New Oxoaporphine Alkaloids from *Hernandia nymphaeifolia*

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Through continuing studies on the constituents of the stem bark of the Formosan plant, *Hernandia nymphaeifolia* Kubitzki (Hernandiaceae), three new oxoaporphine alkaloids, oxohernagine (**1**), oxohernangerine (**2**), and hernanymphine (**3**), were isolated from the tertiary basic parts of the $CHCl_3$-soluble fraction.

Earlier papers[1−11] have shown both aporphine alkaloids and lignans as characteristic constituents of *Hernandia nymphaeifolia* (Presl) Kubitzki (*Hernandia peltata* Meissn.) (*Hernandiaceae*),[12−14] and Formosan species were often mislabeled as *Hernandia ovigera* L. or *Hernandia sonora* L. in Taiwan and Japan for many years. In our chemical investigations of Formosan species collected on Green Island, we were able to isolate six new aporphine alkaloids, 7-formyldehydroovigerine, 7-formyldehydronornantenine, dehydrohernandaline, sonodione, demethylsonodione, and norsonodione, and 10 known compounds from the stem bark.[8,9] Further examination of the tertiary basic fraction of the stem bark of this plant has now resulted in the characterization of three new oxoaporphines, namely oxohernagine (**1**), oxohernangerine (**2**), and hernanymphine (**3**), as additional constituents.

Results and Discussion

Oxohernagine (**1**) was isolated as orange prisms, mp 252−254 °C. The HRFABMS gave an $[M + H]^+$ ion peak at *m*/*z* 338.1041 (calcd 338.1029), consistent with a molecular formula of $C_{19}H_{16}O_5N$. The UV absorption bands at 213, 274, 360, 403 nm and the exhibition of a bathochromic shift on addition of both aqueous KOH and HCl suggested the presence of a phenolic 1,2,10,-11-oxygenated oxoaporphine skeleton.[5,6] The IR spectrum showed a broad band of OH absorption at 3430 cm^{-1} and a conjugated carbonyl group at 1650 cm^{-1}. Analysis of the ^{1}H-NMR spectrum of **1** revealed the presence of two pairs of AB-doublets. One of them at δ 7.77 and 8.87 (J = 5.3 Hz) was assigned to H-4 and H-5, while the other at δ 7.22 and 8.31 (J = 8.5 Hz) was attributed to H-9 and H-8. The other ^{1}H-NMR signals that appeared at δ 7.21 (1H, s) were assigned to H-3 and those at δ 3.54, 3.76, 4.11 (each 3H, s) were assigned to OMe-1, OMe-11, and OMe-2, respectively. The presence of a phenolic hydroxy group in **1** was substantially supported after acetylation of **1** with acetic anhydride in pyridine supplied the acetate **1a**, and a new acetyl signal at δ 2.41 (3H, s) was observed in the ^{1}H-NMR of **1a**. The phenolic hydroxy group could be reasonably located at C-10 because H-9 and OMe-11 in **1** were shifted 0.11 and 0.04 ppm downfield in ^{1}H-NMR of **1a**, respectively. The structural assignment for oxohernagine (**1**) was further supported by the NOE-DIF NMR experiment of **1a** (Figure 1). The previous studies[15,16] have revealed the C-1 methoxy group of oxoaporphine will be located at a higher field in the presence of an oxygenated group in C-11. Thus, both the C-1 methoxy signals of **1** and **1a**, quite high field at δ 3.54 and 3.50, respectively, could be reasonably considered due to the steric effect of the oxygenated groups in C-11 and C-10.

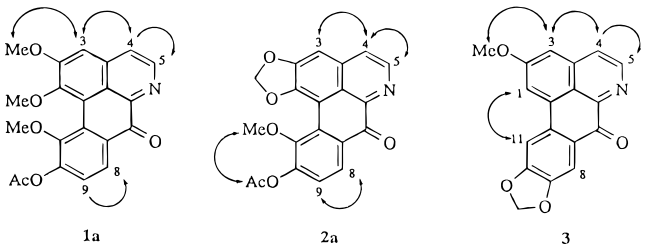

Figure 1. NOE interactions observed for **1a**, **2a**, and **3**.

Oxohernangerine (**2**) was obtained as orange prisms, mp 256-258 °C. The molecular formula was established as $C_{18}H_{11}O_5N$ by HREIMS spectrometry (found 321.0643, calcd 321.0637). The presence of a 1,2,10,11-oxygenated oxoaporphine skeleton in the molecule was easily deduced from the UV spectrum (absorption maxima at 210, 252, 268 sh, 315, 361, 408, 478 nm)[17] and the exhibition of a bathochromic shift in acid, along with a conjugated carbonyl group absorption band at 1640 cm^{-1} in the IR spectrum. The presence of a phenolic hydroxy group in the molecule was indicated by the IR absorption at 3420 cm^{-1} and a bathochromic shift of UV absorption in alkaline solution. The IR spectrum also showed a methylenedioxy group at 1050, 970 cm^{-1}. Further inspection of the ^{1}H-NMR spectrum of **2** revealed the presence of two pairs of AB-doublets. One of them, at δ 7.86 and 8.61 (J = 5.0 Hz), was assigned to H-4 and H-5, while the other, at δ 6.96 and 8.11 (J = 8.8 Hz), was attributed to H-9 and H-8. The ^{1}H-NMR of **2** also included signals at δ 3.75 (3H, s), δ 6.32 (2H, s), and δ 7.31 (1H, s), which were assigned to OMe-11, methylenedioxy (C-1, C-2), and H-3, respectively. Further evidence to support the assignment of **2** was also sought in an acetylation experiment with acetic anhydride in pyridine. The ^{1}H-NMR spectrum of the acetyl derivative (**2a**) included a new acetyl signal at δ 2.41 (3H, s). On the other hand, the phenolic hydroxy group at C-10, neighboring to H-9, was evidenced due to the down shift of H-9 in **2** from δ 6.96 to 7.44 after acetylation of **2**. The structure of oxohernangerine (**2**) was further confirmed by an NOE-DIF NMR experiment on **2a** (Figure 1).

Hernanymphine (**3**) was obtained as yellowish prisms, mp 263−265 °C. Its molecular formula was established as $C_{18}H_{11}O_4N$ by HREIMS spectrometry (found 305.0688, calcd 305.0688). The IR spectrum showed a carbonyl absorption at 1650 cm^{-1} and a methylenedioxy group

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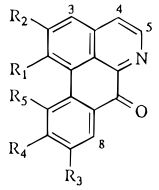
at 1030, 960 cm^{-1}. The UV absorption at 210, 241, 270, 292, 314 sh, 361, 427 nm was similar to that of oxonantenine,[18] and the exhibition of a bathochromic shift in acid suggested the presence of an oxoaporphine skeleton.[19] Analysis of the ^{1}H-NMR spectrum of **3** exhibited a methoxy signal at δ 4.04 (3H, s) assigned to OMe-2 and a methylenedioxy signal at δ 6.15 (2H, s). The aromatic region of the spectrum integrated for six protons: two meta-coupled protons at δ 7.14 and 7.85 (each 1H, d, J = 2.2 Hz) due to H-3 and H-1, two ortho-coupled protons at δ 7.83 and 8.92 (each 1H, d, J = 5.4 Hz) assignable to H-4 and H-5, and two singlet protons at δ 7.58 and 7.93 (each 1H, s) attributed to H-11 and H-8, respectively. According to the above data, hernanymphine (**3**) must be 2-methoxy-9,10-(methylenedioxy)-7-oxoaporphine, and its structure was further confirmed by the NOE-DIF NMR experiment (Figure 1). This structure is exceptionally rare, as there is no oxygenation at C-1 like 1-demethoxy-4,5-dioxodehydroasimilobine.[20] Biogenetically tyrosine is the obvious precursor for the oxygenation at C-1 of the aporphine-type alkaloids. Therefore, we also suppose a joint biogenetic aporphinoid intermediate for **3** and the usual aporphine-type alkaloids and a subsequent deoxygenation process at C-1 in the formation of **3** as Achenbach *et al.* postulated.[20]



1 $R_1 = R_2 = R_5 = OMe$, $R_3 = H$, $R_4 = OH$
1a $R_1 = R_2 = R_5 = OMe$, $R_3 = H$, $R_4 = OAc$
2 $R_1 + R_2 = OCH_2O$, $R_3 = H$, $R_4 = OH$, $R_5 = OMe$
2a $R_1 + R_2 = OCH_2O$, $R_3 = H$, $R_4 = OAc$, $R_5 = OMe$
3 $R_1 = R_5 = H$, $R_2 = OMe$, $R_3 + R_4 = OCH_2O$

Experimental Section

General Experimental Procedures. All mps were determined on a Yanaco micro-melting point apparatus and uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter in $CHCl_3$. IR spectra were taken on a Hitachi 260-30 (KBr) spectrophotometer. UV spectra were obtained on a Hitachi U-200 spectrophotometer in EtOH. EIMS spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HREIMS, FABMS, and HRFABMS were recorded on a JEOL JMX-HX 110 spectrometer. ^{1}H-NMR and NOE-DIF spectra were measured on either a Varian Gemini 200 or a JEOL GSX-400 spectrometer and are given in ppm (δ) downfield from internal TMS.

Plant Material. Stem bark of *H. nymphaeifolia* was collected on Green Island, Taitung Hsien, Taiwan, in August 1992. A voucher sample was deposited in the Herbarium of Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The tertiary phenolic base (fraction A, 10.4 g) and nonphenolic base (fraction B, 26.7 g) afforded from the MeOH extract of the stem bark (7 kg) of *H. nymphaeifolia* were described in the previous paper.[8] Fraction A (10.4 g) was rechromatographed on Si gel, eluting with 10% MeOH−$CHCl_3$, gradually increasing the polarity with MeOH, and eight fractions (fractions A1−A8) were collected. Fraction A4 (1.603 g) was rechromatographed with Si gel by using $CHCl_3$−MeOH (5:1) as eluent to give fractions A4-1−A4-3. Fraction A4-2 (1.032 g) was separated by prep. TLC [$CHCl_3$−MeOH (9:1)] to give fractions A4-2-a−A4-2-d. Fraction A4-2-b (123 mg) was further purified by prep. TLC [$CHCl_3$−MeOH (10:1)] to obtain **1** (10.7 mg) (R_f 0.49) after recrystallization with MeOH. Fraction A5 (2.274 g) was rechromatographed with Si gel by using EtOAc−MeOH (1:1) as eluent to give fractions A5-1−A5-5. Fraction A5-4 (1.285 g) was further separated and purified by Si gel CC and prep. TLC [EtOAc−MeOH (1:1)] to yield **2** (89.5 mg) (R_f 0.69) after recrystallization with MeOH. Fraction B (26.7 g) was washed with $CHCl_3$ to yield hernandonine (971 mg). The washing (25.3 g) was chromatographed over Si gel, eluting with $CHCl_3$, gradually increasing the polarity with MeOH, and nine fractions (B1−B9) were collected. Fraction B3 (10.5 g) was rechromatographed on Si gel using $CHCl_3$ as eluent, and the polarity was gradually increased with Me_2CO to obtain 15 fractions (B3-1−B3-15). Fraction B3-11 (22 mg) was rechromatographed on Si gel eluting with CH_2Cl_2−EtOAc (1:1) to obtain fractions B3-11-1−B3-11-5. Fraction B3-11-4 (7.3 mg) was further purified by prep. TLC [$CHCl_3$−Me_2CO (5:1)] to give **3** (2.3 mg) (R_f 0.44) after recrystallization with $CHCl_3$−MeOH.

7-Oxohernagine (1): orange prisms (MeOH); mp 252−254 °C; $[\alpha]^{24}_D \pm 0°$ (*c* 0.08, $CHCl_3$); HRFABMS, found $[M + H]^+$ 338.1041, $C_{19}H_{16}O_5N$, requires 338.1029; UV λ max (EtOH) (log ϵ) 213 (4.52), 274 (4.42), 360 (3.96), 403 (3.92) nm; (KOH) 208 (4.52), 241 (4.51), 268 (4.47), 331 (4.01), 363 (3.86), 424 (3.86), 484 (3.93) nm; (HCl) 218 (4.50), 251 (4.50), 287 (4.39), 378 (4.04), 434 (3.82) nm; IR ν max (KBr) 3430 (br, OH), 1650 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.54, 3.76, and 4.11 (3H each, s, OMe-1, OMe-11, and OMe-2), 7.21 (1H, s, H-3), 7.22 (1H, d, J = 8.5 Hz, H-9), 7.77 (1H, d, J = 5.3 Hz, H-4), 8.31 (1H, d, J = 8.5 Hz, H-8), 8.87 (1H, d, J = 5.3 Hz, H-5); FABMS *m/z* (rel int) 360 $[M + Na]^+$ (61), 338 $[M + H]^+$ (34).

10-*O*-Acetyl-7-oxohernagine (1a). Ac_2O (0.3 mL) was added to a solution of **1** (2.5 mg) in pyridine (0.3 mL), and the mixture was stirred 8 h at room temperature. The excess pyridine was removed with saturated aqueous $CuSO_4$ solution to give a residue (2.7 mg). The residue was chromatographed on Si gel to give **1a** (2.3 mg), yellowish prisms, mp 152−154 °C ($CHCl_3$−MeOH); UV λ max (EtOH) (log ϵ) 219 (4.55), 245 sh (4.39), 277 (4.31), 413 (3.86) nm; ^{1}H NMR ($CDCl_3$) δ 2.41 (3H, s, OAc-10), 3.50, 3.80, and 4.09 (3H each, s, OMe-1, OMe-11, and OMe-2), 7.19 (1H, s, H-3), 7.33 (1H, d, J = 8.4 Hz, H-9), 7.78 (1H, d, J = 5.2 Hz, H-4), 8.32 (1H, d, J = 8.4 Hz, H-8), 8.87 (1H, d, J = 5.2 Hz, H-5); EIMS *m/z* (rel int) 379 $[M]^+$ (15), 337 (33), 322 (3), 306 (6), 304 (11), 294 (3), 276 (4), 149 (14).

7-Oxohernangerine (2): orange prisms (MeOH); mp 256−258 °C; $[\alpha]^{24}_D \pm 0°$ (*c* 0.12, $CHCl_3$); HREIMS, found $[M]^+$ 321.0643, $C_{18}H_{11}O_5N$, requires 321.0637; UV λ max (EtOH) (log ϵ) 210 (4.56), 252 (4.47), 268 sh (4.43), 315 (3.88), 361 (4.04), 408 (4.03), 478 (3.56) nm; (KOH) 208 (4.56), 248 (4.51), 265 (4.50), 330 (4.00), 364 (3.96), 413 (3.96), 485 (3.92) nm; (HCl) 217 (4.54), 261 (4.53), 332 (3.77), 381 (4.13), 447 (3.92) nm; IR ν max (KBr) 3420 (OH), 1640 (C=O), 1050, 970 (OCH_2O) cm^{-1}; ^{1}H NMR

(CD_3OD) δ 3.75 (3H, s, OMe-11), 6.32 (2H, s, OCH_2O), 6.96 (1H, d, J = 8.8 Hz, H-9), 7.31 (1H, s, H-3), 7.86 (1H, d, J = 5.0 Hz, H-4), 8.11 (1H, d, J = 8.8 Hz, H-8), 8.61 (1H, d, J = 5.0 Hz, H-5); EIMS *m/z* (rel int) 321 $[M]^+$ (100), 306 (36), 278 (47), 264 (11), 250 (19), 236 (11), 164 (23).

10-*O*-Acetyl-7-oxohernangerine (2a). Ac_2O (0.3 mL) was added to a solution of **2** (2.5 mg) in pyridine (0.3 mL), and the mixture was stirred 8 h at room temperature. The excess pyridine was removed with saturated aqueous $CuSO_4$ solution to give a residue (2.8 mg). The residue was chromatographed on Si gel to give **2a** (2.4 mg), yellowish needles, mp 213−215 °C ($CHCl_3$−MeOH); HREIMS, found $[M]^+$ 363.0725, $C_{20}H_{13}O_6N$, requires 363.0743; UV λ max (EtOH) (log ϵ) 217 (4.62), 253 (4.43), 272 (4.35), 413 (4.06) nm; ^1H NMR (CD_3OD) δ 2.41 (3H, s, OAc-10), 3.75 (3H, s, OMe-11), 6.38 (2H, s, OCH_2O), 7.44 (1H, s, H-3), 7.44 (1H, d, J = 8.5 Hz, H-9), 8.00 (1H, d, J = 5.2 Hz, H-4), 8.27 (1H, d, J = 8.5 Hz, H-8), 8.72 (1H, d, J = 5.2 Hz, H-5); EIMS *m/z* (rel int) 363 $[M]^+$ (39), 321 (100), 306 (43), 278 (71), 250 (29), 221 (36), 164 (71).

Hernanymphine (3): yellowish prisms ($CHCl_3$−MeOH); mp 263−265 °C; $[\alpha]^{24}_D \pm 0°$ (*c* 0.04, $CHCl_3$); HREIMS, found $[M]^+$ 305.0688, $C_{18}H_{11}O_4N$, requires 305.0688; UV λ max (EtOH) (log ϵ) 210 (4.57), 241 (4.41), 270 (4.36), 292 (4.21), 314 sh (3.94), 361 (3.86), 427 (3.46) nm; (HCl) 208 (4.51), 278 (4.37), 380 (3.88) nm; IR ν max (KBr) 1650 (C=O), 1030, 960 (OCH_2O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.04 (3H, s, OMe-2), 6.15 (2H, s, OCH_2O), 7.14 (1H, d, J = 2.2 Hz, H-3), 7.58 (1H, s, H-11), 7.83 (1H, d, J = 5.4 Hz, H-4), 7.85 (1H, d, J = 2.2 Hz, H-1), 7.93 (1H, s, H-8), 8.92 (1H, d, J = 5.4 Hz, H-5); EIMS *m/z* (rel int) 305 $[M]^+$ (100), 276 (9), 262 (7), 236 (6), 190 (8), 176 (16).

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