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Alsmaphorazines A and B, Novel Indole Alkaloids from *Alstonia pneumatophora*

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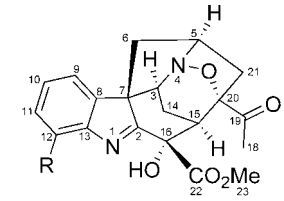
ABSTRACT



alsmaphorazine A (**1**) : R=OH
alsmaphorazine B (**2**) : R=H

Two novel indole alkaloids, alsmaphorazines A and B, were isolated from the leaves of *Alstonia pneumatophora* (Apocynaceae), and their structures were determined on the basis of the 2D NMR and MS spectral analysis. These alkaloids possessed a new skeleton consisting of an 1,2-oxazinane and an isoxazolidine chromophore. The absolute configuration of alsmaphorazine B was determined by using CD spectral analysis. Alsmaphorazine A inhibited the NO production in the LPS-stimulated J774.1 cells dose-dependently without affecting the cell viability.

Alstonia plants growing widely in the tropical regions of Africa and Asia are a well-known rich source of unique heterocyclic alkaloids having a monoterpene indole skeleton. These alkaloids have attracted great attention in the biogenetic[1] and biological fields for their anticancer, antibacterial, anti-inflammatory, antitussive, and antimalarial properties.[2] Previous investigations have shown that the skeleton and the amounts of the monoterpene indole alkaloids in the plants vary greatly depending upon the areas where they grow.[3] For example, picrinine-type indole alkaloids are generally rich in the *Alstonia* species from India, Pakistan, and Thailand, whereas angustilobine-type ones are in species from Indonesia and Philippine.[3] In our present study, we isolated two novel alkaloids, alsmaphorazines A (**1**) and B (**2**) from *Alstonia pneumatophora* (Apocynaceae) collected in Malaysia and elucidated their structures. Of them, **1** was found to inhibit the NO production in J774.1 cells.

Alsmaphorazine A,[4,5] {**1**, $[\alpha]_D^{20}$ −62 (*c* 0.1, MeOH)} showed a pseudomolecular ion peak at *m*/*z* 385 $(M + H)^+$ in the LCESIMS. The molecular formula was established to be $C_{20}H_{20}N_2O_6$ by the LCHRESIMS [*m*/*z* 385.1403 $(M + H)^+$, Δ +0.9 mmu], and its structure was established mainly on the basis of the NMR data. Its ^{13}C NMR spectrum (Table

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Table 1. ^{1}H and ^{13}C NMR Data of Alsmaphorazines A (**1**) and B (**2**) in CD_3OD at 303 K by 600 MHz Cryo Probe NMR

	alsmaphorazine A (**1**)			alsmaphorazine B (**2**)		
position	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2		183.1	6, 15		186.2	6, 15
3	4.14 (1H, m)	68.4	5, 6b, 14a, 15	4.19 (1H, m)	68.2	5, 6b, 14a, 15
5	4.19 (1H, ddd, 10.5, 6.4, 2.1)	67.1	6a, 21a	4.22 (1H, m)	67.3	6a, 21a
6a	2.47 (1H, dd, 14.3, 6.4)	40.6	21	2.54 (1H, dd, 14.3, 6.5)	40.3	21
6b	2.12 (1H, d, 14.3)			2.11 (1H, d, 14.3)		
7		64.1	5, 6, 9, 14a,		64.1	5, 6, 9, 14a
8		146.2	6a, 10		144.4	6a, 10, 12
9	6.85 (1H, dd, 7.6, 0.8)	113.9	11	7.42 (1H, d, 7.2)	123.2	11
10	7.17 (1H, dd, 7.9, 7.6)	129.8		7.35 (1H, dd, 7.4, 7.2)	128.5	12
11	6.82 (1H, dd, 7.9, 0.8)	117.1	9	7.39 (1H, dd, 7.5, 7.4)	129.7	9
12		150.7	10, 11	7.61 (1H, d, 7.5)	121.9	10
13		140.1	9, 11		153.6	9, 11
14a	2.03 (1H, ddd, 14.1, 4.8, 4.1)	18.9		2.05 (1H, ddd, 14.1, 5.4, 4.2)	19.1	15
14b	1.22 (1H, d, 14.1)			1.21 (1H, d, 14.1)		
15	3.19 (1H, m)	45.0	14b, 21a	3.22 (1H, m)	45.4	14, 21
16		78.9	14, 15		79.2	14, 15
18	2.31 (3H, s)	25.0		2.32 (3H, s)	25.0	
19		203.6	15, 18, 21a,		203.8	15, 18, 21a
20		90.1	14b, 18, 21a		90.2	14b, 15, 18, 21a
21a	2.68 (1H, dd, 14.4, 10.5)	39.4	6, 15	2.68 (1H, dd, 14.4, 10.4)	39.5	6, 15
21b	1.83 (1H, dd, 14.4, 2.1)			1.87 (1H, dd, 14.4, 1.8)		
22		172.8	23		172.9	23
23	3.76 (3H, s)	53.1		3.77 (3H, s)	53.2	

1) revealed 20 carbon signals due to four sp^2 quaternary carbons, three sp^2 methines, one ketone, one ester carbonyl, three sp^3 quaternary carbon, three sp^3 methines, three sp^3 methylenes, one methyl, and one methoxy group. The carbon resonance at δ 183.1 due to an imine carbon indicated the presence of an indolenine moiety. The ^{13}C NMR signals of C-12 and C-16 (δ_C 150.7 and 78.9) indicated the presence of a hydroxyl group on each, that of C-20 (δ_C 90.1) the presence of an oxygen atom on it, and those of C-3 (δ_C 68.4) and C-5 (δ_C 67.1) the presence of an *N*-oxide moiety linking to them. The chemical shift of C-20 NMR signal and the molecular formula by LCHRESIMS, $C_{20}H_{20}N_2O_6$, suggested the presence of an oxyamine chromophore.

Partial structures **a** (C-9−C-11), **b** (C-5−C-6 and C-21), and **c** (C-3 and C-14−C-15) shown in heavy lines in Figure 1 were deduced from a detailed analysis of the $^{1}H-^{1}H$ COSY spectrum. The HMBC cross-peaks between H-9/C-7, H-6a/C-8, and H-6b/C-3 indicated that the C-7−C-8 unit connected the three units **a**, **b**, and **c** together. The HMBC correlation between H-5/C-3 and the chemical shifts of C-3 and C-5 NMR signals implied that C-3 and C-5 were both connected to N-4, the correlations between H_3-18/C-19 and H_3-18/C-20 that C-18 and C-20 were connected to C-19, those between H-14/C-20, H-15/C-19, H-21/C-19, and H-21/C-15 that C-15 and C-21 were connected to C-20, and those between H-14/C-16, H-6/C-2, and H-15/C-2 that C-2 and C-6 were connected to C-7, and C-2 and C-15 to C-16. To decide which carbon was connected to an ether oxygen and which to a hydroxyl group, a deuterium shift analysis was performed in CD_3OD and CD_3OH.[6] In those two media, the differences in the chemical shifts were about 0.09 ppm for C-12 and C-16, due to the β effect of hydroxyl group, and

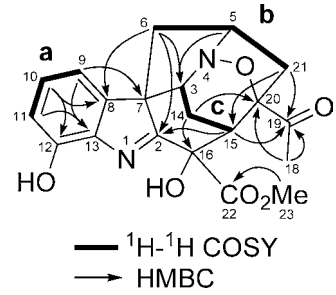

Figure 1. Selected 2D NMR correlations for alsmaphorazine A (**1**).

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(4) A MeOH extract of the leaves of *A. pneumatophora* collected in Malaysia in 2006 was partitioned between EtOAc and 3% aq tartaric acid. The water-soluble fraction was adjusted to pH 9 with saturated Na_2CO_3 and was extracted with $CHCl_3$. The $CHCl_3$-soluble fraction was subjected to silica gel column chromatography (elution, EtOAc/MeOH 1:0 → 0:1). The MeOH fractions were purified by C18 HPLC (MeOH/H2O/TFA solvent system) to afford alsmaphorazines A (**1**, 0.5 mg, 0.00025%) and B (**2**, 0.9 mg, 0.00045%).

(5) Alsmaphorazine A (**1**): colorless amorphous solid; $[\alpha]_D^{20}$ −62 (*c* 0.1, MeOH); IR (film) ν_{max} 3433 and 1642 cm^{-1}; UV (MeOH) λ_{max} 314 (ε 5220), 254 (ε 5450), and 223 (ε 11980) nm; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 385 $(M + H)^+$; HRESIMS *m/z* 385.1403 [$(M + H)^+$, calcd for $C_{20}H_{21}N_2O_6$, 385.1394].

0.06−0.07 ppm for C-2, C-11, C-13, and C-15 due to the γ effect of hydroxyl group, whereas that it was only 0.01 ppm for C-20. Consequently, the ethereal oxygen atom was determined to be on C-20. The gross structure of **1** was thus elucidated to be as shown in Figure 1, possessing a novel ring system in which 1,2-oxazinane and isoxazolidine were fused together.

The relative stereochemistry of **1** was elucidated by the NOESY correlations. The presence of an acetyl group of β-configuration at C-20 was shown by the correlation between H_3-18/H-15, and that of methoxycarbonyl moiety of β-configuration by those between H_3-23/H-21b and H-21b/H-6b. Thus, the relative stereochemistry of **1** was established to be as shown in the computer-generated 3D drawing in Figure 2.

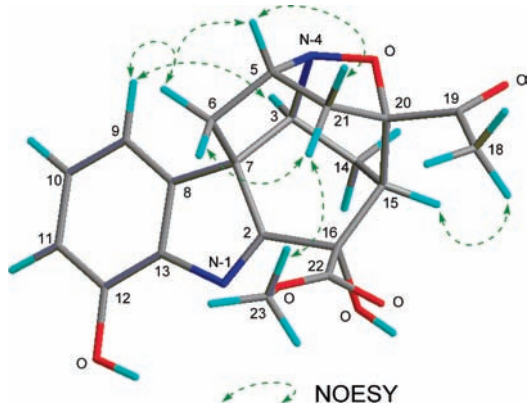

Figure 2. Selected NOESY correlations (arrows) and relative stereochemistry for alsmaphorazine A (**1**).

Alsmaphorazine B (**2**)[7] showed the pseudomolecular ion peak at *m*/*z* 369 $(M + H)^+$ in ESIMS, and the molecular formula was established to be $C_{20}H_{20}N_2O_5$ by HRESIMS [*m*/*z* 369.1446 $(M + H)^+$ Δ +0.1 mmu], which was smaller than that of **1** by 16 Da. Its ^1H and ^{13}C NMR data (Tables 1) disclosed 20 carbon signals that were analogous to those of **1** except for the chemical shift of C-12 sp^2 methine. A detailed analysis of 2D NMR data suggested that the structure of **2** was basically the same as that of **1** except that C-12 of **2** had a hydroxyl group. The relative stereochemistry of **2** was established by the NOESY correlations. The correlations of H_3-18/H-15, H-23/H-21b, and H-21b/H-6b indicated that the acetyl group at C-20 and the methoxycarbonyl moiety at C-16 were both of β-configurations, as in **1**.

The absolute configuration of **2** was studied by comparing its experimental CD spectrum with the calculated CD spectrum, the CD calculations performed by Turbomole 6.1[8] using TD-DFT-B3LYP/TZVPP level of theory on RI-DFT-BP386LYP/TZVPP[9−13] optimized geometries. The conformer used for CD calculation was the model obtained by using MC calculations (MMFF94 force field,[9] Macromodel 9.1.).[14] The CD spectrum of **2** and that calculated for the molecule having 3*S*,5*S*,7*R*,15*R*,16*S*,20*S* were in good agreement (Figure 3). Therefore, the absolute configuration of **2** was deduced to be 3*S*,5*S*,7*R*,15*R*,16*S*,20*S*.

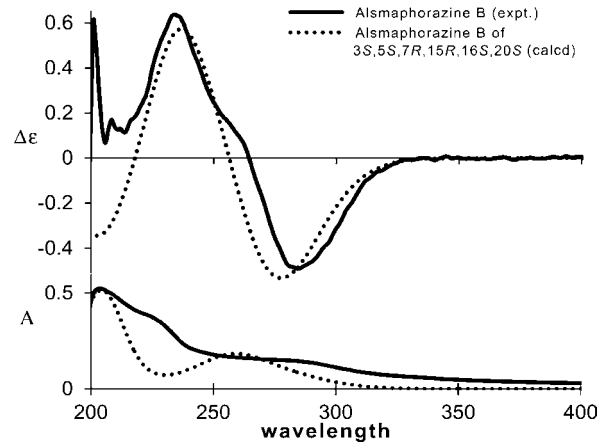

Figure 3. CD and UV spectra of alsmaphorazine B (**2**). Dotted lines indicated simulated CD and UV curves of **2**.

Biogenetic relationships among monoterpene indole alkaloids including stemmadenine and 5-nor-indole derivatives such as vallesamine and apparicine have been discussed.[15] A plausible biogenetic route for the present alkaloids **1** and **2** is proposed in Scheme 1. Compounds **1** and **2** might be derived from scholaricine skeleton[16] that might be derived from pre-acuamicine as follows. Polonovski-type reaction[17] of an *N*-oxidative product might result in an iminium

(6) Kita, M.; Ohishi, N.; Konishi, K.; Kondo, M.; Koyama, T.; Kitamura, M.; Yamada, K.; Uemura, D. *Tetrahedron* **2007**, *63*, 6241–6251.

(7) Alsmaphorazine B (**2**): colorless amorphous solid; $[\alpha]_D^{20}$ −36 (*c* 0.1, MeOH); IR (film) ν_{max} 3434 and 1644 cm^{-1}; UV (MeOH) λ_{max} 281 (ε 4290) and 227 (ε 8220) nm; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 369 $(M + H)^+$; HRESIMS *m*/*z* 369.1446 [$(M + H)^+$, calcd for $C_{20}H_{21}N_2O_5$, 369.1445].

(8) TURBOMOLE V6.1, 2009, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989−2007, TURBOMOLE GmbH, since 2007; available from http://www.turbomole.com.

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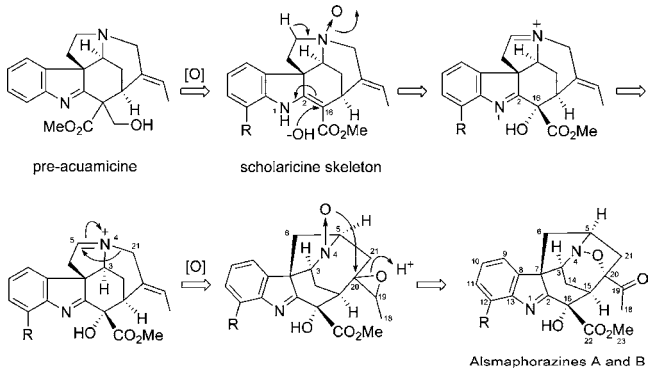
(17) Grierson, D. *Org. React.* **1990**, *39*, 85–295.

Scheme 1. Plausible Biogenetic Route for Alsmaphorazines A (**1**) and B (**2**)



intermediate with a five-membered ring system. Cleavage of the N-4−C-21 bond and linking of N-4 to C-5 followed by oxidation will produce an *N*-oxide derivative. Attack by the N-4 oxygen on C-20 of the epoxide will give alsmaphorazine A (**1**) with an 8-oxa-1-aza-bicyclo[3.2.1]octane ring system.

Alsmaphorazine A was found to dose-dependently inhibit the NO production in LPS-stimulated J774.1, scarcely affecting the cell viability (IC_{50} 49.2 μM).[18] On the other hand, **2** did not show such an inhibitory effect at 50 μM.

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Supporting Information Available: 1D and 2D NMR spectra for compounds **1** and **2** and atom coordinate of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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