Alkaloids from the Leaves of Cryptocarya chinensis HEMSL.

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Investigation of the leaves of Cryptocarya chinensis resulted in the isolation of three new alkaloids, named (−)-isocaryachine-N-oxide, isoboldine-β-N-oxide, and 1-hydroxyprochime, together with seven known compounds. Their structures were elucidated by spectral analysis. The structures of (−)-isocaryachine-N-oxide and 1-hydroxyprochime were further confirmed by X-ray techniques.

Key words Cryptocarya chinensis; Lauraceae; pavine alkaloid; (−)-isocaryachine-N-oxide; isoboldine-β-N-oxide; 1-hydroxyprochime

Results and Discussion

(−)-Isocaryachine-N-oxide (1) was obtained as optically active colorless needles. It exhibited a quasimolecular ion peak \([M+H]^+\) at \(m/z\) 342.1341 in its HR (high resolution)-FAB-MS spectrum consistent with the molecular formula \(C_{19}H_{19}NO_5\). In the \(^1\)H-NMR spectrum of 1, four aromatic proton singlets at \(\delta\) 6.83, 6.69, 6.49 and 6.48, and two AMX protons at \(\delta\) 4.54, 3.91, 2.77 (H-6\(_a\), H-5\(_a\), H-5\(_b\)) and 4.52, 3.53, 2.99 (H-12\(_a\), H-11\(_a\), H-11\(_b\)) are characteristic peaks of pavine alkaloids. \(^{8}\) Beside these signals, one methenedioxy group at \(\delta\) 5.87, 5.83 and one methoxyl group at \(\delta\) 3.84 were observed. These data are similar to those of caryachine, except the peak of NCH\(_3\) \(^{2}\). The 0.7 ppm downfield shift of N-methyl at \(\delta\) 3.27 in 1 compared with that in caryachine suggested that compound 1 is the N-oxide of caryachine. This was further confirmed by the molecular weight, which is 16 amu greater than caryachine. The stereochemistry of 1 was deduced by NOESY (nuclear Overhauser and exchange spectroscopy) and optical rotation. In the NOESY experiment, the presence of a NOE correlations of H-7 (\(\delta\) 6.83) with methoxy (\(\delta\) 3.84) and H-6\(_a\) (Fig. 1) indicated a methoxyl group located on C-8. The optical rotation \([\alpha]_D^{245.08}\) revealed that the stereochemistry of C-6 and C-12 were S, S configurations, respectively. \(^{2}\) The structure of 1 was also confirmed by X-ray single crystal diffraction studies (Fig. 2).

Isoboldine-β-N-oxide (2) was separated from the same fraction of isoboldine as brown powder, after removing the
crystal of isoboldine by their different solubilities on CHCl₃. The UV spectrum at 304, 279, 271 (sh) nm showed that it was an aporphine alkaloid with oxygenated substituents at C-1, 2, 9 and 10. HR-FAB-MS spectrometry showed a quasimolecular ion peak at m/z 344.1494 [M+H]⁺, which established the molecular formula as C₁₉H₂₁NO₅. Furthermore, its ¹H-NMR spectrum showed a similar pattern to isoboldine. It showed three aromatic singlets at δ 8.13 (H-11), 6.77 (H-8) and 6.73 (H-3), two methoxy signals at δ 3.91 (2-OCH₃), 3.87 (10-OCH₃), and signals of CH–CH₂ at δ 5.49 (H-6a), δ 3.33, 2.75 (H-7) and CH₂–CH₂ at δ 4.07, 3.41 (H-5), 3.33, 2.98 (H-4). The greatest difference between 2 and isoboldine is in the chemical shift of NCH₃ (δ 3.08 in 2; δ 2.64 in isoboldine). According to the 16 amu the weight excess in mass spectrum and the downfield methyl of NMe in the ¹H-NMR spectrum, we proposed that oxygen was substituted at nitrogen. The regiochemistry of the methoxyl group was decided by the NOESY experiment (Fig. 1). The presence of NOE correlations of H-3 (δ 6.73) with methoxy (δ 3.91) and H-11 (δ 8.13) with methoxy (δ 3.87) indicated that substituents of the methoxyl group were at C-2, 10, respectively. Due to the negative specific rotation of 2, the stereochemistry of C-6a was confirmed to have a R configuration. Furthermore, since a lack of NOE correlation between H-6a and N–CH₃ was observed, an anti-arrangement must exist between H-6a and N–CH₃. The absolute structure of 2 was assigned as isoboldine-β-N-oxide.

1-Hydroxycryprochine (3) was isolated as colorless needles. It gives a molecular ion peak at m/z 301 [M]⁺ on El (electron impact)-mass. High resolution mass measurement established the molecular formula as C₁₉H₂₁NO₅. The ¹H-NMR spectrum showed a singlet at δ 6.50, a pair of olefinic protons at δ 5.87, 5.80 and twelve complex protons in the aliphatic region. These signals were similar to those of cryprochine,⁵ which was also isolated from the woods of this species. There is only one methoxyl group presented in 3. It is located at C-2 as determined by the existence of NOE of H-3 (δ 6.50) with this methoxy (δ 3.83). Further assignments of these signals with ¹³C, COSY (correlation spectroscopy) and HMQC (¹H-detected heteronuclear multiple quantum coherence) spectra gave these partial structures, CH₂–CH₂, CH–CH₂, and CH=N–CH–CH(OH)–CH₂–CH₂. Based on the result of the ¹H–¹³C long-range correlation described in Table 1, the structure of 3 was assigned as 1-hydroxycryprochine. The X-ray single crystal diffraction further confirmed the structure of 3 (Fig. 2).

The known compounds, (+)-isocaryachine (4),²³ (+)-caryachine (5),¹² (+)-caryachine (5),¹² (+)-caryachine (6),¹² (+)-isocaryachine (7),¹² isoboldine (8),¹¹(--)-munitagine (9),¹² and bisnoragemonine (10)¹³ were also isolated and characterized from the leaves of C. chinensis. Their structures were elucidated 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, single crystal X-ray diffraction pattern and mass spectra were recorded on a Hitachi U-3210 spectrophotometer, Enraf-Nonius CAD4 and VG 70-250S instruments, respectively. The ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were recorded with Varian Unity Plus 400 and Bruker AMX-400 spectrophotometers (CDCl₃ and acetone-d₆ as solvent). Chemical shift values are shown in ppm (δ) with TMS (tetramethylsilane) as an internal standard.

#### Plant Material

The leaves of C. chinensis (9.5 kg) were collected from Kuoh. A voucher specimen is deposited in the Herbarium of National Cheng Kung University, Taiwan.

#### Extraction and Isolation

The plant material was powdered and refluxed with 95% EtOH eight times (20 l). The extract was concentrated then neutralized with (NH₄)₂SO₄ and gave a solid (+) -caryachine 5 (1.2 g). The
resulting solution was then partitioned with CHCl₃. The CHCl₃-soluble portion was evaporated to give phenolic alkaloids, which were chromatographed directly on a silica gel column and eluted with a gradient of (iso-Pr)₂O and MeOH to afford 14 fractions, and then further eluted with a gradient of CHCl₃, and MeOH to afford 4 fractions. The crude crystals in fraction 4 were combined and recrystallized with acetone to give (+)-isocaryachine 4 (10.6 mg). The solid in fraction 6 was determined to be (+)-isocaryachine 4 (12.2 mg), and the mother liquor was chromatographed on silica gel and eluted with CHCl₃/MeOH (19:1) to give (+)-caryachine 5 (3.6 mg). Fraction 7 was chromatographed on silica gel and eluted with CHCl₃/MeOH (19:1) to give (−)-caryachine 6 (28.4 mg), (−)-isocaryachine 7 (34.2 mg), and a mixture of the solid was dissolved in CHCl₃/MeOH (1:1). The resulting solution was then partitioned with CHCl₃. The CHCl₃-soluble liquid was chromatographed on silica gel and eluted with CHCl₃/MeOH (19:1) to give (−)-isocaryachine 6 (3.6 mg) first and then the isoboldine-N-oxide 2 (13.95 mg). The mother liquid was chromatographed on silica gel and eluted with CHCl₃/MeOH (9:1) to give (−)-manungitate 9 (2.13 mg). Fraction 8 was chromatographed on silica gel eluting with CHCl₃/MeOH (19:1) to give bisnoranganetin 10 (6.3 mg) and 1-hydroxycroprochne 3 (32.2 mg). The solid in fraction 11 was collected and recrystallized with MeOH to give (−)-isocaryachine-(5S)-N(10)(1.7 mg).

(−)-Isocaryachine-N-Oxide (1) Colorless needles (acetone): mp 278°C; [α]D = −245.08° (c = 0.0176, MeOH); HR–FAB–MS m/z 334.1341 [(M + H)⁺](Calcd for C₁₉H₁₉NO₅, 334.1341); FAB–MS m/z 342 [M = H⁺] (10), 324 (4), 207 (29), 261 (11), 154 (100), 137 (73); UV λmax(MeOH) nm (log ε): 293 (3.2), 223 (3.7); IR νmax cm⁻¹: KBr: 3535, 1602, 1549, 1427, 1238, 1035; 1H–NMR (CDCl₃, 400 MHz) δ: 8.33 (1H, d, J = 2.0 Hz), 6.94 (2H, d, J = 10.8 Hz), 6.40 (2H, d, J = 10.8 Hz), 5.31 (1H, d, J = 10.8 Hz, H–7), 4.45 (1H, d, J = 10.8 Hz, H–8), 3.87 (3H, s, 2-OCH₃), 3.60 (1H, dd, J = 10.8 Hz, 2.0 Hz, H–5), 3.41 (1H, dd, J = 10.8 Hz, 10.8 Hz, H–7), 3.35 (1H, d, J = 10.8 Hz, H–8), 2.77 (1H, m, H–11). The authors are grateful to the National Science Council of the Republic of China for financial support (NSC 86-2113-M-006-003), and to Prof. C. S. Kuoh (Department of Biology, National Cheng Kung University, Tainan, Taiwan) for plant identification. We also thank Miss S. F. Tung (Regional Institutions Center at the National Cheng Kung University) for the X-ray diffraction measurement.

X-Ray Crystallography of 3 Crystal data: Colorless crystal (0.24 × 0.19 × 0.05 mm) grown from acetone; orthorhombic, space group P2₁₂₁₂, a = 9.7828 (9), b = 11.6025 (14), c = 15.009 (16) Å, V = 1703.6 (19) Å³, Z = 4, Dcalcd = 1.248 g/cm³, F(000) = 1415.81, μ = 0.08 mm⁻¹; λ(MoKα) = 0.70930 Å, 5069 measured intensities (0 ≤ h ≤ 15, k = −32 ≤ 32, l = −25 ≤ 25), 4557 unique (Rint = 0.029), of which 1485 were observed with I≥2.5σ(I).

Data Collection and Structure Refinement The intensity data were collected on a Picker diffractometer, using graphite monochromated MoKα radiation and the (θ–2θ) scan technique up to 44.9°. Cell parameters were refined from 24-well-centered reflections with 10.72° ≤ 2θ ≤ 20.42°. The structure was solved by direct methods using the NRCVAX System and refined by full-matrix least-squares refinement. The last least-squares cycle was calculated with 89 atoms, 189 parameters and 893 out of 2607 reflections. Weights based on counting-statistics were used. The weight modifier K in KF = 2 is 0.000100. Thus, for significant reflections, RF = 0.047, R = 0.042 GoF = 1.53, and for all reflections, RF = 0.056, R = 0.058. In the last D-map, a hole was found between −0.370 to 0.490 e Å⁻³. This phenomenon did not affect the overall geometry of the molecule. The refinement converged to an R-value of 4.04% for all reflections and 5.62% for observed reflections. 

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