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Lignans from the Stem Barks of *Kalopanax septemlobus*

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Abstract – Four lignans were isolated from the CH_2Cl_2-soluble fraction of the stem barks of *Kalopanax septemlobus* and their structures were established as (–)-7*R*,8*S*-dehydrodiconiferyl alcohol (**1**), (–)-simulanol (**2**), (–)-secoisolariciresinol (**3**), and (±)-liriodendrin (**4**) based on the spectroscopic methods including MS, ^{1}H- and ^{13}C-NMR spectral data.

Keywords – *Kalopanax septemlobus*, Araliacaea, (–)-7*R*,8*S*-dehydrodiconiferyl alcohol, (–)-simulanol, (–)-secoisolariciresinol, (±)-liriodendrin

Introduction

Kalopanax septemlobus (Thunb.) Koidz [syn. *Kalopanx pictus* (Thunb.) Nakai], a deciduous tree of the family Araliaceae, is mainly distributed in Korea, China, and Japan. The stem bark of this plant, Kalopanacis Cortex, has been used in traditional medicine as an anti-rheumatic, anti-inflammatory, anti-diabetic, expectorant, and tranquilizer (Jung and Shin, 1990).

Several triterpenoidal saponins including kalopanaxsaponin A-K were previously isolated as main constituents of *K. septemlobus* (Shao *et al.*, 1989a, 1989b, 1990; Sano *et al.*, 1991; Porzel *et al.*, 1992; Kim *et al.*, 2002). Phenylpropanoid glycosides, flavonoids, simple phenolic glycosides, and lignan glycosides were also reported form the genus *Kalopanax* (Sano *et al.*, 1991; Jung *et al.*, 1992). Extensive biological studies have shown that the active constituents on anti-diabetic, cytotoxic, anti-fungal, anti-lipid peroxidation, and anti-inflammatory effects may be hederagenin monodesmoside (Park *et al.*, 1998; 2001; Kim *et al.*, 1998a; 1998b; 2002; Lee *et al.*, 2000; 2001; Choi *et al.*, 2001; Li *et al.*, 2002; 2003).

In the previous paper we reported the isolation and identification of a bisbenzopyran and a neolignan such as 3,3'-bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) and (–)-balanophonin from *K. septemlobus* (Hong *et al.*, 2001). A lignan derivative, (–)-7*R*,8*S*-dihydrodehydrodiconiferyl alcohol, was also isolated from this plant as an inducer of neurite outgrowth in PC12 cells (Shin *et al.*, 2005).

In this paper, we report the isolation and structure determination of four known lignans. Of these, (–)-7*R*,8*S*-dehydrodiconiferyl alcohol (**1**), (–)-simulanol (**2**), and (–)-secoisolariciresinol (**3**) were not identified previously from *K. septemlobus*.

Experimental

Plant material – The stem barks of *K. septemlobus* were collected from Jinbu, Kangwon Province, Korea in August 2004 and identified by emeritus professor Kyong Soon Lee, a plant taxonomist at Chungbuk National University. A voucher specimen of this plant was deposited at the Herbarium of College of Pharmacy, Chungbuk National University (Korea).

General experimental procedures – Melting points were measured on Büchi model B-540 without correction. UV and IR spectra were obtained on a JASCO UV-550 and Perkin-Elmer model LE599 spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX 500 MHz NMR spectrometer using TMS as an internal standard. EI-MS and ESI-MS were recorded on a Hewlett-Packard MS 5989 and a Finnigan Navigator mass spectrometer, respectively. Open column chromatography was performed using a silica gel (Kieselgel 60, 70 - 230 mesh, Merck), Sephadex LH-20 (25 - 100 μm, Pharmacia), and Lichroprep RP-18 (40 - 63 μm, Merck). TLC was conducted on pre-coated silica gel 60 F_{254} plates (0.25 mm, Merck). Semi-preparative HPLC was performed on a Waters HPLC system equipped with three 515

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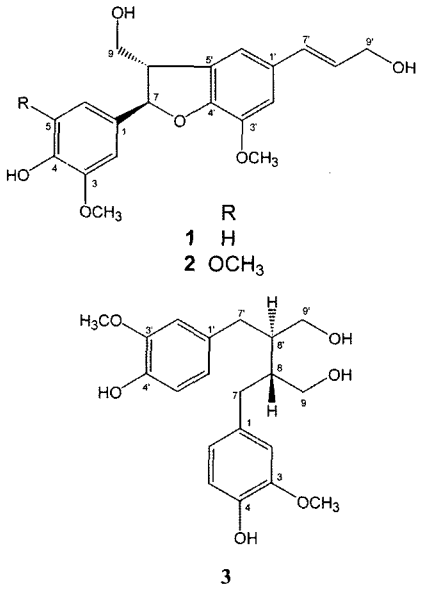

Fig. 1. Structures of compounds **1-3** from the stem barks of *K. septemlobus*.

model pumps and a 2996 photodiode array detector using an ODS column (YMC ODS-H80, 150 × 20 mm, 4 μm).

Extraction and isolation – The dried and powered stem barks of *K. septemlobus* (4.5 Kg) were extracted with 80% MeOH at room temperature. The methanolic extracts were evaporated *in vacuo* to give a dark-brown residue (520 g), which was suspended in water and then successively partitioned with CH_2Cl_2, BuOH, and water. The CH_2Cl_2 extract (62 g) was subjected to silica gel column chromatography eluted with CH_2Cl_2-MeOH (50 : 1, 20 : 1, 10 : 1, 5 : 1, 0 : 1) gradient system, to yield five fractions (KP1-KP5). Fraction KP2 was repeatedly chromatographed on a Sephadex LH-20 (MeOH) to afford five subfractions (KP21-KP25). Fraction KP22 was chromatographed on a silica gel column with CH_2Cl_2-acetone (5 : 1) and on a semi-preparative HPLC column (YMC ODS-H80, 150 × 20 mm, 4 μm) with acetonitrile-H_2O (23 : 77), to give compounds **1** (20.8 mg) and **2** (12.1 mg). Fraction KP3 was further chromatographed on Lichroprep RP-18 column with MeOH-H_2O (30 : 70) to give compound **3** (14.7 mg). Fraction KP5 was chromatographed on a silica gel column with CH_2Cl_2-MeOH-H_2O (70 : 20 : 1) to give compound **4** (4.5 mg).

(–)-7*R*,8*S*-dehydrodiconiferyl alcohol (1) – Pale yellow oil; $[\alpha]_D^{25}$: –15.6° (*c* 0.2, MeOH); EI-MS *m/z* 358 $[M]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.96 (1H, br s, H-6'), 6.94 (2H, br s, H-2, 2'), 6.82 (1H, br d, *J* = 8.1 Hz, H-5), 6.76 (1H, br d, *J* = 8.1 Hz, H-6), 6.53 (1H, d, *J* = 15.8 Hz, H-7'), 6.22 (1H, dt, *J* = 15.8, 5.8 Hz, H-8'), 5.51 (1H, d, *J* = 6.2 Hz, H-7), 4.19 (2H, d, *J* = 5.8 Hz, H-9'), 3.86 (3H, s, 3'-OMe), 3.84 (2H, m, H-9), 3.80 (3H, s, 3-OMe), 3.58 (1H, br q, *J* = 6.2 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD) δ: see Table 1.

Table 1. The ^{13}C-NMR data of compounds **1** and **2** (125 MHz, CD_3OD)

carbon	1	DEPT	2	DEPT
1	134.6	C	133.8	C
2	110.5	CH	104.2	CH
3	149.3	C	149.4	C
4	147.6	C	136.5	C
5	116.1	CH	149.4	C
6	119.7	CH	104.2	CH
7	89.3	CH	89.5	CH
8	55.2	CH	55.3	CH
9	64.9	CH_2	64.9	CH_2
1'	132.6	C	132.7	C
2'	112.1	CH	112.1	CH
3'	145.5	C	145.5	C
4'	149.1	C	149.2	C
5'	132.0	C	130.4	C
6'	116.5	CH	116.5	CH
7'	130.4	CH	132.0	CH
8'	127.5	CH	127.6	CH
9'	63.8	CH_2	63.9	CH_2
3-OMe	56.4	CH_3	56.8	CH_3
5-OMe	–	–	56.8	CH_3
3'-OMe	56.7	CH_3	56.8	CH_3

(–)-simulanol (2) – Colorless oil; $[\alpha]_D^{25}$: –13.7° (*c* 0.11, MeOH); EI-MS *m/z* 388 $[M]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.87 (1H, br s, H-2'), 6.86 (1H, br s, H-6'), 6.58 (2H, s, H-2, 6), 6.44 (1H, d, *J* = 15.8 Hz, H-7'), 6.13 (1H, dt, *J* = 15.8, 5.8 Hz, H-8'), 5.43 (1H, d, *J* = 6.3 Hz, H-7), 4.10 (2H, d, *J* = 5.6 Hz, H-9'), 3.79 (3H, s, 3'-OMe), 3.71 (6H, s, 3,5-OMe), 3.75 (1H, dd, *J* = 11.0, 5.5 Hz, H-9), 3.68 (1H, dd, *J* = 11.0, 5,5 Hz, H-9), 3.40 (1H, br q, *J* = 6.3 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD) δ: see Table 1.

(–)-secoisolariciresinol (3) – Colorless oil; $[\alpha]_D^{25}$: –16.8° (*c* 0.1, MeOH); EI-MS *m/z* 362 $[M]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.56 (2H, d, *J* = 8.0 Hz, H-5, 5'), 6.49 (2H, d, *J* = 2.0 Hz, H-2, 2'), 6.45 (2H, dd, *J* = 8.0, 2.0 Hz, H-6, 6'), 3.63 (6H, s, 3, 3'-OMe), 3.49 (4H, m, H-9, 9'), 2.56 (2H, dd, *J* = 13.7, 6.9 Hz, Hb-7, 7'), 2.46 (2H, dd, *J* = 13.7, 7.8 Hz, Ha-7, 7'), 1.80 (2H, m, H-8, 8'); ^{13}C-NMR (125 MHz, CD_3OD) δ: 133.9 (C-1, 1'), 113.4 (C-2, 2'), 148.8 (C-3, 3'), 145.5 (C-4, 4'), 115.8 (C-5, 5'), 122.7 (C-6, 6'), 36.0 (C-7, 7'), 44.1 (C-8, 8'), 62.1 (C-9, 9'), 56.2 (3, 3'-OMe).

(±)-liriodendrin (4) – White amorphous powder; $[\alpha]_D^{25}$: ± 0° (*c* 0.1, pyridine); ESI-MS *m/z* 765 $[M+Na]^+$; ^{1}H-

NMR (500 MHz, DMSO-d_6) δ: 6.66 (4H, s, H-2, 2', 6, 6'), 4.88 (2H, br d, J = 7.8 Hz, glc H-1 x 2), 4.67 (2H, d, J = 4.5 Hz, H-7, 7'), 4.29 (4H, m, H-9, 9'), 3.76 (12H, s, 3, 3', 5, 5'-OMe); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 152.6 (C-3, 3', 5, 5'), 137.1 (C-4, 4'), 133.7 (C-1, 1'), 104.2 (C-2, 2', 6, 6'), 102.6 (glc C-1, 1'), 85.1 (C-7, 7'), 77.2 (glc C-3, 3'), 77.1 (glc C-5, 5'), 74.1 (glc C-2, 2'), 71.3 (C-9, 9'), 69.9 (glc C-4, 4'), 60.9 (glc C-6, 6'), 56.4 (3, 3', 5, 5'-OMe), 53.6 (C-8, 8').

Results and Discussion

The repeated column chromatographic separation of the CH_2Cl_2-soluble fraction of *K. septemlobus* led to the isolation of (−)-7*R*,8*S*-dehydrodiconiferyl alcohol (**1**), (−)-simulanol (**2**), (−)-secoisolariciresinol (**3**), and (±)-liriodendrin (**4**). Of these, the isolation of (±)-liriodendrin (**4**) from this plant was already reported (Sano *et al.*, 1991).

Compound **1** was obtained as pale yellow oil. The EI-MS spectrum showed a molecular ion peak at *m/z* 358 corresponding to the molecular formula of $C_{20}H_{22}O_6$. The ^{1}H-NMR spectrum of **1** displayed the characteristic signals of a 1,3,4-trisubstituted and a 1,3,4,5-tetrasubstituted aromatic ring at δ_H 6.96 (1H, br s, H-6'), 6.94 (2H, br s, H-2, 2'), 6.82 (1H, br d, J = 8.1 Hz, H-5), and 6.76 (1H, br d, J = 8.1 Hz, H-6), a *trans*-3-hydroxy-1-prophenyl group at δ_H 6.53 (1H, d, J = 15.8 Hz, H-7'), 6.22 (1H, dt, J = 15.8, 5.8 Hz, H-8'), and 4.19 (2H, d, J = 5.8 Hz, H-9'), an oxymethine and a methine proton at δ_H 5.51 (1H, d, J = 6.2 Hz, H-7) and 3.58 (1H, br q, J = 6.2 Hz, H-8), an oxygenated methylene at δ_H 3.84 (2H, m, H-9), and two methoxy groups at δ_H 3.86 and 3.80 (each 3H, s, 3, 3'-OMe). The ^{13}C-NMR and DEPT spectra (Table 1) of **1** showed two methoxy carbons at δ_C 56.7 and 56.4), two oxygenated methylene carbons at δ_C 64.9 and 63.8, nine methine carbons at δ_C 130.4, 127.5, 119.7, 116.5, 116.1, 112.1, 110.5, 89.3, and 55.2, and seven quaternary carbons at δ_C 149.3, 149.1, 147.6, 145.5, 134.6, 132.6, and 132.0. All the above data suggested that compound **1** was dihydrobenzofuran-type neolignan comprising two phenylpropanoid units (Tan *et al.*, 1990; Yuen *et al.*, 1998). The relative configuration of C-7 and C-8 was determined as *trans* on the basis of the coupling constant (J = 6.2 Hz). The negative $[\alpha]_D^{25}$ value [−15.6° (*c* 0.2, MeOH)] and the chemical shifts of H-7 (5.51) and H-9 (3.58) confirmed a 7*R*,8*S*-configuration (Li *et al.*, 1997; Yuen *et al.*, 1998). Thus, the structure of **1** was determined as (−)-7*R*,8*S*-dehydrodiconiferyl alcohol, by comparison of its physicochemical and spectral data with those of literatures (Tan *et al.*, 1990; Yeo *et al.*, 2004).

Compound **2** was obtained as colorless oil, $[\alpha]_D^{25}$ −13.7° (*c* 0.11, MeOH). The EI-MS spectrum displayed the molecular ion peak at *m/z* 388 $[M]^+$, which showed the molecular formula to be $C_{21}H_{24}O_7$ and confirmed by ^{1}H-, ^{13}C-NMR, and DEPT data. The ^{1}H- and ^{13}C-NMR spectra of compound **2** were closely comparable to those of **1**, and suggested compound **2** is also a dihydrobenzofuran-type neolignan. Interpretation of ^{1}H- and ^{13}C-NMR data suggested that the presence of a symmetrical 1,3,4,5-tetrasubstituted aromatic ring in the molecule of **2** instead of 1,3,4-trisubstituted ring in compound **1**. The relative stereochemistry of C-7 and C-8 was also determined to be *trans* on the basis of coupling constant (J = 6.3 Hz) (Li *et al.*, 1997). The absolute configuration were confirmed to be 7*R* and 8*S* from the negative $[\alpha]_D^{25}$ value [−13.7° (*c* 0.11, MeOH)] (Yuen *et al.*, 1998; Yang *et al.*, 2002). Thus, the structure of **2** was determined as (−)-simulanol [7,8-dihydro-7-(4-hydroxy-3,5-dimethoxyphenyl)-4'-(9'-hydroxy-7'-propenyl)-3'-methoxy-8-benzofuranmethanol], by comparison of its physicochemical and spectral data with those of literature (Yang *et al.*, 2002).

Compound **3** was an optically active colorless oil, $[\alpha]_D^{25}$ −16.8° (*c* 0.1, MeOH). The molecular formula was determined to be $C_{20}H_{26}O_6$ on the basis of EI-MS spectrum (*m/z* 362 $[M]^+$). The ^{1}H-NMR spectrum showed the characteristic signal pattern of two 1,3,4-trisubsituted aromatic rings at δ_H 6.56 (2H, d, J = 8.0 Hz, H-5, 5'), 6.49 (2H, d, J = 2.0 Hz, H-2, 2'), and 6.45 (2H, dd, J = 8.0, 2.0 Hz, H-6, 6'), two aliphatic methine protons at δ_H 1.80 (2H, m, H-8, 8'), two oxygenated methylene protons at δ_H 3.49 (4H, m, H-9, 9'), two methoxy protons at δ_H 3.63 (6H, s, 3, 3'-OMe), and a pair of benzylic methylene protons at δ_H 2.46 (2H, dd, J = 13.7, 7.8 Hz) and 2.56 (2H, dd, J = 13.7, 6.9 Hz). The ^{13}C-NMR and DEPT spectra showed only ten carbon signals consisting of three quaternary carbons at δ_C 148.8, 145.5, and 133.9, a methoxy carbon at δ_C 56.2, two methylene carbons at δ_C 62.1 and 36.0, four methine carbons at δ_C 122.7, 115.8, 113.4, and 44.1. This suggested that compound **3** was a symmetrical structure. Thus, the structure of **3** was determined as (−)-secoisolariciresinol, since the negative optical rotation is diagnostic for (2*R*,3*R*)-secoisolariciresinol (Agrawal and Rastogi, 1982; Xie *et al.*, 2003). This is the first report on the isolation of compounds **1**, **2**, and **3** from *Kalopanax* species.

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