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Two New Alkaloids from the Rhizomes of *Sinomenium acutum*

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Sinomenium acutum Rehder et Wilson (Menispermaceae) is a deciduous shrub grown in Asia, particularly in Japan, China, and Korea. The stems and roots of the plant are widely used to treat various rheumatic diseases, allergy, dropsy, dermatophytosis, and acesodyne in traditional Chinese prescriptions.[1] Previous phytochemical investigations of *S. acutum* have isolated various alkaloids, *e.g.* protoberberin-type, aporphin-type, isoaporphin-type, morphine-type, and chlorine-contained alkaloids.[2-12] Also, cyanoglucoside,[13] butenolides,[13,14] and lignan[15] were isolated from this plant. The main alkaloid from this plant has been studied for its effects on anti-inflammation, anticancer, immunosuppression, arthritis amelioration, and protection against hepatitis induced by LPS for sinomenine.[16-18] Column chromatographic purification of the MeOH extract of the rhizome of this source led to isolation of two new alkaloids (**1** and **4**), together with twelve known alkaloids (**2**, **3**, **5**-**14**). The structures of the new compounds (**1** and **4**) were established by spectroscopic and chemical means.

Compound **1** was isolated as a colorless gum, $[\alpha]_D^{25}$ −41.5 (*c* 0.5, MeOH). The molecular formula $C_{21}H_{26}ClNO_7$ was determined by the HR-FAB MS *m/z* 440.1477 $[M+H]^+$ (calcd. 440.1476). The UV, IR, ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those of **2**, except for the presence of signals for an additional acetyl group (δ_H 2.12; δ_C 169.7, 21.3). The downfield shift of the signals of H-1 and C-1 ($\Delta\delta_H$ +1.3; $\Delta\delta_C$ +0.8) in **1**, compared to the corresponding signals in **2**, suggested that the acetyl group should be placed at C-1. The position of the acetyl group was established by an HMBC experiment, in which a long-range correlation was observed between the H-1 (δ_H = 5.93) and a carbonyl carbon (δ_C = 169.7) (Figure 2). The stereochemistry of **1** was established based on NOESY correlation between H-10 and H-14, and no correlation of H-1 with H-14 indicated that the OH group at C-1 was α-oriented (Figure 3),[19] which was in accordance with that of **2**. Moreover, the CD spectrum of **1** showed a negative Cotton effect at 347 nm and positive Cotton effects at 283 nm and 258 nm which were similar with those of **2**, indicating that C-1 has an *R*-configuration in **1**.[20] Alkaline hydrolysis (0.05 M KOH) afforded dauricumine (**2**), which was identified by its ^{1}H-, ^{13}C-NMR, CD and MS spectra.[21] Thus, compound **1** was determined to be 1-*O*-acetyl-dauricumine.

Compound **4** was isolated as a purple gum, $[\alpha]_D^{25}$ +174.0 (*c* 0.25, MeOH). The molecular formula $C_{18}H_{19}NO_3$ was determined by HR-FAB MS *m/z* 297.1365 $[M]^+$ (calcd. 297.1365). The ^{1}H-NMR spectrum of **4** displayed signals for four aromatic protons, comprising a singlet aromatic proton at δ_H = 6.68, and three ABX coupled protons at δ_H = 7.84 (d, *J* = 2.5 Hz), 7.02 (d, *J* = 8.0 Hz), and 6.65 (dd, *J* = 8.0, 2.5 Hz), three methylene protons at δ_H = 3.21, and 2.83 (H-5), 2.94, and 2.62 (H-4), and 2.72 and 2.49 (H-7), a methine proton at δ_H = 3.58 (H-6a), and two methoxy groups at δ_H = 3.80, and 3.61. In the ^{13}C-NMR spectrum, 18 carbon signals appeared, including two methoxy carbons at δ_C = 59.2 (OCH_3-1) and 55.0 (OCH_3-2), twelve aromatic carbons at δ_C = 155.8 (C-10), 152.1 (C-2), 145.0 (C-1), 132.7 (C-11a), 128.9 (C-

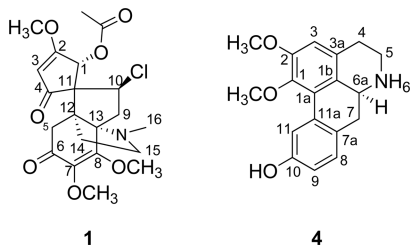

Figure 1. Chemical structures of **1** and **4**.

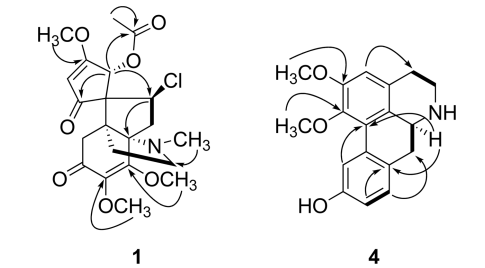

Figure 2. Key ^{1}H-^{1}H COSY (—) and HMBC (→) correlations of **1** and **4**.

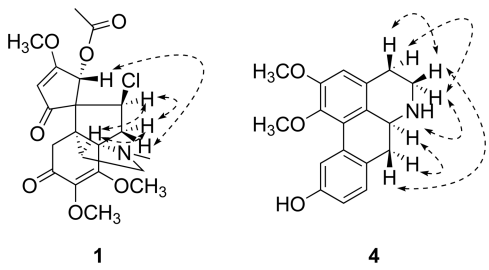

Figure 3. Key NOESY (⇢) correlations of **1** and **4**.

3a), 128.2 (C-1b), 128.1 (C-8), 127.0 (C-7a), 126.3 (C-1a), 114.9 (C-11), 114.0 (C-9), and 111.8 (C-3), three methylene carbons at δ_C = 42.4 (C-5), 35.6 (C-7), and 28.0 (C-4), and a methine carbon at δ_C = 53.6 (C-6a). The ^{1}H- and ^{13}C-NMR, HMQC, ^{1}H-^{1}H COSY, HMBC and NOESY spectra were almost the same with those of tuduranine, which was isolated from the root tuber of *S. rotunda*,[22] except for the optical rotation value. The optical rotation of **4** ($[\alpha]_D^{25}$: +174.0, MeOH) had an opposite sign to that of tuduranine ($[\alpha]_D^{25}$: −156.0, MeOH), which suggested that compound **4** could be a stereoisomer of tuduranine.[23] For the aporphine alkaloids, the *S*-configuration at C-6a showed positive a optical rotation value.[24] Additionally, negative Cotton effects at 323 nm and 217 nm and a positive Cotton effect at 241 nm in the CD spectrum determined the absolute configuration at C-6a to be in the *S* form.[25] Thus, the structure of **4** was determined to be (+) tuduranine.

The known compounds were identified as dauricumine (**2**),[21] acutumine (**3**),[21] magnoflorine (**5**),[21] menisperine (**6**),[26] bianfugenine (**7**),[21] menisporphine (**8**),[21] sinomenine (**9**),[21] sinomenine *N*-oxide (**10**),[6] isosinomenine (**11**),[27] salutaridine (**12**),[28] 8-oxoisocorypalmine (**13**),[29] and *N-trans*-feruloylmethoxytyramine (**14**),[21] by comparison of physicochemical and spectroscopic data with previously reported literature values.

Experimental Section

Plant Material. *Sinomenium acutum* Rehder et Wilson (20 kg) were collected on Jeju Island, Korea in November 2010, and the plant was identified by one of the authors (K.R. Lee). A voucher specimen (SKKU 2011-01) has been deposited at the herbarium in the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The dried and chopped rhizomes of *S. acutum* (20 kg) were extracted with 80% MeOH under reflux and then filtered. The filterate was evaporated under reduced pressure to give a residue (1200 g), which was dissolved in water (18 L) and then successively partitioned with $CHCl_3$, and *n*-BuOH after pre-treatment with 2 N hydrochloric acid (HCl), yielding a $CHCl_3$-fraction (50 g), and *n*-BuOH-fraction (150 g), respectively. Purification of fourteen compounds (**1-14**) was described in the Supplementary material.

1-*O*-Acetyl-dauricumine (1): A colorless gum, $[\alpha]_D^{25}$: −41.5 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 276 (4.0), 276 (3.9) nm; IR (KBr) ν_{max} 3384, 2949, 2834, 1752, 1700, 1662, 1617, 1453, 1364, 1219, 1032 cm^{-1}; CD (MeOH) (Δε): 347 (−19.6), 283 (+7.54), 258 (+10.4), 203 (+4.78) nm; FAB-MS *m/z*: 440 $[M+H]^+$; HR-FAB-MS *m/z*: 440.1477 $[M+H]^+$ (calcd. for $C_{21}H_{27}ClNO_7$, 440.1476); ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.93 (1H, s, H-1), 5.40 (1H, s, H-3), 4.40 (1H, dd, *J* = 12.0, 7.0 Hz, H-10), 4.10 (3H, s, OCH_3-8), 3.86 (3H, s, OCH_3-2), 3.68 (3H, s, OCH_3-7), 2.83 (1H, d, *J* = 16.0 Hz, H_b-5), 2.63 (1H, m, H_b-15), 2.61 (1H, dd, *J* = 12.0, 12.0 Hz, H_b-9), 2.41 (1H, d, *J* = 16.0 Hz, H_a-5), 2.35 (1H, m, H_b-14), 2.34 (1H, m, H_a-15), 2.28 (3H, s, N-CH_3), 2.26 (1H, dd, *J* = 12.0, 7.0 Hz, H_a-9), 2.12 (3H, s, $COCH_3$), 1.58 (1H, m, H_a-14); ^{13}C-NMR (CD_3OD, 125 MHz) δ 201.7 (C-4), 193.7 (C-6), 184.6 (C-2), 169.7 (C=O), 160.3 (C-8), 138.7 (C-7), 107.8 (C-3), 74.4 (C-1), 73.9 (C-13), 67.3 (C-11), 60.7 (OCH_3-8), 60.5 (OCH_3-7), 59.5 (C-10), 59.4 (OCH_3-2) 52.0 (C-12), 51.6 (C-15), 49.0 (C-5), 40.3 (C-9), 40.2 (C-14), 35.9 (N-CH_3), 21.3 ($COCH_3$).

(+) Tuduranine (4): A purple gum, $[\alpha]_D^{25}$: +174.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 284 (3.7) nm; IR (KBr) ν_{max} 3382, 2938, 1726, 1649, 1589, 1460, 1237, 1055, 820 cm^{-1}; CD (MeOH) (Δε): 323 (−3.32), 241 (+13.0), 217 (−4.64) nm; FAB-MS *m/z*: 297 $[M]^+$; HR-FAB-MS *m/z*: 297.1365 $[M]^+$ (calcd. for $C_{18}H_{19}NO_3$, 297.1365); ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.84 (1H, d, *J* = 2.5 Hz, H-11), 7.02 (1H, d, *J* = 8.0 Hz, H-8), 6.65 (1H, dd, *J* = 8.0, 2.5 Hz, H-9), 6.68 (1H, s, H-3), 3.80 (3H, s, OCH_3-2), 3.61 (3H, s, OCH_3-1), 3.58 (1H, dd, *J* = 13.5, 4.5 Hz, H-6a), 3.21 (1H, m, H_b-5), 2.94 (1H, m, H_b-4), 2.83 (1H, m, H_a-5), 2.72 (1H, dd, *J* = 13.5, 4.5 Hz, H_b-7), 2.62 (1H, m, H_a-4), 2.49 (1H, t, *J* = 13.5 Hz, H_a-7); ^{13}C-NMR (CD_3OD, 125 MHz) δ 155.8 (C-10), 152.1 (C-2), 145.0 (C-1), 132.7 (C-11a), 128.9 (C-3a), 128.2 (C-1b), 128.1 (C-8), 127.0 (C-7a), 126.3 (C-1a), 114.9 (C-11), 114.0 (C-9), 111.8 (C-3), 59.2 (OCH_3-1), 55.0 (OCH_3-2), 53.6 (C-6a), 42.4 (C-5), 35.6 (C-7), 28.0 (C-4).

Alkaline Hydrolysis of Compound 1. Compound **1** (3.0 mg) was hydrolyzed with 0.05 M KOH (1 mL) at room temperature for 1 h. And then, H_2O (3 mL) was added and the mixture was extracted with $CHCl_3$ three times, and the $CHCl_3$ extract was evaporated *in vacuo*. The $CHCl_3$ extract (2.5 mg) was purified using RP Silica HPLC (50% MeOH) to afford **1a** (**2**), which was identified by its ^{1}H-, ^{13}C-NMR, CD and MS spectra.

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Supporting Information. Spectral data of compounds **1** and **4**, general experimental procedures and the isolation details are available upon request from the correspondence author.

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