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Knecorticosanones C–H from the fruits of *Knema globularia* (Lam.) warb†

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Six undescribed polyketides, **1–6**, were discovered from the fruits of *Knema globularia* (Lam.) warb. Two known polyketides and three known lignans were also isolated. Cytotoxicities against HepG2 and KKU-M156 cells of all polyketides were evaluated. Compound **1** displayed the most cytotoxic activity against HepG2 and KKU-M156 cell lines with IC_{50} values of 1.57 ± 0.37 and 1.78 ± 0.14 μg mL^{-1}, respectively. The structure of all isolates was identified using spectroscopic methods including NMR, IR, MS and ECD.

1. Introduction

The plant *Knema* genus (Myristicaceae) comprises over 60 species, and they are located in tropical Asia, Africa and Australia.[1] At least 12 species have been found distributed throughout Thailand.[2] Some *Knema* plants have been used to treat sore mouths and throats, pimples and skin diseases.[3] There are many reports on biological activities of *Knema* species such as antimicrobial,[4] antioxidant,[5,6] anticancer,[7–10] antidiabetic,[11] and antimalarial[12] activities.

Knema globularia or Lueat Raet in Thai, is a traditional medicine. An ingredient of external preparation for skin diseases and scabies is made from the oil of the seeds of this plant[2,13] while the bark is used as a blood tonic. The chemical components of this plant are flavonoids, polyketides and steroids.[8,10,14,15] Herein, the chemical constituents from the fruits of this plant are reported, as well as their cytotoxicity against cholangiocarcinoma, KKU-156, and liver cancer cells, HepG2, evaluated using the SRB assay.

2. Discussion

Chemical investigation of the fruits of *Knema globularia* (Lam.) warb using a chromatographic method led to the isolation of eight polyketides (**1–8**) and three lignans (**9–11**). Six new compounds, knecorticosanones C–H (**1–6**) were discovered, and in addition, five known compounds including, knecorticosanones A and B (**7** and **8**),[10] 8,8′-*cis*-cubebin (**9**),[16] (−)-α-cubebin (**10**)[17] and (−)-β-cubebin (**11**)[18] were also found (Fig. 1).

Compound **1** showed a molecular ion at *m*/*z* 393.3013 $[M + H]^+$ corresponding to the molecular formula $C_{24}H_{40}O_4$. The IR spectrum showed absorption bands of a hydroxyl group (3466 cm^{-1}) and carbonyl groups (1664 cm^{-1}). The ^{13}C NMR spectrum displayed three carbonyl carbons at δ_C 195.6 (C-1), 197.9 (C-3) and 206.1 (C-1′) of a 2-acylcyclohex-2-en-1-one moiety. The low field signal at δ_H 18.2 indicated an intramolecular hydrogen bonding (Table 1). The doublet of doublet signal at δ_H 4.03 (J = 13.0, 5.3 Hz) was assigned as H-6, located on the oxygenated carbon at δ_C 71.3. The large coupling constant (J = 13.0 Hz) indicates the axial orientation of this proton, and the hydroxyl group was located at the equatorial position (Fig. 3). The multiplet signals of methylene protons H-5 showed at δ_H 2.32 and δ_H 1.77, while that of H-4 showed at δ_H 2.74, while these protons were located on the carbons at δ_C 26.9 and δ_H 31.3, respectively (Table 2). Cross peaks between H-4 and C-2 (δ_C 110.3), C-3 (δ_C 197.9) and C-6 (δ_C 71.3) were evident (Fig. 2). Long range coupling between H-6 and C-1 (195.6), C-4 (31.3) and C-5 (26.9) were observed. The ^1H–^1H COSY spectrum showed the connection of H-4/H-5/H-6. The ^1H NMR spectrum displayed two olefinic protons at δ_H 5.29 (t, J = 5.0 Hz, H-13′ and H-14′). The ^{13}C NMR data exhibited fourteen methylene carbons around $\delta_C \sim$ 40.3–22.4 (C-2′ to C-12′ and C-15′ to C-17′). Long range correlations between a terminal methyl proton (δ_H 0.84, t, J = 7.0 Hz, H-18′) and C-16′ (δ_C 32.0) and C-17′ (δ_C 22.4) were evident. Correlations of H-17′ (1.28) to C-15′ (δ_C 27.2), of H-14′ to C-15′ (δ_C 27.2) and C-16′ (δ_C 32.0), and of H-13′ to C-12′ (δ_C 27.2) indicated the assignment of a double bond at the C-13′/C-14′ position. *Cis* geometry of double bond was confirmed on the basis of the typical shielded signals of α-olefinic carbons at $\delta_C \sim$ 26–27 while *trans* geometry showed around $\delta_C \sim$ 32–33.[19] The multiplet signal at δ_H 2.96 (H-2′)

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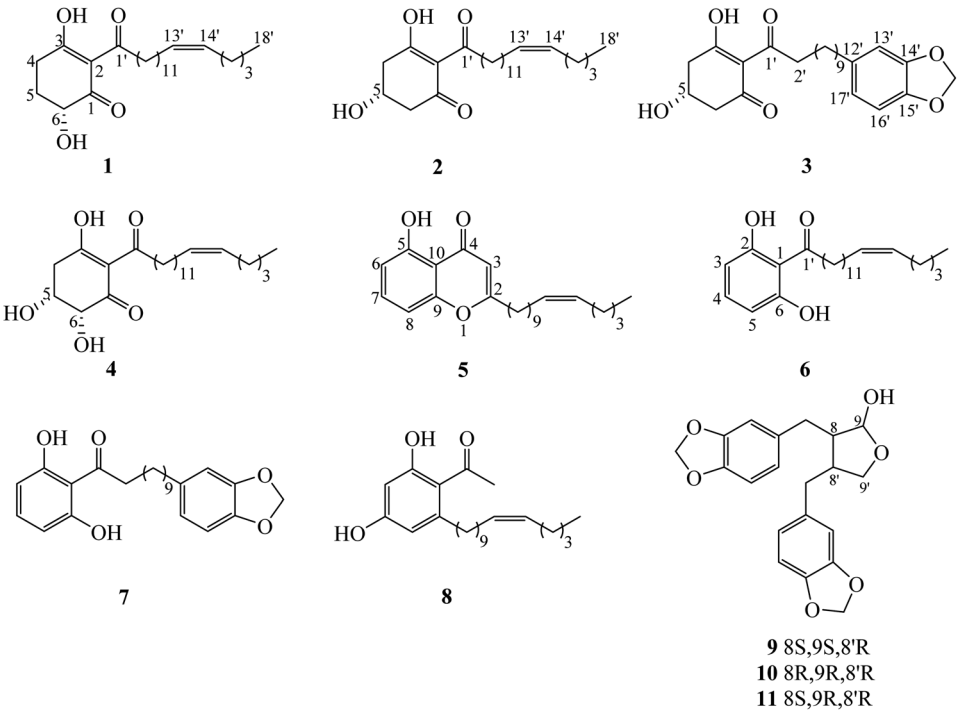

Fig. 1 The structures of all isolated compounds **1–11**.

correlated to the carbonyl carbon at δ_H 206.1 (C-1′) in the HMBC experiment. The specific rotation of this compound was $[\alpha]_D^{20.8}$ = +37.1 (*c* 0.1, MeOH) with the same sign as (+)-trineurone E.[19] The experimental ECD spectrum displayed a negative Cotton effect at 220 nm ($\Delta\varepsilon$ − 41.33) and positive Cotton effect at 237 ($\Delta\varepsilon$ + 7.13) and 288 ($\Delta\varepsilon$ + 11.00) which

Table 1 ^{1}H NMR spectroscopic data of compounds **1–6** (400 MHz, $CDCl_3$, δ in ppm)[a]

Position	1	2	3	4	5	6
3	—	—	—	—	6.00, s	6.38, d (8.2)
4	2.74, m	2.94, dd (18.0, 3.8) 2.78, dd (18.0, 6.0)	2.94, dd (18.0, 4.0) 2.76, dd (18.0, 3.5)	3.05, dd (10.0, 2.7) 2.97, dd (10.0, 2.7)	—	7.21, t (8.2)
5	2.32, m 1.77, m	4.39, m	4.40, m	4.43, q (2.8)	—	6.38, d (8.2)
6	4.03, dd (13.0, 5.3)	2.76, dd (16.5, 4.0) 2.63, dd (16.5, 6.7)	2.79, dd (16.2, 5.4) 2.63, dd (16.2, 6.5)	4.17, d (2.8)	6.75, d (8.4)	—
7	—	—	—	—	7.38, t (8.4)	—
8	—	—	—	—	6.65, d (8.4)	—
1′	—	—	—	—	2.50, t (8.0)	—
2′	2.96, m	3.01, t (7.6)	3.01, t (7.0)	2.99, m	1.63, p (7.0)	3.12, t (7.3)
3′	1.57, m	1.60, p (7.0)	1.26, m	1.62, m	1.20, m	1.69, p
4′–8′	1.25, m	1.30, m	1.26, m	1.29, m	1.20, m	1.29, m
9′	1.25, m	1.30, m	1.26, m	1.29, m	1.92, m	1.29, m
10′	1.25, m	1.30, m	1.26, m	1.29, m	5.25, t (5.4)	1.29, m
11′	1.25, m	1.30, m	2.50, t (7.6)	1.29, m	5.25, t (5.4)	1.29, m
12′	1.97, m	2.01, m	—	2.01, m	1.92, m	2.01, m
13′	5.29, t (5.0)	5.34, t (4.6)	6.66, s	5.34, t (4.7)	1.20, m[a]	5.34, t (4.6)
14′	5.29, t (5.0)	5.34, t (4.6)	—	5.34, t (4.7)	1.20, m[a]	5.34, t (4.6)
15′	1.97, m	2.01, m	—	2.01, m	0.80, t (6.5)	2.01, m
16′	1.28, m[a]	1.31, m[a]	6.71, d (7.5)	1.30, m[a]		1.30, m[a]
17′	1.28, m[a]	1.31, m[a]	6.61, d (7.5)	1.30, m[a]		1.30, m[a]
18′	0.84, t (7.0)	0.89, t (7.0)		0.89, t (7.0)		0.89, t (7.0)
OH	18.2, s	18.2, s	18.2, s	18.3, s	12.4, br s	9.62, br s
OCH_2O	—	—	5.90, s	—	—	—

[a] Overlapping signals.

Table 2 ^{13}C NMR spectroscopic data of compounds **1–6** (100 MHz, $CDCl_3$, δ in ppm)

Position	1	2	3	4	5	6
1	195.6	193.2	192.6	193.8	—	110.1
2	110.3	113.3	112.7	110.7	171.3	161.2
3	197.9	196.5	195.8	196.3	108.4	108.4
4	31.3	42.0	41.4	38.4	183.6	135.6
5	26.9	63.9	63.3	67.0	160.8	108.4
6	71.3	47.7	47.0	74.7	111.1	161.2
7	—	—	—	—	135.0	—
8	—	—	—	—	106.9	—
9	—	—	—	—	156.8	—
10	—	—	—	—	110.6	—
1′	206.1	206.1	205.4	205.4	34.4	208.0
2′	40.3	40.8	40.1	40.2	26.8	44.8
3′	24.6	25.1	31.6–29.2	25.1	29.8–29.0	24.4
4′–8′	29.8–29.3	30.2–29.8	31.6–29.2	30.2–29.8	29.8–29.0	29.8–29.3
9′	29.8–29.3	30.2–29.8	31.6–29.2	30.2–29.8	27.2	29.8–29.3
10′	29.8–29.3	30.2–29.8	31.6–29.2	30.2–29.8	129.9	29.8–29.3
11′	29.8–29.3	30.2–29.8	35.5	30.2–29.8	129.9	29.8–29.3
12′	27.2	27.7	136.7	27.7	27.2	27.2
13′	129.9	130.4	108.7	130.4	32.0	129.9
14′	129.9	130.4	147.2	130.4	22.4	129.9
15′	27.2	27.4	145.2	27.4	14.1	26.9
16′	32.0	32.4	107.8	32.4	—	32.0
17′	22.4	22.8	120.8	22.8	—	22.4
18′	14.0	14.5	—	14.5	—	14.0
OCH_2O	—	—	100.5	—	—	—

appropriately matched the calculated spectrum for the (6*R*) configuration. Thus the structure of **1** was identified as 3,6-dihydroxy-2-(octadec-13′*Z*-enoyl)cyclohex-2-en-1-one which was named knecorticosanone C.

Compound **2**, a yellowish oil, had the molecular formula $C_{24}H_{40}O_4$, determined from *m*/*z* 393.2988 $[M + H]^+$ in the HRESIMS data. The low field singlet signal at δ_H 18.2 (1H, s, OH) indicated intramolecular H-bonding of the enone part. The ^{13}C NMR spectra showed three carbonyl carbons at δ_C 193.2 (C-1), 196.5 (C-3) and 206.1 (C-1′) of the 2-acylcyclohex-2-en-1-one part. A multiplet signal at δ_H 4.39 (H-5) showed correlation with carbon at δ_C 63.9 in the HMQC spectrum, indicating the

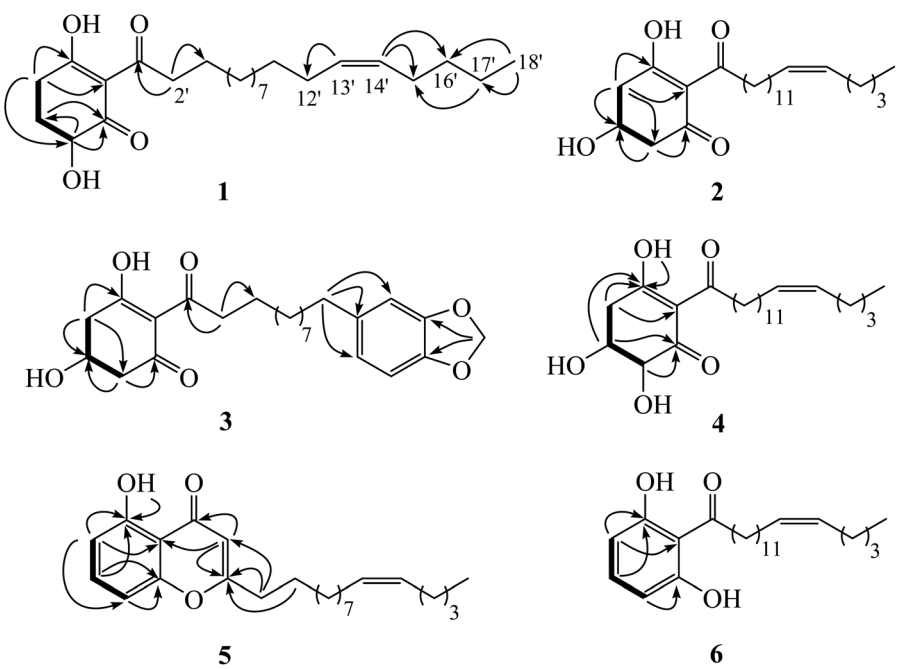

Fig. 2 Key HMBC correlations of compounds **1–6**.

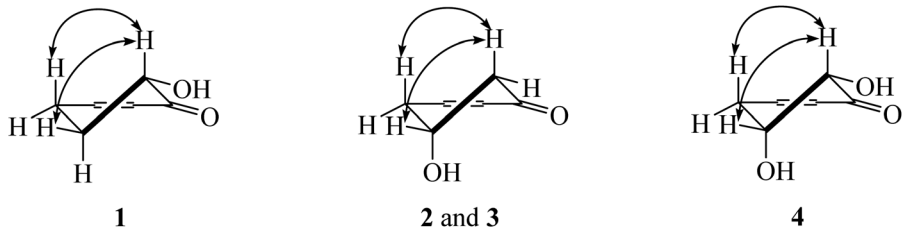

Fig. 3 NOESY correlations of compounds **1–4**.

presence of the hydroxyl group in this position. Two methylene protons displayed at δ_H 2.94 (1H, dd, $J = 18.0$, 3.8 Hz, H-4a), 2.78 (1H, dd, $J = 18.0$, 6.0 Hz, H-4b), 2.76 (1H, dd, $J = 16.5$, 4.0 Hz, H-6a) and 2.63 (1H, dd, $J = 16.5$, 6.7 Hz, H-6b). The HMBC experiment displayed correlations between H-4 and C-3 (δ_C 196.5), C-5 (δ_C 63.9) and C-6 (δ_C 47.7), in addition, between H-6 and C-1 (δ_C 193.2), C-4 (δ_C 42.0) and C-5 (δ_C 63.9). In the ^{1}H–^{1}H COSY spectrum, the connection of H-4/H-5/H-6 was observed. This information confirmed the presence of the 5-hydroxycyclohex-2-en-1-one moiety. The small coupling constants between H-4 and H-5 ($J = 3.8$ and 6.0 Hz) as well as H-5 and H-6 ($J = 4.0$ and 6.7 Hz) indicate the equatorial orientation of H-5 (Fig. 3). The remaining signals in the ^{1}H, ^{13}C NMR and also the HMBC spectra of acylhydrocarbon side chain showed the same as compound **1**. The specific rotation of this compound was $[\alpha]_D^{18.5} = -50.7$ (c 0.1, MeOH). The experimental ECD spectrum displayed a positive Cotton effect at 191 nm ($\Delta\varepsilon$ + 8.91) and negative Cotton effect at 198 ($\Delta\varepsilon$ − 3.88) which appropriately matched the calculated spectrum for the (6*R*) configuration. All data confirmed that the structure of **2** was 3,5-dihydroxy-2-(octadec-13′*Z*-enoyl)cyclohex-2-en-1-one which was named knecorticosanone D.

Compound **3** was obtained as a yellowish oil and showed the molecular ion peak at m/z 439.2124 $[M + Na]^+$ in HRESIMS, consistent with the molecular formula $C_{24}H_{32}O_6$. The IR spectrum displayed absorption bands of a hydroxyl group (3360 cm^{-1}) and carbonyl groups (1716 and 1633 cm^{-1}). The low field singlet signal at δ_H 18.2 (1H, s, OH) indicated intramolecular H-bonding of the enone part. The ^{13}C NMR spectra showed carbonyl carbons at δ_C 192.6 (C-1), 195.8 (C-3) and 205.4 (C-1′) of the 2-acylcyclohex-2-en-1-one moiety. The ^{1}H NMR spectrum showed the ABX pattern of 1,3,4-trisubstituted benzene at δ_H 6.66 (1H, s, H-13′), 6.71 (1H, d, $J = 7.5$ Hz, H-16′) and 6.61 (1H, d, $J = 7.5$ Hz, H-17′) and these protons were attached to carbons at δ_C 108.7 (C-13′), 107.8 (C-16′) and 120.8 (C-17′), respectively. The ^{13}C NMR experiment showed signals at δ_C 147.2 and 145.2 of C-14′ and C-15′, respectively. The signal at δ_H/δ_C 5.90/100.5 was assigned as a methylenedioxy group. The correlations between methylenedioxy protons and carbons C-14′ and C-15′ were evident in the HMBC experiment. The multiplet signal at δ_H 4.40 was assigned as an oxymethine proton at C-5 (δ_C 63.3). The doublet of doublet signals at δ_H 2.94 (1H, $J = 18.0$, 4.0 Hz) and δ_H 2.76 (1H, $J = 18.0$, 3.5 Hz) belonged to H-4 and was located on the carbon at δ_C 41.4. The methylene protons at δ_H 2.79 (1H, $J = 16.2$, 5.4 Hz) and δ_H 2.63 (1H, $J = 16.2$, 6.5 Hz) were assigned as H-6 and attached to the carbon at δ_C 47.0. The equatorial orientation of H-5 was determined by the small coupling constant between H-4 and H-5 ($J = 4.0$ and 3.5 Hz) and between H-5 and H-6 ($J = 6.5$ and 5.4 Hz). The HMBC experiment displayed the correlations between H-4 and C-3 (δ_C 195.8), C-5 (δ_C 63.3) and C-6 (δ_C 47.0), in addition, between H-6 and C-1 (δ_C 192.6), C-4 (δ_C 41.4) and C-5 (δ_C 63.3). The rest of the ^{13}C NMR signals also showed ten methylene carbons around δ_C 40.1 to 29.2. The signals at δ_C 40.1 and 35.5 were assigned as C-2′ and C-11′, respectively. Cross peaks in the HMBC spectrum showed correlations of H-2′ and C-1′, C-3′ and C-4′ while those of H-11′ were C-12′, C-13′ and C-17′. The specific rotation of this compound was $[\alpha]_D^{18.5} = -52.1$ (c 0.1, MeOH). The experimental ECD spectrum displayed a positive Cotton effect at 210 nm ($\Delta\varepsilon$ + 11.48) and negative Cotton effect at 218 nm ($\Delta\varepsilon$ − 1.97). Thus, the structure of compound **3** was 3,5-dihydroxy-2-(11′-phenyl-14′-methylenedioxy-undecanoyl)cyclohex-2-en-1-one, named knecorticosanone E, established as shown.

Compound **4** was found as brown oil and showed the molecular formula of $C_{24}H_{40}O_5$ determined from the molecular ion peak m/z 409.2947 $[M + H]^+$. The ^{1}H NMR spectrum showed a low field shift signal the same as compounds **1–3** at δ_H 18.3. The ^{13}C NMR data displayed carbonyl carbons at δ_C 205.4 (C-1′), 193.8 (C-1) and 196.3 (C-3) and olefinic carbons at δ_C 110.7 (C-2). This information indicates the 3-hydroxycyclohex-2-en-1-one moiety and acyl side chain. The ^{1}H NMR experiment displayed methine oxygenated protons at δ_H 4.43 (q, $J = 2.8$ Hz) and 4.17 (d, $J = 2.8$ Hz) which were assigned as H-5 and H-6, respectively. The small coupling constant ($J = 2.8$ Hz) of H-6 indicated the axial-equatorial coupling of H-6 and H-5. The quartet signal of H-5 ($J = 2.8$ Hz) was observed in the ^{1}H NMR spectrum and indicated the equatorial axis of H-5. The HMQC data insisted that these two protons were located on oxygenated carbons at δ_C 67.0 (C-5) and 74.7 (C-6). The doublet of doublet signals at δ_H 2.97 and 3.05 (each 1H, $J = 10.0$, 2.7 Hz) of H-4 attached to a carbon at δ_C 38.4. The ^{1}H–^{1}H COSY showed the connection of the H-4/H-5/H-6 system. Cross peaks between H-4 and C-2 (110.7) and C-3 (196.3), between H-5 and C-1 (193.8) and C-3 (196.3), and between H-6 and C-1 (193.8) were evident. The rest of ^{1}H and ^{13}C NMR signals showed an alkenoyl side chain which was the same as compounds **1** and **2**. Thus, the structure of **4** was determined as 3,5,6-trihydroxy-2-(octadec-13′*Z*-enoyl)cyclohex-2-en-1-one, named knecorticosanone F, as shown in Fig. 1.

The HRESIMS data of compound **5** indicated a molecular formula of $C_{24}H_{34}O_3$ (m/z 371.2578 $[M + H]^+$). An intramolecular hydrogen bond of a hydroxyl proton displayed low field shift at δ_H 12.4. The HMBC experiment displayed correlation between

hydroxy proton and carbon at δ_C 160.8 (C-5). The ^{1}H NMR showed two doublet signals (J = 8.4 Hz) at δ_H 6.75 and δ_H 6.65 which were assigned as H-6 and H-8, respectively. These protons correlated with aromatic carbons at δ_C 111.1 (C-6) and δ_C 106.9 (C-8), respectively, in the HMQC experiment. The proton H-7 exhibited a triplet signal at δ_H 7.38 and was located on an aromatic carbon at δ_C 135.0. Cross peaks between H-6 and C-5 (δ_C 160.8), C-10 (δ_C 110.6) and C-8 (δ_C 106.9), between H-7 and C-5 (δ_C 160.8) and C-9 (δ_C 156.8) and between H-8 and C-6 (δ_C 111.1), C-9 (δ_C 156.8) and C-10 (δ_C 110.6) were observed in the HMBC spectrum. The ^{13}C NMR signals of C-2 and C-4 appeared at δ_C 171.3 and δ_C 183.6, respectively. The signals at δ_H/δ_C 6.00/108.4 of methine proton H-3 showed long range coupling with C-2 (δ_C 171.3), C-4 (δ_C 183.6) and C-10 (δ_C 110.6). The long chain hydrocarbon at the C-2 position was confirmed by the correlation of H-1′ (δ_H/δ_C 2.50/34.4) and C-2 (δ_C 171.3) and C-3 (δ_C 108.4), in addition, correlation between H-2′ (δ_H/δ_C 1.63/26.8) and C-2 was observed. The *cis* double bond was confirmed by the typical shielded signals of carbon C-9′ and C-12′ at δ_C 27.2. All data confirmed the structure of **5** was 5-hydroxy-2-(10′*Z*-pentadecenyl)-4*H*-chromen-4-one, named knecorticosanone G, as shown.

The molecular formula of compound **6**, $C_{24}H_{38}O_3$, was confirmed on the basis of the molecular ion peak *m*/*z* 397.2710 $[M + Na]^+$. The broad singlet signal at δ_H 9.62 was assigned to the intramolecular hydrogen bond of a hydroxyl group. Three aromatic protons at δ_H 7.21 (1H, t, J = 8.2 Hz, H-4) and δ_H 6.38 (2H, d, J = 8.2 Hz, H-3 and H-5) were observed in the ^{1}H NMR spectrum. Correlations between H-3 and C-1 (δ_C 110.1) and C-2 (δ_C 161.2), between H-4 and C-2 (δ_C 161.2) were evident. The ^{13}C NMR spectrum displayed an acylalkenyl side chain by showing a carbonyl ketone at δ_C 208.0 and *cis* double bond (C-13′ and C-14′) at δ_C 129.9, in addition, α-olefinic C-12′ and C-15′ showed at δ_C 27.2 and 26.9, respectively. Correlations in the HMBC spectrum of protons and carbons at C-14′ to C-18′ were the same as compound **1**. Thus, the structure of compound **6** was 1,3-dihydroxy-2-(octadec-13′*Z*-enoyl)benzene, named knecorticosanone H, established as shown.

Biological activity

Compounds **1–8** were evaluated for cytotoxicity against HepG2 and KKU-M156 cells by using the sulforhodamine B assay and the results are shown in Table 3. Compound **1** exhibited the most cytotoxic activity against HepG2 and KKU-M156 cell lines with IC_{50} values of 1.57 ± 0.37 and 1.78 ± 0.14 μg mL^{-1}, respectively. Compound **2** showed an IC_{50} value around 50 μg mL^{-1} against these two cell lines. Comparing the activity between compounds **2** and **1**, it seems that the hydroxyl group at the C-6 position is important for the cytotoxic activity. Compound **3** exhibited cytotoxicity with IC_{50} values of 6.81 ± 1.15 and 8.83 ± 1.76 μg mL^{-1} against HepG2 and KKU-M156 cell lines, respectively, which was stronger than compound **2**. These results indicate that the aryl moiety is necessary for the cytotoxicity. In the case of compound **6**, it showed stronger activity than **2**. Hence, it was concluded that the 1,3-dihydroxybenzene moiety was important for the activity. In the case of compounds **6** and **7**, the results support that the aryl moiety appeared to increase the activity. Chromone **5** displayed cytotoxicity against these two cell lines with IC_{50} values of 8.17 ± 2.47 and 15.92 ± 3.68 μg mL^{-1}. These results suggest that compound **5** was selective to the HepG2 cell line.

Table 3 Cytotoxicity of isolated compounds from *Knema globularai* (IC_{50} μg mL^{-1})[a]

Compound	HepG2	KKU-M156
1	1.57 ± 0.37	1.78 ± 0.14
3	6.81 ± 1.15	8.83 ± 1.76
5	8.17 ± 2.47	15.92 ± 3.68
6	25.26 ± 7.37	23.14 ± 3.79
7	10.50 ± 3.48	18.66 ± 4.36
8	18.39 ± 6.31	34.19 ± 12.53
The other	Inactive	Inactive
Cisplatin	4.67 ± 1.63	30.09 ± 15.11

[a] Inactive at IC_{50} > 50 μg mL^{-1}.

3. Experimental section

General experimental procedures

Melting points were detected by a Sanyo Gallenkamp melting point apparatus. A JASCO P-1020 digital polarimeter was used to determine optical rotations. The UV spectra were obtained using an Agilent 8453 UV-visible spectrophotometer. A PerkinElmer Spectrum One FT-IR spectrophotometer was used to acquire the IR spectra. A Varian Mercury Plus spectrometer (400 MHz) was used to measure the NMR spectra. HRESIMS was performed on a Micromass Q-TOF 2 hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer. Flash column chromatography (FCC) was performed by using silica gel less than 0.063 mm. Analytical thin-layer chromatography (TLC) was accomplished on Merck Si gel 60 F_{254} plates.

Plant material

The fruits of *K. globularia* were collected from Ban Thawat Buri, Thawat Buri District, Roi-Et Province, Thailand (16°06′00.8″N, 103°49′06.2″E) in April 2019. The plant was identified by Asst. Prof. Dr Suppachai Tiyaworanant, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU022019) was deposited in the Natural Product Research Unit, Khon Kaen University, Khon Kaen.

Extraction and isolation

The dried powdered fruits of *K. globularia* (1.25 kg) were soaked in hexane (3 L × 3), EtOAc (3 L × 3), and MeOH (3 L × 3). Solvent removal was done *in vacuo* to produce crude hexane (126 g), EtOAc (89 g), and MeOH (73 g) extracts. The crude hexane extract (126 g) was subjected to column chromatography, eluted with a gradient system of hexane, hexane–EtOAc and EtOAc–MeOH. On the basis of their TLC characteristics, the fractions which contained the same major compounds were combined to give eight fractions (H1–H8). Fraction H3 was subjected to silica gel column chromatography and eluted with a gradient system of hexane, hexane–EtOAc and EtOAc–MeOH to give 9

subfractions. Subfraction H3.3 was purified by RP-C18 column chromatography and eluted with MeOH to give **5** (38.0 mg, 0.0302%). Gel filtration (sephadex LH-20) was carried out on subfraction H3.8 eluting with MeOH and afforded **6** (52.6 mg, 0.0418%). Subfraction H3.9 was further purified by column chromatography, eluting with 100% CH_2Cl_2 and afforded **3** (10.3 mg, 0.0082%). Moreover, fraction 4 was purified by silica gel CC using 20% EtOAc/hexane to yield 10 subfractions. Further purification of subfraction H4.4 (silica gel CC, 20% EtOAc/hexane) gave 8 subfractions. Subfraction H4.4.4 was purified by silica gel FCC and eluted with 50% CH_2Cl_2/hexane to yield **8** (16.0 mg, 0.0127%). Compound **7** (8.8 mg, 0.0070%) was obtained from fraction H5 (silica gel CC, 20% EtOAc/hexane).

The crude EtOAc extract (89 g) was subjected to column chromatography and afforded 10 fractions. Fraction E3 was purified by silica gel FCC and eluted with 50% CH_2Cl_2/hexane to give 6 subfractions. Subfraction E3.4 was further purified by preparative TLC using pure CH_2Cl_2 to yield **9** (5.2 mg, 0.0058%). Moreover, fraction E6 was further purified by column chromatography, eluting with 100% CH_2Cl_2 and afforded 8 subfractions. Subfraction E6.2 was further purified by sephadex LH-20 to give 4 subfractions. A mixture of compounds **10** and **11** (10.1 mg, 0.0114%) was obtained from the recrystallization of subfraction E6.2.3.

The crude MeOH extract (73 g) was subjected to column chromatography and afforded 5 fractions. Fraction M2 was purified by silica gel CC and eluted with 10% EtOAc/hexane to give **1** (35.6 mg, 0.0488%). Fraction M3 was purified by RP-C18 column chromatography eluted with MeOH to yield 4 subfractions. Moreover, subfraction M3.2 was purified by preperative TLC with 100% CH_2Cl_2 to yield **2** (30.2 mg, 0.0414%). Fraction 4 was further purified by sephadex LH-20 to give 4 subfractions. Subfraction M4.3 was purified by RP-C18 column chromatography eluted with MeOH to give 5 subfractions. Subfraction 4.3.3 was further purified by preparative TLC using pure CH_2Cl_2 to yield **4** (10.8 mg, 0.0302%).

Compound (**1**): yellowish oil; $[\alpha]_D^{20.8}$ + 37.1 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 234 (4.11), 272 (4.27) nm; IR (neat) ν_{max} 3466, 2922, 2853, 1664, 1553, 1457 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 393.3013 $[M + H]^+$ (calcd for $C_{24}H_{41}O_4$, 393.3005).

Compound (**2**): yellowish oil; $[\alpha]_D^{18.5}$ −50.7 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 237 (4.07), 269 (4.34) nm; IR (neat) ν_{max} 3494, 2923, 2854, 1659, 1555, 1452 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 393.2988 $[M + H]^+$ (calcd for $C_{24}H_{41}O_4$, 393.3005).

Compound (**3**): yellowish oil; $[\alpha]_D^{18.5}$ −52.1 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 226 (4.26), 274 (4.14) nm; IR (neat) ν_{max} 3360, 2924, 2853, 1633, 1578, 1445 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 439.2124 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_6Na$, 439.2097).

Compound (**4**): brown oil; $[\alpha]_D^{20.8}$ + 122.2 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 232 (3.97), 273 (4.07) nm; IR (neat) ν_{max} 3433, 2921, 2853, 1725, 1661, 1543, 1462 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 409.2947 $[M + H]^+$ (calcd for $C_{24}H_{41}O_5$, 409.2954).

Compound (**5**): orange oil; UV (CH_3OH) λ_{max} (log ε) 234 (4.28), 326 (3.58) nm; IR (neat) ν_{max} 3290, 2922, 2853, 1650, 1615, 1467, 1411, 1236 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 371.2578 $[M + H]^+$ (calcd for $C_{24}H_{35}O_3$, 371.2586).

Compound (**6**): yellowish oil; UV (CH_3OH) λ_{max} (log ε) 224 (3.86), 269 (3.78), 344 (3.19) nm; IR (neat) ν_{max} 3328, 2923, 2853, 1628, 1590, 1509, 1451 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 397.2710 $[M + Na]^+$ (calcd for $C_{24}H_{38}O_3Na$, 397.2719).

Cytotoxicity assays

A sulforhodamine B assay was performed as formerly described.[20] KKU-M156 and HepG2 cells were placed into 96-well culture plates. The isolated compounds were added into cultured cells and incubated for 24 h. The cytotoxicity was calculated as the percentage absorbance of controls. The IC_{50} value was calculated by a non-linear curve-fitting program from triplicate assay of three separate experiments.[21] The positive control was a cisplatin standard and showed IC_{50} values of 10.0 ± 4.8 and 2.2 ± 0.7 μM against KKU-M156 and HepG2 cell lines, respectively.

ECD calculations

Preliminary conformational analyses were carried out using HyperChem software. These dominant conformers were developed at the B3LYP/6-311g(d,p) basis set by density functional theory.[22] The Gaussian 09 program was used to calculate ECD spectra.[23] The single point energy calculations were computed using time-dependent density functional theory (TD-DFT)[24] at the CAM-B3LYP/6-311++g(d,p) level of theory.[25] The bulk solvent effects were examined using the CPCM polarizable conductor calculation model.[26]

4. Conclusions

Chemical investigation of the fruits of *Knema globularia* (Lam.) warb led to the isolation of six new compounds, knecorticosanones C–H (**1–6**), in addition, three known lignans were also isolated. All isolated polyketides were evaluated for cytotoxicity against HepG2 and KKU-M156 cell lines. Compound **1** exhibited the most cytotoxicity against these two cell lines with IC_{50} values of 1.57 ± 0.37 and 1.78 ± 0.14 μg mL^{-1}, respectively. Cytotoxicity results of compounds **1** and **2** indicate that the hydroxyl group at the C-6 position are important for the activity.

Conflicts of interest

There are no conflicts to declare.

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References

1 W. Salleh and F. Ahmad, *Pharm. Sci.*, 2017, **23**(4), 249–255.
2 T. Smitinand, *Thai Plant Names*, Bangkok, revised edn, 2001.
3 L. M. Perry and J. Metzger, *Medicinal plants of East and Southeast Asia: Attributed properties and uses*, Cambridge, 1st edn, 1980.
4 A. Zahir, A. Jossang, B. Bodo, H. A. Hadi, H. Schaller and T. Sevenet, *J. Nat. Prod.*, 1993, **56**(9), 1634–1637.
5 Vinayachandra and K. R. Chandrashekar, *J. Herbs, Spices Med. Plants*, 2014, **20**(2), 183–195.
6 N. Ismail, M. N. Akhtar, M. Ismail, S. Zareen, S. A. A. Shah, N. H. Lajis and S. N. Tajuddin, *Nat. Prod. Res.*, 2015, **29**(16), 1571–1574.
7 J. Z. Deng, S. R. Starck, S. Li and S. M. Hecht, *J. Nat. Prod.*, 2005, **68**(11), 1625–1628.
8 U. Sriphana, C. Yenjai and M. Koatthada, *Phytochem. Lett.*, 2016, **16**, 129–133.
9 T. H. Giap, P. M. Duc, N. V. The, M. Popova, V. Bankova, C. T. Hue, V. T. K. Oanh, N. T. M. Hang, N. V. Hung and N. L. Thanh, *Nat. Prod. Res.*, 2019, **8**, 1–10.
10 T. V. Pham, H. K. T. Bach, D. V. Ho and B. C. Nguyen, *Nat. Prod. Res.*, 2020, 1–7, DOI: 10.1080/14786419.2020.1777416.
11 F. M. Jaafar, M. J. M. Ridhwan, N. M. A. Mustapha, A. Alias and N. H. Ismail, *Journal Teknologi (Science & engineering)*, 2016, **78**(5–3), 103–108.
12 N. Rangkaew, R. Suttisri, M. Moriyasu and K. Kawanishi, *Fitoterapia*, 2009, **80**(6), 377–379.
13 C. Wiart, *Medicinal plants of Asia and the Pacific*, Boca Raton, 2006.
14 M. Wenli, Y. Yong, N. Wei and C. Changxiang, *Acta Bot. Yunnanica*, 2000, **22**, 358–360.
15 U. Sriphana, C. Yenjai, J. Suthiwong and K. Poopasit, *Nat. Prod. Res.*, 2020, 1–8, DOI: 10.1080/14786419.2020.1815736.
16 G. B. Messiano, L. Vieira, M. B. Machado, L. M. X. Lopes, S. A. D. Bortoli and J. Zukerman-Schpector, *J. Agric. Food Chem.*, 2008, **56**, 2655–2659.
17 D. Pascoli, C. Inara, N. R. Isabele and L. M. X. Lucia, *Phytochemistry*, 2006, **67**(7), 735–742.
18 A. O. Pereira, J. M. Avilab, G. D. Carmob, F. S. Siqueirac, M. M. A. Camposc, D. F. Backb, A. F. Morelb and I. I. Dalcola, *Ind. Crops Prod.*, 2018, **121**, 461–467.
19 E. A. Ferreira, J. B. Reigada, M. V. Correia, M. C. M. Young, E. F. Guimarães, G. C. Franchi Jr, A. E. Nowill, J. H. G. Lago, L. F. Yamaguchi and M. J. Kato, *J. Nat. Prod.*, 2014, **77**, 1377–1382.
20 T. Sribuhom, Y. Thummanant, S. Phusrisom, V. Kukongviriyapan, S. Tontapha, V. Amornkitbamrung and C. Yenjai, *J. Nat. Prod.*, 2019, **82**, 2246–2251.
21 P. Samatiwat, A. Prawan, L. Senggunprai, U. Kukongviriyapan and V. Kukongviriyapan, *Tumor Biol.*, 2016, **37**, 11495–11507.
22 R. G. Parr and W. Yang, *Density Functional Theory of Atoms and Molecules*, Oxford University Press, Oxford, UK, 1989.
23 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, *et al.*, *Gaussian 09, Revision B.01*, Gaussian, Inc., Wallingford, CT, 2010.
24 G. Pescitelli and T. Bruhn, *Chirality*, 2016, **28**, 466–474.
25 T. Yanai, D. P. Tew and N. C. Handy, *Chem. Phys. Lett.*, 2004, **393**, 51–57.
26 A. Mándi, I. W. Mudianta, T. Kurtán and M. J. Garson, *J. Nat. Prod.*, 2015, **78**, 2051–2056.