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Acutaxylines A and B, two novel triterpenes from *Dysoxylum acutangulum*

Intan Safinar Ismail [a,b], Yuta Nagakura [a], Yusuke Hirasawa [a], Takahiro Hosoya [a], Mohd Izwan Mohd Lazim [b], Nordin Hj Lajis [b], Hiroshi Morita [a,*]

[a] Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan
[b] Laboratory of Natural Product, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia

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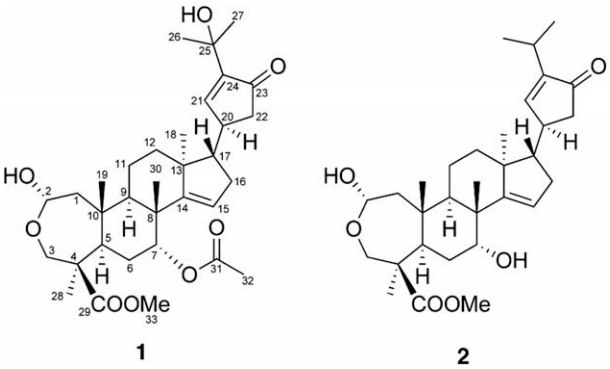
ABSTRACT

Two novel triterpenes, acutaxylines A (**1**) and B (**2**) consisting of a cyclopentenone side chain at C-17 and an oxepan-2-ol, were isolated from the leaves of *Dysoxylum acutangulum*. The relative stereochemistry of **1** and **2** was determined by NOESY correlations. Acutaxyline B showed moderate cytotoxicity against human blood premyelocytic leukemia cells.

Limonoids derived from apotirucallane-type triterpenes have been thoroughly studied due to their significant biological activities such as antifeedants, insecticides, antitumor, and antimalarial activities[1,2] and their diverse structures with the oxidized backbone and the side chain moiety which bonded to ring D have attracted great interest.[3] *Dysoxylum* species (Meliaceae), which are mainly distributed in Southeast Asia, are well-known rich sources of limonoids.[4]

In continuation of our research on Meliaceae family,[5] we have found that the methanol extracts from the leaves of *Dysoxylum acutangulum* Miq. collected in Malaysia showed cytotoxic activity against human cancer cells. We have isolated two novel triterpenes, acutaxylines A (**1**) and B (**2**) with an unprecedented cyclopentenone side chain at C-17 with a *seco*-apotirucallane skeleton. We now wish to report on the isolation and structure elucidation of two novel triterpenes, acutaxylines A (**1**) and B (**2**).



1 **2**

The leaves of *D. acutangulum*, which were collected at Terengganu, Malaysia, were extracted with methanol and the extract (3.5 g) was partitioned with 10% aq MeOH and hexane, and then with EtOAc. The EtOAc-soluble materials (1.0 g) were subjected to an ODS column ($MeOH/H_2O$, 1:4→1:0), in which a fraction eluted with 80% MeOH was further purified on a silica gel column with hexane/acetone and toluene/EtOAc to afford acutaxylines A[6] (**1**, 2.3 mg, 0.0007% yield) and B (**2**, 1.7 mg, 0.0005% yield) as colorless solids.

Acutaxyline A (**1**),[6] colorless amorphous solid, $[\alpha]_D^{23}$ +59 (*c* 1.0, MeOH), showed molecular formula, $C_{33}H_{48}O_8$, which was determined by HRESITOFMS [*m*/*z* 595.3273 $(M+Na)^+$, Δ +2.6 mmu], indicating 10 degrees of unsaturation in the molecule. IR absorption bands were characteristic of hydroxy (3435 cm^{-1}), ester carbonyl (1730 cm^{-1}), and conjugated carbonyl (1700 cm^{-1}) groups. ^1H and ^{13}C NMR data (Table 1) suggested the presence of three carbonyls, two sp^2 quaternary carbons, five sp^3 quaternary carbons, six sp^3 methines, two sp^2 methines, seven sp^3 methylenes, and eight methyls. Among them, one sp^3 quaternary carbon (δ_C 69.6), two sp^3 methines (δ_C 75.6; δ_H 5.16 and δ_C 93.9; δ_H 5.14), one sp^3 methylene (δ_C 67.7; δ_H 3.86 and 3.89), and three sp^2 quaternary carbons (δ_C 170.2, 175.4, and 209.3) were attached to oxygen atoms. Since five of ten degrees of unsaturation were accounted for, **1** was inferred to possess five rings.

Partial structures **a** (C-1–C-2), **b** (C-5–C-7), **c** (C-9 and C-11–C-12), and **d** (C-15–C-17 and C-20–C-22), were deduced from detailed analyses of 2D NMR data (^1H–^1H COSY, HOHAHA, and HMQC spectra) of **1** (Fig. 1). The connection among partial structures **a**, **b**, and **c** was deduced from the HMBC correlations of H_3-19 to C-1, C-5, C-9, and C-10, and H_3-30 to C-7, C-8, and C-9. The presence of an oxepane ring, a methyl, and a methoxy carbonyl groups at C-4, and an acetoxy group at C-7 was elucidated from HMBC correlations of H-3 to C-2, H_3-28 to C-3, C-4, C-5, and C-29, H_3-33 to C-29, and H-7 and H_3-32 to C-31. HMBC correlations from H_3-18 to C-12, C-13, C-14, and C-17, and H-15 to C-13 in partial structures **c** and **d**

* Corresponding author. Tel./fax: +81 3 5498 5778.
E-mail address: moritah@hoshi.ac.jp (H. Morita).

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Table 1
^{1}H and ^{13}C NMR data of acutaxylines A (**1**) and B (**2**) in $CDCl_3$ at 300 K

	1			**2**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1a	1.51 (1H, dd, 15.1, 9.4)	45.1	19	1.52 (1H, m)	44.5	19
1b	2.31 (1H, dd, 15.1, 5.0)			2.28 (1H, dd, 15.1, 3.8)		
2	5.14 (1H, dd, 9.4, 5.0)	93.9	1a, 3	5.12 (1H, br s)	94.0	1, 3
3a	3.86 (1H, d, 12.8)	67.7	28	3.89 (1H, s)	67.7	2, 28
3b	3.89 (1H, d, 12.8)			3.89 (1H, s)		
4		49.9	5, 28		50.0	5, 28
5	1.58 (1H, m)	53.6	1b, 3b, 19, 28	1.82 (1H, br d, 13.4)	52.0	1b, 3, 6b, 19, 28
6a	1.90 (1H, br d, 15.0)	26.0		1.93 (1H, m)	26.5	5
6b	2.68 (1H, dd, 15.0, 14.1)			2.64 (1H, dd, 13.7, 13.4)		
7	5.16 (1H, br s)	75.6	30	3.92 (1H, br s)	71.9	5, 30
8		41.7	30		44.4	15, 30
9	1.95 (1H, m)	41.0	11a, 19, 30	1.97 (1H, m)	38.8	1b, 11a, 19, 30
10		40.8	19		41.2	1b, 5, 19
11a	1.58 (1H, m)	18.5		1.63 (1H, m)	17.9	9
11b	1.85 (1H, m)			1.90 (1H, m)		
12a	1.56 (1H, m)	35.4	18	1.52 (1H, m)	34.4	18
12b	1.84 (1H, m)			1.88 (1H, m)		
13		46.4	15, 18		46.6	15, 17, 18
14		159.4	18, 30		161.8	12b, 15, 16, 18, 30
15	5.30 (1H, br s)	119.2		5.48 (1H, br s)	119.6	16
16a	2.06 (1H, dd, 17.5, 11.0)	35.7		2.21 (1H, m)	35.4	15
16b	2.16 (1H, m)			2.21 (1H, m)		
17	1.57 (1H, m)	60.0	18, 22a	1.56 (1H, m)	60.2	15, 18, 20, 22a
18	1.12 (3H, s)	21.2		1.14 (3H, s)	20.2	
19	1.04 (3H, s)	14.5		1.04 (3H, s)	14.3	5, 9
20	2.92 (1H, m)	39.2	21	2.93 (1H, br t, 7.6)	39.5	21, 22
21	7.39 (1H, d, 2.0)	158.7		7.29 (1H, br s)	157.9	20, 22, 25
22a	2.18 (1H, br d, 18.2)	41.7	21	2.13 (1H, d, 18.8)	41.3	21
22b	2.58 (1H, dd, 18.2, 6.5)			2.55 (1H, dd, 18.8, 6.2)		
23		209.3	21, 22		208.4	21, 22
24		150.2	21, 26, 27		151.8	20, 21, 25, 26, 27
25		69.6	21, 26, 27	2.61 (1H, m)	24.5	21, 26, 27
26	1.43 (3H, s)	28.8	27	1.08 (3H, s)	21.4	25, 27
27	1.43 (3H, s)	28.8	26	1.09 (3H, s)	21.3	25, 26
28	1.08 (3H, s)	24.0		1.20 (3H, s)	24.0	
29		175.4	3a, 28, 33		175.9	3, 5, 28, 33
30	1.21 (3H, s)	27.1		1.15 (3H, s)	27.4	
31		170.2	32			
32	1.98 (3H, s)	21.3				
33	3.68 (3H, s)	51.6		3.69 (3H, s)	51.6	

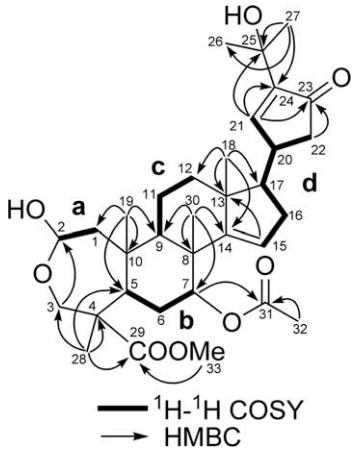


Figure 1. Selected 2D NMR correlations for acutaxyline A (**1**).

established a 2,4,5,6,7,7a-hexahydro-1*H*-indene ring (C-8–C-9 and C-11–C-17). The presence of a cyclopentenone ring with an isopropanol group at C-24 was assigned by the HMBC correlations of H2-22 to C-23, H-21 to C-23, C-24, and C-25, and H_3-27 to C-24, C-25, and C-26. Thus, the gross structure of acutaxyline A was assigned as **1**, a novel *seco*-apotirucallane-type triterpene consisting of a cyclopentenone side chain at C-17 and an oxepan-2-ol.

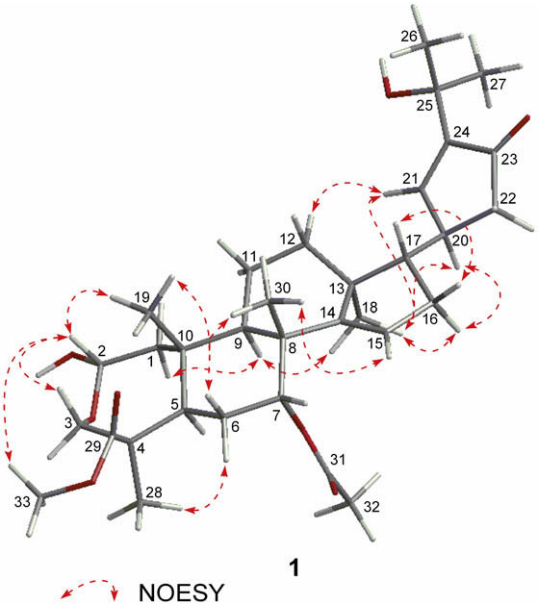
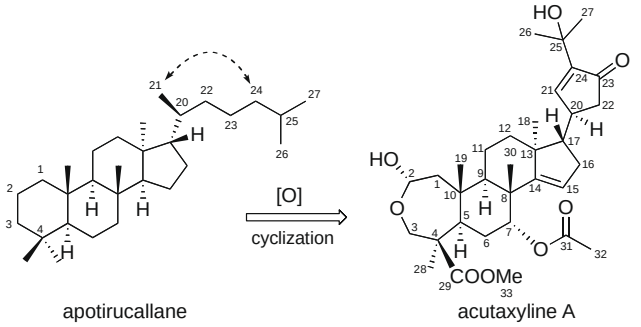


Figure 2. Selected NOESY correlations for acutaxyline A (**1**).



Scheme 1. Plausible biogenetic pathway for acutaxyline A (**1**).

The relative stereochemistry of **1** was deduced from NOESY data (Fig. 2). In the oxepan-2-ol ring (C-1–C-5, C-10, and an oxygen atom), five NOESY correlations (H_3-19/H-2 and H-6b, H-2/H-3a and H_3-33, and H_3-28/H-6a) were observed. These correlations indicated that each 7-membered ring took pseudo-chair form, and a hydroxy at C-2 and methyl at C-4 were α-oriented. Acetoxy group at C-7 was oriented axially because H-7 was observed as broad singlet. Conformation of C-ring took pseudo-boat form by the NOESY correlation between H3-18 and H-9. On the other hands, four NOESY correlations (H-21/H-12b and H_3-18 and H-20/H-16a and H_3-18) were observed in the cyclopentenone moiety (C-20–C-24) at C-17. These correlations supported that H-17 was β-oriented and configuration at C-20 was *R**. Thus, the structure of acutaxyline A (**1**) was assigned as shown.

Acutaxyline B (**2**),[7] colorless amorphous solid, $[\alpha]_D^{23}$ +46 (*c* 0.8, MeOH), showed molecular formula, $C_{31}H_{46}O_6$, which was determined by HRESITOFMS [*m*/*z* 537.3215 (M+Na)$^+$, Δ +2.3 mmu], smaller than that of **1** by a $C_2H_2O_2$ unit. IR absorption bands (3440, 1740, and 1700 cm^{-1}) were characteristic of hydroxy, ester carbonyl, and conjugated carbonyl groups. ^{1}H and ^{13}C NMR data of **2** (Table 1) were analogous to those of **1** without an acetyl and a tertiary hydroxy groups. The following ^{1}H and ^{13}C signals [δ_H 3.92 (1H, br s, H-7) and δ_C 71.9 (C-7); δ_H 2.61 (1H, m, H-25) and δ_C 24.5 (C-25)] indicated the presence of a hydroxy group at C-7 and an isopropyl group at C-24. The gross structure of **2** was elucidated by 2D NMR (^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC) data to be the same skeleton as that of **1**. The conformation and the relative stereochemistry of **2** were deduced from NOESY cross-peaks which were the same correlations as those in **1**. Thus, the structure of acutaxyline B (**2**) was assigned as shown.

Acutaxylines A (**1**) and B (**2**) are two new triterpenes consisting of a cyclopentenone side chain at C-17 and an oxepan-2-ol. A plausible biogenetic pathway for acutaxyline A (**1**) is proposed as shown in Scheme 1. Acutaxyline A (**1**) might be generated from an apotirucallane skeleton via cyclization between C-21 and C-24 and oxidative cleavage between C-2 and C-3. There are many triterpenes, steroids, and limonoids with a furan, a tetrahydrofuran, a pyrane, and so on in a side chain at C-17, but these are the first *seco*-apotirucallane triterpenes with a cyclopentenone ring derived from cyclization of a side chain at C-17.

Acutaxyline B (**2**) showed moderate in vitro cytotoxic activity on human blood premyelocytic leukemia (HL-60) cells (IC_{50} 35 μM for **2**), whereas acutaxyline A (**1**) did not show (IC_{50} >50 μM for **1**).

Acknowledgments

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6. *Acutaxyline A* (**1**): colorless amorphous solid, $[\alpha]_D^{23}$ +59 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3435, 2920, 1730, 1700, and 1245 cm^{-1}; UV (MeOH) λ_{max} 227 (ε 10700) and 202 (13,000) nm; ^{1}H and ^{13}C NMR (Table 1); ESIMS (pos.) *m*/*z* 595 (M+Na)$^+$; HRESITOFMS *m*/*z* 595.3273 (M+Na)$^+$, calcd for $C_{33}H_{48}O_8Na$ 595.3247.
7. *Acutaxyline B* (**2**): colorless amorphous solid, $[\alpha]_D^{23}$ +46 (*c* 0.8, MeOH); IR (KBr) ν_{max} 3440, 2920, 1740, 1700, and 1245 cm^{-1}; UV (MeOH) λ_{max} 227 (ε 6700) and 203 (7000) nm; ^{1}H and ^{13}C NMR (Table 1); ESIMS (pos.) *m*/*z* 537 (M+Na)$^+$; HRESITOFMS *m*/*z* 537.3215 (M+Na)$^+$, calcd for $C_{31}H_{46}O_6Na$ 537.3192.