Dihydrobenzofuran Norlignans from the Leaves of *Cedrela sinensis* A. JUSS

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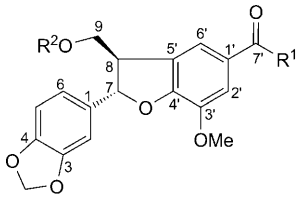
Two new norlignans, cedralins A (**1**) and B (**2**), were isolated from the leaves of *Cedrela sinensis*. Their structures were established by extensive spectroscopic studies and chemical evidence. The absolute configurations of these compounds were determined by comparing their CD spectra with those of known compounds. In addition, their *in vitro* cytotoxic activity against two human-cancer cell lines was evaluated.

Introduction. – *Cedrela sinensis* A. JUSS (Meliaceae) is a broadleaf tree widely distributed and cultivated in China and Korea. The leaves and stems of this plant have been used in oriental medicine for the treatment of itch, dysentery, and enteritis [1]. In addition, the powdered root has been used as a corrective, and the fruits have been used as an astringent and for the treatment of eye infection [2][3]. Recently, it was reported that the crude extract of *C. sinensis* can induce apoptosis of cancer cells [4], enhance lipolysis of differentiated 3T3-L1 adipocyte and its uptake of glucose [5][6], and inhibit Leydig cell steroidogenesis [7]. Previous phytochemical investigations carried out on this plant have resulted in the isolation of flavonoids, phenolics, alkaloids, terpenes, anthraquinones, and limonoids [8–11]. In our continuing phytochemical study on this plant, we have isolated and identified two new norlignans **1** and **2**. Herein, we report the isolation and structure elucidation of these compounds and their *in vitro* cytotoxic activity against two human-cancer cell lines.

Results and Discussion. – The MeOH extract of the leaves of *C. sinensis* was suspended in H_2O and successively partitioned with hexane, AcOEt, and BuOH. The AcOEt-soluble fraction was separated by a series of chromatographic techniques and led to the isolation of two new norlignans **1**[1]) and **2**[1]).

Compound **1** was obtained as an optically active white amorphous powder. The HR-FAB-MS of **1** exhibited a quasimolecular-ion peak at *m/z* 367.0792 ($[M+Na]^+$)

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*



1[1]) R^1 = OH, R^2 = H
2[1]) R^1 = MeO, R^2 = β-D-Glc

corresponding to the molecular formula $C_{18}H_{16}O_7$. The IR spectrum of **1** exhibited absorption bands for an OH group (3360 cm^{-1}), an α,β-unsaturated C=O group (1690 cm^{-1}), and aromatic rings (1610 and 1500 cm^{-1}). Its UV absorption maxima at 278 nm further supported the presence of aromatic-ring systems. The ^{1}H-NMR spectrum (*Table*) of **1** displayed two *meta*-coupled aromatic H-atoms at δ(H) 7.55 (*d*, J = 1.2 Hz, H–C(2′)) and 7.61 (*d*, J = 1.2 Hz, H–C(6′)), three aromatic H-atoms as an

Table. *^{1}H-* (300 MHz) *and ^{13}C-NMR* (75 MHz) *Data of Compounds* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1[a])		**2**[b])	
	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	135.8		136.0	
CH(2)	110.2	6.88 (*d*, J = 1.4)	109.1	6.89 (*d*, J = 1.5)
C(3)	148.1		148.7	
C(4)	149.5		149.1	
CH(5)	116.2	6.78 (*d*, J = 8.0)	116.1	6.75 (*d*, J = 8.0)
CH(6)	120.2	6.87 (*dd*, J = 1.4, 8.0)	120.4	6.87 (*dd*, J = 1.5, 8.0)
CH(7)	89.5	5.66 (*d*, J = 6.4)	89.9	5.74 (*d*, J = 6.5)
CH(8)	54.9	3.54 (*ddd*, J= 5.2, 5.2, 6.4)	52.6	3.67 (*ddd*, J = 5.2, 5.2, 6.5)
CH_2(9)	64.7	3.74 (*dd*, J = 5.2, 10.2), 3.84 (*dd*, J = 5.2, 10.2)	71.9	3.79 (*dd*, J = 5.2, 10.2), 4.22 (*dd*, J = 5.2, 10.2)
C(1′)	125.4		124.5	
CH(2′)	115.1	7.55 (*d*, J = 1.2)	114.9	7.52 (*d*, J = 1.1)
C(3′)	145.2		145.1	
C(4′)	153.9		153.6	
C(5′)	130.2		129.5	
CH(6′)	119.8	7.61 (*d*, J = 1.2)	120.7	7.62 (*d*, J = 1.1)
C(7′)	170.2		168.4	
OCH_2O	102.0	5.92 (*s*)	102.2	5.91 (*s*)
MeO–C(3′)	56.5	3.90 (*s*)	56.7	3.91 (*s*)
MeO–C(7′)			52.4	3.86 (*s*)
β-D-Glc:				
CH(1″)			104.3	4.34 (*d*, J = 8.0)
CH(2″)			74.7	3.24–3.38 (*m*)
CH(3″)			77.9	3.24–3.38 (*m*)
CH(4″)			71.3	3.24–3.38 (*m*)
CH(5″)			77.6	3.24–3.38 (*m*)
CH_2(6″)			62.7	3.71 (*dd* = 5.5, 12.1), 3.88 (*dd* = 2.0, 12.1)

[a]) Measured in $CDCl_3$. [b]) Measured in $CDCl_3$ + MeOD.

ABX system at δ(H) 6.78 (*d*, $J = 8.0$ Hz, H–C(5)), 6.88 (*d*, $J = 1.4$ Hz, H–C(2)), and 6.87 (*dd*, $J = 1.4, 8.0$ Hz, H–C(6)). The H-atoms at δ(H) 5.66 (*d*, $J = 6.4$ Hz), 3.54 (*ddd*, $J = 5.2, 5.2, 6.4$ Hz), and 3.74 and 3.84 (*dd*, $J = 5.2, 10.2$ Hz, each 1 H) were attributed to an aliphatic CH–CH–CH_2 moiety on the basis of ^{1}H,^{1}H-COSY data. In addition, the ^{1}H-NMR spectrum suggested the presence of an O–CH_2–O group due to the CH_2 signal at δ(H) 5.92 (*s*), which correlated with the quaternary C-atom signals at δ(C) 148.1 (C(3)) and 149.5 (C(4)) in the HMBC spectrum (*Figure*), indicating the position of the O–CH_2–O moiety between C(3) and C(4). The above data suggested **1** to be a dihydrobenzofuran-type norlignan, when compared with that of perseal C (=(2*S*,3*R*)-2-(1,3-benzodioxol-5-yl)-2,3-dihydro-3-(hydroxymethyl)-7-methoxybenzofuran-5-carboxaldehyde) isolated from *Persea obovatifolia* [12]. Furthermore, the ^{1}H-NMR spectrum of **1** was closely similar to that of perseal C, except for the absence of a H-atom signal (δ(H) 9.84) corresponding to a CHO group in perseal C. This proposed skeleton was further supported by the ^{13}C-NMR spectrum, which revealed two characteristic C-atom signals at δ(C) 89.5 (C(7)) (δ(H) 5.66 (*d*, $J = 6.4$ Hz)) and 54.9 (C(8)) (δ(H) 3.54 (*ddd*, $J = 5.2, 5.2, 6.4$ Hz)) for a dihydrobenzofuran-type norlignan. The large coupling constant ($J = 6.4$ Hz) between H–C(7) and H–C(8) indicated a *trans*-vicinal coupling of these H-atoms at the dihydrobenzofuran ring [13]. This was further confirmed by the observation of the cross-peaks H–C(7)/H_b–C(9) and H–C(8)/H–C(2) in the NOESY plot. The absolute configuration at C(7) and C(8) was determined to be (7*S*,8*R*) from the circular dichroism (CD) spectrum showing a negative *Cotton* effect around 278 nm, which was compared with those of reported dihydrobenzofuran lignans [14][15]. On the basis of the above data, compound **1** was established as (7*S*,8*R*)-7,8-dihydro-8-(hydroxymethyl)-3′-methoxy-7-[3,4-(methylenedioxy)phenyl]benzofuran-1′-carboxylic acid[1]) and named cedralin A.

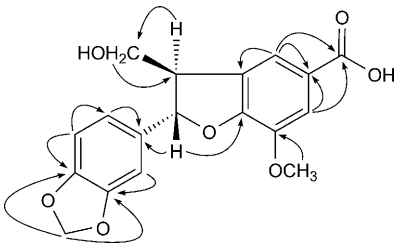


Figure. *Key HMBCs* (H → C) *of compound* **1**

Compound **2** was obtained as an optically active white amorphous powder, whose molecular formula, $C_{25}H_{28}O_{12}$, was determined by the observation of a quasimolecular-ion peak at *m*/*z* 543.1478 ([*M* + Na]$^+$) in the HR-FAB-MS. Its IR (1610 and 1500 cm^{-1}) and UV (280 nm) spectra indicated the presence of aromatic-ring systems. Acid hydrolysis of **2** yielded an aglycone and a monosaccharide unit. The ^{1}H-NMR spectrum (*Table*) of the aglycone part was similar to that of **1**, typical for a dihydrobenzofuran-type norlignan, except for an additional H-atom signal (δ(H) 3.86) corresponding to a Me ester group. In addition, two characteristic C-atom signals at δ(C) 89.9 (C(7)) (δ(H) 5.74 (*d*, $J = 6.5$ Hz)) and 52.6 (C(8)) (δ(H) 3.67 (*ddd*, $J = 5.2, 5.2, 6.5$ Hz)) in the

^{13}C-NMR spectrum of **2** (*Table*) further supported the presence of a dihydrobenzofuran moiety. The C-atom signals at δ(C) 104.3, 77.9, 77.6, 74.7, 71.3, and 62.7 and an anomeric H-atom signal at δ(H) 4.34 of **2** were typical for a glycopyranosyl unit, which was identified as D-glucose after acid hydrolysis of **2** followed by GC analysis. Moreover, the large coupling constant ($J = 8.0$ Hz) of the anomeric H-atom indicated that the glycopyranosyl unit was linked in β-configuration. When the ^{13}C-NMR spectrum of **2** was compared with that of **1**, a downfield shift of 7.2 ppm was observed for C(9), indicating that the glycopyranosyl linkage was at the OH–C(9) group of the aglycone, which was further confirmed by the observation of the HMBC cross-peak between the anomeric H-atom signal at δ(H) 4.34 and the aglycone C-atom signal at δ(C) 71.9 (C(9)). The large coupling constant ($J = 6.5$ Hz) between H–C(7) and H–C(8) was consistent with a norlignan of the *trans*-dihydrobenzofuran type, as found in **1**. The negative *Cotton* effect observed at 280 nm in the CD spectrum indicated that **2** has the same configuration as **1**. Thus, compound **2** was determined as (7*S*,8*R*)-8-[(β-D-glucopyranosyloxy)methyl]-7,8-dihydro-3′-methoxy-7-[3,4-(methylenedioxy)phenyl]-benzofuran-1′-carboxylic acid methyl ester[1]) and named cedralin B.

Previous phytochemical studies of the constituents of *C. sinensis* led to the discovery of many compounds, including flavonoids, phenolics, alkaloids, terpenes, anthraquinones, and limonoids [8–11]. However, there has been no report on the isolation of lignans from this plant to date. To the best of our knowledge, this is the first report of the occurrence of lignans from *C. sinensis* and assumes taxonomic significance.

The isolates **1** and **2** were evaluated for their *in vitro* cytotoxic activity against the two cancer cell lines HL-60 (human leukemia) and K562 (human leukemia) by the MTT-assay method. Cedralin A (**1**) exhibited a weak cytotoxic activity against the HL-60 and K562 cancer cell lines with IC_{50} values of 26.2 ± 1.2 and 22.4 ± 1.4 μg/ml, respectively, whereas cedralin B (**2**) bearing a sugar moiety did not show any significant activity against both cancer cell lines, even at the concentration of 30 μg/ml.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 230–400 mesh; *Merck*); *Sephadex LH-20* (25–100 μm; *Sigma–Aldrich*). Gas Chromatography (GC): *GC-2010* (*Shimadzu*) instrument; FID detector; *TC-1* capillary column (0.25 mm × 30 m; *GL Science, Inc.*); column temp. 230°; programmed increase of 38°/min; carrier gas N_2 (1 ml/min); injection and detector temp. 270°. Optical rotations: *Jasco DIP-370* polarimeter. UV Spectra: *Jasco V-550* UV/VIS spectrometer; λ_{max} (log ε) in nm. CD Spectra: *Jasco J-715* spectrometer; λ ([θ]) in nm. IR Spectra: *Jasco 100* IR spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker DRX-300* or *Bruker Avance-500* spectrometer; δ in ppm rel. to Me_4Si as an internal standard, *J* in Hz. FAB-MS and HR-FAB-MS: *Jeol JMS-DX-300* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The leaves of *C. sinensis* were collected in Daejeon, Korea, in October 2005, and identified by Prof. *K.-H. B.* (Chungnam National University). A voucher specimen (No. CNU-1269) has been deposited with the herbarium at the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Extraction and Isolation. The dried leaves of *C. sinensis* (6.0 kg) were extracted three times with MeOH (3 × 50 l) at r.t. for 7 d, the extracts filtered, and the filtrates concentrated to give a MeOH extract (526.0 g). The MeOH extract was suspended in H_2O (2 l) and then partitioned successively with hexane (3 × 2 l), AcOEt (3 × 2 l), and BuOH (3 × 2 l) to afford hexane- (170 g), AcOEt- (90 g), and BuOH-soluble fractions (150 g), resp. The AcOEt-soluble fraction (90 g) was subjected to CC (SiO_2, $CHCl_3$/

MeOH 100:1 → 1:1): *Fractions A–F. Fr. B* was resubjected to CC (SiO_2, $CHCl_3$/MeOH 50:1 → 40:1): **1** (7 mg). *Fr. D* was further subjected to CC (SiO_2, $CHCl_3$/MeOH 20:1 → 2:1): *Frs. D1–D3*. *Fr. D2* was subjected to CC (*Sephadex LH-20*, 60% aq. MeOH): **2** (15 mg).

Cedralin A (=*(2*S*,3*R*)-2-(1,3-Benzodioxol-5-yl)-2,3-dihydro-3-(hydroxymethyl)-7-methoxybenzofuran-5-carboxylic Acid*; **1**): White amorphous powder. $[\alpha]_D^{25} = -32.0$ ($c = 0.2$, $CHCl_3$). UV (MeOH): 278 (3.42). CD ($c = 0.0078$M, MeOH): 278 (−7735.2). IR: 3360, 2940, 1690, 1610, 1500, 1440, 1035. ^{1}H- and ^{13}C-NMR: *Table*. HR-FAB-MS: 367.0792 ($[M+Na]^+$, $C_{18}H_{16}NaO_7^+$; calc. 367.0794).

Cedralin B (=*(2*S*,3*R*)-2-(1,3-Benzodioxol-5-yl)-3-[(β-*D*-glucopyranosyloxy)methyl]-2,3-dihydro-7-methoxybenzofuran-5-carboxylic Acid Methyl Ester*; **2**): White amorphous powder. $[\alpha]_D^{25} = -6.7$ ($c = 0.2$, MeOH). UV (MeOH): 280 (3.22). CD ($c = 0.0057$M, MeOH): 280 (−5528.9). IR: 3300, 2940, 1695, 1610, 1500, 1435, 1320, 1205, 1040. ^{1}H- and ^{13}C-NMR: *Table*. HR-FAB-MS: 543.1478 ($[M+Na]^+$, $C_{25}H_{28}NaO_{12}^+$; calc. 543.1478).

Acid Hydrolysis. A soln. of **2** (5 mg) in 10% aq. HCl soln. was refluxed for 3 h. On cooling, the mixture was extracted with AcOEt. The aq. phase was neutralized with Na_2CO_3 and concentrated. The sugars were identified as glucose by co-TLC with an authentic sample by using the standard method [16].

Determination of the Absolute Configuration. The soln. of the sugar residue of **2** in pyridine (1.5 ml) was added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60° for 1 h. Then 1-(trimethylsilyl)-1*H*-imidazole (1.5 ml) was added and the mixture kept at 60° for 30 min. The supernatant (4 ml) was analyzed by GC as described previously [17]. The sugar derivative thus obtained had a t_R of 21.30, identical with that of the derivative of authentic D-glucose.

Cytotoxicity Assay. The human-cancer cell lines used in this study were purchased from *KCLB* (*Korean Cell Line Bank*). The cytotoxic activity was determined against the HL-60 (human leukemia) and K562 (human leukemia) cancer cell lines with the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay method. The cytotoxicity assay was performed according to a published procedure [18]. Adriamycin was used as a positive control and exhibited IC_{50} values of 2.8 ± 0.3 and 1.8 ± 0.2 μg/ml against the HL-60 ad K562 cancer cell lines, resp.

REFERENCES

[1] T.-B. Lee, 'Illustrated Flora of Korea', Hyangmunsa, Seoul, 1985, p. 506.
[2] G. A. Stuart, 'Chinese Material Medica: Vegetable Kingdom', American Presbyterian Mission Press, Shanghai, 1911, p. 100.
[3] L. M. Perry, 'Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses', MIT Press, Cambridge (Massachusetts) – London, 1980, p. 263.
[4] H.-C. Chang, W.-C. Hung, M.-S. Huang, H.-K. Hsu, *Am. J. Chin. Med.* **2002**, *30*, 307.
[5] H.-K. Hsu, Y.-C. Yang, J.-H. Hwang, S.-J. Hong, *Kaohsiung J. Med. Sci.* **2003**, *19*, 385.
[6] Y.-C. Yang, J.-H. Hwang, S.-J. Hong, H.-K. Hsu, *Kaohsiung J. Med. Sci.* **2003**, *19*, 327.
[7] S. L. Poon, S.-F. Leu, H.-K. Hsu, M.-Y. Liu, B.-M. Huang, *Life Sci.* **2005**, *76*, 1473.
[8] T.-S. Chen, Z.-P. Luo, H.-A. Cui, X.-Q. Zhen, Z.-Z. Liu, *Shanxi For. Sci. Technol.* **2000**, *20*, 1.
[9] X.-D. Luo, S.-H. Wu, Y.-B. Ma, D.-G. Wu, *Fitoterapia* **2000**, *71*, 492.
[10] X.-D. Luo, S.-H. Wu, Y.-B. Ma, D.-G. Wu, *Chin. Trad. Herbal Drugs* **2001**, *32*, 390.
[11] K. Mitsui, M. Maejima, H. Saito, H. Fukaya, Y. Hitotsuyanagi, K. Takeya, *Tetrahedron* **2005**, *61*, 10569.
[12] I.-L. Tsai, C.-F. Hsieh, C.-Y. Duh, *Phytochemistry* **1998**, *48*, 1371.
[13] K. Ito, K. Ichino, T. Iida, J. Lai, *Phytochemistry* **1984**, *23*, 2643.
[14] O. A. Lima, O. R. Gottlieb, M. T. Magalhães, *Phytochemistry* **1972**, *11*, 2031.
[15] H.-B. Wang, D.-Q. Yu, X.-T. Liang, N. Watanabe, M. Tamai, S. Omura, *J. Nat. Prod.* **1992**, *55*, 214.
[16] E. Bedir, I. A. Khan, *J. Nat. Prod.* **2000**, *63*, 1699.
[17] B.-S. Min, N. Nakamura, H. Miyashiro, Y.-H. Kim, M. Hattori, *Chem. Pharm. Bull.* **2000**, *48*, 194.
[18] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.

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