Journal of Natural Medicines (2018) 72:588–592
https://doi.org/10.1007/s11418-018-1188-8

NOTE

Reinereins A and B, new onocerane triterpenoids from *Reinwardtiodendron cinereum*

Alfarius Eko Nugroho[1] · Daisuke Inoue[1] · Chin Piow Wong[1] · Yusuke Hirasawa[1] · Toshio Kaneda[1] · Osamu Shirota[2] · A. Hamid A. Hadi[3] · Hiroshi Morita[1]

Received: 15 January 2018 / Accepted: 5 February 2018 / Published online: 16 February 2018

Abstract
Bioactivity guided separation of Reinwardtiodendron *cinereum* barks methanol extract led to the isolation of two new onocerane triterpenoids, reinereins A and B (**1** and **2**), together with three known onocerane triterpenoids. Their structures were elucidated on the basis of NMR spectroscopic data. In vitro cytotoxic activities of the isolated compounds against several type of cancer cells were evaluated.

Keywords Triterpenoid · Onocerane · *Reinwardtiodendron cinereum* · Cytotoxicity · Meliaceae

Introduction

Reinwardtiodendron, a genus belonging to the Meliaceae family, is composed of about seven species distributed mainly in Malaysia and Indonesia [1]. In our search for structurally and biologically interesting natural products from tropical plants [2–13], we screened Malaysian plants extracts for cytotoxic activity against human promyelocytic leukemia HL-60 cells, and the bark extract of *R. cinereum* was found to be cytotoxic. To date, chemical constituents of the plants of this genus have never been reported. Thus, we proceeded with bioactivity guided investigation of the extract, yielding two new onocerane triterpenoids, reinereins A and B (**1** and **2**, Fig. 1), together with known compounds lansic acid dimethyl ester (**3**) [14], methyl lansionate (**4**) [15, 16], and lansic acid (**5**) [14, 17]. The structure elucidation and cytotoxic activity of **1** and **2** are described herein.

✉ Hiroshi Morita
moritah@hoshi.ac.jp

[1] Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan

[2] Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan

[3] Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Results and discussion

Reinerein A (**1**) was isolated as an optically active, $[\alpha]_D^{21}$ +26.5 (*c* 1.0, $CHCl_3$), white amorphous solid. The molecular formula of **1** was determined by HRESIMS [*m/z* 483.3471 ($(M + H)^+$; Δ − 0.3 mmu)] to be $C_{31}H_{46}O_4$, and the presence of carbonyl groups in **1** was suggested by the IR absorption at 1740, 1704 and 1669 cm^{-1}. The ^1H and ^{13}C NMR data of **1** (Table 1) were similar to **4**, suggesting **1** was an onocerane triterpenoid related to **4**. Further analysis of the NMR data suggested **1** was different from **4** only in the structure of ring C. The structure of **1**, especially that of ring C was elucidated from 2D (HSQC, ^1H-^1H COSY and HMBC) NMR data (Fig. 2). The HMBC correlations of H_2-12 to C-13 and C-14, H_3-27 to C-13, C-14 and C-15, H_3-28 to C-13, and H-17 to C-15 suggested the presence of a double bond between C-13 and C-14, and a carbonyl C-15, forming an α, β-unsaturated carbonyl moiety in ring C. The relative configuration of **1** was deduced from the value of $^3J_{H\text{-}16a/H\text{-}17}$ (8.7 Hz) and NOESY correlations (Fig. 3), and was confirmed to be similar to **4**. Thus, the structure of **1** was deduced to be a new compound, reinerein A (Fig. 1).

Reinerein B (**2**) was isolated as an optically active white amorphous solid, $[\alpha]_D^{21}$ − 6.5 (*c* 1.0, $CHCl_3$). The molecular formula of **2** was determined by HRESIMS (*m/z* 493.3277 ($[M + Na]^+$; Δ − 1.7 mmu) to be the same as **5** ($C_{30}H_{46}O_4$). However, only 15 carbon signals were observed in the ^{13}C NMR spectrum of **2**, indicating the presence of an internal symmetry in **2**. Analysis of the ^1H and ^{13}C NMR data of **2**

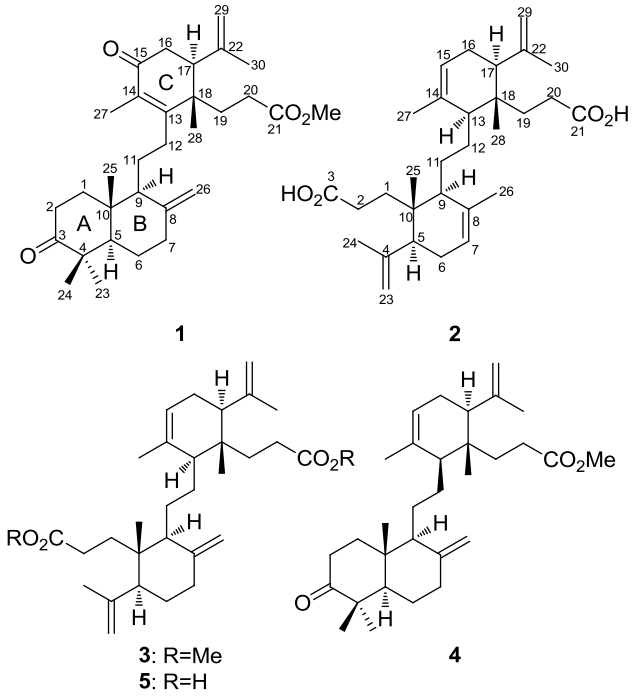

Fig. 1 Structures of reinereins A and B (**1** and **2**), lansic acid dimethyl ester (**3**), methyl lansionate (**4**), and lansic acid (**5**)

(Table 1) showed that they were similar to those of C-12 to C-22 and C-27 to C30 of **5**. Therefore, the structure of **2** was assumed to be comprised of two methylcyclohexene moieties instead of a methylenecyclohexane and a methylcyclohexene as in **5**. Analysis of the 2D NMR (^{1}H-^{1}H COSY, HSQC, and HMBC, Fig. 2) data further supported this assumption, and the NOESY correlations (H-5/H-9, H-5/H-6b and H-6a/H$_3$-25) suggested the relative configuration of **2** to be similar to **5**. Thus, the structure of **2** was deduced to be a new compound, reinerein B (Fig. 1).

Reinwardtiodendron is morphologically and genetically closely related to *Lansium* [1, 18]. In general, previous phytochemical investigation on the members of *Lansium* resulted in the isolation of onocerane triterpenoids and tetranortriterpenoids [16, 19, 20]. Thus, our results shows that *Lansium* and *Reinwardtiodendron* are also closely related phytochemically.

Compounds **1**–**5** were tested for cytotoxic activity against HL-60 cells (Table 2). These onocerane triterpenoids, **3**–**5** in particular, were assumed to be responsible for the cytotoxic activity shown by the bark MeOH extract. In addition, the cytotoxic activity of **1**–**5** against A549, MCF-7, and HepG2 cells was also tested, and **1** and **5** showed cytotoxic activities against all three cancer cell lines (Table 2). Several onocerane triterpenoids have been reported to show anti-bacterial activity [19] and toxicity against brine shrimp (*Artemia salina*) [16]. However, to our knowledge, this is the first

Table 1 ^{1}H & ^{13}C NMR data of **1** and **2** in $CDCl_3$

1						**2**			
	δ_C	δ_H(J, Hz)		δ_C	δ_H(J, Hz)			δ_C	δ_H(J, Hz)
1	27.6	1.58 (1H; m)	16	39.1	2.50 (1H; dd, 17.0, 8.7)	1	19	32.0	1.70 (2H; m)
		2.01 (1H; m)			2.56 (1H; dd, 17.0, 5.2)	2	20	29.4	2.25 (1H; m)
2	34.7	2.41 (1H; m)	17	47.0	2.64 (1H; dd, 8.7, 5.2)				2.41 (1H; m)
		2.61 (1H; ddd, 15.3, 12.4, 6.5)	18	42.3		3	21	181.3	
3	216.4		19	34.0	1.84 (1H; ddd, 14.5, 12.0, 4.8)	4	22	147.5	
4	47.8				1.94 (1H; ddd, 14.5, 12.0, 5.1)	5	17	49.1	2.25 (1H; m)
5	55.2	1.62 (1H; m)	20	29.4	2.10 (1H; ddd, 16.0, 12.0, 4.8)	6	16	29.5	1.83 (1H; m)
6	25.2	1.51 (1H; qd, 12.8, 4.2)			2.30 (1H; ddd, 16.0, 12.0, 5.1)				2.20 (1H; m)
		1.72 (1H; m)	21	173.6		7	15	122.1	5.38 (1H; br.s)
7	37.9	2.04 (1H; td, 12.8, 4.7)	22	145.2		8	14	135.7	
		2.47 (1H; ddd, 12.8, 4.2, 3.7)	23	26.0	1.10 (3H; s)	9	13	48.9	1.86 (1H; m)
8	147.2		24	21.7	1.02 (3H; s)	10	18	38.6	
9	58.0	1.74 (1H; m)	25	14.1	0.84 (3H; s)	11	12	31.2	1.38 (1H; m)
10	39.7		26	107.5	4.66 (1H; s)				1.53 (1H; m)
11	24.0	1.57 (2H; m)			4.97 (1H; s)	23	29	113.9	4.77 (1H; s)
12	30.6	2.00 (1H; m)	27	12.2	1.80 (3H; s)				4.81 (1H; s)
		2.41 (1H; m)	28	21.4	1.13 (3H; s)	24	30	22.9	1.77 (3H; s)
13	162.9		29	115.7	4.75 (1H; s)	25	28	15.6	0.82 (3H; s)
14	133.2				4.91 (1H; s)	26	27	23.3	1.76 (3H; s)
15	198.2		30	22.6	1.67 (3H; s)				
			OMe	51.8	3.66 (3H; s)				

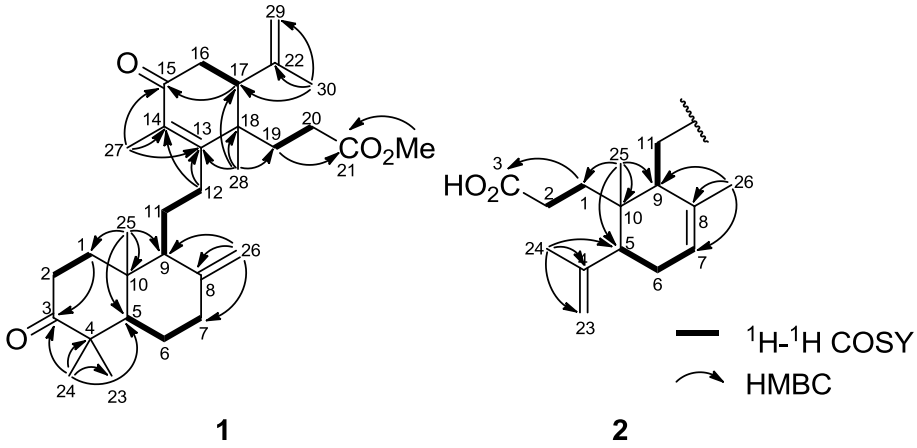

Fig. 2 Selected 2D NMR (^{1}H-^{1}H COSY and HMBC) correlations of **1** and **2**

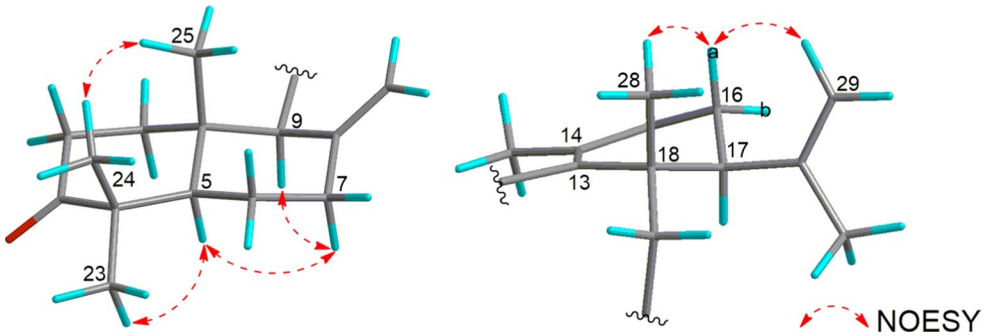

Fig. 3 Selected NOESY correlations of **1**

Table 2 Cytotoxic activity of the isolated compounds

	IC_{50} (μM)					
	1	**2**	**3**	**4**	**5**	Cisplatin
HL-60	14.3	27.8	16.9	23.2	11.5	0.87
A549	16.9	29.7	> 50	> 50	35.5	28.8
MCF7	23.2	> 50	> 50	> 50	35.4	27.8
HepG2	11.5	> 50	> 50	> 50	35.4	12.3

report on the cytotoxicity of **3–5** against human cancer cell lines.

Experimental section

General experimental procedures

Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a JASCO FT/IR-4100 spectrophotometer. High-resolution ESI MS data were obtained on a LTQ Orbitrap XL (Thermo Scientific). ^{1}H and 2D NMR spectra were measured on a 400 or a 600 MHz spectrometers at 300 K, while ^{13}C NMR spectra were measured on a 100 or a 150 MHz spectrometers. The residual $CDCl_3$ chemical shift is used as an internal standard that are δ_H 7.26 and δ_C 77.0. Standard pulse sequences were used for the 2D NMR experiments. Merck silica gel 60 (40–63 μm) and Nacalai Tesque Cosmosil 140C_{18}-OPN were used for the column chromatography, and the separations were monitored by Merck silica gel 60 F_{254}, or Merck silica gel RP C-18 F_{254} TLC plates.

Material

The barks of *R. cinereum* were collected in Pahang, Malaysia in September 1998. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. Voucher specimens (Herbarium No. 4809) are deposited in the Herbarium of the Chemistry Department, University of Malaya.

Extraction and isolation

The barks of *R. cinereum* (100 g) were extracted with methanol to obtain 10.4 g of extract. The methanol extract was successively partitioned with *n*-hexane, ethyl acetate, *n*-butanol and water, and the hexane-soluble materials (3.25 g), which showed cytotoxic activity, were further separated with a silica gel column (*n*-hexane/EtOAc, 1:0–1:1, $CHCl_3$, MeOH) to obtain 20 fractions (Fraction A-T). Fractions showing cytotoxic activity were further separated as follows. Further separation of fraction D by a silica gel column (toluene/EtOAc, 1:0–20:1) yielded lansic acid dimethyl ester (**3**, 46.1 mg, 0.0461%) and methyl lansionate (**4**, 66.1 mg, 0.0661%). Fraction J was further separated by an ODS column (60–100% $MeOH_{(aq)}$), followed by a silica gel column (benzene/EtOAc, 10:1) to obtain **1** (1.9 mg, 0.0019%). Further separation of fraction T by a silica gel column ($CHCl_3$/MeOH, 30:1 with 0.1% HCO_2H) and an ODS column (60–100% $MeCN_{(aq)}$) yielded lansic acid (**5**, 51.2 mg, 0.0512%), and a mixture of **2** and **5** (4.7 mg). The mixture were then separated by HPLC (Nacalai tesque Cholester 4.6 × 250 mm, 70% $MeCN_{(aq)}$) with 0.1% HCO_2H, flow rate 0.5 mL/min, UV detection at 210 nm) to obtain **2** (t_R 80 min, 1.2 mg, 0.0012%).

Reinerein A (1)

White, amorphous solid; $[\alpha]_D^{21}$ +26.5 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 1740, 1704 and 1669 cm^{-1}; UV (MeOH) λ_{max} (ε) 200.5 (15,000) nm; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 483 $(M + H)^+$; HRESIMS *m/z* 483.3471 $(M + H)^+$; calcd. for $C_{31}H_{47}O_4$, 483.3474).

Reinerein B (2)

White, amorphous solid; $[\alpha]_D^{25}$ -6.5 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3405 and 1743 cm^{-1}; UV (MeOH) λ_{max} (ε) 201 (16000) nm; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 493 $(M + Na)^+$; HRESIMS *m/z* 493.3277 $(M + Na)^+$; calcd. for $C_{30}H_{46}O_4Na$, 493.3294).

Cytotoxicity

HL-60, human promyelocyctic leukemia cells were maintained in RPMI-1640 medium; MCF-7, human breast adenocarcinoma; A549, human lung adenocarcinoma; and HepG2, human hepatocellular carcinoma cells were maintained in DMEM medium. Both growth media were supplemented with 10% fetal calf serum and 1% Penicillin–Streptomycin. The cells (5×10^3 cells/well) were cultured in Nunc disposable 96-well plates containing 90 µl of growth medium per well and were incubated at 37 °C in a humidified incubator of 5% CO_2. Serially diluted samples (10 µl, final concentration; 50, 25, 12.5, and 6.25 µM) were added to the cultures at 24 h of incubation. After 48 h of incubation with the samples, MTT solution (15 µl, 5 mg/ml) was added to each of the wells. The cultures were incubated for another 3 h before the cells supernatant was removed. After the removal of the cells supernatant, DMSO (50 µl) was added to each well. The formed formazan crystal was dissolved by re-suspension by pipette. The optical density was measured using a microplate reader (Bio-Rad) at 550 nm with reference wavelength at 700 nm. In all experiments, three replicates were used.

Acknowledgements This work was supported by Grants in-Aid for Scientific Research from JSPS, Japan.

References

1. Mabberley DJ (2011) Meliaceae Flowering plants Eudicots: Sapindales, Cucurbitales, Myrtaceae. Kubitzki K., Springer, p 185-211
2. Nugroho AE, Hirasawa Y, Hosoya T, Awang K, Hadi AHA, Morita H (2010) Bisleucocurine A, a novel bisindole alkaloid from *Leuconotis griffithii*. Tetrahedron Lett 51:2589–2592
3. Hirasawa Y, Shoji T, Arai T, Nugroho AE, Deguchi J, Hosoya T, Uchiyama N, Goda Y, Awang K, Hadi AHA, Shiro M, Morita H (2010) Bisleuconothine A, an eburnane-aspidosperma bisindole alkaloid from *Leuconotis griffithii*. Bioorg Med Chem Lett 20:2021–2024
4. Hirasawa Y, Hara M, Nugroho AE, Sugai M, Zaima K, Kawahara N, Goda Y, Awang K, Hadi AHA, Litaudon M, Morita H (2010) Bisnicalaterines B and C, Atropisomeric Bisindole Alkaloids from *Hunteria zeylanica*, Showing Vasorelaxant Activity. J Org Chem 75:4218–4223
5. Nugroho AE, Hirasawa Y, Wong CP, Kaneda T, Hadi AHA, Shirota O, Ekasari W, Widyawaruyanti A, Morita H (2012) Antiplasmodial indole alkaloids from *Leuconotis griffithii*. J Nat Med 66:350–353
6. Nagakura Y, Nugroho AE, Hirasawa Y, Hosoya T, Rahman A, Kusumawati I, Zaini NC, Morita H (2013) Sanjecumins A and B: new limonoids from *Sandoricum koetjape*. J Nat Med 67:381–385
7. Wong CP, Deguchi J, Nugroho AE, Kaneda T, Hadi AHA, Morita H (2013) Ceramicines from *Chisocheton ceramicus* as lipid-droplets accumulation inhibitors. Bioorg Med Chem Lett 23:1786–1788
8. Morita H, Nugroho AE, Nagakura Y, Hirasawa Y, Yoshida H, Kaneda T, Shirota O, Ismail IS (2014) Chrotacumines G-J, chromone alkaloids from Dysoxylum acutangulum with osteoclast differentiation inhibitory activity. Bioorg Med Chem Lett 24:2437–2439
9. Wong CP, Seki A, Horiguchi K, Shoji T, Arai T, Nugroho AE, Hirasawa Y, Sato F, Kaneda T, Morita H (2015) Bisleuconothine A induces autophagosome formation by interfering with AKT-mTOR signaling pathway. J Nat Prod 78:1656–1662
10. Nugroho AE, Hashimoto A, Wong CP, Yokoe H, Tsubuki M, Kaneda T, Hadi AHA, Morita H (2018) Ceramicines M-P from Chisocheton ceramicus: isolation and structure–activity relationship study. J Nat Med 72:64–72
11. Nugroho AE, Sugiura R, Momota T, Hirasawa Y, Wong CP, Kaneda T, Hadi AHA, Morita H (2015) Dysosesquiflorins A and B, sesquiterpenoids from *Dysoxylum densiflorum*. J Nat Med 69:411–415

12. Mori R, Nugroho AE, Hirasawa Y, Wong CP, Kaneda T, Shirota O, Hadi AHA, Morita H (2014) Opaciniols A-C, new terpenoids from Garcinia opaca. J Nat Med 68:186–191
13. Wong C, Shimada M, Nugroho AE, Hirasawa Y, Kaneda T, Hadi AHA, Osamu S, Morita H (2012) Ceramicines J-L, new limonoids from *Chisocheton ceramicus*. J Nat Med 66:566–570
14. Kiang AK, Tan EL, Lim FY, Habaguchi K, Nakanishi K, Fachan L, Ourisson G (1967) Lansic acid, a bicyclic triterpene. Tetrahedron Lett 8:3571–3574
15. Nishizawa M, Nishide H, Kosela S, Hayashi Y (1983) Structure of lansiosides: biologically active new triterpene glycosides from *Lansium domesticum*. J Org Chem 48:4462–4466
16. Tanaka T, Ishibashi M, Fujimoto H, Okuyama E, Koyano T, Kowithayakorn T, Hayashi M, Komiyama K (2002) New onoceranoid triterpene constituents from *Lansium domesticum*. J Nat Prod 65:1709–1711
17. Habaguchi K, Watanabe M, Nakadaira Y, Nakanishi K, Kiang AK, Lim FY (1968) The full structures of lansic acid and its minor congener, an unsymmetric onoceradienedione. Tetrahedron Lett 9:3731–3734
18. Alexandra NM, Samuel R, Chase MW, Caroline MP, Greger H (2005) *Aglaia* (Meliaceae): an evaluation of taxonomic concepts based on DNA data and secondary metabolites. Am J Bot 92:534–543
19. Dong S-H, Zhang C-R, Dong L, Wu Y, Yue J-M (2011) Onoceranoid-Type triterpenoids from *Lansium domesticum*. J Nat Prod 74:1042–1048
20. Tilaar M, Wih WL, Ranti AS, Wasitaatmadja SM, Suryaningsih Junardy FD, Maily (2008) Review of *Lansium domesticum* correa and its use in cosmetics. Bol Latinoam Caribe Plant Med Aromat 7:183–189