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-hydroxycryprochine, together with seven known compounds. Their structures were elucidated by spectral analysis. The structures of (−)-isocaryachine-*N*-oxide and 1-hydroxycryprochine were further confirmed by X-ray techniques.

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

Cryptocarya chinensis (HANCE) HEMSL. (Lauraceae) is an evergreen tree widely distributed in low altitude forests in Taiwan and southern China.[1] Past studies of this species have found that it contains many pavine and proaphorphine alkaloids.[2—6] The pavine alkaloids have been noted to possess such biological activities as antiviral and immunological activity,[7] behavioral and electrophysiological effects, and antiarrhythmic potential.[4] These results promoted us to study this species again. So far, three new alkaloids, (−)-isocaryachine-*N*-oxide (**1**), isoboldine-β-*N*-oxide (**2**), and 1-hydroxycryprochine (**3**), together with seven known compounds, were isolated from the ethanol extract of the leaves of *C. chinensis* by the usual alkaloid extraction procedure. Among them, two were novel *N*-oxide alkaloids which have been found rarely in a natural source. Here we report the isolation and structural elucidation of these three new compounds.

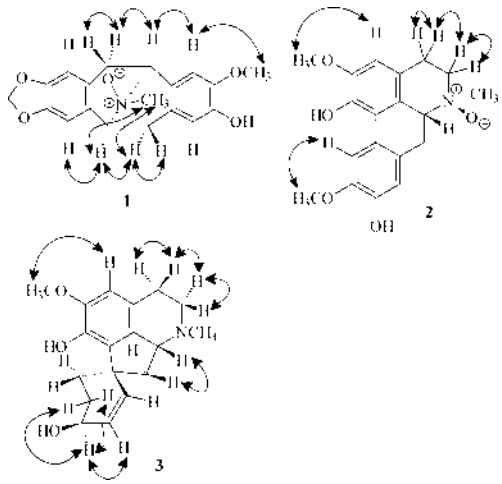

Fig. 1. NOESY Correlations of **1**, **2** and **3**

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 δ 6.83, 6.69, 6.49 and 6.48, and two AMX protons at δ 4.54, 3.91, 2.77 (H-6α, H-5α, H-5β) and 4.52, 3.53, 2.99 (H-12α, H-11α, H-11β) are characteristic peaks of pavine alkaloids.[8] Beside these signals, one methenedioxy group at δ 5.87, 5.83 and one methoxyl group at δ 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 δ 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 (δ 6.83) with methoxy (δ 3.84) and H-6α (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).

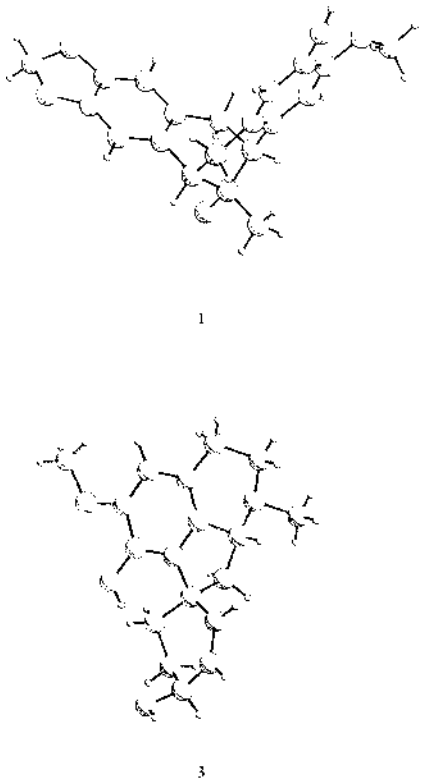

Fig. 2. X-Ray Single Crystal Diffractions of **1** and **3**

Isoboldine-β-*N*-oxide (**2**) was separated from the same fraction of isoboldine as brown powder, after removing the

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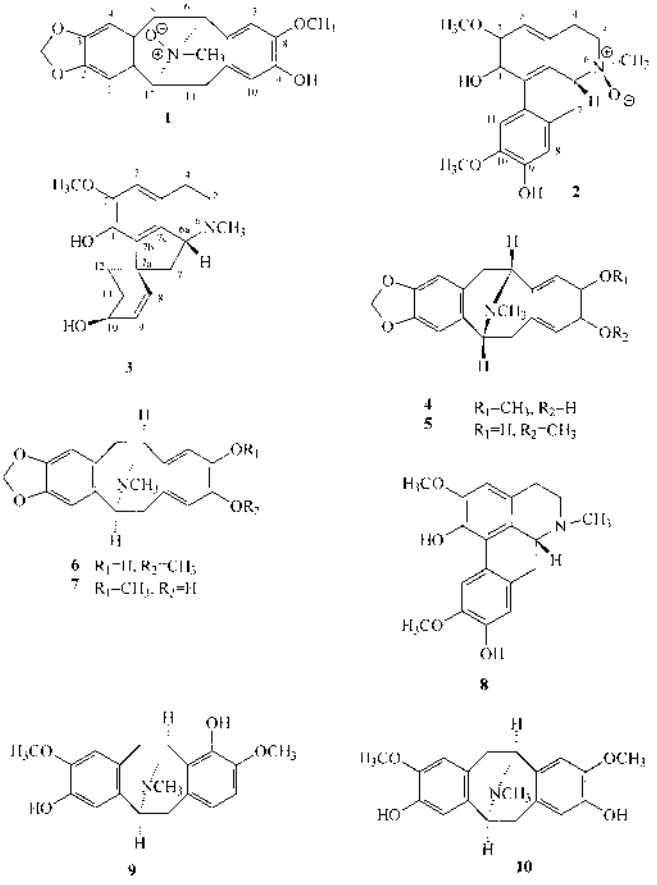
Table 1. $^{2}J,^{3}J$-Correlations of 1-Hydroxycryprochine (**3**)

C	δ, ppm	H
1	140.4	3 (6.50)
2	147.1	OCH_3 (3.83), 3 (6.50)
3	108.6	4α (2.73)
3a	121.8	4 (2.73, 2.98), 5β (3.12)
4	26.8	5β (3.12)
5	54.9	NCH_3 (2.38), 4β (2.98)
6a	65.3	7β (1.86), NCH_3 (2.38), 5β (3.12)
7	49.5	8 (5.80)
7a	47.6	11β (1.89), 7β (1.86)
7b	135.1	7β (1.86)
7c	130.4	3 (6.50)
8	135.1	11β (1.89)
9	127.7	
10	63.6	8 (5.80), 9 (5.87)
11	29.0	9 (5.87)
12	28.5	11β (1.89)
OCH_3	56.5	
NCH_3	43.0	

crystal of isoboldine by their different solubilities on $CHCl_3$. The UV spectrum at 304, 279, 271 (sh), 219 nm showed that it was an aporphine alkaloid with oxygenated substituents at C-1, 2, 9 and 10.[9] HR-FAB-MS spectrometry showed a quasimolecular ion peak at *m/z* 344.1494 $[M+H]^+$, which established the molecular formula as $C_{19}H_{21}NO_5$. Furthermore, its ^{1}H-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-OCH_3), 3.87 (10-OCH_3), and signals of CH–CH_2 at δ 4.59 (H-6a), δ 3.33, 2.75 (H-7) and CH_2–CH_2 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 NCH_3 (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 ^{1}H-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 substitutions 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.[10] Furthermore, since a lack of NOE correlation between H-6a and N–CH_3 was observed, an anti-arrangement must exist between H-6a and N–CH_3. 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 EI (electron impact)-mass. High resolution mass measurement established the molecular formula as $C_{18}H_{23}NO_3$. The ^{1}H-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,[6] 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 ^{13}C, COSY (correlation spectroscopy) and HMQC (^{1}H-detected heteronuclear multiple quantum coherence) spectra gave these partial structures,



CH_2–CH_2, CH–CH_2, and CH=CH–CH(OH)–CH_2–CH_2–. Based on the result of the ^{1}H–^{13}C 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**),[2] (+)-caryachine (**5**),[2] (−)-caryachine (**6**),[2] (−)-isocaryachine (**7**),[2] isoboldine (**8**),[11] (−)-munitagine (**9**),[12] and bisnorargemonine (**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 ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with Varian Unity Plus 400 and Bruker AMX-400 spectrophotometers ($CDCl_3$ and acetone-d_6 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 Kaohsiung Hsien in Taiwan in July, 1986, and identified by Prof. C. S. 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 treated with 3% HOAc. The acidic water-soluble part was neutralized with NH_4OH (aq.) and partitioned with $CHCl_3$. The $CHCl_3$ layers containing total alkaloids, was extracted by 2% NaOH solution. The aqueous solution was neutralized with $(NH_4)_2SO_4$ and gave a solid (+)-caryachine **5** (1.2 g). The

resulting solution was then partitioned with $CHCl_3$. The $CHCl_3$-soluble portion was evaporated to give phenolic alkaloids, which were chromatographed directly on a silica gel column and eluted with a gradient of $(iso\text{-}Pr)_2O$ and MeOH to afford 14 fractions, and then further eluted with a gradient of $CHCl_3$ and MeOH to afford 4 fractions. The crude crystals in fraction 4 and fraction 5 were combined and recrystallized with acetone to give (+)-isocaryachine **4** (10.6 mg). The solid in fraction 6 was determined to be (+)-isocaryachine **4** (1.2 mg), and the mother liquid was chromatographed on silica gel and eluted with $CHCl_3$/MeOH (19 : 1) to give (+)-caryachine **5** (3.6 mg). Fraction 7 was chromatographed on silica gel and eluted with $CHCl_3$/MeOH (19 : 1) to give (−)-caryachine **6** (24.8 mg), (−)-isocaryachine **7** (34.2 mg), and a mixture of the solid was dissolved in $CHCl_3$, then the solvent was evaporated gradually to give isoboldine **8** (3.62 mg) first and then the isoboldine-*N*-oxide **2** (13.95 mg). The mother liquid was chromatographed on silica gel and eluted with $CHCl_3$/MeOH (9 : 1) to give (−)-munitagine **9** (2.13 mg). Fraction 8 was chromatographed on silica gel eluting with $CHCl_3$/MeOH (19 : 1) to give bisnorargemonine **10** (6.3 mg) and 1-hydroxycryprochine **3** (32.2 mg). The solid in fraction 11 was collected and recrystallized with MeOH to give (−)-isocaryachine-(*S*)-*N*-oxide **1** (10.7 mg).

(−)-Isocaryachine-*N*-oxide (1) Colorless needles (acetone): mp >280 °C; $[\alpha]_D$ −245.08° (c=0.1076, MeOH); HR-FAB-MS m/z 342.1341 ($[M+1]^+$) (Calcd for $C_{19}H_{20}NO_5$, 342.1341); FAB-MS m/z 342 $[M+H]^+$ (10), 324 (4), 307 (26), 289 (11), 154 (100), 137 (73); UV λ_{max} (MeOH) nm (log ε): 293 (3.2), 223 (3.7); IR ν_{max} cm^{-1} (KBr): 3535, 1602, 1529, 1498, 1427, 1261, 1238, 1035; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 6.83 (1H, s, H-10), 6.70 (1H, s, H-7), 6.50 (1H, s, H-1), 6.48 (1H, s, H-4), 5.87, 5.83 (each 1H, d, J=1.2 Hz, –OCH_2O–), 4.54 (1H, d, J=6.4 Hz, H-6α), 4.52 (1H, d, J=6.4 Hz, H-12α), 3.91 (1H, dd, J=16.4, 6.4 Hz, H-5α), 3.84 (3H, s, OMe), 3.53 (1H, dd, J=16.4, 6.4 Hz, H-11α), 3.27 (3H, s, NMe), 2.98 (1H, d, J=16.4 Hz, H-11β), 2.77 (1H, d, J=16.4 Hz, H-5β).

X-Ray Crystallography of 1 Crystal data: Colorless crystal (0.24×0.19×0.53 mm) grown from acetone; $C_{19}H_{19}NO_5$, MW=341, orthorhombic, space group $P2_12_12_1$ a=9.7828 (9), b=11.6025 (14), c=15.009 (16) Å, V=1703.6 (19) Å^3, Z=4, D_c=1.401 g/cm^3, F(000)=760.36, μ=0.10 mm^{-1}, λ (Mo$K\alpha$)=0.70930 Å, 2127 measured intensities ($-11 \leqq h \leqq 11$), k: $-13 \rightarrow 13$, l: $-17 \rightarrow 17$), 1724 unique (R_{int}=0.019) of which 1343 observed with $I \geqq 2.5\sigma(I)$.

Data Collection and Structure Refinement The intensity data were collected on a Picker diffractometer, using graphite monochromated Mo$K\alpha$ radiation and the (θ–2θ) scan technique up to 49.8°. Cell parameters were refined from 18 well-centered reflections with $6.82° \leqq \theta \leqq 22.92°$. The structure was solved by direct methods using the NRCVAX System[14] and refined by full-matrix least-squares refinement. The last least squares cycle was calculated with 47 atoms, 236 parameters and 1343 out of 1724 reflections. Weights based on counting-statistics were used. The weight modifier K in KFo**2 is 0.000100. Thus, for significant reflections, RF=0.038, R_w=0.044 GoF=1.00, and for all reflections, RF=0.055, R_w=0.044. In the last D-map, a hole was found between −0.230 to 0.250 e A^{-3}.

(−)-Isoboldine-β-*N*-oxide (2) Brown powder, mp 177—179 °C; $[\alpha]_D$ −90.32° (c=0.1395, MeOH); HR-FAB-MS m/z 344.1494 ($[M+1]^+$) (Calcd for $C_{19}H_{22}NO_5$, 344.1497); FAB-MS m/z 344 $[M+H]^+$ (7), 328 (90), 307 (18), 176 (20), 154 (100); UV λ_{max} (MeOH) (log ε) nm: 304 (2.7), 279 (2.7), 271 (2.68) (sh), 219 (3.08); IR ν_{max} cm^{-1} (KBr): 3347, 1596, 1519, 1465, 1245, 1112. ^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.13 (1H, s, H-11), 6.77 (1H, s, H-8), 6.73 (1H, s, H-3), 4.59 (1H, br s, H-6a), 4.07 (1H, m, H-5), 3.91 (3H, s, 2-OCH_3), 3.87 (3H, s, 10-OCH_3), 3.41 (1H, m, H-5), 3.33 (2H, m, H-7, 4), 3.08 (3H, br s, NCH_3), 2.98 (1H, br d, J=16 Hz, H-4), 2.75 (1H, br d, J=13.6 Hz, H-7).

1-Hydroxycryprochine (3) Colorless needles (acetone); mp 117—119 °C; $[\alpha]_D$ +65.32° (c=0.322, $CHCl_3$); HR-FAB-MS m/z 302.1755 ($[M+1]^+$) (Calcd for $C_{18}H_{24}NO_3$, 302.1756); EI-MS m/z (rel. int.): 301 (78) $[M]^+$, 300 (100), 282 (89), 240 (58), 218 (56), 191 (56); UV λ_{max} (MeOH) (log ε) nm: 288 (3.27), 227 (sh) (3.83); IR ν_{max} (KBr) cm^{-1}: 3432, 1488, 1458, 1288, 1110, 1031; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.50 (1H, s, H-3), 5.87 (1H, dd, J=9.6, 4 Hz, H-9), 5.80 (1H, d, J=9.6 Hz, H-8), 4.12 (1H, dd, J=8, 4 Hz, H-10), 3.83 (3H, s, 2-OMe), 3.32 (1H, dd, J=10.8, 6.8 Hz, H-6a), 3.12 (1H, dd, J=11.6, 6.8 Hz, H-5β), 2.98 (1H, ddd, J=17.2, 11.2, 6.8 Hz, H-4β), 2.87 (1H, ddd, J=16.4, 13.2, 3.2 Hz, H-12β), 2.73 (1H, dd, J=17.2, 6.8 Hz, H-4α), 2.50 (1H, ddd, J=17.2, 11.6, 6.8 Hz, H-5α), 2.45 (1H, dd, J=10.8, 6.8 Hz, H-7α), 2.38 (3H, s, NMe), 2.06 (1H, m, H-11α), 1.89 (1H, m, H-11β), 1.86 (1H, d, J=10.8 Hz, H-7β), 1.56 (1H, dt, 14, 4.4 Hz, H-12α); ^{13}C-NMR ($CDCl_3$) δ: 147.1 (C-1), 140.4 (C-2), 135.1 (C-7b, 8), 130.4 (C-7c), 127.7 (C-9), 121.8 (C-3a), 108.6 (C-3), 65.3 (C-6a), 63.6 (C-10), 56.5 (2-OMe), 54.9 (C-5), 49.5 (C-7), 47.6 (C-7a), 43.0 (NMe), 29.0 (C-11), 28.5 (C-12), 26.8 (C-4).

X-Ray Crystallography of 3 Crystal data: Colorless crystal (0.31×0.11×0.13 mm) grown from acetone; orthorhombic, space group $P2_12_12_1$ a=10.125 (5), b=14.2020 (20), c=24.319 (6) Å, V=3496.8 (20) Å^3, Z=4, D_c=1.248 g/cm^3, F(000)=1415.81, μ=0.08 mm^{-1}, λ (Mo$K\alpha$)=0.70930 Å, 5069 measured intensities ($0 \leqq h \leqq 10$), k: $-1 \rightarrow 15$, l: $-25 \rightarrow 26$), 4557 unique (R_{int}=0.029), of which 1485 were observed with $I \geqq 2.5\sigma(I)$.

Data Collection and Structure Refinement The intensity data were collected on a Picker diffractometer using graphite monochromated Mo$K\alpha$ radiation and the (θ–2θ) scan technique up to 44.9°. Cell parameters were refined from 24-well-centered reflections with $10.72° \leqq \theta \leqq 20.42°$. The structure was solved by direct methods using the NRCVAX System[14] 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 KFo**2 is 0.000100.

Thus, for significant reflections, RF=0.047, R_w=0.042 GoF=1.53, and for all reflections, RF=0.056, R_w=0.058. In the last D-map, a hole was found between −0.370 to 0.490 e A^{-3}.

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