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Modified limonoids from the leaves *of Sandoricum koetjape*

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Abstract

Three trijugin-type limonoids, sandrapins A, B and C, were isolated from the leaves of *Sandoricum koetjape* and their structures, which are related to capensolactones, were elucidated by a detailed 2D-NMR spectroscopic analysis.
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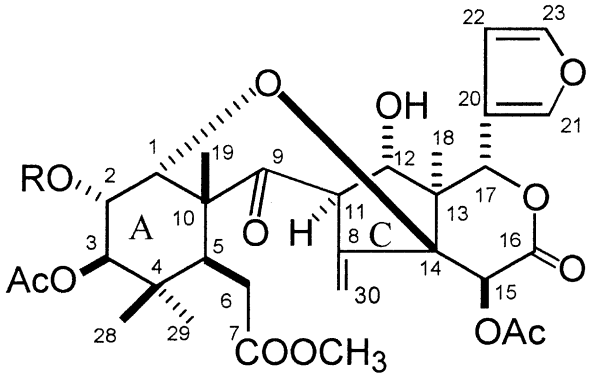
Keywords: *Sandoricum koetjape*; Meliaceae; Leaf; Limonoids; Trijugin-type skeleton; Sandrapins A–C

1. Introduction

Plants in the Meliaceae are rich sources of a variety of sesquiterpenoids and triterpenoids, including limonoids with diverse structures (Hegnauer, 1969, 1990). One member of the Meliaceae native to India and Malaysia, *Sandoricum koetjape* Merr. (syn. *Sandoricum indicum* Cav.), is now widely distributed in Southeast Asia. A decoction of its bark is used traditionally as a tonic after childbirth in Malaysia (Burkill, 1966), while Indonesians use this plant to treat colic and leucorrhoea (Perry; 1980). Triterpenoids have been found in its heartwood and bark, including katonic acid and indicic acid (King and Morgan, 1960), koetjapic acid, 3-oxo-olean-12-en-29-oic acid (Kaneda et al., 1992), secobryononic acid (Kosela et al., 1995) and sandorinic acids A–C (Tanaka et al., 2001). Andirobin-type limonoids with antifeedant effects have been also obtained from its seeds (Powell et al., 1991). The fruit hulls yield triterpenoids (bryononic acid and bryonolic acid) and polyalcohols (*meso*-inositol and dimethyl mucate) (Sim and Lee, 1972). However, no phytochemical study on the leaves of this plant has been reported. Here, we describe the isolation and structure elucidation of three new acylated trijugin class limonoids from its leaves: sandrapins A (**1**), B (**2**), and C (**3**).



1 : R=$COCH_3$

2 : R=$COCHCH_2CH_3$ (CH_3)

3 : R=$COCHCH_3$ (CH_3)

2. Results and discussion

The MeOH extract of the dried leaves was partitioned with hexane, EtOAc, and H_2O. The EtOAc-soluble portion was subjected to CC over Toyopearl HW-40C and MCI-gel CHP-20P with aq. MeOH, successively, and further purified by preparative TLC followed by HPLC to afford sandrapins A (**1**), B (**2**), and C (**3**) in yields of 0.006, 0.002, and 0.001% from dried leaves, respectively.

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Sandrapin A (**1**) was obtained as colourless needles, mp 252–255 °C, $[\alpha]_D$ +6.6° ($CHCl_3$). The molecular formula was determined to be $C_{33}H_{40}O_{14}$ from the $[M+NH_4]^+$ ion peak at *m/z* 678.2804 in the high-resolution electrospray ionization mass spectrum (HRESIMS) in the positive ion mode. The ^1H and ^{13}C NMR spectra of **1** (Table 1) contained signals due to three acetyl methyls, four quaternary methyls, one carbomethoxyl, one exomethylene, and one methylene group, as well as a monosubstituted furan ring and a lactone group, which are characteristic of limonoids with a cleaved ring B, such as ekebergins (Taylor, 1981), mexiconolides (Adesida et al., 1971), trijugins (Purushothaman et al., 1987), and capensolactones (Mulholland and Iourine, 1998). The ^{13}C NMR spectrum coupled with HMQC also showed the presence of a quaternary carbon bearing an oxygen atom, and eight methine carbons, six of which bore oxygen atoms. In addition to five ester (or lactone) carbonyl carbons, a ketonic carbon was observed at δ 206.1. These spectral features and similarity to known compounds implied that **1** had the same carbon skeleton as trijugins and capensolactones. The planar structure was confirmed by the HMBC spectrum as summarized in Table 1. On the addition of D_2O in the ^1H NMR spectrum, the signal at δ 5.59 *(dd, J* = 3.5, 5.0 Hz) collapsed to a doublet (*J* = 3.5 Hz) accompanying the disappearance of the signal at δ2.92 (*d, J* = 5.0 Hz, OH). This signal was assigned to H-12 based on its vicinal coupling with δ 3.40 (*d, J* = 3.5 Hz), which was assigned to H-11 by the HMBC correlations with the ketonic carbonyl (δ 206.1, C-9) and a lower carbon (δ 140.3, C-8) signal of exomethylene carbon signals. The presence of a free hydroxyl group at C-12 was thus confirmed. The small coupling constant (*J* = 3.5 Hz) of H-11 with H-12, and the absence of ROE enhancement between these proton signals indicated a *trans*-relationship between the C_{12}-hydroxyl and C_{11}-substituents on the cyclopentane ring. The HMBC correlations of H-2, H-3, and H-15 is to the acetyl carbonyl carbon signals located the acetoxy groups at C-2,

Table 1
^1H and ^{13}C NMR spectroscopic data of **1** in $CDCl_3$[a]

	δ_H	δ_C	HMBC (to carbon)
1	4.43 *d* (3.5)	76.4	2, 3, 5, 14
2	5.11 *dd* (3.5,11.5)	69.1	1, 3, $COCH_3$
3	5.30 *d* (11.5)	74.6	4, $COCH_3$
4	–	40.2	–
5	2.71 *m*	41.2	6, 10, 28, 29, 9
6	2.72 *dd* (17.5, 7.5) 2.37 *br d* (17.5)	30.0	4, 7, 10
7	–	175.2	–
8	–	140.3	–
9	–	206.1	–
10	–	54.9	–
11	3.40 *d* (3.5)	71.5	8, 9, 10, 12
12	5.59 *dd* (3.5, 5.0)	76.7	8, 13
OH	2.92 *d* (5.0)		13
13	–	52.9	–
14	–	90.7	–
15	5.94 *s*	69.4	8, 14, 16, $COCH_3$
16	–	166.4	–
17	6.15 *s*	80.7	12, 13, 18, 20, 21, 22
18	1.05 *s*	11.0	12, 13, 14, 17
19	1.15 *s*	19.0	1, 9, 10
20	–	121.3	–
21	7.59 *brs*	140.7	22,23
22	6.57 *brs*	109.5	20, 21
23	7.43 *t* (1.5)	143.2	20, 21, 22
28	0.93 *s*	18.0	3, 5, 29
29	0.93 *s*	29.2	3, 5, 28
30	5.28 *s*, 5.07 *s*	116.1	8, 9, 14
OCH_3	3.68 *s*	52.5	7
$COCH_3$	2.13 *s*	21.4	2, $COCH_3$
		171.5	–
	2.04 *s*	20.8	2, $COCH_3$
		169.8	–
	2.22 *s*	20.3	15, $COCH_3$
		169.9	–

[a] ^1H NMR, 500 MHz, coupling constants (*J* in Hz) in parentheses; ^{13}C NMR, 125 MHz.

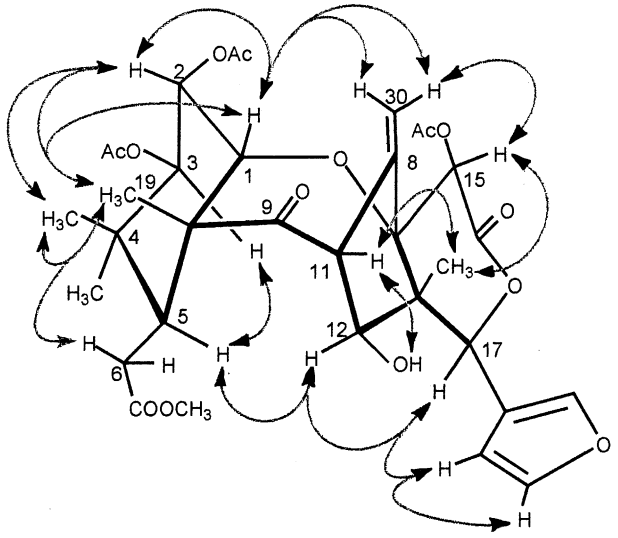

Fig. 1. Selected ROEs for **1**.

Table 2
^{13}C NMR spectroscopic data for **2** and **3** in $CDCl_3$

C(No)	2[a]	3[b]	C(No)	2	3
1	76.9	76.8	19	18.8	18.8
2	68.6	68.5	20	121.1	121.1
3	74.2	74.2	21	140.5	140.5
4	40.4	40.4	22	109.3	109.3
5	40.9	40.9	23	143.1	143.1
6	29.9	29.9	28	17.8	17.8
7	175.1	175.1	29	29.1	29.1
8	140.2	140.2	30	115.9	115.9
9	205.7	205.7	OCH_3	52.3	52.3
10	54.9	54.9	$COCH_3$	20.9	20.9
11	71.3	71.3		169.9	169.61
12	76.4	76.5		20.2	20.4
13	52.8	52.8		169.7	169.64
14	89.9	89.9	COO	176.3	176.9
15	69.2	69.3	CH	39.8	33.3
16	166.0	166.1	CH_2	25.1	–
17	80.3	80.5	CH_3	10.9	18.0
18	10.9	10.9		15.2	19.2

[a] 125 MHz.
[b] 150 MHz.

C-3, and C-15, respectively, and the large coupling constant between H-2 and H-3 ($J=11.5$ Hz) suggested their *trans*-diaxial orientation. The three-bond correlations between H-1/C-14, H-11/ C-10, H-19/C-9, and H-5/C-9 gave definite evidence for connecting rings A and C. The ROESY spectrum of **1** (Fig. 1) revealed the relative stereostructure. Clear cross peaks between H-1/H-2, H-2/H-19, H-1/H-19, and H-6/H-19 were observed, while no ROE was detected between H-2 and H-3. The ROE between H-3 and H-5 indicated their 1,3-diaxial orientation. Therefore, the C-3 acetoxyl group was β-oriented. Clear ROE correlations were also observed between H-12/H-5 and H-30/H-1. All of these correlations satisfied the gross stereostructure shown in formula **1**.

Sandrapin B (**2**) was obtained as colourless fine needles, mp 210–213 °C, $[\alpha]_D$ +6.0 ° ($CHCl_3$). The ^{1}H and ^{13}C NMR (Table 2) spectra of **2** were almost identical to those of **1**, except for the presence of 2-methylbutyloyl signals (δ_H 2.57, 1.76, 1.48 (each *m*), 0.95 (3H, *t*, $J=7.0$ Hz) and 1.11 (3H, *d*, $J=7.0$ Hz); δ 39.8, 25.1, 15.2, 10.9 and 176.3) instead of the acetyl signal in **1**. The molecular formula $C_{36}H_{46}O_{14}$ deduced from the pseudomolecular ion $[M+N/H_4]^+$ at *m/z* 720.3192 in the HRESIMS was consistent with the assumption that one methylbutyrate and two acetate esters are present in **2**. The 2-methylbutyloyl group was located at C-2 from an HMBC correlation between H-2 (δ 5.20) and the ester carbonyl carbon resonance at δ 176.3, which was also correlated with methyl signal at δ 1.11 (*d*). The other HMBC correlations, including those of H-3 (δ 5.37, *d*, $J=11.0$ Hz) and H-15 (δ 5.94, *s*) to acetyl carbonyl carbons (δ 169.7 and δ 169.9), were the same as observed for **1**. The ROESY correlations and negative Cotton effect at 300 nm (n–π* of carbonyl) in the CD spectrum were also consistent with structure **2** for sandrapin B.

Sandrapin C (**3**) was obtained as colourless fine needles, mp 205–208 °C, $[\alpha]_D$ +7.0 ° ($CHCl_3$). Comparison of the ^{1}H and ^{13}C NMR (Table 2) spectral data, including the HMBC correlations, with those of **1** and **2** indicated that **3** had the same trijugin-type limonoid carbon skeleton as **1**. The HRESIMS showed an $[M+NH_4]^+$ at *m/z* 706.3090 corresponding to the molecular formula $C_{35}H_{44}O_{14}$, which is 14 mass units (CH_2) smaller than that of **2**. From the ^{1}H NMR spectrum, it was evident that **3** had one 2-methylpropionoyl [δ 2.80 (1H, *qui*, $J=6.0$ Hz), and 1.14, 1.16 (3H each *d*, $J=6.0$ Hz)] and two acetyl (δ 2.03 and 2.17) groups. The acyl groups were determined to be at C-2, C-3, and C-15 by the respective three-bond couplings of H-2, H-3, and H-15 with the ester carbonyl carbons in the HMBC spectrum. Taking the ROESY data similar to those of **1** into consideration, sandrapin C was represented as formula **3**.

Trijugin-type limonoids with a novel carbon skeleton having a contracted ring C are postulated to be produced biogenetically via a pinacol–pinacolone rearrangement of methyl 9,11-dihydroxyangolensate, an ekebergin-type limonoid (Purushothaman et al., 1987). After their isolation in 1987, several analogues including capensolactones have been reported (Kehrli et al., 1990). To our best knowledge, however, sandrapins are the first examples of trijugin-type limonoids with 2α,3β-acyloxy substituents.

3. Experimental

3.1. General procedures

Optical rotations were measured on a JASCO DIP-4 digital polarimeter. ^{1}H and ^{13}C NMR spectra were

recorded on a Varian VXR-500 or UNITY INOVA AS600NB (500 or 600 MHz for ^{1}H and 125 or 150 MHz for ^{13}C) in $CDCl_3$. The chemical shifts are given in δ (ppm) values relative to that of the solvent $CDCl_3$ (δ H 7.26; δ_C 77.0)] on a tetramethylsilane scale. The standard pulse sequences programmed for the instruments were used for each 2D measurement. J_{CH} was set at 6 Hz in the HMBC spectra. HRESIMS and ESIMS were obtained on a Micromass Auto Spec OA-Tof spectrometer using 50% aq. MeOH containing 0.1% NH_4OAc as the solvent (flow rate 0.02 ml/min). CD spectra were measured on a JASCO J-720W spectrometer. Preparative HPLC was performed on a YMC-Pack ODS-A A-324 (YMC Co. Ltd., Japan) column (i.d. 10.0×300 mm), developed with CH_3CN–H_2O (50:50) (flow rate, 1.5 ml/min; detection, UV 230 nm) at 40 °C. CC was performed with Toyopearl HW-40C (Tosoh Co. Ltd., Japan) and MCI-gel CHP-20P (Mitsubishi Kasei Co. Ltd., Japan). Kieselgel $60F_{254}$ plates (0.2 mm thick, Merck) were used for analytical and preparative TLC, and the chromatogram was developed in a *n*-hexane–Me_2CO–EtOAc (4:2:1) solvent system.

3.2. Materials

Leaves of *S. koetjape* were bought in May, 2000 from a Malaysian traditional medicine company (Juaro Sdn. Bhd., Alor Setar City, Kedah) and identified by the company's chemist, Mr. Amir Syarif Toha (BSc). The voucher specimen is deposited in the company's herbarium under code number RM061.

3.3. Extraction and isolation

The dried *S. koetjape* leaves (500 g) were crushed and macerated in MeOH (6 l×4), for 24 h each time at room temperature. The extracts were combined and filtered through Celite 545. The concentrated filtrate (2 l) was extracted with *n*-hexane (5×500 ml) and EtOAc (6×500 ml), successively, to give *n*-hexane (19.1 g) and EtOAc (13.5 g) extracts and an aqueous residue (13.6 g). A portion (5 g) of the EtOAc extract was subjected to CC over Toyopearl HW-40C (2.2 i.d.×50 cm) with step-wise MeOH–H_2O gradient mode from 10% → 20% → 30% → 40% → 50% MeOH, and 50% → 100% MeOH, and 70% Me_2CO. The 40% MeOH fraction (800 mg) was further purified by CC on MCI-gel CHP-20P with 10–70% aqueous MeOH in increasing MeOH. Fractionation was achieved by monitoring the eluates with reversed-phase HPLC. Elution with MeOH–H_2O (7:3) afforded a brown solid (316 mg), which was then further purified by preparative TLC and HPLC to give **1** (32.4 mg), **2** (8.3 mg) and **3** (3.7 mg).

3.4. Sandrapin A (*1*)

Colourless needles, mp 252–255 °C, $[\alpha]_D^{25}$ +6.6° ($CHCl_3$ *c* 1.1). HRESIMS *m/z:* 678.2804 $[M + NH_4]^+$ (calc. for $C_{33}H_{40}O_{14} + NH_4$, *m/z:* 678.2762). CD (MeOH): $[\theta]_{300}$ -2.0×10^4, UV λ_{max} (MeOH) nm (log ϵ): 230 (sh) (2.99). for ^{1}H and ^{13}C NMR spectra: Table 1.

3.5. Sandrapin B (*2*)

Colourless needles, mp 210–213 °C $[\alpha]_D^{25}$ +6.0° ($CHCl_3$ *c* 0.8). HRESIMS *m/z:* 720.3192 $[M + NH_4]^+$ (calc. for $C_{36}H_{46}O_{14} + NH_4$, *m/z*: 720.3231). CD (MeOH): $[\theta]_{300}$ -2.0×10^4, UV λ_{max} (MeOH) nm (log ϵ): 230 (sh) (2.26). ^{1}H NMR (500 MHz): δ 7.59 (1H, *br s,* H-21), 7.43 (1H, *br s,* H-23), 6.57 (1H, *br s,* H-22), 6.17 (1H, *s,* H-17), 5.94 (1H, *s,* H-15), 5.62 (1H, *br t, J* = 3.5 Hz, H-12), 5.37 (1H, *d, J* = 11.0 Hz, H-3), 5.28 (1H, *s,* H-30), 5.20 (1H, *dd, J* = 3.5, 11.0 Hz, H-2), 5.06 (1H, *s,* H-30), 4.42 (1H, *d, J* = 3.5 Hz, H-1), 3.66 (3H, *s,* –OCH_3), 3.41 (1H, *d, J* = 3.5 Hz, H-11), 2.90 (1H, *br d, J* = 4.5 Hz, OH-12), 2.72 (2H, *m,* overlapped, H-5 and H-6), 2.37 (1H, *br d,* 16.5 Hz, H-6), 2.17, 2.03 (3H each, *s,* $COCH_3$), 1.17 (3H, *s,* H-19), 1.11 (3H, *d, J* = 7.0 Hz), 2.57 (1H, *m*), 1.76 and 1.48 (1H each, *m*), 0.95 (3H, *t, J* = 7.0 Hz) (methylbutyoyl), 1.06 (3H, *s,* H-18), 0.94 (3H, *s,* H-29), 0.92 (3H, *s,* H-28). For ^{13}C NMR spectrum: Table 2.

3.6. Sandrapin C (*3*)

Colourless needles, mp 205–208 °C, $[\alpha]_D^{25}$ +7.0° ($CHCl_3$ *c* 0.8). HRESIMS *m/z:* 706.3090 $[M + NH_4]^+$ (calc. for $C_{35}H_{44}O_{14} + NH_4$, *m/z:* 706.3075). CD (MeOH): $[\theta]_{300}$ -2.0×10^4, UV λ_{max} (MeOH) nm (log ϵ): 230 (sh) (1.88). ^{1}H NMR (600 MHz): δ 7.60 (1H, *dd, J* = 1.0, 1.5 Hz, H-21), 7.43 (1H, *t, J* = 1.5 Hz, H-23), 6.57 (1H, *dd, J* = 1.0, 1.5 Hz, H-22), 6.17 (1H, *s,* H-17), 5.94 (1H, *s,* H-15), 5.61 (1H, *d, J* = 3.0 Hz, H-12), 5.37 (1H, *d, J* = 9.5 Hz, H-3), 5.28 (1H, s, H-30), 5.22 (1H, *dd, J* = 3.0, 9.5 Hz, H-2), 5.06 (1H, *s,* H-30), 4.40 (1H, *d, J* = 3.0 Hz, H-1), 3.67 (3H, *s,* –OCH_3), 3.40 (1H, *d, J* = 3.0 Hz, H-11), 2.89 (H4, *br s,* OH-12), 2.73 (1H, *m,* overlapped, H-6), 2.71 (1H, *m,* overlapped, H-5), 2.37 (1H, *m,* H-6), 2.17, 2.03 (3H each, *s,* $COCH_3$), 1.17 (3H, *s,* H-19), 2.80 (1H, *qui, J* = 6.0 Hz), 1.16, 1.14 (3H, each *d, J* = 6.0 Hz) (methylpropionoyl), 1.06 (3H, *s*, H-18), 0.94 (3H, *s*, H-29), 0.92 (3H, *s,* H-28). For ^{13}C NMR spectrum: Table 2.

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