N-FORMYL- AND N-HYDROXYAPORPHINE ALKALOIDS
FROM FORMOSAN HERNANDIA NYMPHAEIFOLIA

Ih-Sheng Chen*, Jih-Jung Chen, and Ian-Lih Tsai

Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical College,
Kaohsiung, Taiwan, R.O.C.

Abstract — Three new aporphine alkaloids, (+)-N-formylovigerine (1), (+)-N-formylhernangerine (2), (+)-N-hydroxyovigerine (3) and one known aporphine alkaloid, (+)-N-formylnormantenine (4), have been isolated from the trunk bark of Hernandia nymphaeifolia.

INTRODUCTION

Hernandia nymphaeifolia (Presl) Kubitzki (Hernandia peltata Meissn.) (Hernandiaceae), is an evergreen tree, widespread in tropics of the Old World. In the previous papers, we have reported sixteen new compounds, mainly aporphine alkaloids and ten known compounds including several antiplatelet agents from the trunk bark of Formosan H. nymphaeifolia. Further extension of studies on the tert. basic fraction of trunk bark of this plant has led to the isolation of three new aporphine alkaloids including two N-formylaporphines: (+)-N-formylovigerine (1), (+)-N-formylhernangerine (2); one N-hydroxyaporphine: (+)-N-hydroxyovigerine (3) and one known aporphine alkaloid, (+)-N-formylnormantenine (4) as additional constituents. In this paper, we deal with the isolation and structural elucidation of these new compounds.
RESULTS AND DISCUSSION

(+)-N-Formylovigerine (1) was obtained as colorless prisms. The molecular formula, \( \text{C}_{19}\text{H}_{15}\text{NO}_3 \), was determined by EI-mass (\( M^+, m/z \) 337) and HR-mass spectrometry (found 337.0959, calcd 337.0950). The uv absorptions at 227, 271, 278 sh, 313 nm were similar to those of ovigerine and were characteristic of the 1,2,10,11-tetrasubstituted aporphine nucleus. The ir spectrum showed an amidocarbonyl absorption at 1660 cm\(^{-1}\) and a methylenedioxy group at 1055, 935 cm\(^{-1}\). The \(^1\)H nmr spectrum of 1 showed two rotational isomers in a ratio of 2.5:1, due to the existence of an \( N \)-formyl group which was supported by a base peak m/z 279 due to loss of (CH\(_2\)-N-CHO + H) from the molecular ion in EI-mass spectrum. The \(^1\)H nmr spectra for the two isomers (Figure 1) could be clearly differentiated, even though the isomers could not be separated. The major isomer 1a showed the C-3 proton at \( \delta \) 6.61 (1H, s), the \( N \)-formyl group at \( \delta \) 8.25 (1H, s) and two methylenedioxy groups at \( \delta \) 5.95, 5.96, 6.08, 6.09 (each 1H, d, \( J = 1.4 \) Hz). On the other hand, for the minor isomer 1b, the corresponding C-3 proton was relatively downfield at \( \delta \) 6.64 (1H, s), the \( N \)-formyl group at \( \delta \) 8.38 (1H, s) and two methylenedioxy groups at \( \delta \) 5.96, 5.98, 6.09, 6.11 (each 1H, d, \( J = 1.4 \) Hz).
But for both isomers, the ortho-coupled aromatic protons at δ 6.80 and 6.75 (each 1H, d, J = 7.9 Hz, H-8 and H-9) in ring D were not affected by this rotational isomerism. The other aliphatic protons of two isomers (1a and 1b) including C-4, C-5, C-6a and C-7 protons could be well assigned comparative to those of (+)-N-formylnorbornantene.9 The NOESY experiment (Figure 2) was fully supported the above assignments and also evidenced the proximity of the N-formyl proton to H-5β (δ 3.82) in the major isomer and to H-6a (δ 4.47) in the minor isomer as in the case of (+)-N-formylnorbornantene. With dextrorotatory optical activity, the structure of 1 was elucidated as (+)-N-formylovigerine.

Figure 1 ¹H Nmr chemical shifts (ppm) of 1a and 1b

Figure 2 NOESY correlations for 1a and 1b
(+)-N-Formylhernangereine (2) was isolated as colorless prisms. The molecular formula, $C_{19}H_{17}NO_5$, was determined by El-mass ($M^+$, $m/z$ 339) and HR-mass spectrometry (found 339.1104, calc. 339.1106). The uv absorption bands at 223, 270, 309 nm were similar to those of hernangereine and were characteristic of the 1,2,10,11-oxygenated aporphine skeleton. The presence of a phenolic hydroxy group in the molecule was indicated by the ir absorption at 3150 cm$^{-1}$ and a bathochromic shift of uv absorption in alkaline solution. The ir spectrum also showed an amidocarbonyl absorption at 1645 cm$^{-1}$ and a methylenedioxy group at 1040, 950 cm$^{-1}$. The $^1$H nmr spectrum of 2 was also
composed of 2 species in a ratio of 2.6 : 1, due to rotational isomerism like compound (1). The \textsuperscript{1}H nmr spectrum of 2 was similar to those of 1 except that a hydroxy group [\( \delta \) 5.93 (1H, s, disappeared with D\textsubscript{2}O) in major isomer, \( \delta \) 5.98 (1H, s, disappeared with D\textsubscript{2}O) in minor isomer] and a methoxy group (\( \delta \) 3.63 in major isomer, \( \delta \) 3.67 in minor isomer) of 2 replaced a methylenedioxy group of 1. Because the methoxy group was at relatively high field and the Gibbs test was negative, the methoxy and hydroxy group were reasonably assigned at C-11 and C-10 position, respectively.\textsuperscript{11,12} The existence of an \( N\)-formyl group (\( \delta \) 8.26 in major isomer, \( \delta \) 8.38 in minor isomer) was also evidenced by the base peak (m/z 281) due to loss of (CH\textsubscript{2}-N-CHO + H) from the molecular ion (m/z 339) in the EI-mass spectrum. The assignments for each proton in the \textsuperscript{1}H nmr spectra of 2a and 2b (Figure 3) were substantiated by the NOESY experiments (Figure 4). With dextrorotatory optical activity, the structure of 2 was elucidated as (+)-\( N\)-formylhernangerine.

(+)-\( N\)-Hydroxyovigerine (3) was obtained as grayish prisms. The molecular formula was established as C\textsubscript{18}H\textsubscript{15}N\textsubscript{0}5 by EI-mass (M\textsuperscript{+}, m/z 325) and HR-mass spectrometry (found 325.0949, calcd 325.0951). The uv absorption bands at 228, 270, 278 sh, 312 nm were similar to those of ovigerine and were characteristic of the 1,2,10,11-tetrasubstituted aporphine skeleton.\textsuperscript{8} The ir spectrum showed a hydroxy function at 3250 cm\textsuperscript{-1} and a methylenedioxy group at 1055, 935 cm\textsuperscript{-1}. The \textsuperscript{1}H nmr spectrum of 3 showed three mutually coupling aliphatic protons at \( \delta \) 2.62 (1H, br t, \( J = 12.0 \) Hz, H-7\( \beta \)), \( \delta \) 3.33 (1H, br d, \( J = 12.0 \) Hz, H-7\( \alpha \)) and \( \delta \) 3.57 (1H, br d, \( J = 12.0 \) Hz, H-6\( \alpha \)). In addition, four mutually coupling aliphatic protons at \( \delta \) 2.77 (1H, m, H-4\( \alpha \)), \( \delta \) 3.02 (1H, m, H-5\( \alpha \)), \( \delta \) 3.21 (1H, m, H-4\( \beta \)) and \( \delta \) 3.51 (1H, m, H-5\( \beta \)) in ring B, and two methylenedioxy signals at \( \delta \) 5.92, 5.94, 6.07, 6.08 (each 1H, d, \( J = 1.3 \) Hz) were observed. The aromatic region of the spectrum showed the presence of three protons, one at \( \delta \) 6.58 (1H, s) was assigned to H-3, the other \textit{ortho}-coupled protons at \( \delta \) 6.74 and 6.79 (each 1H, d, \( J = 7.8 \) Hz) to H-9 and H-8. Besides, a hydroxy group was attached at nitrogen atom which could be supported by the important fragment ion at m/z 308 (M\textsuperscript{+}—OH) and
m/z 280 (M⁺— CH₂-N-OH) in the EI-mass spectrum. The above assignments were further confirmed by nOe-DIF and NOESY experiments (Figure 5). On the basis of the above results and with the dextrorotatory optical activity, structure (3) was assigned to (+)-N-hydroxyovigerine.

\[ \text{Figure 5 NOESY correlations for 3} \]

(+)-N-Formylnormantenine (4) was a known compound and identified by comparison of its spectral data (uv, ir, \(^1\)H nmr, mass) and melting point with the reported data.⁹

**EXPERIMENTAL**

All mps were determined on a Yanaco micro-melting point apparatus and uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter in CHCl₃. Ir spectra were taken on a Hitachi 260-30 (KBr) spectrophotometer. Uv spectra were obtained on a Shimadzu UV-160A spectrophotometer in EtOH. Elms spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HRElms spectra 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 JEOL GSX-400 spectrometer and are given in ppm (δ) downfield from internal TMS. Silica gel (60-230 mesh) (Merck) was used for CC and silica gel 60 F-254 for tlc.
Plant Material.

Trunk bark of *H. nymphaefolia* was collected from Green Island, Taitung Hsien, Taiwan in August 1992. A voucher sample was deposited in the herbarium of School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation.

The tert. phenolic base (fraction A, 10.4 g) and nonphenolic base (fraction B, 26.7 g) afforded from the MeOH extract of the trunk bark (7 kg) of *H. nymphaefolia* were described in the previous paper. Fr. A (10.4 g) was rechromatographed on silica gel eluting with MeOH-CHCl₃ (9:1) and gradual increase in proportion of MeOH; 8 frs (A1~A8) were collected. Fr. A2 (134 mg) was purified by prep. tlc (CH₂Cl₂-MeOH, 10:1) to yield 2 (7.4 mg) (Rf 0.85) after recrystallization from MeOH. Fr. B (26.7 g) was washed with CHCl₃ and filtered to yield hemandonine (971 mg). The washings (25.3 g) were chromatographed over silica gel and elution with CHCl₃ and CHCl₃-MeOH mixts gave 9 frs (B1~B9). Fr. B3 (10.5 g) was rechromatographed on silica gel using CHCl₃ and CHCl₃-Me₂CO mixts to yield 15 frs (B3-1~B3-15). Fr. B3-5 (403 mg) was rechromatographed on silica gel and eluted with CHCl₃ to give 3 frs, the first fr. (92.7 mg) and the second fr. (120.5 mg) further purified by prep. tlc (CHCl₃-Me₂CO, 10:1) to afford 1 (21.6 mg) (Rf 0.66) after recrystallization from MeOH. Fr. B3-7 (89.6 mg) was chromatographed on silica gel and eluted with CHCl₃ to give 3 frs and the last fr. (33.1 mg) further purified by prep. tlc (CHCl₃-Me₂CO, 10:1) to afford 4 (5.1 mg) (Rf 0.62) after recrystallization from MeOH. Fr. B3-13 (3.595 g) was rechromatographed on a silica gel column and eluted with CHCl₃-Me₂CO (10:1) to afford frs B3-13-1~B3-13-6. Fr. B3-13-1 (872 mg) was further purified by prep. tlc (CHCl₃-Me₂CO, 10:1) to give 3 (12.3 mg) (Rf 0.46) after recrystallization from CHCl₃-MeOH.

(+)-N-Formyllovigerine (1)

Colorless prisms (MeOH), mp 105-107 °C. Anal. Calcd for C₁₉H₁₅NO₅·1/3H₂O: C, 66.47; H, 4.60;
N, 4.08. Found: C, 66.29; H, 4.47; N, 4.03. Uv λ<sub>max</sub> nm (log ε) : 206 (4.43), 227 (4.40), 271 (4.15), 278sh (4.11), 313 (3.83). Ir ν<sub>max</sub> cm<sup>-1</sup> : 1660 (C=O), 1055, 935 (OCH<sub>2</sub>O). Elms m/z (rel. int.) : 337 (M<sup>+</sup>, 56), 292 (14), 280 (19), 279 (100), 250 (2), 221 (2), 191 (3), 163 (12); HRMs : C<sub>19</sub>H<sub>15</sub>NO<sub>5</sub>, found : 337.0959, calcd : 337.0950.

'H Nmr (400 MHz) : major isomer (1a) and minor isomer (1b) see Figure 1. [α]<sub>D</sub> +321° (c = 0.11, CHCl<sub>3</sub>).

(+)-N-Formylhernangerine (2)

Colorless prisms (MeOH), mp 233-235 °C. Anal. Calcd for C<sub>19</sub>H<sub>17</sub>NO<sub>5</sub>·1/2H<sub>2</sub>O : C, 65.51; H, 5.21; N, 4.02. Found: C, 65.54; H, 5.16; N, 4.21. Uv λ<sub>max</sub> nm (log ε) : 223 (4.48), 270 (4.12), 309 (3.80); Uv λ<sub>max</sub>·KOH nm (log ε) : 203 (4.77), 234 (4.44), 254 sh (4.22), 278 sh (4.04), 312 (3.72), 338 sh (3.46). Ir ν<sub>max</sub> cm<sup>-1</sup> : 3150 (OH), 1645 (C=O), 1040, 950 (OCH<sub>2</sub>O). Elms m/z (rel. int.) : 339 (M<sup>+</sup>, 91), 294 (13), 282 (15), 281 (100), 266 (35), 238 (6), 209 (5), 180 (7), 152 (16); HRMs : C<sub>19</sub>H<sub>15</sub>NO<sub>5</sub>, found : 339.1104, calcd : 339.1106. 'H Nmr (400 MHz) : major isomer (2a) and minor isomer (2b) see Figure 3. [α]<sub>D</sub> +461° (c = 0.10, CHCl<sub>3</sub>).

(+)-N-Hydroxyovigerine (3)

Grayish prisms (CHCl<sub>3</sub>-MeOH), mp 102-104 °C. Anal. Calcd for C<sub>18</sub>H<sub>15</sub>NO<sub>5</sub>·3/4H<sub>2</sub>O : C, 63.80; H, 4.91; N, 4.13. Found : C, 63.74; H, 4.99; N, 3.96. Uv λ<sub>max</sub> nm (log ε) : 228 (4.39), 270 (4.19), 278 sh (4.13), 312 (3.82); Ir ν<sub>max</sub> cm<sup>-1</sup> : 3250 (OH), 1055, 935 (OCH<sub>2</sub>O). Elms m/z (rel. int.) : 325 (M<sup>+</sup>, 81), 309 (36), 308 (57), 281 (25), 280 (100), 279 (67), 250 (39), 223 (23), 222 (55), 191 (19), 165 (6), 164 (32), 163 (62); HRMs : C<sub>18</sub>H<sub>15</sub>NO<sub>5</sub>, found : 325.0949, calcd : 325.0951. 'H Nmr (400 MHz) : δ 2.62 (1H, br t, J = 12.0 Hz, H-7β), 2.77 (1H, m, H-4α), 3.02 (1H, m, H-5α), 3.21 (1H, m, H-4β), 3.33 (1H, br d, J = 12.0 Hz, H-7α), 3.51 (1H, m, H-5β), 3.57 (1H, br d, J = 12.0 Hz, H-6α), 5.92, 5.94, 6.07, 6.08 (each 1H, d, J = 1.3 Hz, OCH<sub>2</sub>O × 2), 6.58 (1H, s, H-3), 6.74 (1H, d, J = 7.8 Hz, H-9), 6.79 (1H, d, J = 7.8 Hz, H-8). [α]<sub>D</sub> +184° (c = 0.10, CHCl<sub>3</sub>).
ACKNOWLEDGEMENT

This research was kindly supported by a grant (NSC 82-0420-B-037-007-M13) from the National Science Council of the Republic of China.

REFERENCES


Received, 23rd October, 1995