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A New Neolignan from *Cephalotaxus koreana*

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Cephalotaxus koreana Nakai (Cephalotaxaceae), commonly called Korean Plum Yew, is an upright, slow growing shrub with broad, relatively coarse, black-green needles, and found at low to middle elevations in Korea, northern and central Japan, and northeastern China.[1,2] Previous investigations for *C. koreana* have resulted in the isolation of flavonoids, biflavonoids,[3-7] and alkaloids.[8-10] The present study described the isolation of seven alkaloids, cephalotaxine (**2**),[11,12] 4-hydroxycephalotaxine (**3**),[13] desmethylcephalotaxinone (**4**),[14] deoxyharringtonine (**5**),[12] isoharringtonine (**6**),[12] harringtonine (**7**)[12] and homoharringtonine (**8**),[12] along with a new neolignan (**1**) and three phenylpropanoids, junipediol A (**9**),[15] junipediol B (**10**)[16] and junipediol B 8-*O*-β-D-glucopyranoside (**11**).[15] The structures of ten known compounds (**2**-**11**) were confirmed as shown in Fig. 1, by interpreting the measured 1D and 2D NMR spectroscopic data and comparing those data with the published values. All the isolates except compound **8** were isolated from this plant for the first time.

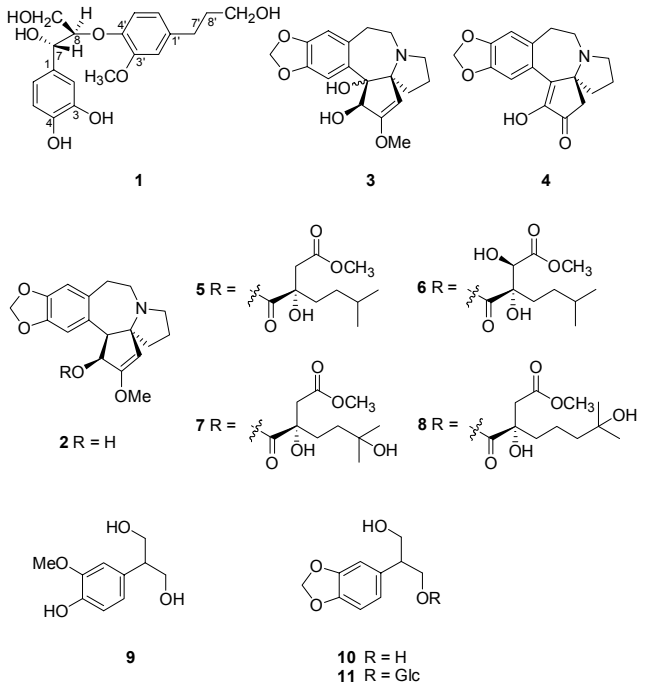

Figure 1. Alkaloids and phenylpropanoids from *Cephalotaxus koreana* Nakai.

Compound **1** was isolated as an amorphous powder, and its molecular formula was assigned as $C_{19}H_{24}O_7$ by the observed pseudomolecular ion peak at *m*/*z* 365.1598 $[M+H]^+$ in the HRFABMS. The ^{1}H NMR spectrum of **1** displayed resonances of two 1,3,4-trisubstituted benzene units [δ 6.99 (1H, d, J = 1.8 Hz, H-2), 6.74 (1H, d, J = 8.1, H-5), 6.83 (1H, dd, J = 8.1, 1.8 Hz, H-6), δ 6.65 (1H, d, J = 2.0 Hz, H-2'), 6.69 (1H, d, J = 8.2 Hz, H-5'), 6.52 (1H, dd, J = 8.2, 2.0 Hz, H-6'), four methylene protons [δ 2.53 (2H, t, J = 7.5 Hz, H-7'), 1.76 (2H, m, H-8'), 3.53 (2H, t, J = 6.5 Hz, H-9'), 3.72 (1H, dd, J = 11.8, 3.6 Hz, H-9b), 3.83 (1H, dd, J = 11.8, 6.6 Hz, H-9a)], two methine protons [δ 4.85 (1H, d, J = 5.3 Hz, H-7), 4.12 (1H, m, H-8)], and a methoxy group [δ 3.80 (3H, s, 3'-OCH_3)]. The ^{13}C NMR displayed nineteen carbon signals arising from two C_6-C_3 units and a methoxy carbon, indicating that **1** possessed lignan type skeleton. The ^{1}H-^{1}H COSY, HMQC, and HMBC data revealed two partial structures of Ar-CH-CH-CH_2-O- and Ar-CH_2-CH_2-CH_2-O-, and the HMBC correlations of both δ_H 4.12 (H-8) and 6.52 (H-6') to δ_C 146.5 (C-4') suggested that the chemical structure of **1** was a type of 8-*O*-4' type neolignan (Fig. 2). Furthermore, the observed long range correlations of δ_H 6.69 (H-5') and 3.80 (-OCH_3) to δ_C 150.3 (C-3') enabled to locate a methoxy group on C-3'. The small $J_{7\text{-}8}$ coupling constant (J = 5.3 Hz) in the ^{1}H NMR of **1** suggested C-7 and C-8 to be relatively *erythro* configuration, when compared with the coupling constants (*ca.* J = 8.0 Hz) of *threo* configuration.[17,18] The absolute configurations of C-7 and C-8 were confirmed, by a negative cotton effect at 239 nm in the CD spectrum, as being 7*S* and 8*R* configuration.[18,19] Therefore, **1** was determined to be 7*S*,8*R*-3,4,7,9,9'-pentahydroxy-3'-methoxy-8-*O*-4'-neolignan, and this compound was isolated for the first time as a natural product. Even though there is a paper covering the occurrence of dibenzylbutyrolactone type lignan from Cephalotaxaceae,[7] isolation of 8-*O*-4' type neolignan from this family is reported for the first time.

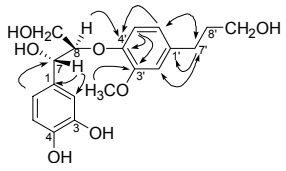

Figure 2. Key HMBC correlations of compound **1**.

Experimental Section

General Procedures.

Plant materials: The aerial parts (leaves and twigs) of *C. koreana* were collected in the Kwanak Arboretum, Seoul National University, located in Anyang-Si in August 2004, and were identified by Prof. Jong Hee Park, College of Pharmacy, Pusan National University. Voucher specimens (SNUPH-0821) have been deposited in the Medicinal Herb Garden, Seoul National University.

Extraction and isolation: The air-dried and milled leaves and twigs of *C. koreana* (1.2 kg) were extracted with methanol. The methanol extract (128 g) was partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH sequentially. The BuOH-soluble fraction (67 g) was subjected to silica gel column chromatography (CC) (230 - 400 mesh, 10 × 40 cm) using gradient elution of CH_2Cl_2-MeOH system (10:1 → MeOH) to afford ten fractions (A1-A10). Fractions F1-F4 (18 g) were rechromatographed on silica gel CC [230 - 400 mesh, 5 × 25 cm, CH_2Cl_2-MeOH (10:1)] to give twenty subfractions (B1-B20). Subfractions B4-B8 (1.6 g) were combined and subjected to reversed-phase HPLC [J'sphere ODS H80, 20 × 250 mm, 4 μm, 4 mL/min, MeCN-0.03M ammonium carbonate (50:50 → 80:20)] to give deoxyharringtonine (**5**, 21 mg). Subfractions A9-A16 (4.1 g) were combined and chromatographed on reversed-phase HPLC [J'sphere ODS H80, 20 × 250 mm, 4 μm, 4 mL/min, MeCN-0.03M ammonium carbonate (50:50 → 80:20)] to give isoharringtonine (**6**, 26 mg)**,** harringtonine (**7**, 12 mg) and homoharringtonine (**8**, 35 mg), respectively. Fractions A5-A7 (26 g) were subjected to Sephadex LH-20 CC (2.5 × 65 cm) with methanol to afford twenty five subfractions (C1-C25). Subfractions C6-C14 (5.2 g) were combined and rechromatographed on HPLC [J'sphere ODS H80, 20 × 250 mm, 4 μm, 4 mL/min, MeCN-0.03M ammonium carbonate (30:70 → 50:50)] to yield cephalotaxine (**2**, 24 mg), 4-hydroxycephalotaxine (**3**, 4 mg), and desmethylcephalotaxinone (**4**, 9 mg), respectively. Subfractions C16-C20 (3.8 g) were combined followed by HPLC chromatography [J'sphere ODS H80, 20 × 250 mm, 4 μm, 4 mL/min, MeCN-H_2O (20:80 → 35:65)] to give 7*S*,8*R*-3,4,7,9,9'-pentahydroxy-3'-methoxy-8-*O*-4'-neolignan (**1**, 5.6 mg), junipediol A (**9**, 6 mg), junipediol B (**10**, 14 mg) and junipediol B 8-*O*-β-D-glucopyranoside (**11**, 8 mg). The structures of compounds **2**-**11** were identified by combination of spectroscopic data (^{1}H and ^{13}C NMR, IR, UV, MS and optical rotation) and comparing with those of literature values (Fig. 1).

7*S*,8*R*-3,4,7,9,9'-pentahydroxy-3'-methoxy-8-*O*-4'-neolignan (**1**): amorphous powder; $[\alpha]_D^{24}$ 1.3 (*c* 0.33, MeOH); UV (MeOH): λ_{max} (log ε) 227 (1.79), 278 (1.41) nm; IR (neat) ν_{max} 3396, 2922, 1515, 1458, 1268 cm^{-1}; HRFABMS (positive ion mode): *m*/*z* 365.1598 $[M+H]^+$ (Calcd for $C_{19}H_{25}O_7$, 365.1600); CD (*c* = 0.006, MeOH): $[\theta]_{239}$ −17,233; ^{1}H-NMR (CD_3OD, 500 MHz) δ 6.99 (1H, d, *J* = 1.8 Hz, H-2), 6.83 (1H, dd, *J* = 8.1, 1.8 Hz, H-6), 6.74 (1H, d, *J* = 8.1 Hz, H-5), 6.69 (1H, d, *J* = 8.2 Hz, H-5'), 6.65 (1H, d, *J* = 2.0 Hz, H-2'), 6.52 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 4.85 (1H, d, *J* = 5.3 Hz, H-7), 4.12 (1H, m, H-8), 3.83 (1H, dd, *J* = 11.8, 6.6 Hz, H-9a), 3.80 (3H, s, 3'-OC*H*$_3$), 3.72 (1H, dd, *J* = 11.8, 3.6 Hz, H-9b), 3.53 (2H, t, *J* = 6.5 Hz, H-9'), 2.53 (2H, t, *J* = 7.5 Hz, H-7'), 1.76 (2H, m, H-8'); ^{13}C-NMR (CD_3OD, 125 MHz) δ 150.3 (C-3'), 149.6 (C-3), 147.9 (C-4), 146.5 (C-4'), 139.5 (C-1'), 134.6 (C-1), 121.6 (C-6), 121.4 (C-6'), 120.9 (C-5'), 118.0 (C-2'), 116.0 (C-5), 112.5 (C-2), 88.6 (C-8), 74.8 (C-7), 63.1 (C-9'), 62.8 (C-9), 57.1 (3'-OCH_3), 36.3 (C-8'), 33.3 (C-7').

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