

HETEROCYCLES, Vol. 80, No. 2, 2010, pp. 1471 - 1477. © The Japan Institute of Heterocyclic Chemistry
 Received, 4th August, 2009, Accepted, 18th September, 2009, Published online, 24th September, 2009
 DOI: 10.3987/COM-09-S(S)106

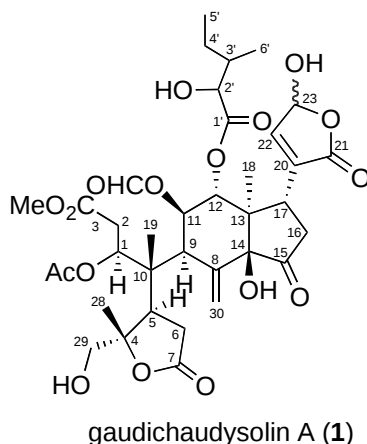
GAUDICHAUDYSOLIN A, A NEW LIMONOID FROM THE BARK OF *DYSOXYLUM GAUDICHAUDIANUM*

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Abstract – A new limonoid, gaudichaudysolin A (**1**) was isolated from the bark
 of *Dysoxylum gaudichaudianum* (Meliaceae) and the structure was elucidated by
 spectroscopic analysis.

Limonoids, highly oxidative unique secondary metabolites obtained from Meliaceae are produced by a
 unique biosynthetic route through tetranortriterpenoid nucleus,^{1,2} and are known to show various
 biological activities such as insecticidal, insect antifeedant, antibacterial, antifungal, antimalarial,
 anticancer, and antiviral activities.³ Recently, we have isolated new limonoids, ceramicines A – D⁴ with
 an unique tetranortriterpenoid skeleton from *Chisocheton ceramicus* and walsogyne A⁵ with a ring
 C–*seco* limonoid from *Walsura chrysogyne*. They showed an antiplasmodial and cytotoxic activities.^{4,5}



[†]Dedicated to Professor Emeritus Akira Suzuki, Hokkaido University, on the occasion of his 80th birthday.

In continuation of our research on limonoids containing in the plants belonging to Meliaceae family, we have isolated a new limonoid, gaudichaudysolin A (**1**) from the bark of *Dysoxylum gaudichaudianum*. Herein we report the structure elucidation of gaudichaudysolin A (**1**) by spectroscopic methods.

The bark of *D. gaudichaudianum* was extracted with MeOH, and the MeOH extract was in turn partitioned between EtOAc and H₂O. EtOAc-soluble materials were subjected to a silica gel column (hexane/EtOAc, 1:0→1:1; CHCl₃/MeOH, 1:0→0:1) and the fractions eluted by hexane/EtOAc (1:1) were subjected to a silica gel column (Toluene/EtOAc, 1:0→5:5; CHCl₃/MeOH, 1:0→5:5; CHCl₃/MeOH/H₂O, 5:5:1) followed by C₁₈ HPLC (40% CH₃CN/0.1%TFA) to afford gaudichaudysolin A (**1**, 0.00002 %).

Gaudichaudysolin A {**1**, [α]_D²³ -126 (*c* 0.2, MeOH)} was obtained as a colorless solid and was revealed to have the molecular formula C₃₆H₄₈O₁₇, by HRESITOFMS [*m/z* 775.2780 (M+Na)⁺, Δ -0.9 mmu]. IR absorptions implied the presence of hydroxyl (3425 cm⁻¹) and carbonyl (1755 and 1680 cm⁻¹) groups. UV spectrum (230 nm) indicated the presence of an unsaturated carbonyl group. ¹H and ¹³C NMR data (Table 1) revealed thirty six carbon resonances due to seven carbonyls, two sp² quaternary carbons, three sp³ quaternary carbons, one sp² methine, ten sp³ methines, one sp² methylene, five sp³ methylenes, and seven methyl groups. Among them, eight sp³ carbons (δ_C 67.3, 70.0, 72.0, 75.2, 76.3, 81.4, 93.1, and 98.8) and seven sp² carbons (δ_C 163.5, 172.1, 172.9, 173.2, 173.3, 177.8, and 208.0) were ascribed to those bearing an oxygen atom.

Six partial structures **a** (C-1 to C-2), **b** (C-5 to C-6), **c** (C-9 and C-11 to C-12), **d** (C-16 to C-17), **e** (C-22 to C-23), and **f** (C-2' to C-6') were deduced from ¹H-¹H COSY analysis of **1** in CD₃OD (Figure 1). Unit **A** composed of the partial structures **a** and **b** was assigned as shown in Figure 1 with a γ -lactone ring by using HMBC and NOESY correlations as follows. HMBC correlations of H₃-28 (δ_H 1.58) to C-4 (δ_C 93.1), C-5 (δ_C 43.8), and C-29 (δ_C 67.3), H₂-6 (δ_H 2.80) to C-4 and C-7 (δ_C 177.8), and H₂-29 (δ_H 3.72 and 3.78) to C-4 revealed the presence of a γ -lactone ring⁶ with a hydroxymethyl and a methyl groups at C-4. Acetoxy group at C-1 and a methyl carboxylate at C-2 were assigned by the HMBC correlations as shown in Figure 1. Connection between the partial structures **a** and **b** through C-10 (δ_C 49.6), was deduced by an HMBC correlation of H₂-6 to C-10 and the NOESY correlations as shown in Figure 2. Unit **B** composed of the partial structures **c** and **d** was assigned as an octahydroinden-1-one ring system with a methyl, an exo-methylene, a hydroxyl, and two ester functions as follows. These functions can be connected by the HMBC correlations of H₃-18 (δ_H 1.02) to C-12 (δ_C 75.2), C-13 (δ_C 51.5), C-14 (δ_C 81.4), and C-17 (δ_C 36.8), H-9 (δ_H 3.29) to C-8 (δ_C 142.0) and C-14, and H₂-30 (δ_H 5.75 and 5.84) to C-9 and C-14. This ring system and functions were also supported by the comparison of the ¹H and ¹³C NMR chemical shifts [C-14 (δ_C 81.4), C-15 (δ_C 208.0), and C-16 (δ_C 40.8)] with those [C-14 (δ_C 79.5), C-15 (δ_C 209.3), and C-16 (δ_C 42.0)] of rohituka 14⁷ isolated from the seeds of *Aphanamixis polystacha*. The presence of a formate at C-11 was indicated by an HMBC correlation of an aldehyde proton to C-11 (δ_C

72.0). Connection between the units **A** and **B** was indicated by HMBC correlations of H-9 to C-5 and C-10. Unit **C** composed of the partial structure **e**, which was attached at C-17 in the unit **B**, was assigned as an α -substituted γ -lactone ring system with a hydroxyl at γ position by HMBC correlations of H-17 (δ_{H} 3.86) and H-22 (δ_{H} 7.19) to C-20 (δ_{C} 136.5), H-23 (δ_{H} 6.10) to C-21 (δ_{C} 172.9), and IR absorption at 1755 cm^{-1} . Unit **D** composed of the partial structure **f** was assigned as a 2-hydroxy-3-methylpentanoic acid by the ^1H - ^1H COSY correlation in Figure 1 and the comparison of NMR data in turrupubesin D,⁸ which might be attached at the hydroxy group at C-12 (δ_{C} 75.2). Thus, the gross structure of **1** was assigned as A,B-*seco*-tetranorlimonoid skeletal system with a γ -butanolide at C-17.

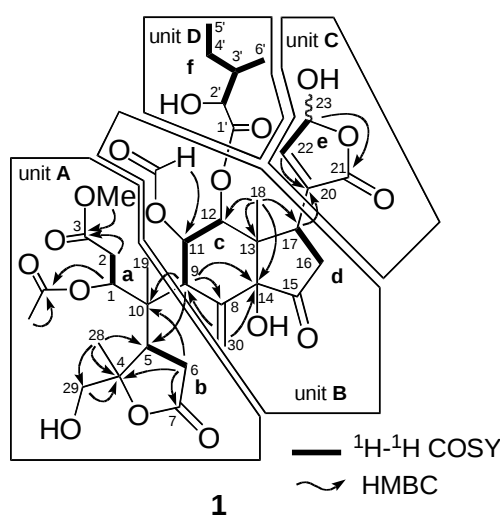


Figure 1. Selected 2D NMR correlations for **1**

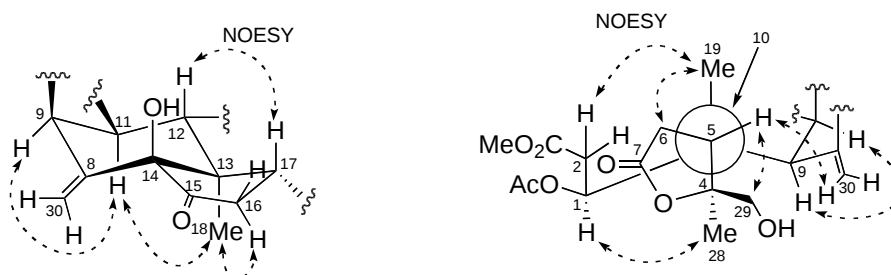


Figure 2. Selected NOESY correlations in unit **B** and rotation model of C-5/C-10 bond in unit **A** for **1**

The relative stereochemistry with selected NOESY correlations in unit **B** and rotation model of C-5/C-10 bond in unit **A** for **1** were elucidated by NOESY correlations as shown in Figure 2. Configurations of C-9, C-11, C-12, C-13, and C-17 in the unit **B** were elucidated by NOESY correlations of H₃-18/H-11 and H-16a, H-12/H-17, and H-9/H-11. As you can see the rotation model of C-5/C-10 bond, NOESY correlations of H₃-19/H-2 and H₂-6, H₃-28/H-1, and H-5/H₂-29 and H-30 indicated connectivity of C-5/C-10 bond and the relative stereochemistry in the unit **A** as shown in Figure 2. Thus, the relative configuration of **1** was assigned to be shown in computer-generated 3D drawing in Figure 3 except for

C-2' and C-3' in the 2-hydroxy-3-methylpentanoic acid.

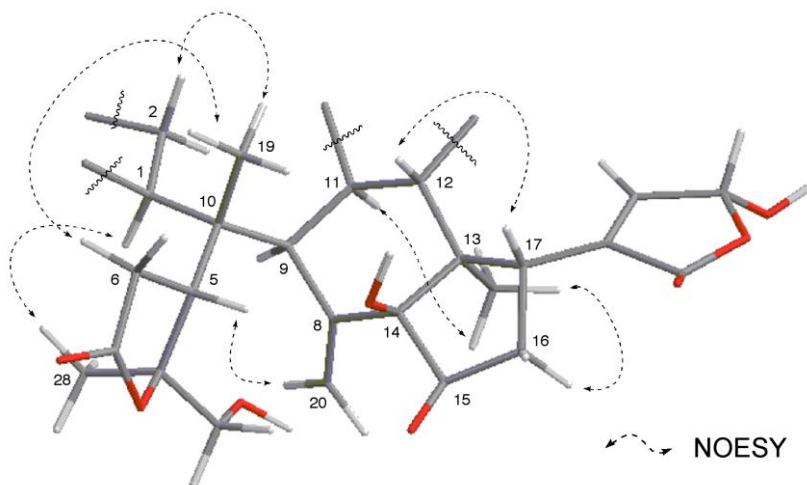


Figure 3. Selected NOESY correlations and relative configurations for **1**

From the bark of *Dysoxylum gaudichaudianum*, a series of dysoxylins A – D with a tetranortriterpenoid nucleus have already been isolated.⁹ Biogenetically, gaudichaudysolin A (**1**) may be derived by a unique oxidative route through this tetranortriterpenoid nucleus. Gaudichaudysolin A (**1**) was evaluated *in vitro* for cytotoxicity against five human cancer cell lines, HL60 (human blood premyelocytic leukemia), RPMI8226 (multiple myeloma), NCI-H226 (non-small cell lung carcinoma), HCT116 (human colon cancer), and MCF7 (human breast adenocarcinoma) cells, using MTT assay, but showed no inhibitory activity against five tested cell lines ($IC_{50} > 50 \mu M$).

EXPERIMENTAL

General Experimental Procedures. 1H and 2D NMR spectra were recorded on a Bruker AV600 spectrometer and chemical shifts were reported using residual CD_3OD (δ_H 3.31 and δ_C 49.0) as internal standards. HSQC experiments were optimized for $^1J_{CH}=145$ Hz and HMBC experiments for $^nJ_{CH}=8$ Hz. Mass spectra were recorded on a Micromass LCT spectrometer.

Plant Material. The bark of *D. gaudichaudianum* was collected at Alas Purwo, Indonesia in 2007. A voucher specimen is deposited at the Purwodadi Botanical Garden, Indonesia.

Extraction and Isolation. The bark of *D. gaudichaudianum* (1370 g) was extracted with MeOH, and the MeOH extract was partitioned between EtOAc and H_2O . Water-soluble materials were extracted with BuOH. EtOAc-soluble materials were subjected to a silica gel column (hexane/EtOAc, 1:0→1:1; $CHCl_3$ /MeOH, 1:0→0:1) and the fractions eluted by hexane/EtOAc (1:1) were subjected to a silica gel column (toluene/EtOAc, 1:0→5:5; $CHCl_3$ /MeOH, 1:0→5:5, $CHCl_3$ /MeOH/ H_2O 5:5:1) followed by C_{18} HPLC (40% CH_3CN /0.1%TFA) to afford gaudichaudysolin A (**1**, 1.3 mg, 0.00002 %).

Table 1. ^1H and ^{13}C NMR Data [δ_{H} (J, Hz) and δ_{C}] of Gaudichaudysolin A (**1**) in CD_3OD at 300K

Position	δ_{H}	δ_{C}
1	5.70 (1H, m)	70.0
2a	2.70(1H, dd, 15.0, 11.4)	36.0
2b	3.05 (1H, d, 14.4)	
3		173.2
4		93.1
5	3.16 (1H, m)	43.8
6	2.80 (2H, d, 10.2)	35.7
7		177.8
8		142.0
9	3.29 (1H, m)	55.4
10		49.6
11	5.38 (1H, t, 10.2)	72.0
12	5.97 (1H, d, 10.8)	75.2
13		51.5
14		81.4
15		208.0
16a	2.51 (1H, m)	40.8
16b	2.86 (1H, m)	
17	3.86 (1H, m)	36.8
18	1.02 (3H, s)	13.0
19	1.44 (3H, s)	20.0
20		136.5
21		172.9
22	7.19 (1H, br s)	148.6
23	6.10 (1H, br s)	98.8
28	1.58 (3H, s)	20.0
29a	3.72 (1H, d, 13.2)	67.3
29b	3.78 (1H, d, 13.2)	
30a	5.52 (1H, br s)	123.3
30b	5.84 (1H, br s)	
1-OAc	2.04 (3H, s)	21.1
		172.1
3-OMe	3.65 (3H, s)	52.4
11-OCHO	8.15	163.5
1'		173.3
2'	3.86 (1H, m)	76.3
3'	1.62 (1H, m)	39.3
4'a	1.28 (1H, m)	24.4
4'b	1.20 (1H, m)	
5'	0.86 (3H, s)	11.9
6'	0.93 (3H, s)	15.9

Gaudichaudysolin A (1): a colorless amorphous solid; $[\alpha]_D^{23}$ -126 (*c* 0.2, MeOH); IR (KBr) $\nu_{\max}$ 3425, 1755, 1680, 1630, 1585, 1440, 1390, 1200, 1135, 1075, 840, and 801 cm^{-1} ; UV (MeOH) $\lambda_{\max}$ 230 (ϵ 9000); ^1H and ^{13}C NMR data (Table 1); ESIMS m/z 775 ($\text{M}+\text{Na}^+$); HRESITOFMS m/z 775.2780 [$(\text{M}+\text{Na})^+$, calcd for $\text{C}_{36}\text{H}_{48}\text{O}_{17}$, 775.2789].

Cytotoxic Activity. Each cell line [HL60 (human blood premyelocytic leukemia), RPMI8226 (multiple myeloma), NCI-H226 (non-small cell lung carcinoma), HCT116 (human colon cancer), and MCF7 (human breast adenocarcinoma) cells] was seeded onto 96-well microtiter plates at 1×10^4 cells per well for HL60 and RPMI8226 and 5×10^3 cells per well for NCI-H226, HCT116, and MCF7, respectively. Cells were preincubated for 24 h at 37°C in humidified atmosphere of 5% CO_2 . Different concentrations of each compound (10 μL) were added to the cultures, and then the cells were incubated at 37°C for 48 h. On the third day, 15 μL MTT solution (5 mg/mL) was added into each well of the cultured medium. After further 2 h of incubation, 100 μL of 10% SDS-0.01N HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. The optical density measurements were made using a micropipette reader (Benchmark Plus microplate spectrometer, BIO-RAD) equipped with a two wavelengths system (550 and 700 nm). In each experiment, three replicate of wells were prepared for each sample. The ratio of the living cells was determined based on the difference of the absorbance between those of samples and controls. These differences are expressed in percentage and cytotoxic activity was indicated as an IC_{50} value.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and grants from the Research Foundation for pharmaceutical Sciences and The Open Research Center Project.

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