38.1	33.9	36.0	35.9	31.9	31.8	32.3
2	20.9	21.1	21.0	21.1	20.0	20.4	19.0	18.3	18.3	19.0	20.0
3	35.5	36.9	38.4	36.2	34.8	37.7	37.1	36.8	36.6	30.6	34.1
4	45.6	49.1	49.4	49.7	55.9	45.0	48.1	47.6	51.4	60.7	58.7
5	45.2	44.0	47.9	44.2	46.7	46.3	48.5	47.4	49.9	48.1	50.1
6	23.5	23.3	23.1	23.8	25.2	23.8	24.9	25.0	23.1	23.0	24.2
7	29.6	30.8	30.0	30.7	29.1	28.2	29.9	30.1	30.8	31.1	31.2
8	36.6	37.3	36.3	37.0	36.0	38.5	36.2	37.1	36.7	36.8	36.9
9	42.6	42.0	41.3	42.0	40.9	43.4	48.7	49.4	44.3	44.1	44.2
10	38.6	36.0	35.4	36.0	35.4	40.9	47.9	45.8	47.3	50.8	51.4
11	22.5	22.6	22.5	22.5	22.6	71.1	69.3	68.7	23.0	23.2	23.5
12	149.5	149.2	148.9	149.0	147.9	148.0	147.0	148.3	148.2	147.8	148.1
13	122.4	122.2	122.1	122.2	122.5	129.0	128.0	127.3	122.3	122.4	122.4
14	31.5	31.7	31.5	31.6	31.3	31.8	32.2	32.1	31.5	31.4	31.5
15	109.8	109.6	109.5	109.6	109.5	109.2	109.3	109.1	109.4	109.4	109.4
16	140.3	140.6	140.6	140.5	141.0	143.2	142.8	142.8	140.7	140.8	140.8
17	16.8	17.2	17.1	17.2	17.2	14.0	14.4	14.7	16.9	17.1	17.0
18	175.8	174.7	176.5	174.9	172.0	174.4	179.0	178.5	178.2	173.1	171.2
19	61.6	94.8	66.9	102.6	170.5	70.0	17.3	15.8	15.8	198.5	172.8
20	97.2	63.5	101.1	62.7	73.6	104.5	103.3	70.3	208.4	206.2	206.4
18-OMe	51.5	51.7	51.6	51.5	52.3	51.6	51.9	52.0	52.0	52.7	52.1
19-OMe				55.5							52.7
20-OMe			57.4								

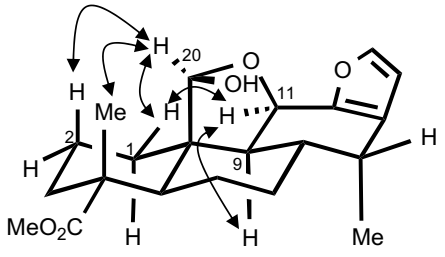


Fig. 3. Selected NOESY cross-peak for compound **7**.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Fisher–John melting point apparatus. Optical rotation $[\alpha]_D$ values were determined using a JASCO P-1020 polarimeter. IR spectra were measured with a Perkin–Elmer FTS FT-IR spectrophotometer, whereas ^{1}H and ^{13}C NMR spectra were recorded using 300 MHz Bruker FTNMR Ultra Shield™ spectrometer. Chemical shifts were recorded in parts per

million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as a internal reference. Quick column chromatography (QCC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F_{254} and reversed-phase (RP-18 F_{254}) were used for analytical purposes.

3.2. Plant material

Caesalpinia sappan L. was collected from Khonkaen province, Thailand in October 2005. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. SC07) was deposited at the Prince of Songkla University Herbarium.

3.3. Extraction and Isolation

Air-dried seeds (700 g) of *C. sappan* were extracted with CH_2Cl_2 and acetone successively (each 2×2.5 l, for 5 days) at room temperature. The crude extracts were evaporated under reduced pressure to afford brownish CH_2Cl_2 (77.8 g) and acetone (6.4 g) extracts, respectively. The crude CH_2Cl_2 extract was further purified by QCC using hexane as eluent and increasing polarity with EtOAc to give nine fractions (C1–C9). Fraction C2 (900.5 mg) was further purified by CC with EtOAc–hexane (1:9, v/v) to give **9** (307.5 mg). Fraction C4 (3.12 g) was subjected to CC with EtOAc–hexane (3:17, v/v) to afford nine subfractions (C4a–C4i). Subfraction C4b (84.5 mg) was separated by CC with EtOAc–CH_2Cl_2–hexane (1:1:8, v/v) to give **8** (26.3 mg). Subfraction C4d (60.8 mg) was purified by CC with CH_2Cl_2–hexane (7:3, v/v) to give **11** (10.4 mg). Fraction C5 (7.3 g) was recrystallized from CH_2Cl_2 to give **1** (832.3 mg), with the mother liquor (6.4 g) further subjected to QCC with CH_2Cl_2–hexane (8:2, v/v) to afford seven subfractions (C5a–C5g). Subfraction C5c (117.2 mg) was purified by CC with CH_2Cl_2 to give **6** (6.6 mg) and **7** (8.2 mg). Fraction C6 (3.9 g) was separated by CC with EtOAc–CH_2Cl_2 (1:19, v/v) to afford 10 subfractions (C6a–C6j). Subfraction C6b (211.5 mg) was recrystallized from CH_2Cl_2 to give **10** (156.3 mg). Subfraction C6h (345.4 mg) was purified by CC with acetone–hexane (1:9, v/v) and followed by prep TLC with acetone–hexane (1:9, v/v) to give **2** (3.2 mg), **3** (6.0 mg) and **4** (31.7 mg). Fraction C8 (2.4 g) was separated by CC with CH_2Cl_2 and followed by prep TLC with CH_2Cl_2–hexane (4:1, v/v) to give **5** (10.5 mg).

3.3.1. Phanginin A (*1*)

White solid; m.p. 110–112 °C; $[\alpha]_D^{30} - 41.4$ ($CHCl_3$; *c* 0.76); UV (MeOH) λ_{max} ($\log\varepsilon$): 218 (3.85) nm; IR (neat) υ_{max}: 3433, 1726, 755 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 1 and 3; HREIMS: *m/z* $[M]^+$ 360.1949 (calcd. for $C_{21}H_{28}O_5$, 360.1937).

3.3.2. Phanginin B (*2*)

Viscous oil; $[\alpha]_D^{30} + 48.7$ ($CHCl_3$; *c* 0.14); UV (MeOH) λ_{max} ($\log\varepsilon$): 214 (3.84) nm; IR (neat) υ_{max}: 3394, 1730, 762 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 1 and 3; HREIMS: *m/z* $[M]^+$ 360.1927 (calcd. for $C_{21}H_{28}O_5$, 360.1937).

3.3.3. Phanginin C (*3*)

Viscous oil; $[\alpha]_D^{30} - 40.4$ ($CHCl_3$; *c* 0.25); UV (MeOH) λ_{max} ($\log\varepsilon$): 216 (3.88) nm; IR (neat) υ_{max}: 1728, 757 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 1 and 3; HREIMS: *m/z* $[M]^+$ 374.2116 (calcd. for $C_{22}H_{30}O_5$, 374.2093).

3.3.4. Phanginin D (*4*)

Viscous oil; $[\alpha]_D^{30} + 44.8$ ($CHCl_3$; *c* 0.61); UV (MeOH) λ_{max} ($\log\varepsilon$): 215 (2.78) nm; IR (neat) υ_{max}: 1731, 755 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 1 and 3; HREIMS: *m/z* $[M]^+$ 374.2110 (calcd. for $C_{22}H_{30}O_5$, 374.2093).

3.3.5. Phanginin E (*5*)

Viscous oil; $[\alpha]_D^{30} - 24.8$ ($CHCl_3$; *c* 0.18,); UV (MeOH) λ_{max} ($\log\varepsilon$): 215 (3.26) nm; IR (neat) υ_{max}: 1726, 824 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 1 and 3; HREIMS: *m/z* $[M]^+$ 358.1744 (calcd. for $C_{21}H_{26}O_5$, 358.1780).

3.3.6. Phanginin F (*6*)

White solid; m.p. 105–107 °C; $[\alpha]_D^{30} - 1.8$ ($CHCl_3$; *c* 0.52); UV (MeOH) λ_{max} ($\log\varepsilon$): 215 (4.05) nm; IR (neat) υ_{max}: 3436, 1725, 756 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 1 and 3; HREIMS: *m/z* $[M-H_2O]^+$ 358.1765 (calcd. for $C_{21}H_{28}O_6$, 376.1886).

3.3.7. Phanginin G (*7*)

Viscous oil; $[\alpha]_D^{30} + 31.1$ ($CHCl_3$; *c* 0.61); UV (MeOH) λ_{max} ($\log\varepsilon$): 219 (3.94) nm; IR (neat) υ_{max}: 3395, 1721, 755 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 2 and 3; HREIMS: *m/z* $[M]^+$ 360.1916 (calcd. for $C_{21}H_{28}O_5$, 360.1937).

3.3.8. Phanginin H (*8*)

Viscous oil; $[\alpha]_D^{30} + 3.6$ ($CHCl_3$; *c* 0.39); UV (MeOH) λ_{max} ($\log\varepsilon$): 216 (3.72) nm; IR (neat) υ_{max}: 1726, 755 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 2 and 3; HREIMS: *m/z* $[M]^+$ 344.1996 (calcd. for $C_{21}H_{28}O_4$, 344.1988).

3.3.9. Phanginin I (*9*)

White solid; m.p. 121–122 °C; $[\alpha]_D^{30} + 76.9$ ($CHCl_3$; *c* 0.91); UV (MeOH) λ_{max} ($\log\varepsilon$): 220 (3.80) nm; IR (neat) υ_{max}: 2930, 1725, 737 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see

Tables 2 and 3; HREIMS: m/z $[M]^+$ 344.1983 (calcd. for $C_{21}H_{28}O_4$, 344.1988).

*3.3.10. Phanginin J (**10**)*

White solid; m.p. 148–150 °C; $[\alpha]_D^{30} + 41.5$ ($CHCl_3$; c 0.28); UV (MeOH) λ_{max} ($\log\varepsilon$) 219 (3.93) nm; IR (neat) υ_{max}: 2865, 1717, 824 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 2 and 3; HREIMS: m/z $[M]^+$ 358.1779 (calcd. for $C_{21}H_{26}O_5$, 358.1780).

*3.3.11. Phanginin K (**11**)*

Viscous oil; $[\alpha]_D^{30} + 1.8$ ($CHCl_3$; c 0.55); UV (MeOH) λ_{max} ($\log\varepsilon$): 215 (3.87) nm; IR (neat) υ_{max}: 2929, 1727, 755 cm^{-1}; For ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectra, see Tables 2 and 3; HREIMS: m/z $[M]^+$ 388.1881 (calcd. for $C_{22}H_{28}O_6$, 388.1886).

*3.4. X-ray crystallographic analysis of **1***

$C_{21}H_{28}O_5$, $M = 360.43$, orthorhombic, $P2_12_12_1$, $a = 7.5063(5)$ Å, $b = 8.1013(5)$ Å, $c = 30.554(2)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 1858.0(2)$ Å^3, $Z = 4$, $T = 100.0(1)$ K. Two thousand five hundred and ninety-one independent reflections (2591 independent, $R_{int} = 0.057$) were collected. Largest electron density residue: 0.39 eÅ^{-3}, R_1 (for $I > 2\sigma(I) = 0.0560$ and $wR_2 = 0.1854$ (all data) with $R_1 = \sum||F_o| - |F_c|/\sum|F_o|$ and $wR_2 = (\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2)^{0.5}$. Data were collected on a Bruker SMART APEX2 CCD area detector diffractometer with Mo Kα radiation ($\lambda = 0.71073$ Å) equipped with an Oxford Cryosystem Cobra low-temperature attachment. Cell refinement: *APEX2*; data reduction: *SAINT* (Bruker, 2005); programs used to solve structures: *SHELXTL* (Sheldrick, 1998); molecular graphics: *SHELXTL*; softwares used to prepare material for publication: *SHELXTL* and *PLATON* (Spek, 2003). The structure was solved by direct methods *SHELXTL* (Sheldrick, 1998) and all non-hydrogen atoms were refined anisotropically using the least-squares method on F^2 *SHELXTL* (Sheldrick, 1998). Hydroxyl H and H atom attached to C9 were located from the difference map and isotropically refined. The remaining H atoms were positioned geometrically and allowed to ride on their parent atoms, with C—H distances in the range 0.93–0.97 Å. The U_{iso} values were constrained to be $1.5U_{eq}$ of the carrier atom for methyl H atoms and $1.2U_{eq}$ for the remaining H atoms.

4. Supplementary material

The crystallographic data for the structure of **1** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication (Deposition No. CCDC 650908). This data can be obtained free of charge, by request to the Director, via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; email deposit@ccdc.cam.ac.uk).

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