Wrightiamines A and B, Two New Cytotoxic Pregnane Alkaloids from *Wrightia javanica*

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Two new pregnane alkaloids, wrightiamines A (1) and B (2), were isolated from the extract of the tropical Apocynaceous plant *Wrightia javanica* collected in Thailand, and their structures were elucidated by spectral data. Wrightiamine B (2) was preparaed from 3β-hydroxy-5α-pregnan-20-one to establish the configuration of the C-20 position as *S*. Wrightiamine A (1) exhibited cytotoxic activity against vincristine-resistant murine leukemia P388 cells.

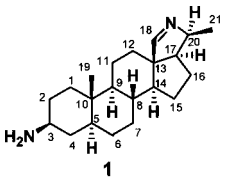
Key words Apocynaceae; *Wrightia javanica*; pregnane alkaloid; cytotoxicity

Wrightia javanica DC. (Apocynaceae) is a small tree widely distributed over the northern Malayan peninsula and other south Asian areas, and its milky lotion has been used as a folk medicine.[1)] During our search for bioactive natural products from tropical plants,[2)] we investigated the chemical constituents of leaves of *W. javanica* collected in Thailand. Here we describe the isolation and structure elucidation of two new pregnane alkaloids, wrightiamines A (**1**) and B (**2**), and preparation of compound **2** to establish the C-20 configuration. **1** exhibited cytotoxic activity against vincristine-(VCR)-resistant murine leukemia P388 cells, while the cytotoxicity of **2** was weak.

The leaves of *W. javanica*, collected in Thailand, were extracted with MeOH, and the MeOH extract was subjected to solvent partitioning to give hexane-, EtOAc-, *n*-BuOH-, and water-soluble fractions. The *n*-BuOH-soluble fraction containing Dragendorff reagent-positive spots on TLC examination was subjected to repeated chromatography on silica gel and Sephadex LH-20, followed by further purification with HPLC on ODS to give **1** and **2**.

1 was obtained as colorless amorphous solid and was suggested to have the molecular formula $C_{21}H_{34}N_2$ based on its high resolution (HR)-FAB-MS data (*m/z* 315.2790, M+H, Δ +1.1 mmu). The IR spectrum of **1** showed absorption bands due to an amino (3400 cm^{-1}) and an imino group (1650 cm^{-1}). The presence of an imino group was also indicated from the ^{1}H- and ^{13}C-NMR signals [δ_H 7.67, 1H, s (H-18); δ_C 169.9 (C-18)]. The ^{1}H-NMR spectrum of **1** showed signals due to one tertiary methyl [δ_H 0.83, 3H, s (H_3-19)], one secondary methyl [δ_H 1.39, 3H, d, J=7.0 Hz (H_3-21)], and two nitrogen-bearing sp^3 methines [δ_H 3.58, 1H, br t, J=11.0 Hz (H-3) and 4.07, 1H, m (H-20)]. In addition to these groups, the ^{13}C-NMR spectrum aided by ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) experiments revealed the presence of nine sp^3 methylenes, five sp^3 methines, and two sp^3 quaternary carbons. Since one of six unsaturation degrees was accounted for by the imino group and no other sp^2 carbon signals were observed in the ^{13}C-NMR spectrum, **1** was inferred to have five rings. The ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectra of **1** showed correlations consistent with the pregnane skeleton. The ^{1}H–^{1}H COSY spectrum suggested the presence of an amino group on C-3, and the methine proton on C-3 (δ_H 3.58) was observed as a broad triplet (J=11 Hz), indicating that H-3 is α-axial and that the C-3 amino group has β-equatorial orientation. The imino proton (δ_H 7.67, H-18) showed HMBC correlations to C-13, C-17, and C-20, thus suggesting that the imino carbon (C-18) was attached at the C-13 position and the nitrogen of the imino group was connected to the C-20 position to construct a 1-pyrroline ring. Substantial nuclear Overhauser effect (NOE) correlations were observed for H-20/H-17 and H_3-21/H-16β, indicating that H-20 is α and the methyl group (C-21) has β orientation. From these results, the structure of wrightiamine A was concluded to be **1**, which corresponds to a 5,6-dihydro derivative of conkurchine (**3**).[3)] Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of **3** in the literature[3)] also supported the structure of **1**.

Compound **2** was suggested to have the molecular formula $C_{21}H_{35}NO$ based on the HR-FAB-MS data (*m/z* 318.2812, M+H, Δ +1.5 mmu). The ^{1}H-NMR spectrum of **2** showed signals due to two tertiary methyl [δ_H 0.63, 3H, s (H_3-18); 0.89, 3H, s (H_3-19)] and one secondary methyl [δ_H 1.59, 3H, d, J=6.5 Hz (H_3-21)] groups, and its ^{13}C-NMR spectrum revealed the presence of a ketone (δ_C 209.4) and a nitrogen-bearing methine carbon (δ_C 50.4). The IR spectrum of **2** showed absorption bands due to an amino (3450 cm^{-1}) and a carbonyl (1690 cm^{-1}) group. Since one of five unsaturation degrees was due to the ketone group and no other sp^2 carbon



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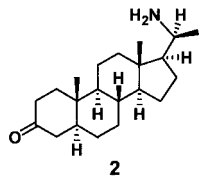

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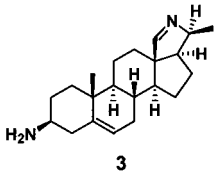

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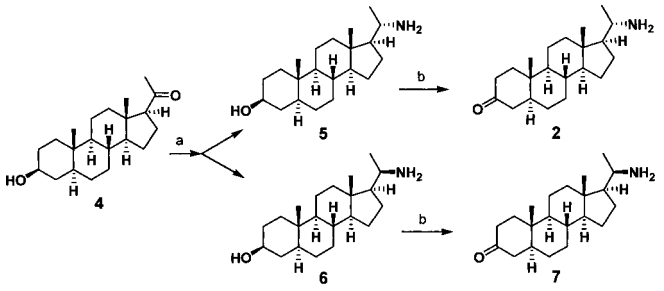

(a) Refs. 5, 6 [(i) $NH_2OH{\cdot}HCl$, pyridine, EtOH, reflux, 5h, 92%; (ii) H_2, PtO_2, AcOH, 16h; (iii) silica gel column, ether saturated with NH_3/MeOH (85:15), **5**: 63%; **6**: 20%]; (b) CrO_3, H_2SO_4, acetone, **2**: 86%; **7**: 71%.

Chart 1

Table 1. ^{13}C-NMR Spectral Data of Compounds **1** and **2** in C_5D_5N

Position	**1**	**2**
	δ_C	δ_C
1	37.4	37.7
2	27.6	37.4
3	51.3	209.4
4	33.9	43.9
5	45.4	45.7
6	28.7	28.0
7	32.9	30.8
8	37.6	34.2
9	54.4	52.6
10	36.1	34.2
11	23.4	20.5
12	33.7	38.3
13	66.9	41.7
14	55.2	55.0
15	29.4	23.4
16	24.7	26.5
17	49.3	54.7
18	169.9	11.2
19	17.7	10.3
20	69.2	50.4
21	12.5	19.1

signals were observed, **2** was suggested to have four rings. The HMQC and distortionless enhancement by polarization transfer (DEPT) data revealed that **2** has three methyls, nine methylenes, six methines, and three quaternary carbons. The HMBC spectral data of **2** were indicative of the pregnane skeleton, and the ketone group was placed on C-3 (HMBC correlations: H_2-1/C-3, H_2-2/C-3, H_2-4/C-3, and H_2-5/C-3), and the amino group on C-20 (HMBC correlations: H_3-21/C-20 and H-17/C-20). The ^{13}C-NMR chemical shift of the C-19 methyl carbon (δ_C 10.3) suggested that **2** has the 5α-H configuration, since the C-19 of 5α- and 5β-pregnan-3,20-dione resonated at δ_C 10.3 and 22.6, respectively.[4] To determine the configuration of the C-20 amino-bearing methine carbon, compound **2** and its 20-epimer were prepared as shown in Chart 1. 3β-Hydroxy-5α-pregnan-20-one (**4**) was converted into the known 20*S*- and 20*R*-amino-5α-pregnan-3β-ol (**5**, **6**) using procedures reported in the literature.[5,6] The absolute configurations of the C-20 of **5** and **6** have been firmly established based on chemical evidence.[7] Jones oxidation of **5** and **6** afforded 20*S*- and 20*R*-amino-5α-pregnan-3-one (**2**, **7**), respectively, and wrightiamine B proved to be identical to the 20*S*-isomer (**2**) on the basis of the comparison of TLC and ^{1}H-NMR spectral data. Thus the structure of wrightiamine B was established to be 20*S*-amino-5α-pregnan-3-one (**2**).

The cytotoxic activities of **1** and **2** against VCR-resistant murine leukemia P388 cells were examined, and the IC_{50} values in the presence and absence of VCR 12.5 ng/ml were 2.0 and 3.1 μg/ml, respectively, for **1**, and 22 and *ca.* 25 μg/ml, respectively, for **2**. Thus compound **1** was cytotoxic but had no reversal effect of multidrug resistance,[8] while the cytotoxicity of **2** was weak.

Experimental

General Optical rotations were recorded on a JASCO J-20. IR spectra were measured on KBr disks in a Hitachi 260-10 infrared spectrophotometer. NMR spectra were recorded on JEOL JNM GSX-A400, A500, and ecp600 spectrometers. HR-FAB mass spectra were acquired on a JMS HX-110 mass spectrometer.

Plant Materials Leaves of *W. javanica* were collected in Khon Kaen, Thailand. A voucher specimen is maintained in the Department of Horticulture, Faculty of Agriculture, Khon Kaen University.

Extraction and Isolation The air-dried leaves (200 g) were extracted with MeOH. The MeOH extract (68 g) was partitioned between hexane (200 ml×3) and 10% aqueous MeOH (200 ml), and the aqueous phase was further extracted with EtOAc (200 ml×3) and *n*-BuOH (200 ml×2) to give four corresponding fractions (4.7, 8.9, 17.9, 25.5 g, respectively). Part of the *n*-BuOH-soluble fraction (8.3 g) was subjected to silica gel column chromatography (3.8×33 cm) eluted with 0—100% MeOH/$CHCl_3$. The fraction eluted with MeOH/$CHCl_3$ (1 : 1) was further separated by gel filtration with Sephadex LH-20 (2.4×33 cm) eluted with MeOH, followed by purification on a second silica gel column (1.4×13 cm; eluent: $CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5 : 6 : 1 : 1) and a second Sephadex LH-20 column (1.4×38 cm; eluent: MeOH) to afford **1** (29.2 mg). Another part of the *n*-BuOH-soluble fraction (9.6 g) was subjected to silica gel column chromatography (3.6×25 cm) eluted with 0—100% MeOH/$CHCl_3$. The fraction eluted with MeOH/$CHCl_3$ (1 : 1) was further separated by gel filtration on Sephadex LH-20 (1.5×27 cm) eluted with MeOH, followed by purification with a second silica gel column (1.5×15 cm; eluent: $CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5 : 6 : 1 : 1) and ODS flash column chromatography (1.5×15 cm; eluent: 60—100% MeOH with 0.1% trifluoroacetic acid (TFA)), and finally with ODS HPLC (Develosil ODS UG-5, 10×250 mm; eluent: 70% MeOH with 0.1% TFA) to give **2** (3.4 mg).

Wrightiamine A (**1**): Colorless amorphous solid; $[\alpha]_D^{25}$ −14° (*c*=0.2, MeOH); IR (KBr) ν_{max} 3400 and 1650 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ_H 7.67 (1H, s; H-18), 4.07 (1H, m; H-20), 3.58 (1H, br t, *J*=11.0 Hz; H-3), 2.31 (1H, br t, *J*=11.0 Hz; H-2a), 2.10 (2H, m; H-2b and H-4a), 1.95 (1H, t, *J*=11.0 Hz; H-4b), 1.39 (3H, d, *J*=7.0 Hz; H_3-21), and 0.83 (3H, s; H_3-19); ^{13}C-NMR (Table 1); electron impact (EI)-MS *m/z* 314 (M^+); FAB-MS *m/z* 315 $(M+H)^+$; HR-FAB-MS *m/z* 315.2790 [Calcd. for $C_{21}H_{34}N_2$, (M+H) 315.2779].

Wrightiamine B (**2**): Colorless amorphous solid; $[\alpha]_D^{25}$ +5° (*c*=0.04, MeOH); CD (MeOH) λ_{ext} 289 ($\Delta\varepsilon$ 0.42), 237 (−0.053), 222 (0.17), and 209 nm (−0.19); IR (KBr) ν_{max} 3450 and 1685 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ_H 3.48 (1H, m; H-20), 2.35 (1H, m; H-2a), 2.34 (1H, m; H-2b), 2.24 (1H, m; H-4a), 2.07 (1H, m; H-4b), 1.78 (1H, m; H-17), 1.59 (3H, d, *J*=6.5 Hz; H_3-21), 0.86 (3H, s; H_3-19), and 0.63 (3H, s; H_3-18); ^{13}C-NMR (Table 1); EI-MS *m/z* 317 (M^+); FAB-MS *m/z* 318 $(M+H)^+$; HR-FAB-MS *m/z* 318.2812 [Calcd. for $C_{21}H_{36}NO$, (M+H) 318.2797].

Preparation of 20*S*- and 20*R*-Amino-5α-pregnan-3-one (2, 7) Commercially available 3β-hydroxy-5α-pregnan-20-one (**4**, 721 mg) was converted into known 20*S*-amino-5α-pregnan-3β-ol (**5**, 326 mg) and 20*R*-amino-5α-pregnan-3β-ol (**6**, 104 mg) using the procedures reported in the literature[5,6] [(i) $NH_2OH.HCl$, pyridine, EtOH, reflux, 5 h, 92%; (ii) H_2, PtO_2, AcOH, 16 h; (iii) silica gel column, ether saturated with NH_3/MeOH (85 : 15), **5**: 63%; **6**: 20%]. 20*S*-aminoalcohol (**5**, 6.6 mg) dissolved in acetone (1.0 ml) was treated with 6 μl of Jones reagent (CrO_3 2.67 g, conc. H_2SO_4 2.3 ml, and H_2O *ca.* 7.7 ml) for 5 min at room temperature. After addition of 4 N NaOH (10 ml), the reaction mixture was extracted with $CHCl_3$ (3 ml×10), dried over $MgSO_4$, and purified with silica gel column chromatography (9×35 mm; 5—10% MeOH/$CHCl_3$) to give 20*S*-amino-5α-pregnan-3-one (**2**, 5.1 mg). 20*R*-Aminoalcohol (**6**, 6.4 mg) was converted into 20*R*-amino-5α-pregnan-3-one (**7**, 5.0 mg). **7**: $[\alpha]_D^{25}$ −3° (*c*=0.2, MeOH); IR (KBr) ν_{max} 3450 and 1685 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ_H 3.54 (1H, m; H-

20), 2.49 (1H, m; H-2a), 2.07 (1H, m; H-2b), 2.20 (1H, m; H-4a), 2.06 (1H, m; H-4b), 1.70 (1H, m; H-17), 1.49 (3H, d, J=6.5 Hz; H_3-21), 0.87 (3H, s; H_3-18), and 0.78 (3H, s; H_3-19); EI-MS m/z 317 (M^+); FAB-MS m/z 318 $(M+H)^+$; HR-FAB-MS m/z 318.2796 [Calcd. for $C_{21}H_{36}NO$, (M+H) 318.2797].

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