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Two new cycloartane-type triterpenoids and one new flavanone from the leaves of *Dasymaschalon dasymaschalum* and their biological activity

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ABSTRACT

Two new cycloartane-type triterpenoids, 3β-hydroxy-21-*O*-acetyl-24-methylenecycloartane (**3**) and 3β,21-dihydroxy-24,31-epoxy-24-methylenecycloartane (**4**), one new flavanone, 7-hydroxy-6,8-dimethoxyflavanone (**5**), two new natural products, 2-hydroxybenzyl benzoate (**7**) and 2-phenyl-2-acetoxyethyl benzoate (**8**), and ten known compounds, 3β-hydroxy-24-methylenecycloartane (**1**), 3β,21-dihydroxy-24-methylenecycloartane (**2**), desmosdumotin B (**6**), artabotrene (**9**), (−)-senepoxide (**10**), (+)-crotepoxide (**11**), (−)-1,6-desoxypipoxide (**12**), rotundol (**13**), cassipourol (**14**) and (+)-spathulenol (**15**) were isolated from the leaves of *Dasymaschalon dasymaschalum*. The structures of the new compounds were elucidated by spectroscopic analysis and of the known compounds by comparison of their physical, UV, IR, ^{1}H and ^{13}C NMR data with those of published compounds. Antimycobacterial, antiplasmodial and cytotoxic activities of the isolates, except **8** and **10** were evaluated. Compounds **1**, **4**, **5**, **11**, **12** and **15** exhibited potent cytotoxic activities against human lung cancer cell lines (NCI-H187) with respective IC_{50} values of 4.67, 7.82, 1.85, 6.33, 3.07 and 6.68 μg/mL.

1. Introduction

The genus *Dasymachalon* (Annonaceae) contains about 40 species native to tropical Asia and Africa. Twelve species are found in Thailand (Chalermklin, 2001). Previous phytochemical studies of *Dasymachalon* plants have reported the isolation of flavonol glycosides, acetogenins and alkaloids from the leaves of *D. sootepensa* (Sinz et al., 1998a; Sinz et al., 1998b; Sinz et al., 1998a,b,c). In addition, alkaloids were found from the stems, leaves and twigs of *Dasymaschalon dasymaschalum* (synonym: *Dasymaschalon blumei* Finet & Gagnep var. *suratense* Bân) (Wang et al., 2009) with potentially cytotoxic activities (Chanakul et al., 2011). In continuation of our work on bioactive substances from Thai medicinal plants, preliminary screenings of the ethyl acetate extract of the leaves of *D. dasymaschalum* revealed its cytotoxicity against human epidermoid carcinoma (KB), human breast cancer (BC) and human small cell lung cancer (NCI-H187) cell lines with the IC_{50} valued of 40.84, 17.79 and 4.35 μg/mL, respectively. We are now reporting the isolation of two new cycloartane-type triterpenoids, 3β-hydroxy-21-*O*-acetyl-24-methylenecycloartane (**3**) and 3β,21-dihydroxy-24,31-epoxy-24-methylenecycloartane (**4**), a new flavanone, 7-hydroxy-6,8-dimethoxyflavanone (**5**), two new natural products, 2-hydroxybenzyl benzoate (**7**) (Sekine et al., 1981) and 2-phenyl-2-acetoxyethyl benzoate (**8**) (Wu et al., 1999), and ten known compounds, 3β-hydroxy-24-methylenecycloartane (**1**) (De Pascual-Teresa et al., 1987), 3β,21-dihydroxy-24-methylenecycloartane (**2**) (Arthur et al., 1974; Purushothaman et al., 1983), desmosdumotin B (**6**) (Wu et al., 2001; Nguyen et al., 2009), artabotrene (**9**) (Murphy et al., 2008), (−)-senepoxide (**10**) (Hollands et al., 1968), (+)-crotepoxide (**11**) (Pancharoen et al., 1984), (−)-1,6-desoxypipoxide (**12**) (Schulte et al., 1982), rotundol (**13**) (Stevenson et al., 2007), cassipourol (**14**) (Chaturvedula et al., 2006) and (+)-spathulenol (**15**) (Tringali et al., 1995) from the leaves of *D. dasymaschalum* (Fig. 1). The structures of the known compounds were elucidated from comparisons with literature spectroscopic data. The polyoxygenated cyclohexenes, the benzyl benzoate esters and the cycloartanes were isolated for the first time from the *Dasymachalon* genus. The antimycobacterial, antiplasmodial and cytotoxic activities of the isolates, except **8** and **12** are also reported. Herein, this paper describes the isolation, structural elucidation and biological activities of the isolates.

2. Results and discussion

Compound **2** was obtained as colorless needles, m.p. 160 °C, $[\alpha]_D^{27}$ + 39.5 ($CHCl_3$; *c* 0.57) (lit. m.p. 167–168; $[\alpha]_D^{30}$ + 40 (*c* 1.0, $CHCl_3$) (Purushothaman et al., 1983)), and identified as 3β,21-dihydroxy-24-methylenecycloartane by comparing its spectral data with the literature data for **2**. This compound was previously isolated from *Lithocarpus* sp. and *Heynia trijuga* (Arthur et al., 1974;

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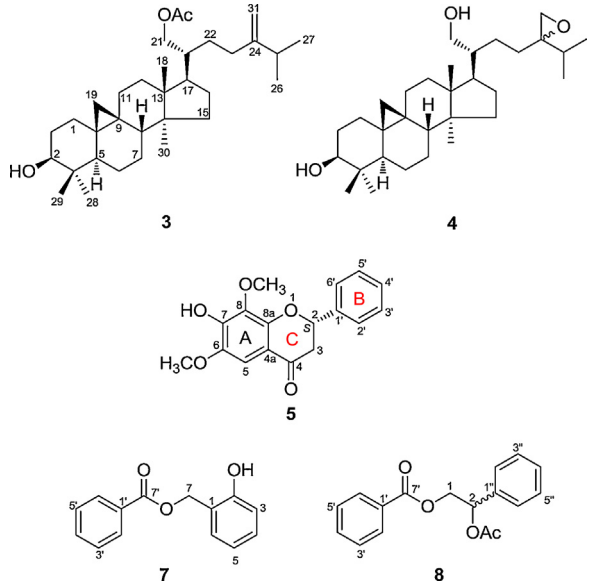

Fig. 1. Structures of compounds **3**–**5**, **7** and **8**.

Purushothaman et al., 1983). This is the first report of the isolation of **2** from the genus *Dasymachalon* as well as its complete ^{1}H and ^{13}C NMR (Tables 1 and 2) assignments on the basis of the HMQC and HMBC spectra.

Compound **3** was isolated as a colorless oil with $[\alpha]_D^{27}$ + 26.2 (*c* 0.58, $CHCl_3$) and shown to have the molecular formula $C_{33}H_{54}O_3$ by HR-TOF-APCI-MS. The structure of compound **3** was closely related to compound **2** based on the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2). The difference in 42 mass units between **2** and **3**, IR spectrum of **3**, which showed an ester carbonyl absorption at 1740 cm^{-1}, and the appearance of a three-proton singlet at δ 2.00 and a methyl carbon at δ 21.0 and an ester carbonyl at δ 171.3 in the ^{1}H and ^{13}C NMR spectra of **3**, respectively, indicating the presence of one extra acetyl group. The long-range HMBC correlations of 21–CH_2O (δ 3.89 and 4.16) to the ester carbonyl (δ 171.3), C-17 (δ 39.6), C-20 (δ 46.6) and C-22 (δ 29.0) (Fig. 2) confirmed the substitution of the acetyl group at C-21. Compound **3** was thus identified as 3β-hydroxy-21-*O*-acetyl-24-methylenecycloartane.

Table 1
The ^{1}H NMR (400 MHz) data of **2**–**4** ($CDCl_3$, δ in ppm, *J* in Hz).

Position	2	3	4
1			1.25, m
3	3.29, dd (11.0, 4.4)	3.22, m	3.28, m
5	1.31, m	1.23, dd (12.7, 4.4)	1.30, m
8	1.54, m	1.44, dd (12.5, 4.8)	1.51, m
17	1.51, m	1.62, m	1.50, m
18	0.99, s	0.93, s	1.00, s
19	0.34, d (4.2)	0.26, d (4.1)	0.35, d (4.1)
	0.56, d (4.2)	0.48, d (4.1)	0.56, d (4.1)
20	1.94, m	1.87, m	1.60, m
21	3.63, dd (11.1, 4.4)	3.89, dd (11.3, 4.4)	3.22, dd (11.4, 1.0)
	3.74, dd (11.1, 2.5)	4.16, dd (11.3, 3.2)	3.82, ddd (11.4, 4.0, 1.3)
23	2.15, m	2.03, m	1.60, m
			1.81, m
25	2.25, septet (6.8)	2.16, septet (6.8)	2.37, septet (7.0)
26	1.03, d (6.8)	0.96, d (6.8)	0.90, d (7.0)
27	1.04, d (6.8)	0.95, d (6.8)	0.85, d (7.0)
28	0.97, s	0.90, s	0.97, s
29	0.81, s	0.74, s	0.81, s
30	0.92, s	0.84, s	0.88, s
31	4.70, d (0.9)	4.61, d (1.1)	3.28, d (11.0)
	4.74, br.s	4.67, br.s	3.57, d (11.0)
21–$OCOCH_3$		2.00, s	

Table 2
The ^{13}C NMR (100 MHz) data of **2**–**4** in $CDCl_3$.

Position	2	3	4
1	30.3	30.4	32.0
2	26.0	26.0	25.0
3	78.8	78.8	78.8
4	40.5	40.5	40.5
5	47.0	47.1	47.1
6	21.1	21.1	21.1
7	31.9	31.8[a]	29.7
8	47.9	48.0	47.9
9	19.9	19.9	19.9
10	26.2	26.1	26.1
11	26.4	26.4	26.0
12	32.1	32.0[a]	32.3
13	45.2	45.1	45.2
14	48.9	48.9	48.8
15	35.4	35.4	35.4
16	27.6	27.6	26.3
17	42.6	39.6	38.5
18	18.3	18.2	18.4
19	29.8	29.9	29.9
20	46.2	46.6	48.8
21	62.4	64.9	65.0
22	28.2	29.0	30.4
23	31.3	30.9	25.8
24	156.6	156.3	76.0
25	33.8	33.8	26.6
26	21.9[a]	21.8[b]	15.7
27	22.0[a]	21.9[b]	17.2
28	25.4	25.4	25.4
29	14.0	14.0	14.0
30	19.4	19.4	19.3
31	106.2	106.4	63.5
21–*OCOCH*$_3$		171.3	
21–*OCOCH*$_3$		21.0	

a, b: may be interchangeable.

Compound **4** was obtained as a colorless oil with $[\alpha]_D^{28}$ + 166.1 (*c* 0.05, $CHCl_3$). The HR-TOF-APCI-MS of **4** exhibited a pseudo molecular ion $[M+Na]^+$ at *m*/*z* 495.3807 consistent with the molecular formula $C_{31}H_{52}O_3$. The structure of **4** was closely related to **2** based on the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2). However, **4** had 16 mass units more than **2**, indicating the presence of one extra oxygen atom. The appearances of methylene protons as two doublets at δ 3.28 (*J* = 11.0 Hz) and 3.57 (*J* = 11.0 Hz) and an oxymethylene carbon at δ 63.5 and an oxyquaternary carbon at δ 76.0 in the ^{1}H and ^{13}C NMR spectra of **4**, respectively, suggested an epoxy moiety. The long-range HMBC correlations between C-24 (δ 76.0) and H-25 (δ 2.37), H-26 (δ 0.90), H-27 (δ 0.85) and Ha-31 (δ 3.57) and C-23 (δ 25.8) and Ha-31 (δ 3.57) and Hb-31 (δ 3.28) (Fig. 2) indicated that the epoxide ring located between C-24 and C-31. Compound **4**, therefore, assigned as 3β,21-dihydroxy-24,31-epoxy-24-methylenecycloartane.

Compound **5** was isolated as a pale yellow oil with $[\alpha]_D^{27}$ − 1.6 (*c* 0.14, $CHCl_3$). The HR-TOF-APCI-MS of **5** exhibited a pseudo molecular ion $[M+H]^+$ at *m*/*z* 301.1062, corresponding to the molecular formula $C_{17}H_{16}O_5$. The UV spectrum of **5** exhibited absorptions at 344, 278 and 238 nm and the IR spectrum showed bands for hydroxyl (3413 cm^{-1}) and carbonyl (1672 cm^{-1}) functions. The ^{1}H NMR spectrum of **5** (Table 3) showed five aromatic protons [δ 7.46 (m, 2H), 7.45 (m, 2H), 7.39 (m, 1H)], characteristic of a monosubstituted phenyl ring (ring B), and an ABX system at δ 2.80 (dd, *J* = 16.8, 2.9 Hz, Ha-3), 3.03 (dd, *J* = 16.8, 13.4 Hz, Hb-3) and 5.41 (dd, *J* = 13.4, 2.9 Hz, H-2) (ring C), suggesting the flavanone with an unsubstituted in ring B. The ^{1}H NMR spectrum of **5** also exhibited an aromatic methine proton as a singlet at δ 6.39 (H-5), two aromatic methoxy groups at δ 3.92 (s, 6-OCH_3) and 3.95 (s, 8-OCH_3) and one phenolic hydroxyl group

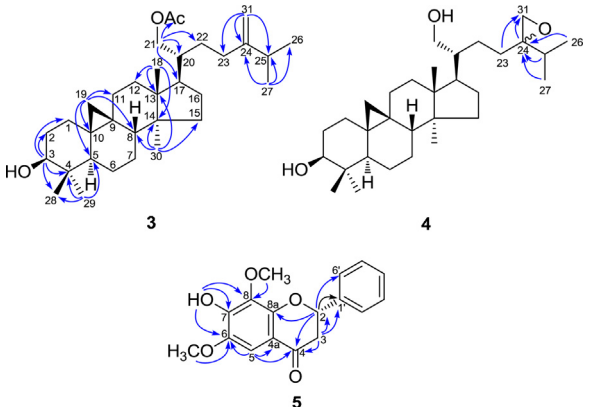

Fig. 2. Selected HMBC correlations of **3**–**5**.

at δ 5.56 (s, 7-OH). The ^{13}C NMR spectrum of **5** (Table 3) exhibited signals of one monosubstituted aromatic ring (ring B) at δ 138.7 (C-1′), 126.1 (C-2′ and C-6′), 128.9 (C-3′ and C-5′) and 128.8 (C-4′), one pentasubstituted aromatic ring (ring A) at δ 108.5 (C-4a), 96.4 (C-5), 154.0 (C-6), 133.9 (C-7), 146.0 (C-8) and 157.2 (C-8a), two methoxy carbons at δ 56.3 (6–OCH_3) and 61.8 (8–OCH_3), one oxygenate methine carbon at δ 79.5 (C-2), one methylene carbon at δ 45.5 (C-3) and one carbonyl carbon at δ 189.5 (4–C=O). The positions of the two methoxy groups at C-6 and C-8 and the phenolic hydroxyl group at C-7 were established from the connectivities indicated in the 2D HMBC experiments (Fig. 2). The methoxy protons at δ 3.92 and 3.95 showed the long-range HMBC correlations to the signals of C-6 (δ 154.0) and C-8 (δ 146.0), respectively. The phenolic hydroxyl group at δ 5.56 was correlated with C-7 (δ 133.9), C-6 (δ 154.0) and C-8 (δ 146.0). The important long-rang correlations were also observed between H-5 (δ 6.39) and 4–C=O (δ 189.5), C-4a (δ 108.5), C-6 (δ 154.0) and C-7 (δ 133.9), H-3 (δ 2.80 and 3.03) and 4–C=O (δ 189.5), C-4a (δ 108.5), C-2 (δ 79.5) and C-1′ (δ 138.7) and H-2 (δ 5.41) and 4–C=O (δ 189.5), C-8a (δ 157.2), C-1′ (δ 138.7) and C-2′ and C-6′ (δ 126.1). The levorotatory nature of **5** indicated the normal flavanone *S* absolute configuration at C-2 by comparing the optical rotation value with literature data for 6-geranyl-5,7-dihydroxy-3′,4′-dimethoxyflavanone (Asai et al., 2008). Compound **5** was thus identified as (2*S*)-7-hydroxy-6,8-dimethoxyflavanone.

Table 3
The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of **5** ($CDCl_3$, δ in ppm, *J* in Hz).

Position	δ_H	δ_C
2	5.41, dd (13.4, 2.9)	79.5
3	2.80, dd (16.8, 2.9)	45.5
	3.03, dd (16.8, 13.4)	
4		189.5
4a		108.5
5	6.39, s	96.4
6		154.0
7		133.9
8		146.0
8a		157.2
1′		138.7
2′	7.46, m	126.1
3′	7.45, m	128.9
4′	7.39, m	128.8
5′	7.45, m	128.9
6′	7.46, m	126.1
6–OCH_3	3.92, s	56.3
7–OH	5.56, s	
8–OCH_3	3.95, s	61.8

Compounds **7** and **8** were identified as 2-hydroxybenzyl benzoate and 2-phenyl-2-acetoxyethylbenzoate ($[\alpha]_D^{28}$ – 6.5 (*c* 0.16, $CHCl_3$)), respectively, by comparing their spectroscopic data with literature values (Sekine et al., 1981; Wu et al., 1999). These compounds were previously synthesized by the reaction between dialkylacylphosphonate and 2-hydroxybenzyl alcohol (Sekine et al., 1981) and the reaction of cyclic ketene acetals with carboxylic acid (Wu et al., 1999). The absolute configuration of **8** (C-2) was not determined due to the inadequacy of sample. However, these are the first report of **7** and **8** from a natural source.

All of the isolated compounds except **8** and **10** were evaluated for cytotoxic, antiplasmodial and antimycobacterial activities as summarized in Table 4. Compounds **1**, **5** and **12** showed good

Table 4
Biological activities of compounds **1**–**7**, **9** and **11**–**15**.

Compounds[a]	Cytotoxicity (IC_{50}, μg/mL)			Antiplasmodial (IC_{50}, μg/mL)	Antimycobacterial (MIC, μg/mL)
	KB[c]	BC[d]	NCI-H187[e]		
1	15.85	31.65	4.67	NA	NA
2	NA	30.62	NA	NA	NA
3	17.80	NA	17.54	NA	NA
4	18.21	NA	7.82	NA	50
5	15.24	44.60	1.85	NA	NA
6	NA	NA	49.74	NA	NA
7	25.49	48.42	48.61	NA	NA
9	31.46	NA	18.72	NA	NA
11	7.44	32.43	6.33	NA	NA
12	19.87	17.76	3.07	NA	NA
13	NA	NA	NA	NA	NA
14	48.83	30.84	15.63	NA	NA
15	26.26	NA	6.68	NA	NA
Ellipticin[b]	0.667	0.128	0.522		
Doxorubicin[b]	0.096	0.065	0.031		
Dihydroartemisinin[b]				0.0044	
Rifampicin[b]					0.019
Kanamicin[b]					1.250
Isoniazid[b]					0.050

NA = not active (IC_{50} > 50 μg/mL or MIC > 200 μg/mL).
[a] Purity (%) of tested compounds were >98%.
[b] The compounds were used as a positive control (95%).
[c] Human epidermoid carcinoma in the mouth.
[d] Human breast cancer cells.
[e] Human lung cancer cells.

cytotoxicity in NCI-H187 cells with IC_{50} values in the range of 1.85–4.67 μg/mL, while compounds **3**, **4**, **9**, **11**, **14** and **15** were moderately active with an IC_{50} ranging from 6.33 to 18.72 μg/mL and compounds **2**, **6**, **7** and **13** were either weakly active or inactive. Compounds **1, 3**–**5** and **11** showed moderate cytotoxicity in KB cells with an IC_{50} in the range of 7.44–18.21 μg/mL, whereas compounds **2, 7, 9** and **12**–**15** were either weakly active or inactive. Only compound **12** showed moderate cytotoxicity in BC cell with an IC_{50} value of 17.76 μg/mL, while compounds **1**–**7, 9, 11** and **13**–**15** were either weakly active or inactive. All of tested compounds were inactive against antiplasmodial activity and were also inactive against antimycobacterial activity, except compound **4** which exhibited activity with the MIC value of 50 μg/mL.

3. Experimental

3.1. General experimental procedures

Melting points were determined by Buchi melting point B-540 apparatus and are reported without correction. Optical rotations $[\alpha]_D$ were measured in $CHCl_3$ solution at the sodium D line (590 nm) on a JASCO DIP-370 digital polarimeter. UV spectra were recorded with a Shimadzu UV-VIS 2001S Spectrophotometer. IR spectra were recorded with a Perkin Elmer Spectrum One FT-IR Spectrophotometer using UATR technique and Shimudzu FTIR-8900 instrument with KBr disks. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ solution on a Bruker AVANCE 400 (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. The signals in the ^1H and ^{13}C NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC and HMBC. EIMS were recorded on a MS Finnigan Polaris spectrometer. HRMS were recorded on a Bruker MicrOTOF Mass spectrometer. Column chromatography (CC) was carried out on silica gel 60 (Scharlau, 70–230 mesh or 230–400 mesh) and 60 silanized (Merck, 40–230 mesh) Sephadex LH 20. Vacuum liquid chromatography (VLC) (Coll and Bowden, 1989) was carried out on silica gel 60H (Merck, 5–40 μm). TLC were performed on precoated silica gel 60 F_{254} plates (Merck); spots were detected by UV or spraying with 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4 following by heating. All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

3.2. Plant material

The leaves of *D. dasymaschalum* were collected in Trang Province, Thailand in 2008 and were identified by Dr. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU2008003) is deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

3.3. Extraction and isolation

Ground fresh leaves (2.83 kg) of *D. dasymaschalum* was exhaustively extracted with EtOAc at room temperature, filtered and concentrated to give a green solid (208 g). Amount of EtOAc extract (207 g) was adsorbed onto 415 g of silica gel 60H and fractionated by VLC over a sintered glass filter column of silica gel 60H (1.84 kg, diameter × height: 19 × 13 cm) using increasing amount of EtOAc in hexane (1% EtOAc–100% EtOAc) and increasing amounts of MeOH in EtOAc (1% MeOH–100% MeOH) to yield 14 fractions. Fraction 4 (4.45 g) was separated by VLC over silica gel 60H (98 g, diameter × height: 10.0 × 2.5 cm), eluted with 1% EtOAc in hexane to give 4 subfractions (4-1–4-4). Subfraction 4-3 (367 mg) was further separated by CC over silanized silica gel 60, eluted with MeOH to give 7 subfractions (4-3-1–4-3-7). Subfraction 4-3-2 (52 mg) was further purified by CC on silanized silica gel 60 and eluted with 20% H_2O in MeOH to give **8** (5 mg) and **15** (12 mg). Subfraction 4-3-4 (29 mg) was chromatographed by CC over silica gel 60 (230–400 mesh), eluted with hexane–CH_2Cl_2–EtOAc (9:7:1) to give **14** (10 mg). Subfraction 4-3-6 (33 mg) separated by CC on silica gel 60 (230–400 mesh), eluted with 5% MeOH in 50% CH_2Cl_2 in hexane to give **1** (14 mg). Fraction 8 (7.26 g) was undergone series of chromatographic separations on silica gel using hexane–CH2Cl2–EtOAc (10:7:1, 7:7:1 and 5:7:1) as the eluent to give **12** (15 mg). Fraction 9 (6.58 g) was separated by VLC on silica gel 60H (72 g, diameter × height: 4.8 × 2.0 cm), eluted with hexane –CH_2Cl_2–EtOAc (15:7:1) to give 11 subfractions (9-1–9-11). Subfraction 9-4 (128 mg) was purified by CC over silica gel 60 (230–400 mesh), eluted with 2% MeOH in CH_2Cl_2 to give **3** (11 mg). Subfraction 9-6 (79 mg) was chromatographed by CC on Sephadex LH-20, eluted with MeOH–CH_2Cl_2 (1:1) to give 4 subfractions (9-6-1–9-6-4). Subfraction 9-6-2 (21 mg) was further separated by CC over silanized silica gel 60, eluted with 20% H_2O in MeOH to give **10** (1 mg). Subfraction 9-6-3 (16 mg) was further purified by CC over silanized silica gel 60, eluted with 20% H_2O in MeOH to give **6** (7 mg). Subfraction 9-8 (1.25 g) was chromatographed by CC over Sephadex LH-20, eluted with MeOH–CH_2Cl_2 (1:1) to give 5 subfractions (9-8-1–9-8-5). Subfraction 9-8-2 was identified as **2** (322 mg). Subfraction 9-7-3 (33 mg) and subfraction 9-8-4 (34 mg) were combined and separated by CC over silica gel 60 (230–400 mesh), eluted with 3% EtOAc in CH_2Cl_2 to yield **7** (3.4 mg). Subfraction 9-10 (247 mg) was undergone series of chromatographic separation on silica gel 60 (230–400 mesh) using 30% EtOAc in hexane as the eluent and on silanized silica gel 60 using MeOH as the eluent to give **13** (23.8 mg). Fraction 10 (5.52 g) was separated by CC over silica gel 60 (70–230 mesh), eluted with 1% MeOH in CH_2Cl_2 to give 8 subfractions (10-1–10-8). Subfraction 10-4 (598 mg) was chromatographed by CC on Sephadex LH-20, eluted with MeOH–CH_2Cl_2 (1:1) to give **2** (374 mg). Subfraction 10-5 (633 mg) was separated by CC on Sephadex LH-20 using MeOH–CH_2Cl_2 (1:1) as the eluent to give 4 subfractions (10-5-1–10-5-4). Subfraction 10-5-2 (92 mg) was further purified by CC over silica gel 60 (230–400 mesh) using hexane–CH_2Cl_2–EtOAc (5:10:1) as the eluent to yield **2** (17 mg) and **4** (11 mg). Subfraction 10-6 (90 mg) was chromatographed by CC on Sephadex LH-20, eluted with MeOH–CH_2Cl_2 (1:1) to give 4 subfractions (10-6-1–10-6-4). Subfraction 10-6-3 (55 mg) was further purified by CC over silica gel 60 (230–400 mesh) using 40% EtOAc in hexane as the eluent to yield **9** (9.6 mg). Fraction 11 (6.38 g) was identified as **11**. Fraction 12 (10.61 g) was crystallized from CH_2Cl_2–hexane as colorless needle (1.74 g) which was identified as **11**. Fraction 13 (15.53 g) was separated by CC over silica gel 60 (70–230 mesh) using 1% MeOH in CH_2Cl_2 as the eluent to give 6 subfractions (13-1–13-6). Subfraction 13-4 (79 mg) was undergone series of chromatographic separation by CC on Sephadex LH-20 using MeOH–CH_2Cl_2 (1:1) as the eluent and on silanized silica gel 60 using 40% H_2O in MeOH as the eluent to yield **5** (8 mg). All purified compounds had a degree of purity >98%, based on the TLC analyzed (all compounds exhibited one spot both under UV radiation and when sprayed with H_2SO_4) and NMR spectra (the baseline was smooth without impurity peaks).

3.3.1. 3β-Hydroxy-21-O-acetyl-24-methylenecycloartane (**3**)

A colorless oil, $[\alpha]_D^{27}$ + 26.2 (*c* 0.58, $CHCl_3$); IR (UATR) ν_{max}: 3461, 2935, 1740, 1640, 1370, 1239, 887 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; HR-TOF-APCI-MS: *m/z* 499.4136 $[M+H]^+$ (calcd. for $C_{33}H_{55}O_3$ 499.4146).

*3.3.2. 3β,21-Dihydroxy-24,31-epoxy-24-methylenecycloartane (**4**)*

A colorless oil; $[\alpha]_D^{28}$ + 166.1 (*c* 0.05, $CHCl_3$); IR (UATR) ν_{max}: 3439, 2935, 1070, 1045, 1023, 736 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; HR-TOF-APCI-MS: *m/z* 495.3807 $[M+Na]^+$ (calcd. for $C_{31}H_{52}O_3Na$ 495.3809).

*3.3.3. (2S)-7-Hydroxy-6,8-dimethoxyflavanone (**5**)*

A pale yellow oil; $[\alpha]_D^{27}$ − 1.6 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 344 (3.39), 278 (3.83), 238 (3.98) nm; IR (UATR) ν_{max}: 3413, 2936, 1672, 1494, 1455, 1364, 1236, 1200, 1095, 1094, 905, 701 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): Table 3; HR-TOF-APCI-MS: *m/z* 301.1062 $[M+H]^+$ (calcd. for $C_{17}H_{17}O_5$ 301.1070).

3.4. Bioassays

3.4.1. Antimycobacterial assay

Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). Standard drugs, isoniazid (>99%) and rifampicin (>95%), were obtained from Sigma and used as the reference compounds (Table 4).

3.4.2. Antiplasmodial assay

Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen (1976). Quantitative assessments of malarial activity *in vitro* were determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al. (1979). The inhibition concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* incorporation of [^{3}H]-hypoxanthine by *Plasmodium falciparum*. The standard compound was dihydroartemisinin (99%) was in house supplied by Dr. Bongkoch Tarnchompoo, BIOTEC (Table 4).

3.4.3. Cytotoxicity assay

Cytotoxic assays against human epidermoid carcinoma (KB), human breast cancer (BC) and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method described by Skehan et al. (1990). The reference substances were ellipticin (>95%) and doxorubicin (>95%) from Sigma and Ebewe, respectively (Table 4).

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