New Pavine *N*-Oxide Alkaloids from the Stem Bark of *Cryptocarya chinensis* HEMSL.

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Three new pavine *N*-oxide alkaloids, (−)-isocaryachine-*N*-oxide B, (+)-caryachine-*N*-oxide, (−)-caryachine-*N*-oxide, and a new isoquinoline alkaloid, 6,7-methylenedioxy-*N*-methylisoquinoline together with 11 known alkaloids were isolated and characterized from the stem bark of *Cryptocarya chinensis*. The structures of the isolated compounds were determined by spectral methods. The stereochemistry of pavine-*N*-oxide alkaloids is also discussed.

Key words *Cryptocarya chinensis*; Lauraceae; alkaloid; (−)-isocaryachine-*N*-oxide B; (+)-caryachine-*N*-oxide; (−)-caryachine-*N*-oxide

Cryptocarya chinensis (HANCE) HEMSL. (Lauraceae) is an evergreen tree and widely distributed in low-altitude forests of Taiwan and southern China.[1)] Earlier studies on this species noted their profuse production of pavine alkaloids.[2—6)] In a previous paper, we reported several alkaloids from the leaves of this species.[7)] Among them, a pavine-*N*-oxide alkaloid called (−)-isocaryachine-*N*-oxide was isolated and characterized by spectral and X-ray analysis. Past studies on the pavine-*N*-oxide identified (−)-argemonine *N*-oxide, (−)-eschscholtzine *N*-oxide, and (−)-thalimonine *N*-oxide A and B.[8)] In ongoing studies of the alkaloids of *C. chinensis*, three new pavine *N*-oxide alkaloids, (−)-isocaryachine-*N*-oxide B (**1**), (+)-isocaryachine-*N*-oxide (**2**), (−)-caryachine-*N*-oxide (**3**) and a new isoquinoline alkaloid, 6,7-methylenedioxy-*N*-methylisoquinoline (**4**) together with 11 known compounds were isolated and characterized from the stem bark of *C. chinensis*. This paper deals with the isolation and structural elucidation of these rare pavine-*N*-oxide alkaloids based on their spectroscopic evidence.

Results and Discussion

Silica gel column chromatography followed by preparative TLC of a $CHCl_3$-soluble fraction of the ethanol extract of stem bark of *C. chinensis* resulted in the isolation of three new pavine *N*-oxide alkaloids, a new isoquinoline alkaloid, and 11 known compounds.

(−)-Isocaryachine-*N*-oxide B (**1**) was obtained as optically active colorless needles with levorotation. It has the molecular formula $C_{19}H_{19}NO_5$ established by high-resolution fast-atom bombardment mass spectrometry (HR-FAB-MS). The ^{1}H-NMR spectrum of **1** showed four aromatic singlets at δ 6.84, 6.71, 6.52, and 6.50 and two AMX resonances at δ 4.63 (1H, d, J=5.6 Hz), 3.90 (1H, dd, J=17.2, 5.6 Hz), 2.81 (1H, d, J=17.2 Hz) and δ 4.61 (1H, d, J=5.6 Hz), 3.58 (1H, dd, J=17.2, 5.6 Hz), 3.02 (1H, d, J=17.2 Hz) assigned by correlation spectroscopy (COSY) and ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) experiments were characteristic signals of the pavine skeleton (Table 1).[2—8)] In addition, it showed signals for one methylenedioxy at δ 5.88, 5.85 (each 1H), one methoxyl at δ 3.84, and one NCH_3 at δ 3.35. The connection of the aromatic ring with the aliphatic AMX system was shown by heteronuclear multiple bond connectivity (HMBC) experiments to be at H-7 (δ 6.84) and to have ^3J correlations with C-6 (δ_C 70.0), C-10a (δ_C 119.9), and C-8/9 (δ_C 147.0) and at H-1 (δ 6.71) and to have ^3J correlations with C-12 (δ_C 70.5), C-4a (δ_C 122.7), and C-2/3 (δ_C 147.7) (Fig. 1). As shown in Fig. 1, the ^2J and ^3J correlations of H-1, H-4 (δ 6.50) and OCH_2O (δ 5.88, 5.85) with C-2/3 (δ_C 147.7) specified that they were in the same benzene ring whereas H-7 and H-10 (δ 6.52) were in the other. These data are similar to those of (−)-isocaryachine except for NCH_3 (δ 3.35; δ_C 54.6). The signals of NCH_3 appear to shift downfield from those of (−)-isocaryachine (δ 2.54; δ_C 40.6) in both ^{1}H- and ^{13}C-NMR spectra. This suggests that **1** is an *N*-oxide derivative of (−)-isocaryachine. This was further confirmed by the 16 atomic mass unit (amu) excess in molecular weight compared with that of (−)-isocaryachine. The relative stereochemistry of *N*-oxide was further determined by nuclear Overhauser effect spectroscopy (NOESY) experiments, as shown in Fig. 2. The presence of nuclear Overhauser effect (NOE) correlations of

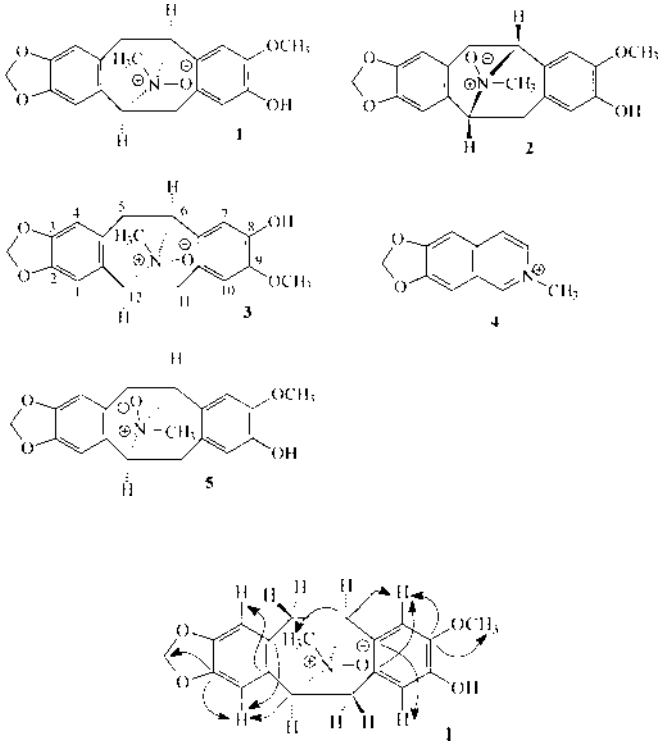

Fig. 1. HMBC Correlations of **1**

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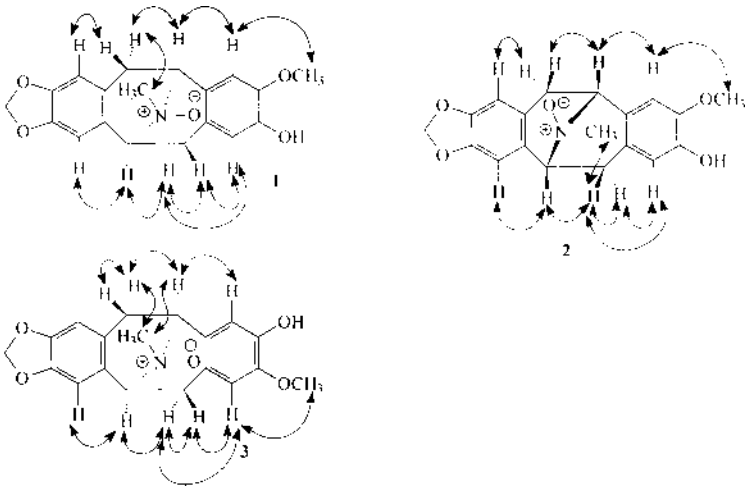

Fig. 2. NOESY Correlations of **1**—**3**

Table 1. ^{1}H-NMR Spectral Data for **1**—**3** and **5** (400 MHz, δ, Multiplicity, *J*, Hz)

	1[a)]	**2**[a)]	**3**[a)]	**5**[a)]
H-1	6.71, s	6.70, s	6.57, s	6.69, s
OCH_2O	5.88, 5.85, d, 1.6	5.88, 5.85, d, 1.2	5.92, 5.87, d, 1.6	5.87, 5.84, d, 1.2
H-4	6.50, s	6.49, s	6.51, s	6.48, s
H-5α	3.90, dd, 17.2, 5.6	2.77, d, 16.4	3.50, dd, 12.4, 6	3.91, dd, 16.4, 5.6
H-5β	2.81, d, 17.2	3.92, dd, 16.4, 5.6	2.97, d, 12.4	2.77, d, 16.4
H-6	α: 4.63, d, 5.6	β: 4.54, d, 5.6	α: 4.65, d, 6	α: 4.54, d, 5.6
H-7	6.84, s	6.82, s	6.76, s	6.83, s
OCH_3	3.85, s	3.84, s	3.84, s	3.84, s
H-10	6.52, s	6.51, s	6.81, s	6.49, s
H-11α	3.58, dd, 18, 5.6	3.00, d, 18.4	3.55, dd, 12.4, 6	3.54, dd, 17.6, 5.6
H-11β	3.02, d, 18	3.55, dd, 18.4, 5.6	2.83, d, 12.4	2.99, d, 17.6
H-12	α: 4.61, d, 5.6	β: 4.54, d, 5.6	α: 4.65, d, 6	α: 4.52, d, 5.6
N-CH_3	3.35, s	3.28, s	2.92, s	3.26, s

a) CD_3OD.

H-7 with methoxy (δ 3.85) and H-6α (δ 4.63) indicated that the presence of a methoxyl group on C-8. The cross-peaks between NCH_3 (δ 3.35) and H-5α (δ 3.90) confirmed that NCH_3 is close to H-5. Based on the above spectral data, the structure **1** was assigned for (−)-isocaryachine-*N*-oxide B.

Compound **2** was isolated as optically active colorless needles with dextrorotation and its molecular formula was determined to be $C_{19}H_{19}NO_5$ by HR-FAB-MS. The ^{1}H-NMR spectrum of **2** also showed four aromatic singlets and two AMX resonances characteristic of pavine alkaloids (Table 1). The UV, IR, mass, and ^{1}H-NMR spectral data of **2** were very close to those of (−)-isocaryachine-*N*-oxide (**5**) which has been isolated from the leaves of this species and confirmed by X-ray techniques.[7)] The NOESY experiment with **2** confirmed the location of an OCH_3 group at C-8 by the correlations of H-7 (δ 6.82) with OCH_3 (δ 3.84) and H-6β (δ 4.54). The NOE correlation between NCH_3 (δ 3.28) and H-11β (δ 3.55), indicating the relative configuration of NCH_3, is shown in Fig. 2. Based on the similarities of the NMR and MS spectra with those of (−)-isocaryachine-*N*-oxide (**5**) except for the optical rotation, it is assumed that compound **2** is (+)-isocaryachine-*N*-oxide.

Compound **3** was obtained as optically active colorless needles with levorotation. Its molecular formula $C_{19}H_{19}NO_5$ was determined by HR-FAB-MS. The ^{1}H-NMR spectrum of **3** showed the characteristic signal pattern of a pavine alkaloid (Table 1), and appeared very similar to that of compound **1** with few exceptions. In the NOESY spectrum, the correlations of H-10 (δ 6.81) with OCH_3 (δ 3.84) and H-11β (δ 2.83) confirmed the position of a methoxy group at C-9. The NOE correlation between NCH_3 (δ 2.92) and H-5α (δ 3.50) showed that the stereochemistry of **3** at NCH_3 was the same as that in **1** (Fig 1). Thus the structure of **3** was assigned to be (−)-caryachine-*N*-oxide.

Compound **4** was isolated as yellow syrup. FAB-MS showed a molecular ion peak at *m*/*z* 188 which corroborated the molecular formula of $C_{11}H_{10}NO_2$. UV absorption maxima at 338, 296, 287, and 253 nm indicated that it is an isoquinoline derivative.[9)] In the ^{1}H-NMR spectrum of **4**, two aromatic singlet signals at δ 7.44, 7.36 (each 1H) and a methylenedioxy group at δ 6.22 indicated that a methylenedioxy group is located at C-6 and C-7. In addition to those, only a pair of heterocyclic aromatic protons at δ 8.11 and 7.99 (each 1H, d, J=6.4 Hz), a singlet at δ 9.07, and a methyl signal (δ 4.26) were observed. The downfield shift of signals at δ 9.07 (H-1) and δ 4.26 (NCH_3) was induced by the positive-charged nitrogen atom. Thus the structure of **4** was elucidated to be 6,7-methylenedioxy-*N*-methylisoquinoline, which is often used as a substrate for the synthesis of benzylisoquinoline derivatives. To our knowledge, this was the

first time it was isolated from natural sources.[9]

The known compounds, (−)-neocaryachine,[2] (+)-caryachine,[2] (+)-cinnamolaurine,[11] (−)-caryachine,[2] (−)-mutagenine,[12] (−)-isocaryachine,[2] 1-hydroxycryprochine,[8] 4-(6,7-dimethyloxyisoquinoline-1-ylmethylphenol,[10] (−)-2-*O*-norargemonine,[13] (−)-*N*,*N*-dimethylcaryachine,[5] and (−)-eschscholtzidine[2] were also isolated and identified from the stem bark of *C. chinensis* by comparison of their spectroscopic data (UV, IR, NMR, mass spectrometry) with values in the literature.

Experimental

General Procedures Melting points were determined on a Yanagimoto MP-S3 micro melting point apparatus and are uncorrected. Optical rotations were obtained on a Jasco Dip-370 polarimeter. The IR spectra were recorded on a Shimadzu FT IR-8501 spectrophotometer as KBr disks. The UV spectra were recorded on a Hitachi U-3210 spectrophotometer. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with Varian Unity plus 400 and Bruker AMX-400 spectrophotometer ($CDCl_3$ and CD_3OD as solvent). Chemical shift values are shown in ppm (δ) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a VG 70-250S mass spectrometer.

Plant Material The stem bark of *C. chinensis* was collected from Kaohsiung Hsien, Taiwan, in July 1986, and was identified by Professor C. S. Kuoh. A voucher specimen is deposited in the Herbarium of National Cheng Kung University, Taiwan.

Extration and Isolation The plant material (22 kg) was powdered and refluxed 10 times with 95% EtOH (20 l). The filtrate was concentrated and treated with 3% HOAc, and the acidic water-soluble portion was neutralized with NH_4OH (aq.) and partitioned with $CHCl_3$. The $CHCl_3$ condensate containing total alkaloids was extracted with 2% NaOH solution to afford phenolic alkaloids. The nonphenolic base gave a solid (−)-eschscholtzidine (15 g). The water-soluble portion was neutralized with $(NH_4)_2SO_4$ and then partitioned with $CHCl_3$. The $CHCl_3$ layer suspension was filtered to afford a solid (±)-caryachine (10 g), and the filtrate was evaporated and recrystallized in acetone to give (−)-caryachine (8 g). To the mother liquid of (−)-caryachine 2% H_2SO_4 was added to acidify it, then it was partitioned with $CHCl_3$, the water-soluble portion was collected and neutralized with 10% NaOH (aq.), and further partitioned with ether to afford a tertiary base and ether extract. The condensed ether extract (20 g) was chromatographed directly on silica gel and eluted with a gradient of $CHCl_3$ and MeOH to afford seven fractions. Fraction 3 was rechromatographed on silica gel and eluted with iso-Pr_2O–MeOH (19 : 1) and the sixth fraction further chromatographed on TLC with $CHCl_3$–MeOH (9 : 1) to give 4-(6,7-dimethyloxyisoquinoline-1-ylmethyl)phenol (1.2 mg). Fraction 4 was recrystallized in acetone to give (+)-cinnamolaurine (12 mg). Fraction 6 was chromatographed on silica gel and eluted with iso-Pr_2O–MeOH (9 : 1) to give (+)-caryachine (1.2 mg), (−)-isocaryachine (11 mg), and (−)-caryachine (3 mg). Fraction 7 was chromatographed on silica gel and eluted with $CHCl_3$–MeOH–H_2O (9 : 1 : 1 dropwise) to give nine subfractions. Each subfraction was rechromatographed on a silica gel column or TLC to afford (+)-isocaryachine-*N*-oxide (**2**, 0.9 mg), 1-hydroxycryprochine (20 mg), (−)-caryachine-*N*-oxide (**3**, 0.7 mg), (−)-isocaryachine-*N*-oxide B (**1**, 1.1 mg), (−)-mutagenine (1.5 mg), and (−)-2-*O*-norargemonine (1.3 mg). The tertiary base (15 g) was chromatographed on a silica gel column to afford 6,7-methylenedioxy-*N*-methylisoquinoline (**4**, 2.5 mg) and (−)-*N*,*N*-dimethylcaryachine (13.5 g).

(−)-Isocaryachine-*N*-oxide B (**1**): Colorless needles (acetone); mp >280 °C; $[\alpha]_D$ −26.2° (c=0.2175, MeOH); HR-FAB-MS m/z 342.1342 ($[M+H]^+$) (Calcd for $C_{19}H_{20}NO_5$, 342.1341); UV λ_{max} (MeOH) nm (log ε): 288 (3.3), 225 (3.6); IR ν_{max} cm^{-1} (KBr): 3253, 1602, 1502, 1425, 1242; FAB-MS m/z 342 $[M+H]^+$ (8), 324 (5), 307 (26), 154 (100), 137 (73); ^{13}C-NMR δ (100 MHz, CD_3OD): 147.7, 147.6 (C-2, 3), 147.1, 147.0 (C-8, 9), 126.8 (C-6a), 125.0 (C-12a), 122.7 (C-4a), 119.9 (C-10a), 114.4 (C-4), 109.9 (C-7), 107.6 (C-10), 106.6 (C-1), 101.2 (OCH_2O), 70.5 (C-12), 70.0 (C-6), 55.3 (OCH_3), 54.6 (NCH_3), 36.1 (C-5), 34.6 (C-11).

(+)-Isocaryachine-*N*-oxide (**2**): Colorless needles (acetone); mp >280 °C; $[\alpha]_D$ +72.60° (c=0.073, MeOH); HR-FAB-MS m/z 342.1345 ($[M+H]^+$) (Calcd for $C_{19}H_{20}NO_5$, 342.1341); UV λ_{max} (MeOH) nm (log ε): 280 (3.2), 219 (3.7); IR ν_{max} cm^{-1} (KBr): 3347, 1600, 1515, 1460, 1247; FAB-MS m/z 342 $[M+H]^+$ (10), 307 (19), 176 (100), 154 (93).

(−)-Caryachine-*N*-oxide (**3**): Colorless needles (acetone); mp >280 °C; $[\alpha]_D$ −86.87° (c=0.099, MeOH); HR-FAB-MS m/z 341.1261 ($[M]^+$) (Calcd for $C_{19}H_{19}NO_5$, 341.1263); UV λ_{max} (MeOH) nm (log ε): 284 (3.2), 223 (sh) (3.7); IR ν_{max} cm^{-1} (KBr): 3535, 1602, 1508, 1427, 1244, 939; FAB-MS m/z 342 $[M+H]^+$ (10), 324 (4), 307 (26), 289 (11), 154 (100), 137 (73).

6,7-Methylenedioxy-*N*-methylisoquinoline (**4**): Yellow syrup; $C_{11}H_{10}NO_2$; UV λ_{max} (MeOH) nm: 338, 296, 287, 253; IR ν_{max} cm^{-1} (KBr): 3347, 1460, 1480; FAB-MS m/z 188 (M^+), 116 (10); ^{1}H-NMR (D_2O, 400 MHz) δ: 9.07 (1H, s), 8.11 (1H, d, J=6.4 Hz), 7.99 (1H, d, J=6.4 Hz), 7.44 (1H, s), 7.36 (1H, s), 6.22 (2H, s), 4.26 (3H, s, NMe).

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