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ALKALOIDS FROM *LYCOPODIUM CASUARINOIDES*

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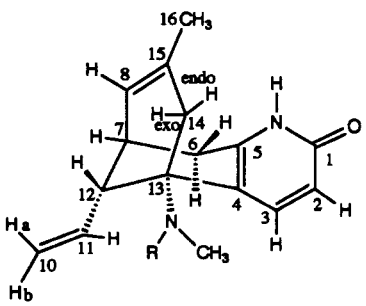
ABSTRACT.—In addition to huperzinine [**1**], a new alkaloid, *N*-demethylhuperzinine [**2**], was isolated from the aerial parts of *Lycopodium casuarinoides* by bioassay-directed fractionation. The structure of **2** was established by spectral analysis and extensive nOe difference nmr studies as well as spectral comparison with huperzinine. *N*-Demethylhuperzinine [**2**] exhibited anticholinesterase activity in pharmacological studies.

Lycopodium alkaloids are a group of chemically and pharmacologically interesting secondary metabolites occurring in the Lycopodiaceae (1). Previous studies include the isolation of huperzines A and B, two potent cholinesterase inhibitors from *Huperzia serrata* (Thunb.) Trev. (2), and the isolation of 6β-hydroxyhuperzine from *L. selago* (3). The present clinical application of huperzine A in the treatment of myasthenia gravis, Alzheimer's dementia, and for the improvement of senile memory loss makes the search for *Lycopodium* alkaloids more important. In a preliminary pharmacological screen, we have found that an EtOH extract of *L. casuarinoides* Spring (Lycopodiaceae) possessed significant anticholinesterase activity. To explore the biological activity, a study was initiated to investigate the alkaloidal constituents of *L. casuarinoides*, a species distributed over the mountainous area of Taiwan (4).



1 R=Me
2 R=H

To our knowledge, no study has been carried out on the constituents of this species. Here, we wish to report the isolation of huperzinine [**1**] and the structure elucidation of a new alkaloid, *N*-demethylhuperzinine [**2**], from this species.

The EtOH/$(CH_3)_2CO$ extract of *L. casuarinoides* was fractionated by acid-base and solvent partition. Extensive bioassay-guided chromatographic separation over Si gel, LH-20 and C-18 furnished compound **1** and the new alkaloid, *N*-demethylhuperzinine [**2**].

Compound **1**, $C_{17}H_{22}ON_2$ [M^+] *m/z* 270, showed characteristic signals [310, 230 nm in the uv spectrum; 1673, 1614, 1556, and 1473 cm^{-1} in the ir spectrum; two doublets (δ 7.70, 6.41, J=9.4 Hz) in the ^{1}H-nmr spectrum] for α-pyridone absorption (5–7). The olefinic methyl singlet (δ 1.56), two methyl groups (δ 2.44) and the AMX spin system in the vinylic moiety (δ 6.04, 5.26, and δ 5.11) as well as the corresponding carbon signals indicated that compound **1** is huperzinine, which was first isolated from *Huperzia serrata* (1). The complete assignment of ^{1}H- and ^{13}C-nmr signals was established by using COSY, DEPT, and extensive nOe experiments.

N-Demethylhuperzinine [**2**] has the molecular formula of $C_{16}H_{20}ON_2$, as deduced from the molecular ion (*m/z* 256) in its eims and also from the DEPT nmr spectrum. The uv data (230, 310 nm) and

the ir bands (1652, 1615, 1556, 1463 cm^{-1}) as well as the eims fragmentation pattern (*m/z* 241, 213, and *m/z* 175) resembled those of huperzinine [**1**], suggesting a similar structure. In addition, the olefinic signals of the α-pyridone and vinylic groups are similar to those of **1**. However, a signal at δ 2.40 exhibited only one *N*-methyl singlet indicating a loss of one methyl moiety from **1**. The ^{13}C-nmr spectrum of **2** was almost superimposable with that of **1** except for the carbon signals near the *N*-methyl side-chain. In comparison with **1**, the signal of the *N*-methyl carbon appeared at δ 28.61 (upfield by 11 ppm) and the signal for C-13 was shifted to δ 57.3 (upfield by 4 ppm), suggesting that the *N*-methyl group was located at the C-13 position. The relative stereochemistry of **2** was determined by extensive nOe studies. Irradiation of the *N*-methyl protons caused significant enhancements of signal intensity of H-3, H-11, H-12, H-10_a and H-14_{exo} (3.5%, 3.7%, 4%, 2%, and 7.4%, respectively). Irradiation of the signal of H-11 increased the intensity of H-10_b (2%), but did not increase the intensity of H-10_a, clearly determining the relative locations of H-10_a and H-10_b. Because the signal of H-8 was enhanced by irradiation of the methylene proton at δ 2.22, this helped to locate H-6β. The absence of an nOe between H-14_{exo} and the vinylic side-chain protons (H-10_a, H-10_b, and H-11) suggested that the chirality of C-12 is identical to that of **1**. The peak enhancement of H-12 (7.2%) by irradiation of H-10_a and enhancement of H-14_{exo} (2.6%) by irradiation of H-12 reflect their close spatial relationship. The nOe observation and similar corresponding coupling constants between **1** and **2** as well as chemical correlation confirmed the structural assignment of **2**.

Preliminary pharmacological screening revealed that *N*-demethylhuperzinine [**2**] showed inhibition of cholinesterase activity as tested on isolated mouse skeletal muscle at a concentration of 2 μM/ml. The control, neostigmine, showed activity at 0.3 μM/ml, while compound **1** showed no activity at 10 μM/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir and uv spectra were measured on Perkin-Elmer 577 and Hitachi 150-20 spectrophotometers, respectively. Eims and cims spectra were recorded on a Finnigan 4023 mass spectrometer. The ^{1}H-, ^{13}C-nmr, and nOe spectra were recorded on a Bruker AM 300 MHz instrument.

EXTRACTION AND ISOLATION.—The aerial part of *L. casuarinoides* Spring was collected near the Ali mountain in Taiwan in July 1988. A voucher specimen is kept in the School of Pharmacy, National Taiwan University. The dried plant (160 g) was ground and repeatedly extracted with EtOH and $(CH_3)_2CO$. The combined extracts were concentrated to a green tar, which was stirred with 5% HOAc (100 ml) and extracted with an equal volume of EtOAc. The acid-soluble fraction was collected and adjusted to pH 9 with NH_4OH solution. After exhaustive extraction with $CHCl_3$, the alkaloid fraction (302 mg) was chromatographed over a Si gel column (10 g) and eluted with a solvent mixture of $CHCl_3/(CH_3)_2CO$ to provide four fractions, A, B, C, and D. Both fraction A (110 mg) and D (30 mg) showed anticholinesterase activity at a concentration of 3 μg/ml. These two fractions were applied on a LH-20 column and eluted with MeOH to give an alkaloid-containing solid. Purification by reversed-phase chromatography (C-18, developed with equal volumes of MeOH and H_2O) and further recrystallization furnished huperzinine [**1**] (0.01%). *N*-Demethylhuperzinine [**2**] (0.002%) was obtained from C-18 cc purification (0.5 g) using a solvent mixture of MeOH and H_2O (4:6).

Huperzinine [**1**].—Isolated as colorless crystals; ir ν max (neat) 1673, 1614, 1556, 1473, 920 (-CH=CH_2) cm^{-1}; uv λ max (MeOH) (log ε) 310 (3.88), 230 (3.99), 202 (4.08) nm; ^{1}H nmr (CD_3OD) δ 6.41 (1H, d, *J*=9.4 Hz, H-2), 7.70 (1H, d, *J*=9.4 Hz, H-3), 2.91 (1H, dd, overlap, H-6α), 2.22 (1H, d, *J*=17.5 Hz, H-6β), 2.41 (1H, m, H-7), 5.41 (1H, d, *J*=5.2 Hz, H-8), 5.26 (1H, dd, *J*=17 and 1.9 Hz, H-10_a), 5.11 (1H, dd, *J*=10 and 1.9 Hz, H-10_b), 6.04 (1H, ddd, *J*=17, 10, and 10.3 Hz, H-11), 2.91 (1H, dd, J=10.3 and 4.1 Hz, H-12), 2.89 (1H, d, overlap, H-14_{exo}), 1.65 (1H, d, *J*=17.3 Hz, H-14_{endo}), 1.56 (3H, s, H-16), 2.44 (6H, s, N-CH_3); ^{13}C nmr (CD_3OD) δ 165.5 (s, C-1), 118.4 (d, C-2), 143.9 (d, C-3), 120.2 (s, C-4), 144.4 (s, C-5), 30.0 (t, C-6), 40.2 (d, C-7), 125.5 (d, C-8), 117.3 (t, C-10), 141.4 (d, C-11), 47.1 (d, C-12), 61.5 (s, C-13), 45.3 (t, C-14), 135.5 (s, C-15), 22.9 (q, C-16), 39.9 (q, $N(CH_3)_2$);

cims *m/z* 271 $[M+1]^+$ (100); eims *m/z* 270 $[M]^+$ (68), 255 $[M-15]^+$ (43), 229 (100), 215 (25), 202 (54), 189 (49), 175 (32).

N-*Demethylhuperzinine* [**2**].—Isolated as a amorphous powder; ir ν max (neat) 3310, 2922, 1652, 1615, 1556, 1463, 924, 754 cm^{-1}; uv λ max (MeOH) (log ϵ) 310 (3.88), 230 (4.14), 203 (4.10) nm; ^1H nmr (CD_3OD) δ 6.41 (1H, d, J=9.5 Hz, H-2), 7.81 (1H, d, J=9.5 Hz, H-3), 2.85 (1H, dd, J=17.3 and 5.5 Hz, H-6a), 2.34 (1H, d, J=17.7 Hz, H-6b), 2.55 (1H, m, H-7), 5.49 (1H, d, J=5.3 Hz, H-8), 5.37 (1H, dd, J=17 and 1.8 Hz, H-10_a), 5.23 (1H, dd, J=10.2 and 1.8 Hz, H-10_b), 5.84 (1H, ddd, J=17.1, 10.2, and 9.5 Hz, H-11), 2.76 (1H, dd, J=9.5 and 4.2 Hz, H-12), 2.65 (1H, d, overlap, H-14_{exo}), 1.74 (1H, d, J=17.3 Hz, H-14_{endo}), 1.60 (3H, s, H-16), 2.40 (3H, s, N-CH_3); ^{13}C nmr (CD_3OD) δ 165.5 (s, C-1), 118.6 (d, C-2), 142.1 (d, C-3), 120.4 (s, C-4), 144.4 (s, C-5), 30.2 (t, C-6), 38.2 (d, C-7), 126.1 (d, C-8), 119.5 (t, C-10), 138.1 (d, C-11), 45.5 (d, C-12), 57.3 (s, C-13), 42.5 (t, C-14), 134.4 (s, C-15), 22.9 (q, C-16), 28.6 (q, N-CH_3); eims *m/z* 256 $[M]^+$ (51), 241 (80), 227 (44), 213 (100), 199 (57), 187 (48), 175 (70).

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