New Multiflorane-Type Triterpenoid Acids from *Sandoricum indicum*

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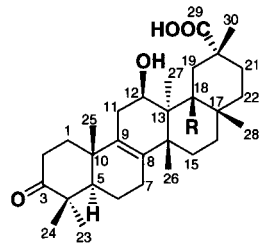
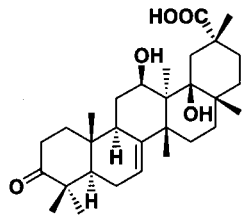
Three new 12β-hydroxymultiflorane triterpenoid acids, sandorinic acids A−C (**1**−**3**), were isolated from the stem bark of *Sandoricum indicum* together with five known triterpenes (**4**−**8**). The structures of **1**−**3** were elucidated by spectral data interpretation. Compounds **1**−**8** were evaluated for their inhibiting activity against several tumor cell lines and for their effects on lymphocyte proliferation.

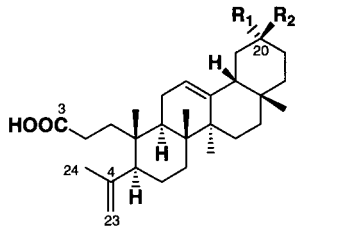
Sandoricum indicum Cav. (syn. *Sandoricum koetjape*) (Meliaceae) is a large tree found in the forests of southern Asia. This plant is used against colic and diarrhea by local populations, and decoctions of the bark are drunk after childbirth.[1] Previous studies have revealed that this plant contains several triterpenoids[2−4] with some having cytotoxic[5] and DNA polymerase-inhibitory activities.[6] As part of a search for bioactive natural products from tropical plants, we have investigated the chemical constituents of the stem bark of this plant collected in Thailand. Here we describe the isolation and structure elucidation of three new triterpenoid acids (**1**−**3**) together with five known triterpenoids (**4**−**8**).



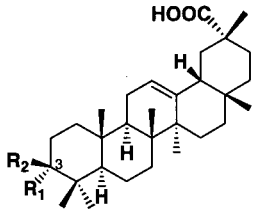
1 R=OH
3 R=H



2



4 R_1=Me, R_2=COOH
5 R_1=COOH, R_2=Me



6 R_1=OH, R_2=H
7 R_1,R_2=O
8 R_1=H, R_2=OH

The EtOAc-soluble fraction of the MeOH extract of this plant, which was active on preliminary screening against lyphocyte proliferation,[7] was subjected to repeated chromatography on Si gel and/or Sephadex LH-20, followed by further purification by HPLC on ODS to give eight triterpenoids (**1**−**8**). Among them, five were identified as koetjapic acid (**4**),[5] 20-epikoetjapic acid (**5**),[8] katonic acid (**6**),[2,5] 3-oxoolean-12-en-29-oic acid (**7**),[5] and 3-epikatonic acid (**8**),[9] on the basis of comparison of their spectral data with literature values. The remaining three triterpenes, to our knowledge, are new compounds and were named sandorinic acids A−C (**1**−**3**).

Sandorinic acid A (**1**) was obtained as colorless needles, mp 257−260 °C, and was shown to have the molecular formula $C_{30}H_{46}O_5$ by HRFABMS (*m*/*z* 487.3466, $[M + H]^+$). The IR absorption bands of **1** at 3440, 1710, and 1690 cm^{-1} indicated the presence of hydroxyl, ketone, and carboxyl groups. The ^1H NMR spectrum of **1** (Table 1) showed seven methyl singlets, and its ^{13}C NMR spectrum aided by DEPT experiments revealed the presence of a tetrasubstituted olefin (δ_C 136.6 and 129.8), a ketone (δ_C 216.5), and a carboxyl group (δ_C 181.6). Since three out of eight unsaturation degrees were thus accounted for, **1** was inferred to have a pentacyclic skeleton, and the spectral features of **1** were similar to those of bryononic acid (3-oxomultiflora-8-en-29-oic acid) previously isolated from the same plant.[3,4] Compound **1**, however, contained two oxygenated carbons, one tertiary [δ_H 4.91 (1H, d, J = 5.4 Hz); δ_C 72.6] and the other quaternary (δ_C 80.9). The HMBC spectrum of **1** showed correlations (Table 1) consistent with a multiflorane skeleton possessing a 3-oxo-8-ene moiety. This spectrum also suggested the presence of OH-12 and OH-18 functions as exemplified by the correlations of H_3-27 to C-12 (δ 72.6, d) and C-18 (δ 80.9, s). NOE difference experiments showed correlations for H_3-27/H-12 and H-12/H-19α (δ_H 3.49),[10] thus locating H-12α at δ 4.91. Hence, the 12-hydroxy group is β-oriented. From these results, the structure of sandorinic acid A was concluded to be 12β,18-dihydroxy-3-oxomultiflora-8-en-29-oic acid (**1**).[11]

Sandorinic acid B (**2**), colorless needles, mp 296−299 °C, has a molecular formula of $C_{30}H_{46}O_5$ as determined by its HRFABMS data (*m*/*z* 487.3437, $[M + H]^+$), identical with that of **1**. The ^1H and ^{13}C NMR data of **2** (Table 1) closely resembled those of **1**. However, **2** contained a trisubstituted double bond, as evidenced by the ^1H NMR spectrum exhibiting an olefinic proton signal at δ_H 5.51, and the ^{13}C NMR spectrum, two olefinic carbon signals at δ_C 117.1, d and 147.4, s. This double bond was located between the C-7 and C-8 positions, designated by the comparison with the corresponding carbon signals of secoisobryononic acid

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Table 1. ^{13}C NMR Spectral Data (δ/ppm, m[a]) of Sandorinic Acids A−C (**1**−**3**) in C_5D_5N

	1		**2**		**3**	
position	δ_C	HMBC correlations	δ_C	HMBC correlations	δ_C	HMBC correlations
1	35.4 t	H_3-25	34.5 t	H_3-25	35.3 t	H_3-25
2	34.5 t		34.8 t		30.5 t	
3	216.5 s	H_3-23, H_3-24	215.1 s	H_3-23, H_3-24	216.8 s	H_3-23, H_3-24
4	47.1 s	H_3-23, H_3-24	47.5 s	H_3-23, H_3-24	47.0 s	H_3-23, H_3-24
5	51.1 d	H_3-23, H_3-24, H_3-25	51.8 d	H_3-23, H_3-24, H_3-25	50.9 d	H_3-23, H_3-24, H_3-25
6	27.0 t	H-5	24.7 t	H-5	27.3 t	H-5
7	20.7 t		117.1 d	H-9	20.6 t	
8	136.6 s	H_3-26	147.4 s	H_3-26	135.5 s	H_3-26
9	129.8 s	H-12, H_3-25	47.2 d	H_3-25	130.8 s	H_3-25
10	37.3 s	H_3-25	31.0 s	H_3-25	37.1 s	H_3-25
11	33.1 t		37.9 t		34.4 t	
12	72.6 d	H_3-27	76.7 d	H_3-27	70.6 d	H_3-27
13	45.2 s	H_3-26, H_3-27	45.1 s	H_3-26, H_3-27	41.4 s	H_3-26, H_3-27
14	42.2 s	H-12, H_3-26, H_3-27	43.1 s	H-12, H_3-26, H_3-27	31.2 s	
15	27.9 t	H_3-26	31.6 t	H_3-26	27.3 t	H_3-26
16	35.7 t	H_3-28	34.7 t		31.8 t	H_3-28
17	38.2 s	H_3-28	34.9 s	H_3-28	35.2 s	H-18, H_3-28
18	80.9 s	H_3-27, H_3-28	79.8 s	H_3-27, H_3-28	38.0 d	H_3-27
19	44.8 t	H_3-30	45.0 t	H_3-30	30.9 t	
20	43.8 s	H_3-30	43.8 s	H_3-30	40.5 s	H-18, H_3-30
21	29.6 t	H_3-30	28.9 t	H_3-30	29.8 t	H_3-30
22	37.3 t		38.1 t	H_3-28	37.3 t	H_3-28
23	26.7 q	H_3-24	24.5 q	H_3-24	26.6 q	H-5, H_3-24
24	21.1 q	H_3-23	21.1 q	H_3-23	21.0 q	H-5, H_3-23
25	19.5 q		12.6 q		19.3 q	
26	24.2 q		28.0 q		23.1 q	
27	21.2 q		26.8 q		19.4 q	
28	27.6 q		27.8 q		31.6 q	
29	181.6 s	H_3-30	181.8 s	H_3-30	181.8 s	H_3-30
30	34.2 q		35.0 q		33.2 q	

[a] m: multiplicity from DEPT experiments.

methyl ester ($\Delta^{7,8}$; δ_C 117.2 and 145.7).[4] This location was supported by HMBC correlations observed between H_3-26 and C-8 (Table 1). The hydrogen on C-9 was suggested to have an α-orientation since on irradiation of H_3-25 the NOE enhancement was observed not at H-9 but at H_3-26. Thus, the structure of sandorinic acid B was established as 12β,18-dihydroxy-3-oxomultiflora-7-en-29-oic acid (**2**). This structure for **2** was further supported by NOED experiments and analysis of its HMBC spectrum. It is noted that H-12 in the ^1H NMR spectrum of **1** and **2** showed different coupling patterns (**1**, brd, J = 5.4 Hz; **2**, t, J = 8.4 Hz), which are ascribable to the conformation change of the C-ring due to different double-bond positions ($\Delta^{8,9}$ in **1** or $\Delta^{7,8}$ in **2**).

The molecular formula of sandorinic acid C (**3**) was determined as $C_{30}H_{46}O_4$ from its HRFABMS data (m/z 470.3389, $[M]^+$), possessing one less oxygen atom than that of **1** or **2**. The oxygenated quaternary carbon signal was not observed in the ^{13}C NMR spectrum of **3**, while one more sp^3 methine carbon (δ_C 38.0) was observed instead. These results indicated that compound **3** did not have a tertiary hydroxyl group at C-18. The other ^1H and ^{13}C NMR spectral data of **3** (Table 1) corresponded well to those of sandorinic acid A (**1**), suggesting the presence of a tetrasubstituted olefin at C-8,C-9 and a secondary hydroxyl group on C-12, which were supported by the HMBC correlation data (Table 1). The oxymethine proton (H-12) of **3** was observed as a broad doublet (J = 6.3 Hz), which was similar to that of **1**, and NOE experiments afforded correlations between H-12 and H_3-27, thus indicating that H-12 has an α-orientaion. Thus, sandorinic acid C was established as 12β-hydroxy-3-oxomultiflora-8-en-29-oic acid (**3**).

Although many oleanane or multiflorane-type triterpenoids were obtained from this plant,[2−6] 12- or 18-hydroxymultiflorane derivatives have never been isolated from the plant kingdom before. Pentacyclic triterpenoids (**1**, **2**, and **6**−**8**) showed inhibitory activity against lymphocyte proliferation (IC_{50}: 25−50 μg/mL for **1**, **6**, and **7**; 50−75 μg/mL for **2** and **8**),[12] whereas two seco-oleanane derivatives (**4** and **5**) did not show such activity (IC_{50} >100 μg/mL). Compounds **1** and **7** exhibited cytotoxic activity against human leukemia HL-60 cells with an IC_{50} value of 15 μg/mL for both compounds. The cytotoxic activity of these triterpenoids against murine leukemia P388 cells was examined, and the IC_{50} values (μg/mL) against adriamycin (ADR)- and vincristine (VCR)-resistant P388 cells (P388/ADR and P388/VCR, respectively) as well as those against a sensitive P388 strain (P388/S) are presented in Table 2. Although these triterpenoids had no reversal effect of multidrug resistance,[13] compounds **6** and **7** exhibited selective cytotoxicity against P388/VCR cells, while compound **1** was cytotoxic against all cell lines and compound **2** showed more cytotoxic potency against ADR- and VCR-resistant P388 cells than against sensitive P388 strain. Compounds **3**, **4**, **5**, and **8** were inactive against all the cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO J-20 instrument. UV spectra were obtained on a Hitachi U-3400 spectrometer. IR spectra were measured from samples on KBr disks on a Hitachi 260-10 infrared spectrophotometer. NMR spectra were recorded on JEOL JNM GSX-A400, A500, and ECP600 spectrometers. HRFAB mass spectra were acquired on a JMS HX-110 mass spectrometer.

Plant Material. The bark of *Sandoricum indicum* was collected at Khon Kaen, Thailand, in February 2000. A voucher specimen (accession number 6-288) is maintained at the Department of Horticulture, Faculty of Agriculture, Khon Kaen University.

Table 2. Cytotoxic Activity (IC_{50} Values, μg/mL) of Compounds **1**−**8**[a]

compound	P388/S	P388/VCR(−)	P388/VCR(+)	P388/ADR(−)	P388/ADR(+)
1	16	10	9	9	9
2	>25	16	16	18	>25
3	>25	>25	>25	>25	>25
4	>25	>25	>25	>25	>25
5	>25	>25	>25	>25	>25
6	>25	25	16	>25	>25
7	>25	12	12	>25	>25
8	>25	>25	>25	>25	>25

[a] P388/ADR and P388/VCR are adriamycin- and vincristine-resistant P388 cell lines, respectively, while P388/S is a sensitive P388 cell line. Tests toward P388/ADR and P388/VCR cell lines were carried out in the absence (−) or presence (+) of 0.1 μg/mL of ADR and 0.004 μg/mL of VCR, respectively, which did not affect the growth of the cells, respectively.

Extraction and Isolation. The air-dried stem bark (319 g) was extracted with MeOH (1.5 L × 2). The MeOH extract (46.3 g) suspended in 10% aqueous MeOH (600 mL) was partitioned against hexane (200 mL × 2), EtOAc (200 mL × 2), and *n*-BuOH (200 mL × 2). The EtOAc-soluble fraction (3.6 g) was subjected to Si gel column chromatography (column A; 3.2 × 20 cm) eluted with 0−100% MeOH in $CHCl_3$. The fraction (1.8 g) eluted with 8% MeOH was dissolved in MeOH (100 mL), and the solution stood overnight to give an insoluble material (0.45 g), which was identified as koetjapic acid (**4**).[5] Part of the residue from the mother liquid (0.3 g out of 1.4 g) was separated by gel filtration with Sephadex LH-20 (column B; 1.5 × 33 cm) eluted with MeOH. The fraction (169 mg) in the 125−140 mL elution was further separated by Si gel column chromatography (column C; 3.6 × 18 cm) eluted with hexanes−EtOAc (2:1 to 1:2). The fraction (53 mg) from hexane−EtOAc (1:1) elution was finally purified with HPLC on ODS (Develosil ODS-HG5, 10 × 250 mm; eluent, 90% MeOH; detection, UV at 220 nm and RI; flow rate, 2.0 mL/min) to give sandorinic acid C (**3**, 4.6 mg, t_R 16.8 min), 20-epikoetjapic acid[8] (**5**, 2.2 mg, t_R 38.4 min), and katonic acid[2,5] (**6**, 2.8 mg, t_R 62.4 min). The rest of the residue from the mother liquid (1.1 g) was repeatedly purified by HPLC on ODS (Senshu Pak ODS-5251-S, 20 × 250 mm; eluent, 90% MeOH; detection, UV at 220 nm; flow rate, 8.0 mL/min) to give 20-epikoetjapic acid (**5**, 54.7 mg, t_R 51.5 min),[8] katonic acid[2,5] (**6**, 46.2 mg, t_R 85.3 min), 3-oxoolean-12-en-29-oic acid[5] (**7**, 12.0 mg, t_R 62.8 min), 3-epikatonic acid[9] (**8**, 11.2 mg, t_R 66.5 min), sandorinic acid A (**1**, 5.2 mg, t_R 20.4 min), and sandorinic acid B (**2**, 25.8 mg, t_R 22.1 min). The structures of compounds **5**−**8** were identified by their spectral data, being identical with those published in the literature.[2,5,8,9]

Sandorinic acid A (1): colorless needles from acetone; mp 257−260 °C; $[\alpha]^{25}_D$ +15° (*c* 0.15, MeOH); IR (KBr) ν_{max} 3440, 1710, 1690, 1460, 1380 cm^{-1}; ^1H NMR (C_5D_5N) δ_H 4.91 (1H, d, J = 5.4 Hz, H-12), 3.49 (1H, d, J = 15.0 Hz, H-19α), 2.01 (1H, d, J = 15.0 Hz, H-19*β*), 1.94 (3H, s, H_3-26), 1.53 (1H, m, H-5), 1.41 (3H, s, H_3-30), 1.33 (3H, s, H_3-28), 1.23 (3H, s, H_3-27), 1.10 (3H, s, H_3-23), 0.98 (3H, s, H_3-24), 0.93 (3H, s, H_3-25); ^{13}C NMR (Table 1); FABMS *m*/*z* 487 $[M + H]^+$, 469 $[M - H_2O + H]^+$, 451 $[M - 2H_2O + H]^+$; HRFABMS *m*/*z* 487.3466 [calcd for $C_{30}H_{47}O_5$, $[M + H]^+$ 487.3424].

Sandorinic acid B (2): colorless needles from acetone; mp 296−299 °C; $[\alpha]^{25}_D$ −34° (*c* 0.82, MeOH); IR (KBr) ν_{max} 3490, 3190, 1730, 1630, 1470, 1370, 1220 cm^{-1}; ^1H NMR (C_5D_5N) δ_H 5.51 (1H, br d, J = 2.8 Hz, H-7), 4.48 (1H, t, J = 8.4 Hz, H-12), 3.72 (1H, d, J = 15.5 Hz, H-19α), 2.12 (1H, m, H-9), 2.07 (1H, d, J = 15.5 Hz, H-19*β*), 1.84 (3H, s, H_3-26), 1.57 (1H, m, H-5), 1.46 (3H, s, H_3-30), 1.38 (3H, s, H_3-28), 1.32 (3H, s, H_3-27), 1.06 (3H, s, H_3-23), 1.02 (3H, s, H_3-24), 0.94 (3H, s, H_3-25); ^{13}C NMR (Table 1); FABMS *m*/*z* 487 $[M + H]^+$, 469 $[M - H_2O + H]^+$, 451 $[M - 2H_2O + H]^+$; HRFABMS *m*/*z* 487.3437 [calcd for $C_{30}H_{47}O_5$, $[M + H]^+$ 487.3424].

Sandorinic acid C (3): colorless amorphous solid; $[\alpha]^{25}_D$ +14° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3330, 1700, 1690, 1510, 1460 cm^{-1}; ^1H NMR (C_5D_5N) δ_H 4.53 (1H, d, J = 6.3 Hz, H-12), 2.97 and 1.87 (each 1H, d, J = 16.0 Hz, H_2-19), 2.73 (1H, d, J = 8.0 Hz, H-18), 1.55 (3H, s, H_3-26), 1.56 (1H, m, H-5), 1.37 (3H, s, H_3-30), 1.19 (3H, s, H_3-27), 1.13 (3H, s, H_3-28), 1.08 (3H, s, H_3-23), 1.00 (3H, s, H_3-24), 0.97 (3H, s, H_3-25); ^{13}C NMR (Table 1); FABMS *m*/*z* 470 $[M^+]$; HRFABMS *m*/*z* 470.3389 [calcd for $C_{30}H_{46}O_4$, 470.3396 $[M]^+$].

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(10) H-19*β* of **1** (δ_H 2.01) and **2** (δ_H 2.07) showed NOE correlations with *β*-oriented methyl groups (H_3-28 and H_3-30), while H-19*β* of **3** (δ_H 1.87) also showed a NOE correlation with H_3-30.
(11) The ^{13}C NMR chemical shifts of C-23 and C-24 of **1** were assigned as δ_C 26.7 and 21.1, respectively, since the H_3-23 (δ_H 1.10, 3H, s) signal showed an NOE correlation with H-5 (δ_H 1.53, 1H, m). In the HMQC spectrum of **1**, correlations were observed from H_3-23 to the signal resonating at δ_C 26.7 (C-23) and from H_3-24 (δ_H 1.10, 3H, s) to the ^{13}C NMR signal at δ_C 21.1 (C-24). In a previous report,[4] the ^{13}C NMR chemical shifts of C-23 and C-24 of bryononic acid (**9**) were assigned as δ_C 21.1 and 26.8, respectively; these assignments may be transposed.
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