1′,2′,3′,4′-Tetradehydrotubulosine, a Cytotoxic Alkaloid from *Pogonopus speciosus*

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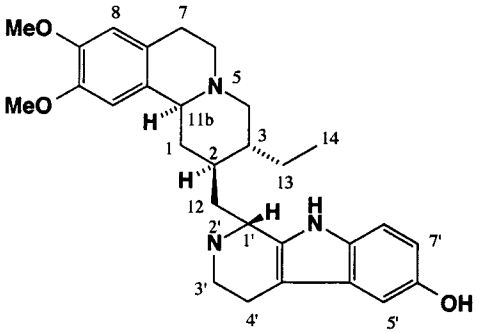
Received May 28, 1999

Bioassay-guided phytochemical investigation of the stems of *Pogonopus speciosus*, using human oral epidermoid carcinoma (KB) cells as a monitor, led to the isolation of a novel alkaloid, 1′,2′,3′,4′-tetradehydrotubulosine (**1**), along with tubulosine (**2**) and psychotrine (**3**) as bioactive constituents. The structure of the novel compound was elucidated through 1D- and 2D-NMR spectroscopic methods. Alkaloids **1** and **3** showed weak cytotoxic activity against a panel of human cancer cell lines, with the potency of these compounds being markedly less than that of tubulosine (**2**).

Pogonopus speciosus (Jacq.) K. Schum. (Rubiaceae), found in moist tropical rain forests throughout Central and South America, is a tree about 6 m high with thin obvate acuminate leaves and cymose flowers.[1] *Pogonopus* is a small genus comprised of only three species,[2] and *P. speciosus* is the sole species found in Panama.[3] In previous phytochemical work on *P. speciosus*, an extract of the sap extract afforded the known alkaloids tubulosine (**2**) and psychotrine (**3**). The structure of **2** was confirmed by single-crystal X-ray crystallography, and the cytotoxic activities of **2** and **3** were evaluated against a small panel of human tumor cell lines, with tubulosine (**2**) being broadly cytotoxic.[4] A series of emetine derivatives including tubulosine was examined in the in vivo L1210 and P388 leukemia test systems; tubulosine (**2**) showed good activity, exhibiting 30% and 80% ILS (increase in life span) values, respectively, at a dose of 2 mg/kg in each case.[5,6] Compounds **2** and **3** have been studied for various other biological activities, such as inhibition of protein biosynthesis,[7−13] and have amebicidal,[14,15] antimalarial,[16] and HIV reverse-transcriptase inhibitory activities.[17]

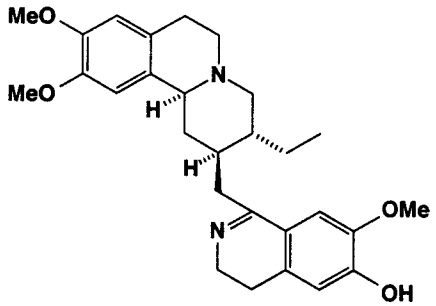
As a part of our ongoing program for the discovery of the anticancer agents from plants, supported by a National Cooperative Natural Products Drug Discovery Group grant, an alkaloidal fraction of the stems of *P. speciosus* was found to exhibit significant cytotoxic activity against a panel of human cancer cell lines. Bioassay-guided phytochemical investigation of this alkaloid fraction, using cytotoxicity with human oral epidermoid carcinoma (KB) cells in culture as a monitor, led to the isolation of the novel alkaloid **1**, along with tubulosine (**2**) and psychotrine (**3**), as active constituents. Alkaloids **2** and **3** were identified on the basis of physical and spectral data comparison with literature values.[4,18,19] The structure of compound **1** was elucidated using 1D- and 2D-NMR spectroscopic methods, and the three pure compounds were evaluated against a human cancer cell line panel.

The HREIMS of alkaloid **1** showed a molecular ion peak at *m/z* 471.2503, indicating an elemental molecular formula of $C_{29}H_{33}N_3O_3$. The EIMS showed a molecular ion peak at *m/z* 471, along with major fragment ions at *m/z* 287, 273, 272, 271, 258, 246, 244, 205, 198, and 191. The ^{1}H NMR spectrum of compound **1** was similar to that of **2**, except for the presence of signals consistent with the unsaturation of H-1′ through H-4′. In the ^{13}C NMR spectrum of **1**, when compared with that of **2**, a quaternary carbon at δ_C 149.2 (C-1′) and double bond signals at δ_C 137.1 and δ_C 114.2 (C-3′ and C-4′) were observed, instead of a methine signal and two methylene signals of C-1′, C-3′, and C-4′, respectively. In the ^{1}H NMR spectrum of **1**, signals at δ_H 8.20 (1H, d, J = 5.4 Hz) and δ_H 7.87 (1H, d, J = 5.4 Hz) were assigned to the aromatic protons at C-3′ and C-4′, respectively. The structure of the side chain of **1** was confirmed by HMBC NMR spectral observations which showed cross-peaks between H-12 and C-1′; H-3′ and C-1′; H-4′ and C-4a′;



1 $\Delta^{1',2'}, \Delta^{3',4'}$

2 $N_{2'}$-H



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10.1021/np990255u CCC: $18.00
Published on Web 08/27/1999

Table 1. Cytotoxic Activity of Alkaloids **1**−**3**[a,b]

compound	BC1	Lu1	Col2	KB	KB−V$^+$	KB−V$^-$	LNCaP	SW626	SKNSH	M109
1	3.9	3.6	2.8	2.2	9.0	8.8	6.8	6.1	4.0	4.4
2	0.1	<0.001	0.05	<0.16	0.1	0.1	<0.16	0.22	0.1	0.1
3	3.4	5.7	4.5	2.8	9.0	9.4	2.3	13.3	4.0	9.0

[a] Results are expressed as ED_{50} values (μg/mL). [b] Key: BC1 = human breast cancer; Lu1 = human lung cancer; Col2 = human colon cancer; KB = human epidermoid carcinoma of the nasopharynx; KB−V$^+$ = multidrug-resistant KB assessed in the presence of vinblastine (1 μg/mL); KB−V$^-$ = multidrug-resistant KB assessed in the absence of vinblastine; LNCaP = hormone-dependent human prostate cancer; SW626 = human ovarian cancer; SKNSH = human neuroblastoma cancer, M109 = mouse lung cancer.

H-5′, C-5a′, and C-6′; H-7′, C-6′, and C-8′; and H-8′, C-7′, and C-8a′. These data suggested that the side-chain moiety was a carboline unit. Therefore, the structure of **1** was characterized as 1′,2′,3′,4′-tetradehydrotubulosine.

Alkaloids **1**−**3** were evaluated with a panel of human tumor cell lines (Table 1). The most potent activity was observed by tubulosine (**2**), with the Lu1 (lung cancer, ED_{50} < 0.001 μg/mL) cell line being particularly susceptible. 1′,2′,3′,4′-Tetradehydrotubulosine (**1**) and psychotrine (**3**) were only weakly cytotoxic in comparison to tubulosine (**2**). Accordingly, the presence of a pyridine ring in the carboline unit, as in **1**, led to a reduction in the observed cytotoxicity of **2** by about two orders of magnitude. The cytotoxicity results of **2** and **3** were in good agreement with earlier values reported in the literature.[4] In a recent study, structural requirements were discussed for the biological activity of emetine-type alkaloids, including the importance of the bond angles between C-9 and N-5 and between N-2′ and N-5 for the inhibition of protein synthesis.[20] The present bioassay results are consistent with these observations because the configuration at position C-1′ and protonation of N-2′ could affect the preferred bond angles. In the future, 1′,2′,3′,4′-tetradehydrotubulosine (**1**) could be a useful negative control for biological experiments involving the potent cytotoxin tubulosine (**2**).

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus, and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. IR spectra were taken on a Nicolet MX-1 FT-IR spectrophotometer. ^{1}H NMR, ^{13}C NMR, and ^{1}H−^{1}H COSY (including APT) spectra were measured on a Varian XL-300 instrument operating at 300 and 75.6 MHz, respectively. Compounds were analyzed in $CDCl_3$, with tetramethylsilane (TMS) as internal standard. A General Electric Omega 500 NMR spectrometer, operating at 499.9 MHz, was used to perform HMQC and HMBC experiments. ^{13}C NMR multiplicity was determined using APT and DEPT experiments. The DEPT experiment was conducted on a Nicolet NMC-360 instrument, operating at 90.8 MHz for the determination of ^{13}C. EIMS and HREIMS were recorded on a Finnigan MAT-90 instrument. Silica gel (Merck 60A, 70−230 mesh ASTM) and neutral alumina (Brockman Activity 1, 80−200 mesh, Fisher, Pittsburgh, PA) were used for column chromatography.

Plant Material. The stems of *P. speciosus* were collected in a tropical rain forest, at Chilibre, Panama in November 1991. Voucher specimens representing this collection have been deposited at the Field Museum of Natural History, Chicago, IL (No. A3107) and the University of Panama, Panama, Republic of Panama (No. Florpan 949).

Extraction and Isolation. The dried stems of *P. speciosus* (1.3 kg) were extracted three times with MeOH (3 × 3 L) at room temperature, and the solution was evaporated *in vacuo*. The dried MeOH extract (35.3 g) was treated with aqueous HCl (3%) and extracted with $CHCl_3$ (3 × 300 mL). The acidic solution was then basified with K_2CO_3 and extracted with $CHCl_3$ (3 × 300 mL). Evaporation of the organic solvent under reduced pressure led to a crude alkaloid fraction (8.7 g). In the cytotoxicity assay, this alkaloid fraction showed an IC_{50} value of 0.015 μg/mL against KB cells in culture. The alkaloid fraction was subjected to silica gel column chromatography, and eluted using mixtures of $CHCl_3$−MeOH (50:1→4:1) to give three fractions. Fraction 2 was active in the KB cytotoxicity assay (IC_{50} < 0.1 μg/mL). Additional chromatographic separation of active fraction 2 over alumina using 15% MeOH in $CHCl_3$ as solvent yielded three subfractions (2A−2C). After a series of further chromatographic purification steps on subfraction 2A over silica gel, using mixtures of $CHCl_3$−MeOH (50:1→9:1) for elution, alkaloids **1**−**3** were obtained in crude form. Alkaloid **1** (5 mg, 0.014% w/w) was finally purified by silica gel column chromatography eluted with $CHCl_3$−MeOH (24:1), while **2** (18 mg, 0.051% w/w) was finally purified using Sephadex LH-20 column chromatography eluted with MeOH−H_2O (9:1) followed by recrystallization from MeOH. Alkaloid **3** (12 mg, 0.034% w/w) was purified by silica gel column chromatography ($CHCl_3$−MeOH, 19:1) and preparative TLC (silica gel plate, 0.25 mm layer, $CHCl_3$−MeOH, 17:3, R_f 0.5).

1′,2′,3′,4′-Tetradehydrotubulosine (1). Amorphous powder; $[\alpha]_D$ −20.4° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.13), 231 (2.88), 296 (2.60), 367 (2.20) nm; IR (NaCl) ν_{max} 3278, 2958, 1604, 1518 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.20 (1H, d, J = 5.4 Hz, H-3′), 7.87 (1H, d, J = 5.4 Hz, H-4′), 7.51 (1H, d, J = 2.3 Hz, H-5′), 7.41 (1H, d, J = 8.8 Hz, H-8′), 7.10 (1H, dd, J = 8.8, 2.3 Hz, H-7′), 6.59 (1H, s, H-8), 6.06 (1H, s, H-11), 3.71 (3H, s, OMe-10), 3.49 (1H, dd, J = 13.3, 3.8 Hz, H-12), 3.17 (3H, s, OMe-9), 3.14−3.00 (4H, m, overlapped, H-4, 6, 7, 11b), 2.93 (1H, dd, J = 13.3, 9.6 Hz, H-12), 2.67 (1H, m, H-7), 2.56 (1H, m, H-6), 2.20 (1H, dd, J = 11.6, 11.6 Hz, H-4), 2.00−1.90 (3H, m, overlapped, H-1, 2, 13), 1.68 (1H, m, H-3), 1.41−1.28 (3H, m, overlapped, H-1, 13), 1.01 (3H, t, J = 7.4 Hz, H-14); ^{13}C NMR (CD_3OD, 75.6 Hz) δ 152.4 (s, C-6′), 149.2 (s, C-1′), 148.6 (s, C-10), 145.9 (s, C-9), 137.7 (s, C-9′a), 137.5 (s, C-8′a), 137.1 (d, C-3′), 129.8 (s, C-11a), 127.1 (s, C-8a), 123.2 (s, C-4′a), 119.7 (d, C-9′), 114.2 (d, C-4′), 113.5 (d, C-10′), 112.8 (d, C-9), 109.0 (d, C-12), 106.7 (d, C-6′), 63.6 (d, C-11b), 61.6 (t, C-4), 56.3 (q, OMe-10), 55.8 (q, OMe-9), 53.4 (t, C-6), 43.1 (d, C-3), 42.8 (d, C-2), 37.9 (t, C-12), 37.2 (t, C-1), 28.9 (t, C-7), 24.5 (t, C-13), 11.5 (q, C-14); EIMS *m*/*z* [M]$^+$ 471 (74), 287 (15), 273 (63), 272 (88), 271 (6), 258 (47), 246 (11), 244 (46), 205 (20), 198 (100), 192 (14), 191 (31), 190 (19); HREIMS *m*/*z* 471.2503 (calcd for $C_{29}H_{33}N_3O_3$, 471.2521).

Tubulosine (2): needles (MeOH); mp 257−259 °C (lit.[19] 259−261 °C); $[\alpha]_D$ −46.9° (*c* 0.3, pyridine) [lit.[4] −58.8° (*c* 0.2, pyridine)], and UV, IR, ^{1}H NMR, ^{13}C NMR, and EIMS consistent with literature values.[4]

Psychotrine (3): yellow needles (MeOH); mp 119−120 °C (lit.[4] 122 °C); $[\alpha]_D$ +50.2° (*c* 0.2, MeOH) [lit.[4] +70° (*c* 0.36, MeOH)], and UV, IR, ^{1}H NMR, ^{13}C NMR, and EIMS consistent with literature values.[4]

Cytotoxic Evaluation. Alkaloids **1**−**3** were evaluated for cytotoxicity against a panel of human cancer cell lines, according to established protocols.[21] ED_{50} values of >5 μg/mL are regarded as inactive.

Acknowledgment. This investigation was supported by Grant U19-CA-52956, funded by the National Cancer Institute, NIH, Bethesda, MD. We thank Mr. R. B. Dvorak of the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, for recording the mass spectral data, and the Nuclear Magnetic

Resonance Laboratory of the Research Resources Center, University of Illinois at Chicago, for the provision of certain NMR spectral facilities used in this investigation.

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NP990255U