A New Diarylheptanoid Glycoside from the Stem Bark of *Alnus hirsuta* and Protective Effects of Diarylheptanoid Derivatives in Human HepG2 Cells

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To search for secondary metabolites of *Alnus hirsuta* (Betulaceae), various chromatographic separations of the ethyl acetate soluble fraction of the stem bark of *A. hirsuta* led to the isolation of a new diarylheptanoid glycoside, (3*R*)-1,7-bis-(4-dihydroxyphenyl)-3-heptanol 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-xylopyranoside (13) and twelve diarylheptanoid derivatives, namely, oregonin (1), rubranoside A (2), hirsutanonol 5-*O*-*β*-D-glucopyranoside (3), rubranoside B (4), rubranoside C (5), hirsutanonol (6), hirsutenone (7), (5*S*)-*O*-methylhirsutanonol (8), platyphylloside (9), platyphyllonol 5-*O*-*β*-D-xylopyranoside (10), aceroside VII (11) and platyphyllenone (12). Isolates were assessed for their hepatoprotective effects against *tert*-butylhydroperoxide (*t*-BHP)-induced toxicity in HepG2 cells. Of these isolates, compounds 1—8 showed significant hepatoprotective effects on *t*-BHP-induced damage to HepG2 cells, with 8 exhibiting the greatest protective effect (50.7±3.7% at a concentration of 10 μM).

Key words *Alnus hirsuta*; diarylheptanoid derivative; hepatoprotective effect; *tert*-butylhydroperoxide; HepG2 cell

Alnus hirsuta Turcz. (Betulaceae) is indigenously distributed in Korea, China, Japan, and Russia. *Alnus* species have been used in Oriental traditional medicine as remedies for fever, emorrhage, burn injuries, diarrhea, and alcoholism. Several triterpenes, tannins, flavonoids and diarylheptanoids have been isolated from these species.[1—7] Pharmacological studies on this natural medicine have shown that its extracts have anti-inflammatory, antitumor, antiobesity and antioxidative effects.[8—10]

tert-Butylhydroperoxide (*t*-BHP) is an organic hydroperoxidant that is metabolized to free radical intermediates, which can subsequently initiate lipid peroxidation, affect cellular integrity, and form covalent bonds with cellular molecules.[11] Furthermore, *t*-BHP causes leakage of lactate dehydrogenase (LDH) from hepatocytes.[12] Moreover, these effects are similar to those of oxidative stress in cells and/or tissues. Therefore, we investigated the hepatoprotective effects of natural products and their constituents by measuring their effects on the toxicity of *t*-BHP in human liver-derived HepG2 cells. The present study examined the hepatoprotective effects of the ethyl acetate (EtOAc) soluble fraction prepared from the stem bark of *A. hirsuta* on *t*-BHP-induced toxicity in HepG2 cells. We also isolated and attempted to identify phytochemical components in the EtOAc soluble fraction. The structure–activity relationships of the isolates are also discussed with respect to their hepatoprotective effects.

Results and Discussion

A bioassay-guided phytochemical investigation of the EtOAc soluble fraction of *Alnus hirsuta* led to the isolation of 13 diarylheptanoid derivatives (Fig. 1) by column chromatography and preparative HPLC. The structures of **1**—**12** were identified by comparing their physical and spectroscopic data with previously reported results, as follows, for oregonin (**1**),[13] rubranoside A (**2**),[14] hirsutanonol 5-*O*-*β*-D-glucopyranoside (**3**),[15] rubranoside B (**4**),[13] rubranoside C (**5**),[14] hirsutanonol (**6**), hirsutenone (**7**),[15] (5*S*)-*O*-methylhirsutanonol (**8**),[10] platyphylloside (**9**), platyphyllonol 5-*O*-*β*-D-xylopyranoside (**10**), aceroside VII (**11**)[16,17] and platyphyllenone (**12**).[18]

Compound **13** was isolated as a brown amorphous powder. The molecular formula of $C_{30}H_{42}O_{12}$ was supported by the presence of a quasi-molecular ion at *m*/*z* 593.2610 in a negative HR-FAB-MS spectrum. The ^{1}H- and ^{13}C-NMR spectra of **13** were similar to those of rubranoside C (**5**) except for the presence of signals corresponding to the phenyl moiety in **13**. The ^{1}H-NMR spectrum of **13** contained two 1,4-disubstituted aromatic protons at δ 6.95 (2H, d, J=8.3 Hz), 6.96 (2H, d, J=8.3 Hz), 6.66 (2H, d, J=8.3 Hz) and 6.67 (2H, d, J=8.3 Hz), and two β-pyranosyl units, in which anomeric proton signals appeared as a doublet at δ 4.55 (1H, d, J=7.8 Hz, glc-1), 4.27 (1H, d, J=7.5 Hz, xyl-1). The assignment of the sugar as a glucopyranosyl unit was supported by

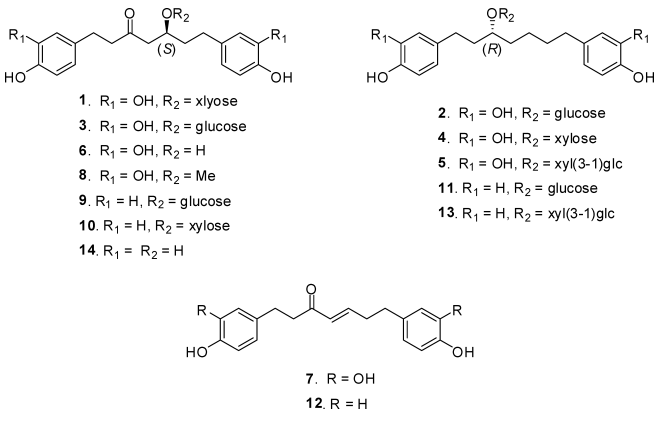

Fig. 1. Compounds **1**—**13** Isolated from *Alnus hirsuta*

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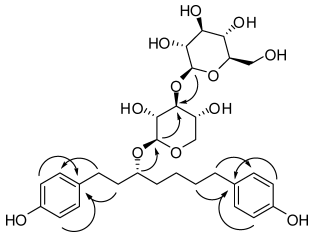

Fig. 2. Selected HMBC Correlation of **13**

observations of signals for oxygenated carbons at δ 104.9 (glc-1), 75.3 (glc-2), 77.9 (glc-3), 71.4 (glc-4), 77.6 (glc-5) and 62.5 (glc-6), and the presence of a xylopyranosyl unit was supported by signals for oxygenated carbons at δ 103.5 (xyl-1), 74.1 (xyl-2), 87.3 (xyl-3), 69.8 (xyl-4) and 66.1 (xyl-5).[14] In the heteronuclear multiple bond correlation (HMBC) spectrum, the signal at δ 4.55 due to the anomeric proton of glucose was found to be correlated with the signal at δ 87.3 (C-3) of xylose, indicating that the glucose was at C-3 of xylose. Furthermore, the signal at δ 4.27 (H-1) assigned to the anomeric proton of xylose was found to be correlated with the signal at δ 79.8 (C-3) (Fig. 2). These data showed that the xylose unit was located at C-3 of the aglycone. The absolute configuration of **13** was established by ^{13}C-NMR spectroscopy. By comparing the ^{13}C-NMR chemical shifts of the glycoside (**13**) with those of the aglycone,[17] it was found that the glycosylation shift at C-4 (-3.5 ppm) was larger than that at C-2 (-2.5 ppm) (Table 1). The application of the glycosylation shift rule led to the determination of an *R* configuration at C-3 of **13**.[19] The remaining issue was identification of the absolute configurations of the sugars. The optical rotation of **13** exhibited a negative value, which was the same direction as that of rubranoside C (**5**). The D-forms of the glucose and xylose in **13** were clearly indicated by its optical rotation. Compound **13** is a relatively simple diglycoside in which optical rotation may be considered as the contributions of two sugars as their methyl β-glycosides on the basis of Klyne's rule.[20,21] According to the molecular rotation calculations shown in Experimental, the absolute configuration of all sugars in **13** should be D-form, which is the common forms for glucose and xylose existing in nature. Based on these results, the structure of **13** was assigned to (3*R*)-1,7-bis-(4-dihydroxyphenyl)-3-heptanol 3-*O*-β-D-glucopyranosyl(1→3)-β-D-xylopyranoside, a novel (−)-centrolobol diglycoside.

All isolated compounds were tested with respect to their hepatoprotective effects on *t*-BHP-induced toxicity and cytotoxicity in a human hepatoma cell line (HepG2). Quercetin and silybin (a well-known hepatic antioxidant and chemopreventive agent, respectively) were used as positive controls.[22] The results shown in Table 2 are expressed as relative percent protection of HepG2 cell viabilities against *t*-BHP-induced toxicity at concentrations of 1, 10 and 100 μM. Among the 13 diarylheptanoid derivatives (**1**—**13**), (5*S*)-*O*-methylhirsutanol (**8**) exerted the greatest hepatoprotective effect in HepG2 cells (50.7±3.7%), which was comparable to the effects of quercetin (49.1±0.3%) at a concentration of 10 μM. However, quercetin was more effective (87.0±0.5%) than **8** at a concentration of 100 μM (67.4±3.0%). Compounds **1**—**3** and **6** exhibited moderate hepatoprotective effects against *t*-BHP-induced toxicity, with relative percent protection effects ranging from 42.8 to 53.5%. Compounds **4** and **7** at 100 μM also displayed moderate effects against *t*-BHP-induced toxicity with protection effect values of 37.1% and 37.7%, respec-

Table 1. ^{1}H- and ^{13}C-NMR Spectra of **13** and (−)-Centrolobol from *A. hirsuta* in Methanol-d_4

Carbon	**13**			(−)-Centrolobol
	δ_H	δ_C	δ_C *a)*	δ_C *b)*
1	2.54 (2H, m)	31.5	31.4	31.9
2	1.73 (2H, m)	38.0	38.0	40.7
3	3.63 (1H, overlapped)	79.8	79.0	70.3
4	1.53 (2H, overlapped)	34.7	34.7	38.4
5	1.39 (2H, m)	25.3	25.1	26.0
6	1.53 (2H, overlapped)	32.8	32.7	32.5
7	2.50 (2H, t, 7.4)	35.7	35.5	35.5
1′, 1″		134.5	133.7, 133.6	133.4, 133.7
2′, 2″	6.95, 6.96 (each 2H, d, 8.3)	130.0, 130.1	130.1, 130.2	129.8 ,129.9
3′, 3″	6.66, 6.67 (each 2H, d, 8.3)	115.8	116.3	116.1
4′, 4″		156.0	157.1	156.8
5′, 5″	6.66, 6.67 (each 2H, d, 8.3)	115.8	116.3	116.1
6′, 6″	6.95, 6.96 (each 2H, d, 8.3)	130.0, 130.1	130.1, 130.2	129.8 ,129.9
1‴	4.27 (1H, d, 7.5)	103.5	103.9	
2‴	3.37 (1H, overlapped)	74.1	74.0	
3‴	3.47 (1H, t, 8.6)	87.3	88.1	
4‴	3.62 (1H, overlapped)	69.8	69.8	
5‴	3.22 (1H, t, 11.0)	66.1	66.5	
	3.90 (1H, dd, 5.4, 11.3)			
1⁗	4.55 (1H, d, 7.8)	104.9	105.8	
2⁗	3.26 (1H, overlapped)	75.3	75.8	
3⁗	3.38 (1H, t, 9.0)	77.9	78.8	
4⁗	3.32 (1H, overlapped)	71.4	71.8	
5⁗	3.33 (1H, overlapped)	77.6	78.4	
6⁗	3.62 (1H, overlapped)	62.5	62.7	
	3.89 (1H, d, 11.9)			

a) Measured in pyridine-d_5. *b*) Reference data in pyridine-d_5.[17]

Table 2. Hepatoprotective Effects of Isolates (**1**—**13**) against *t*-BHP-Induced Toxicity in HepG2 Cells

Sample	Relative protection (%) *a)*		
	1 μM	10 μM	100 μM
Control	100±0.77	100±0.77	100±0.77
t-BHP-treated	0.0±1.0	0.0±1.0	0.0±1.0
Oregonin (**1**)	23.5±1.9*	39.8±0.3*	51.7±0.8*
Rubranoside A (**2**)	24.1±9.2	40.7±5.1*	51.2±3.8*
Hirsutanonol 5-*O*-β-D-glucopyranoside (**3**)	22.3±3.1	34.4±1.7*	42.8±1.5*
Rubranoside B (**4**)	21.5±4.7	28.3±3.7	37.1±3.1*
Rubranoside C (**5**)	33.2±2.6*	45.5±3.2*	49.1±2.3*
Hirsutanonol (**6**)	23.1±3.3	36.1±0.1*	53.5±2.5*
Hirsutenone (**7**)	7.5±3.3	24.4±6.2	37.7±1.1*
(5*S*)-*O*-Methylhirsutanolol (**8**)	19.7±0.6	50.7±3.7*	67.4±3.0*
Platyphylloside (**9**)	−14.6±2.5	−20.6±0.8	−43.7±2.9
Platyphyllonol 5-*O*-β-D-xylopyranoside (**10**)	10.7±2.6	−0.7±3.3	−1.9±5.7
Aceroside VII (**11**)	5.5±0.9	12.9±1.2	32.6±6.3
Platyphyllenone (**12**)	1.0±1.0	2.0±6.0	−33.6±2.9
(−)-Centrolobol 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside (**13**)	6.9±12.4	7.9±13.3	15.4±8.5
Quercetin	36.7±3.8*	49.1±0.3*	87.0±0.5*
Silybin	—	−9.6±9.3	−3.1±13.9

* Significantly different from the vehicle (*t*-BHP group)-treated group by *t*-test ($p<0.05$). Each value represents the mean±S.E.M. of two individual experiments ($n=6$/experiment). *a*) The relative percent protection (%) is calculated as 100×(value of *t*-BHP-treated−value of sample)/(value of *t*-BHP-treated−value of control).

tively. Compound **13** and the *p*-hydroxyphenyl moiety-containing compounds **9**—**12** were inactive but all isolates did not show any cytotoxicity at a concentration below 10 μM (cytotoxicity dada not shown), whereas compounds **9** and **12** exhibited negative protection effects due to the cytotoxicity at a high concentration (Table 2). Isolated compounds (**1**—**8**), which contain the catechol moiety, showed significant hepatoprotective effects on *t*-BHP-induced damage, and these effects were dose-dependent. These findings suggest that the presence of a catechol moiety enhances the cytoprotective effect of these diarylheptanoid derivatives in HepG2 cells. In this *t*-BHP assay system, silybin was found to be inactive, which is consistent with previous findings, although it has also been reported to have a hepatoprotective effect.[23—26] It has also been reported that silybin may act in different ways when used to treat hepatic diseases.[27]

Experimental

General Experimental Procedures Optical rotations were determined using an Autopol III automatic polarimeter (Rudolph Research Co., Flanders, NJ, U.S.A.). NMR spectra were recorded on a Bruker AMX-400 (400 MHz) spectrometer. ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC) spectral data were processed using standard Bruker software. HR-FAB-MS and EI-MS were measured using a JEOL JMS-600 mass spectrometer and on a Waters Quattro Micro LC/MS system, respectively. Preparative HPLC was performed using a Waters pump (model 510), a photodiode array detector (PDA, Waters model 996) and a ChiraSper (10×250 mm, Merck) column.

Plant Material The stem bark of *Alnus hirsuta* was collected at Namyangju, Gyeong-gi, Korea in April 2007. The plant was identified by Emeritus Professor Chang-Soo Yook at Kyung Hee University (Seoul), one of the authors. A voucher specimen (960-2C) has been deposited at the Korea Institute of Science and Technology (KIST).

Extraction and Isolation The dried stems of *A. hirsuta* (4.7 kg) were cut into small pieces and extracted three times with MeOH at room temperature. The methanol extract so obtained (993.2 g, 21.1%) was suspended in water and then partitioned sequentially with CH_2Cl_2, EtOAc, and *n*-butanol. The EtOAc extract (20.1 g, 0.43%) was then subjected to Sephadex LH-20 column chromatography and eluted with MeOH to yield 10 fractions (Fr. 1—Fr. 10). Fraction 6 (4.3 g) was subjected to column chromatography over silica gel using CH_2Cl_2–MeOH–H_2O (7 : 1 : 0.1→5 : 1 : 0.1→3 : 1 : 0.1) as an eluent to give 17 fractions (Fr. 6a—Fr. 6q). Fraction 6m (1.7 g) was purified by LiChroprep RP-18 column chromatography using MeOH–H_2O (5 : 5) to yield compounds **1** (1.3 g) and **4** (53.0 mg). Fraction 6c (57.5 mg) was purified by HPLC using 25% CH_3CN at 2.3 ml/min flow rate to yield compounds **7** (t_R=25.3 min, 40.9 mg) and **8** (t_R=30.9 min, 7.7 mg), and fraction 6p (98.3 mg) was purified by HPLC using an aqueous MeOH (40→70%) gradient system at 2.3 ml/min to yield compounds **2** (t_R=36.2 min, 89.6 mg) and **3** (t_R=10.9 min, 3.2 mg). Fraction 6g (61.7 mg) was purified by preparative TLC (5×10 cm) on RP-18 using 45% aqueous MeOH to yield compound **6** (3.1 mg). Fraction 6j (241.1 mg) was subjected to LiChroprep RP-18 column chromatography and eluted with MeOH–H_2O (38 : 62→5 : 5) to give 11 fractions (Fr. 6j1—Fr. 6j11). Fraction 6j6 was subjected to LiChroprep RP-18 column chromatography using 22% CH_3CN to give three fractions (Fr. 6j6a—Fr. 6j6c). Fraction 6j6b (32.6 mg) was purified by silica gel column chromatography using cyclohexane–EtOAc–MeOH (3 : 3 : 1) to yield compound **12** (6.7 mg). Fraction 2 (1.32 g) was purified by LiChroprep RP-18 column chromatography using a MeOH–H_2O (2 : 3→1 : 1) gradient system to give 10 fractions (Fr. 2a—Fr. 2j). Fraction 2f (247 mg) was purified by LiChroprep RP-18 column chromatography using a MeOH–H_2O (1 : 1→1 : 4) gradient system to yield compound **9** (294.6 mg). Fraction 2g (107.1 mg) was purified by LiChroprep RP-18 column chromatography using aqueous MeOH (40→45%) to yield **10** (40.7 mg) and **11** (51.0 mg). Finally, fraction 2j (20.6 mg) was purified by silica gel column chromatography using CH_2Cl_2–MeOH (5 : 1) to yield **5** (8.9 mg) and **13** (6.0 mg).

Rubranoside C (**5**): Brown amorphous powder; $[\alpha]_D^{24}$ −23.7° (*c*=0.43, MeOH); FAB-MS (Negative-ion mode): *m/z* 625 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 1.40 (2H, m, H-5), 1.55 (4H, m, overlapped, H-4, 6), 1.74 (2H, m, H-2), 2.46 (2H, t, *J*=7.6 Hz, H-7), 2.54 (2H, m, H-1), 3.22 (1H, t, *J*=11.1 Hz, H-5‴), 3.26 (1H, overlapped, H-4‴), 3.27 (1H, overlapped, H-2‴), 3.29 (1H, overlapped, H-5‴), 3.32 (1H, overlapped, H-2‴), 3.39 (1H, t, *J*=9.8 Hz, H-3‴), 3.50 (1H, t, *J*=8.7 Hz, H-3‴), 3.62 (3H, overlapped, H-3, 4‴, 6‴), 3.90 (1H, dd, *J*=5.0, 11.5 Hz, H-5‴), 3.91 (1H, dd, *J*=2.4, 11.5 Hz, H-6‴), 4.27 (1H, d, *J*=7.5 Hz, H-1‴), 4.59 (1H, d, *J*=7.2 Hz, H-1‴), 6.48, 6.50 (each 2H, br s, H-6′, 6″), 6.61, 6.62 (each 2H, d, *J*=1.9 Hz, H-2′, 2″), 6.65, 6.67 (each 2H, d, *J*=8.0 Hz, H-5′, 5″); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 25.5 (C-5), 31.9 (C-1), 32.9 (C-6), 35.0 (C-4), 36.1 (C-7), 38.1 (C-2), 62.6 (C-6‴), 66.3 (C-5‴), 70.0 (C-4‴), 71.6 (C-4‴), 74.3 (C-2‴), 75.5 (C-2‴), 77.8 (C-5‴), 78.1 (C-3‴), 80.1 (C-3), 87.3 (C-3‴), 103.7 (C-1‴), 105.1 (C-1‴), 116.3 (C-5′, 5″), 116.6 (C-2′, 2″), 120.7 (C-6′, 6″), 135.6 (C-1′, 1″), 144.0 (C-4′, 4″), 146.0 (C-3′, 3″).

(5*S*)-*O*-Methylhirsutanonol (**8**): Brown amorphous powder, $[\alpha]_D^{28}$ +3.8° (*c*=0.29, MeOH); EI-MS: *m/z* 360 $[M]^+$; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 1.68 (2H, m, H-6), 2.43 (2H, m, H-7), 2.48 (1H, dd, *J*=5.2, 16.0 Hz, H-4), 2.64 (1H, dd, *J*=7.2, 16.0 Hz, H-4), 2.68 (4H, br s, H-1, 2), 3.24 (3H, s, OMe), 3.62 (1H, m, H-5), 6.45 (1H, dd, *J*=2.0, 8.0 Hz, H-6″), 6.46 (1H, dd, *J*=2.1, 8.0 Hz, H-6′), 6.58 (1H, d, *J*=2.0 Hz, H-2″), 6.59 (1H, d, *J*=2.1 Hz, H-2′), 6.63 (1H, d, *J*=8.0 Hz, H-5′), 6.66 (1H, d, *J*=8.0 Hz, H-5″); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 30.1 (C-1), 31.6 (C-7), 36.9 (C-6), 46.4 (C-2), 48.2 (C-4), 57.1 (C-OMe), 77.9 (C-5), 116.3 (C-5′, 5″), 116.5 (C-2′, 2″), 120.5, 120.6 (C-6′, 6″), 134.0, 134.8 (C-1′, 1″), 144.3, 144.5 (C-4′, 4″), 146.1 (C-3′, 3″), 211.6 (C-3).

(3*R*)-1,7-Bis-(4-dihydroxyphenyl)-3-heptanol 3-*O*-β-D-Glucopyranosyl-(1→3)-β-D-xylopyranoside (**13**): Brown amorphous powder, $[\alpha]_D^{26}$ −25.7° (*c*=0.17, MeOH); HR-FAB-MS (Negative-ion mode): *m/z* 593.2610 $[M-H]^-$ (Calcd for $C_{30}H_{41}O_{12}$, 593.2598); ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz): see Table 1.

Acid Hydrolysis of 1 Compound **1** (10.1 mg) was hydrolyzed in 5% H_2SO_4 at 50 °C for 3 h. After neutralization with $BaCO_3$, the reaction mixture was extracted with EtOAc and evaporated to dryness. The residue was column chromatographed over silica gel using $CHCl_3$–MeOH–H_2O= 7 : 1 : 0.1 as an eluent to yield hirsutanol (**6**, 1.7 mg), hirsutenone (**7**, 2.4 mg) and unreacted compound **1** (2.4 mg). The aqueous layer was evaporated *in vacuo* to give a residue, which was subjected to silica gel column chromatography ($CHCl_3$–MeOH–H_2O=7 : 3 : 0.6) to yield D-xylose (3.0 mg). The sugar was compared with authentic sample on TLC and by measuring optical rotation.

Enzymatic Hydrolysis of 9 Compound **9** (8.2 mg) was treated with β-glucosidase (1.3 mg) in H_2O–EtOAc (1 : 1, 2 ml) at 37 °C for 1 d. The reaction mixture was extracted with EtOAc and evaporated to dryness. The residue was column chromatographed over silica gel using CH_2Cl_2–MeOH= 7 : 1 as eluent to yield platyphyllonol (**14**) (5.1 mg).[16] The aqueous layer was evaporated *in vacuo* to give a residue, which was subjected to silica gel column chromatography (CH_2Cl_2–MeOH–H_2O=6 : 4 : 1) to yield D-glucose (3.0 mg). The sugar was compared with authentic sample on TLC and by measuring optical rotation. Platyphyllonol (**14**): pale yellow oil, $[\alpha]_D^{25}$ −1.8° (*c*=0.27, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 1.65 (2H, m, H-6), 2.52 (3H, m, H-4, 7), 2.62 (1H, m, H-4), 2.73 (4H, br s, H-1, 2), 3.99 (1H, m, H-5), 6.67, 6.68 (each 2H, d, *J*=8.3 Hz, H-3′, 5′ and 3″, 5″), 6.98 (4H, d, *J*=8.3 Hz, H-2′, 6′ and 2″, 6″); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 29.8 (C-1), 31.9 (C-7), 40.5 (C-6), 46.4 (C-2), 51.3 (C-4), 68.2 (C-5), 116.1, 116.2 (C-3′, 5′ and 3″, 5″), 130.3 (C-2′, 6′ and 2″, 6″), 133.3, 134.1 (C-1′,1″), 156.4, 156.6 (C-4′, 4″), 211.9 (C-3).

Estimation of Sugar Absolute Configuration According to Klyne's Rule Molecular rotation $[M]_D^\alpha$=[specific optical rotation ($[\alpha]_D$)×molecular weight]/100. $[M]_D^\alpha$ value of **13** is −152.6°. The difference of molecular rotation $\Delta[M]_D^\alpha$, {$[M]_D^\alpha$ of **13**−$[M]_D^\alpha$ of (−)-centrolobol}, showed −126.8° indicating that two sugars had a levorotatory rotation that was comparable to those of methyl-β-D-xylopyranoside ($[M]_D^\alpha$=−107°) and methyl-β-D-glucopyranoside ($[M]_D^\alpha$=−62°).[28]

The Cytoprotective Effects of Isolates against *t*-BHP-Induced Toxicity *t*-BHP-induced toxicity assay was conducted using a minor modification of the method described by Yau *et al.*[29] Briefly, HepG2 cells (a hepatocellular carcinoma cell line) were obtained from the Korean Cell Bank (Seoul). Cells were placed in 96-well culture plates at 2×10^4 cells/well in complete medium consisting of RPMI supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin-100 μg/ml streptomycin, 1 mM sodium pyruvate and 1.5 g/l sodium bicarbonate and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

To examine the cytotoxicity and *t*-BHP-induced toxicity, 24 h after seeding cells in medium, the medium was replaced with fresh medium containing *t*-BHP or without treatment (control). Cell viability was determined using tetrazolium dye colorimetric assays [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assays]. MTT assays are based on the ability of

functional mitochondria to catalyze the reduction of MTT bromide to insoluble formazan, the concentration of which can be measured spectrophotometrically.[30)]

To evaluate the cytoprotective and cytotoxic effects of the diarylheptanoid derivatives and two positive controls, the test samples were added into FBS-free medium containing HepG2 cells and treated for 2 h. After pretreatment, the plate was washed twice and treated with new medium containing 200 μM *t*-BHP (except control and cytotoxicity tests), followed by a second 3 h incubation period. The cells were then subjected to MTT assay.

Statistical Analysis Data were expressed as the mean±S.E.M. of two individual experiments (n=6/experiment). Statistical comparisons were performed using the ANOVA test. p-values of <0.05 were considered significant.

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