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LIMONOIDS FROM THE SEEDS OF *Chisocheton macrophyllus*

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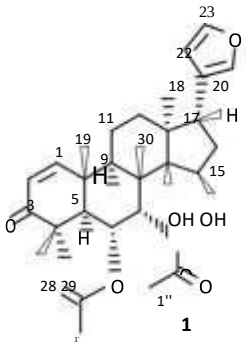
*A new limonoid compound, dysobinol (**1**), along with three known limonoid compounds, 7 - hydroxyneotricilenone (**2**), dysobinin (**3**), and nimonol (**4**), was isolated from the seeds of* Chisocheton macrophyllus *(Meliaceae). Their structures were established by spectroscopic techniques such as UV, IR, MS, 1D, and 2D NMR. Compounds **1–4** showed cytotoxic activity against P-388 murine leukemia cells with IC_{50} values of 49.7, 79.4, 19.5, and 64.5 g/mL, respectively.*

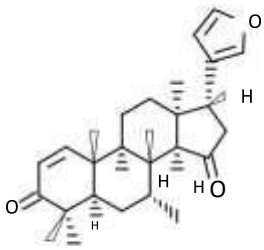
Keywords: dysobinol, limonoid, *Chisocheton macrophyllus*, P-388 murine leukemia cells.

The *Chisocheton* genus, a member of the Meliaceae family, consists of approximately 50 species that are distributed mainly in India, Thailand, Malaysia, and Indonesia [1, 2]. The genus *Chisocheton* belongs to the subtropical and tropical plant family widely known for its insecticidal limonoid constituents [3]. Previous phytochemical studies on *Chisocheton* species have yielded a number of interesting compounds, including limonoids [4, 5], antifungal meliacin-type compound [6], dammarane triterpenoids [7], and spermidine alkaloids [8]. As part of our studies on novel compounds from Indonesian Meliaceae plants [9, 10], we carried out a study on *Chisocheton macrophyllus* seeds. *C. macrophyllus* is a higher plant found growing in the rain forest in the northern part of Sulawesi Island, Indonesia [2, 11].The plant is known as Ma-aa in Indonesia, and the seed oil from this plant is used in Indonesia for lighting [12]. Its leaves have been reported to yield dammarane triterpenoids [7], but the seeds of this plant have never been phytochemically investigated. In this communication, we describe the isolation and structure elucidation of a new limonoid (**1**) and three known limonoids (**2–4**) from the seeds of *C. macrophyllus* along with their cytotoxic activity against P-388 murine leukemia cells.

The dried and powdered seeds of *C. macrophyllus* (3.5 kg) was extracted with methanol at room temperature and filtered. After removal of the solvent *in vacuo*, the residue was partitioned between water and *n*-hexane, EtOAc, and *n*-butanol.



1



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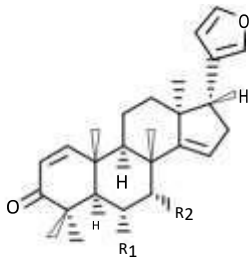

3, 4

3: $R_1 = R_2 = OAc$; **4:** $R_1 = OH$, $R_2 = OAc$

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TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for **1** ($CDCl_3$, , ppm, J/Hz)

C atom	H	C	C atom	H	C
1	7.15 (1H, d, J = 10.4)	157.8 (d)	15	3.44 (1H, dd, J = 3.2, 6.4)	67.4 (d)
2	5.94 (1H, d, J = 10.4)	126.4 (d)	16	1.58 (1H, m)	32.3 (t)
3	–	204.8 (s)		2.11 (1H, m)	–
4	–	43.0 (s)	17	2.04 (1H, dd, J = 7.3, 11.2)	51.0 (d)
5	2.51 (1H, d, J = 12.3)	48.7 (d)	18	0.90 (3H, s)	20.8 (q)
6	5.36 (1H, dd, j = 2.6, 12.3)	70.3 (d)	19	1.16 (3H, s)	28.2 (q)
	–	–	20	–	123.8 (s)
7	5.04 (1H, d, J = 2.6)	73.7 (d)	21	7.10 (1H, s)	139.8 (d)
8	–	42.0 (s)	22	7.37 (1H, dd, J = 1.9, 1.1)	111.1 (d)
9	1.41 (1H, m)	39.5 (d)	23	6.16 (1H, dd, J = 1.9, 1.3)	143.1 (d)
10	–	40.7 (s)	28	1.19 (3H, s)	19.1 (q)
11	1.27 (1H, m)	16.5 (t)	29	1.22 (3H, s)	20.8 (q)
	1.52 (1H, m)	–	30	1.47 (3H, s)	22.0 (q)
12	1.24 (1H, m)	29.4 (t)	1	2.11 (3H, s)	21.5 (q)
	1.50 (1H, m)	–	2	–	170.1 (s)
13	–	45.4 (s)	1	2.02 (3H, s)	21.4 (q)
14	–	72.9 (s)	2	–	170.2 (s)

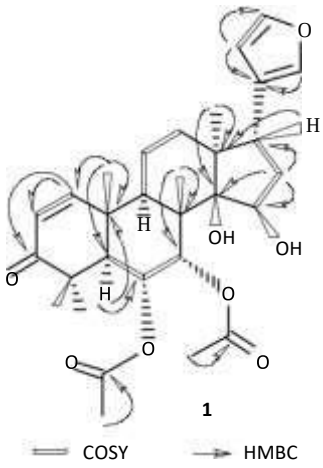

Fig. 1. Selected COSY and HMBC correlations for **1**.

A portion of the *n*-hexane soluble fraction (60 g) was subjected to a silica gel vacuum-liquid chromatography (VLC) column packed with silica gel G 60 by gradient elution. The VLC fractions were repeatedly subjected to column chromatography on silica gel and crystallization to afford two limonoids, dysobinol (**1**) and 7 -hydroxyneotricilenone (**2**). The EtOAc-soluble fraction (104 g) was separated as described for the *n*-hexane soluble fraction to yield two known limonoids, dysobinin (**3**) and nimonol (**4**).

Dysobinol (**1**) was isolated as colorless crystals from chloroform–methanol. The $[M + Na]^+$ peak at *m/z* 551.3343 (calcd for $C_{30}H_{40}O_8Na$, 551.2287) in the HR-ESI-TOF/MS spectrum indicated the molecular formula $C_{30}H_{40}O_8$, thus requiring eleven degrees of unsaturation. The UV spectrum indicated the presence of an , -unsaturated ketone at $_{max}$ 240 nm, while the IR spectrum of compound **1** showed the presence of hydroxyl ($_{max}$ 3448 cm^{-1}), ester carbonyls ($_{max}$ 1755 and 1743 cm^{-1}), conjugated carbonyl ($_{max}$ 1680 cm^{-1}), and olefinic ($_{max}$ 1606 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table 1) showed the presence of five tertiary methyl groups (0.90, 1.16, 1.19, 1.22, and 1.47, each 3H, s), two acetoxyl groups (2.02 and 2.11, each 3H, s), three oxygenated protons (3.44, 5.04, and 5.36), a -furan moiety [6.16 (1H, dd, J = 1.9, 1.3 Hz), 7.10 (1H, s), and 7.37 (1H, dd, J = 1.9, 1.1 Hz)], and two olefinic protons [5.94 (1H, d, J = 10.4 Hz) and 7.15 (1H, d, J = 10.4 Hz)]. A total of 30 carbon resonances was observed in the ^{13}C NMR spectrum (Table 1). These were assigned by DEPT and HMQC experiments to one carbonyl (C 204.8), two acetyl carbons (C 21.4, 21.5, 170.1, and 170.2), six sp^2 carbons (C 111.1, 123.8, 126.4, 139.8, 143.1, and 157.8), five methyls, three sp^3 ethylenes, four sp^3 oxygenated carbons (C 67.4, 70.3, 72.9, and 73.7), three sp^3 methines, and four sp^3 quartenary carbons. These functionalities accounted for six out of the total eleven degrees of unsaturation.

TABLE 2. Cytotoxicity Activity of Compounds **1–4** against P-388 Murine Leukemia Cells

Compound	IC_{50}, g/mL	Compound	IC_{50}, g/mL
1	49.7 0.10	**3**	19.5 0.12
2	79.4 0.09	**4**	64.5 0.12

The remaining five degrees of unsaturation were consistent with a pentacyclic limonoid structure [5, 13]. The ^{1}H and ^{13}C NMR data of **1** resembled those of dysobinin [14, 15], except for the absence of olefinic signals at C-14/C-15 and, instead, the appearance of oxygenated signals [H 3.44 (1H, dd, J = 3.2, 6.4 Hz); C 67.4 (d) and 72.9 (s)], thus suggesting that **1** was a 14,15-dihydroxy derivative of dysobinin. The position of these hydroxyl groups at C-14 and C-15 was determined through ^{1}H–^{1}H COSY and HMBC experiments (Fig. 1). In the HMBC spectrum, the oxymethine proton signal (H 3.44) showed ^{2}J correlations with C-14 (C 72.9) and C-16 (C 32.3) and ^{3}J correlations with C-13 (C 45.4) and C-17 (51.0), indicating that both hydroxyl groups were located at C-14/C-15. The relative stereochemistry of hydroxyl groups at C-14 and C-15 of **1** was presumed to be the same as epoxyazadiradione [14, 16] because of the high similarity of the NMR chemical shifts of the backbone skeleton and on the basis of the coupling constant values (H 3.44 (1H, dd, J = 3.2, 6.4 Hz) along with the biogenetic presence of mexicanolide type limonoids [5, 13–17], indicating that the hydroxyl groups at C-14 and C-15 are -oriented. Therefore, compound **1**, was established as a new limonoid derivative, 24-nor-5 ,13 ,17 -chola-1,20,22-trien-3-one-6,7-bis(acetyloxy)-21,23-epoxy-14 ,15 ,6 ,7 -tetrahydroxy-4,4,8-trimethyl and was named dysobinol.

The known compounds were identified to be 7 -hydroxyneotricilenone (**2**) [15, 16], dysobinin (**3**) [16, 18], and nimonol (**4**) [15, 16] on the basis of NMR and MS spectra, as well as by comparison of their spectral data with those reported previously.

The cytotoxicity effects of the four isolated compounds **1–4** against P-388 murine leukemia cells were conducted according to the method described in previous papers [9, 10, 19, 20], which used artonin E (IC_{50} 0.3 g/mL) as a positive control [21]. The cytotoxicity activities of the isolated compounds **1–4** are shown in Table 2. The cytotoxic activity of dysobinin (**3**) having double bond at C-14 and 6,7-diacetyl moieties showed stronger activity than other limonoids, suggesting that the presence of double bond and 6,7-diacetyl moieties may be an important structural feature for cytotoxic activity in the limonoid structure.

EXPERIMENTAL

General. Melting points were measured on a Fisher-John micro melting point apparatus and are uncorrected. Optical rotations were recorded on a ATAGO AP-300 automatic polarimeter. The IR spectra were recorded on a PerkinElmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with JEOL JMS-700 and a SynaptG2 mass spectrometer instruments. ^{1}H and ^{13}C NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as internal standard. Chromatographic separations were carried out on silica gel 60 (Merck). PTLC glass plates were precoated with silica gel GF_{254} (Merck, 0.25 mm). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm), and detection was achieved under UV light at 254 and 367 nm and by spraying with 10% H_2SO_4 in ethanol followed by heating.

Plant Material. Seeds of *C. macrophyllus* were collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in August 2011. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. Bo-1295452) was deposited at the Herbarium.

Extraction and Isolation. Seeds of *C. macrophyllus* (3.5 kg) were extracted with methanol over a period of 3 days at room temperature. The extract was filtered and concentrated under reduced pressure to provide a viscous concentrated MeOH extract (414 g). The crude extract was first suspended in H_2O and then partitioned with *n*-hexane, EtOAc, and *n*-butanol, successively. Evaporation resulted in a crude extract of *n*-hexane (197 g), EtOAc (108 g), and *n*-BuOH (8 g). A portion of the *n*-hexane-soluble fraction (60 g) was subjected to vacuum liquid chromatography using gradient elution of *n*-hexane–EtOAc (10:0–0:10) to afford seven fractions (A1–A7). Fraction A2 (16.5 g) was subjected to silica gel column chromatography using a mixture of *n*-hexane–EtOAc (10:0–5:1) as eluting solvent to afford five fractions (B1–B5). Fractions B3 and B4 were combined (225 mg) and chromatographed on a column of silica gel, eluted with *n*-hexane–acetone (10:0–1:1), to give 10 subfractions (C1–C10). Subfraction C5 (50 mg) was recrystallized with chloroform–methanol to give **1** (30 mg). Fraction C8 (120 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane–EtOAc (10:1 to 7:3), to give

20 subfractions (C8.1–C8.20). Subfraction C8.14 was chromatographed on a column of silica gel and further recrystallized with chloroform to give **2** (14 mg). The EtOAc-soluble fraction (104 g) was fractionated by column chromatography on silica gel 60 using gradient *n*-hexane and EtOAc to give five fractions (D–H). Fraction E (350 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of $CHCl_3$–MeOH (20:1 to 1:2), to give 10 fractions (E1–E10). Fractions E2–E5 were combined (142.5 mg) and chromatographed on a column of silica gel to give **3** (16.1 mg). Fractions E8–E10 were combined (96 mg) and chromatographed on a column of silica gel to give **4** (18 mg).

Dysobinol (1). Colorless crystals, mp 194–195 C, $[\ ]_D^{20}$ –21.8 (*c* 0.05, $CHCl_3$). IR (KBr, $_{max}$, cm^{-1}): 3448, 1755, 1743, 1680, 1606. For ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1. HR-ESI-TOF/MS *m/z* 551.2287 $[M + Na]^+$

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