Jineol, a Cytotoxic Alkaloid from the Centipede *Scolopendra subspinipes*

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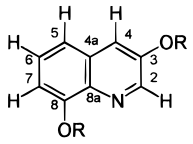
Jineol (**1**), a new quinoline alkaloid, was isolated from the centipede *Scolopendra subspinipes*, and its structure was elucidated by 2D NMR experiments. Jineol exhibited modest cytotoxic activity *in vitro* against the growth of human tumor cell lines: A-549, SKOV-3, SK-Mel-2, XF-498, and HCT-15 cells.

The centipede *Scolopendra subspinipes mutilans* L. Koch (Scolopendridae) has been traditionally employed to treat spasm, childhood convulsion, seizure, poisonous nodules, and diphtheria.[1] Pulverized materials of the roasted dry centipede have been used as folk medicine to dissipate back pain or treat furuncles and sores in Korea. Despite the alleged medicinal usages, however, chemical investigations have been limited. The crude venom of the centipede was reported to be toxic in mice (LD_{50} 22.5 mg/kg)[2] and to induce platelet aggregation.[3] The hydrolyzed lipid portion of the centipede extract gave oleic, linoleic, linolenic, and palmitic acids.[4] Toxin-S from the tropical centipede *S. subspinipes dehaani* Brandt has been isolated by others as a cardiotoxic protein toxin (LD_{50} 41.7 μg/kg in male mice) that was acidic and heat labile, with a molecular weight of 60 000 Da.[5] In our search for bioactive compounds from folk medicines we found the ethanol extract of *S. subspinipes mutilans* showed strong cytotoxic activities against five human cancer cell lines. Here we present the isolation and characterization of cytotoxic jineol (**1**), a novel quinoline alkaloid possessing a 3-hydroxyl group.



1 R = H
2 R = Me
3 R = Ac

Jineol was isolated as an orange amorphous solid (mp 139−141 °C) from the ethanol extract of the live centipede by solvent partition followed by silica gel and sephadex LH-20 chromatography. The molecular formula $C_9H_7NO_2$ was established by HREIMS (M^+, m/z 161.0485, calcd 161.0477). The UV spectrum in MeOH showed maximum absorptions at 254 and 267 nm. The IR spectrum (KBr pellet) exhibited absorptions at 3370, 1595, 1562, 1355, 1313, and 1200 cm^{-1}. These observations suggest a hydroxyquinoline moiety. The ^{13}C NMR spectrum indicated the presence of nine aromatic carbon atoms: five methine and four quaternary. The methine protons were determined to be H-2, H-4, H-5, H-6, and H-7 protons by a combination of H,H-COSY and HMQC experiments. The methine proton (d, J = 2.5 Hz) at δ 8.44 was coupled to the methine proton (d, J = 2.5 Hz) at δ 7.41, which was weakly coupled to the methine proton at δ 7.14. The methine proton (d, J = 8.0 Hz) at δ 7.14 was strongly coupled to the methine proton (t, J = 8.0 Hz) at δ 7.29, which in turn was coupled to the methine proton (d, J = 8.0 Hz) at δ 6.85.

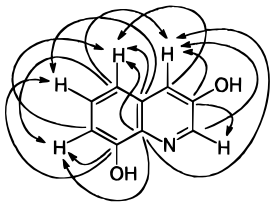


Figure 1. HMBC correlations of jineol (C → H).

Signal assignments for the remaining quaternary carbon atoms and their connectivities were unambiguously determined by HMBC and NOE experiments. In the HMBC experiments, the carbon signal (C-3) at δ 153.0 showed correlations with the proton signals (H-2, H-4) at δ 8.44 and 7.41, respectively, and the carbon signal (C-8) at δ 154.4 with the proton signals (H-6, H-7) at δ 7.29 and 6.85, respectively. Correlations were also shown between C-4a and both H-5 and H-6; C-8a and H-4, H-5, and H-7; C-4 and H-5; C-2 and H-4; and C-5 and H-4 (Figure 1). In addition, a significant NOE was observed between the H-4 and H-5 protons when either the H-4 or the H-5 proton was irradiated. These observations established the connectivities in the ring system.

Methylation of jineol (**1**) with diazomethane confirmed the positions of two hydroxyl groups. When the H-7 proton (dd, J = 7.9, 1.0 Hz) of **2** at δ 7.02 was irradiated, NOEs were observed on both the 8-methoxy protons at δ 4.02 and the H-6 proton (t, J = 7.9 Hz) at δ 7.46. The H-4 proton (d, J = 2.6 Hz) at δ 7.64 showed NOEs with the 3-methoxy protons at δ 3.95 and the H-5 proton (d, J = 2.6 Hz) at δ 8.48. The presence of two hydroxyl groups was further confirmed by acetylation of jineol (**1**). Two acetyl groups of **3** appeared at δ 2.44 and 2.38 in the ^1H NMR. Thus, jineol (**1**) was unambiguously determined to be 3,8-dihydroxyquinoline. It is worth noting that the two proton signals of H-4 and H-7 shift downfield by 0.75 and 1.0 ppm, respectively, upon acetylation, whereas the H-2 proton shifts downfield only by 0.25 ppm in the ^1H NMR.

Quinoline alkaloids bearing oxygen at the 3-position are rare in nature.[6] Recently, 3-hydroxyquinoline from

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Table 1. Cytotoxicity (ED_{50}, μg/mL) of **1**−**3** against Human Tumor Cell Lines

	cell line[a]				
compd	A-549	SKOV-3	SK-Mel-2	XF-498	HCT-15
1	5.8	4.5	5.6	10	1.9
2	38	32	21	29	42
3	8	14	11	12	8
carboplatin	22	13	10	13	47
cisplatin	1.0	1.0	0.7	0.4	1.4
adriamycin	0.05	0.11	0.05	0.1	1.8

[a] Cell lines: A-549, non-small cell lung cancer; SKOV-3, ovarian cancer; SK-Mel-2, melanoma; XF-498, central nervous system cancer; and HCT-15, colon cancer.

the aerial parts of *Ruta montana*[7] and an antitumor cyclic depsipeptide[8] having the 3-hydroxyquinoline moiety have been reported. 3-Hydroxyquinoline-2-carboxylate is known from *Streptomyces*[9] and was also found as a part of the antitumor antibiotic depsipeptide, sandramycin.[10,11] To our knowledge, jineol (**1**), 3,8-dihydroxyquinoline, is the first example of an atypical 3-hydroxyquinoline alkaloid with cytotoxic activity isolated from an animal.

Jineol is cytotoxic *in vitro* in several human tumor cellular models by the SRB assay.[12,13] ED_{50} values of jineol were 5.8, 4.5, 5.6, 10, and 1.9 μg/mL against A-549 non-small cell lung cancer, SKOV-3 ovarian cancer, SK-Mel-2 melanoma, XF-498 central nervous system cancer, and HCT-15 colon cancer cell lines, respectively. Methylated jineol (**2**) and acetylated jineol (**3**) were lower in toxicity, as compared to jineol (**1**) (Table 1). It is notable that the cytotoxicity against the XF-498 central nervous system cancer cell line was relatively unaffected by chemical modifications, whereas cytotoxicity against the HCT-15 colon cancer cell line decreased ca. 20 and 4 times by methylation and acetylation, respectively. Jineol also showed a strong inhibitory effect against *Trycophyton mentagrophytes* by an agar diffusion assay (MIC value was not determined). Other biological activities of jineol and derivatives are presently under evaluation.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Unity-500 spectrometer. The chemical shifts given in ppm (δ) were referenced to solvent peaks: δ_H 3.30 (residual CHD_2OD) and δ_C 49.0 for CD_3OD. Melting points were measured on an Electrothermal IA 9100 digital melting point apparatus and are uncorrected. High and low resolution mass spectra were obtained using a JEOL HX110A-HX110A Tandem HR mass spectrometer by electron impact or electron spray (ES). IR spectra were recorded on a Shimazu IR-408 infrared spectrophotometer. UV spectra were recorded in MeOH on a Hitachi U-3300 ultraviolet spectrophotometer. Analytical TLC was carried out on Merck plastic plates precoated with Si gel 60 F254 (0.2 mm layer thickness) and visualized by a UV lamp. Chromatography was performed on Merck Si gel 60 (70−230 mesh).

Animal Material. *Scolopendra subspinipes mutilans* L. Koch was collected in November 1992 in the mountainous areas near the Kongju National University, Kongju, Korea. The live centipede was immediately placed in EtOH and soaked in EtOH for 2 weeks.

Extraction and Isolation. The animals (3.1 g) were extracted with EtOH (200 mL × 2). The extract was concentrated and partitioned between hexane and aqueous MeOH. The MeOH phase was concentrated and partitioned between H_2O/EtOAc. The EtOAc phase was fractionated by Si gel chromatography with an elution gradient from 1:1 hexane/EtOAc to EtOAc, guided by the SRB assay[12,13] using five human cancer cell lines: A-549 non-small cell lung cancer, SKOV-3 ovarian cancer, SK-Mel-2 melanoma, XF-498 central nervous system cancer, and HCT-15 colon cancer. The active fractions (mainly EtOAc eluate) were further purified on sephadex LH-20. Elution with MeOH gave jineol (**1**) (2 mg, 0.07% of the animals) as a yellowish red solid: mp 139−141 °C; EIMS *m/z* 161 (M^+, 100), 133 (47), 104 (14); HREIMS *m/z* 161.0485 (M^+, $C_9H_7NO_2$ requires 161.0477); (−) ESMS *m/z* 160 $[M - H]^+$; IR (KBr) ν max 3370, 1595, 1562, 1355, 1313, 1200, 1087, 885, 743 cm^{-1}; UV (MeOH) λ max 254, 267 nm; ^1H NMR (CD_3OD, 500 MHz) δ 8.44 (1 H, d, J = 2.5 Hz, H-2), 7.41 (1 H, d, J = 2.5 Hz, H-4), 7.29 (1 H, t, J = 8.0 Hz, H-6), 7.14 (1 H, d, J = 8.0 Hz, H-5), 6.85 (1 H, d, J = 8.0 Hz, H-7); ^{13}C NMR (CD_3OD, 125 MHz) δ 154.4 (s, C-8), 153.0 (s, C-3), 142.2 (d, C-2), 134.9 (s, C-8a), 131.9 (s, C-4a), 129.1 (d, C-6), 117.7 (d, C-5), 117.2 (d, C-4), 109.1 (d, C-7); HMBC correlations: C-2 to H-4; C-3 to H-2, H-4; C-4 to H-5; C-4a to H-5, H-6; C-5 to H-4, H-7; C-7 to H-5; C-8 to H-6, H-7; C-8a to H-4, H-5, H-7.

Jineol Methyl Ether (2). To a solution of jineol (**1**) (4.5 mg) in Et_2O (2 mL) was added an ethereal solution of diazomethane that was generated from a reaction of Diazald (Aldrich Chemical Co.) with KOH. The reaction mixture was flushed with N_2 and chromatographed on Si gel with EtOAc to give jineol methyl ether (**2**) (4.2 mg, 80%) as an oil: UV (MeOH) λ max 219, 250 nm; ^1H NMR (CD_3OD, 500 MHz) δ 8.48 (1 H, d, J = 2.6 Hz, H-2), 7.64 (1 H, d, J = 2.6 Hz, H-4), 7.46 (1 H, t, J = 7.9 Hz, H-6), 7.38 (1 H, dd, J = 7.9, 1.0 Hz, H-5), 7.02 (1 H, dd, J = 7.9, 1.0 Hz, H-7), 4.02 (3 H, s, 8-OMe), 3.95 (3 H, s, 3-OMe).

Jineol Acetate (3). A solution of jineol (**1**) (5.0 mg) in acetic anhydride (0.5 mL) and pyridine (0.5 mL) was stirred at room temperature for 9 h. The reaction mixture was diluted with EtOAc, washed with saturated Na_2CO_3 solution and brine, and dried over anhydrous $MgSO_4$. Removal of solvent and Si gel chromatography (6:4 hexane/EtOAc to EtOAc) gave jineol acetate (**3**) (5.9 mg, 78%) as a white solid: mp 101−102 °C; UV (MeOH) λ max 212, 253 nm; ^1H NMR CD_3OD, 500 MHz) δ 8.69 (1 H, d, J = 2.6 Hz, H-2), 8.16 (1 H, d, J = 2.6 Hz, H-4), 7.85 (1 H, dd, J = 7.9, 1.5 Hz, H-7), 7.63 (1 H, t, J = 7.9 Hz, H-6), 7.48 (1 H, dd, J = 7.9, 1.5 Hz, H-5), 2.44 (3 H, s, 8-OAc), 2.38 (3 H, s, 3-OAc).

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