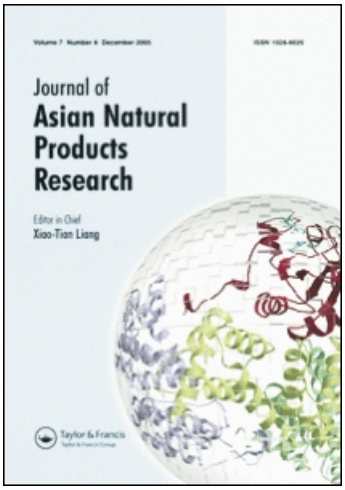

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new diarylheptanoid from the bark of *Alnus japonica*

Nguyen Huu Tung[a]; Jeong Chan Ra[b]; Dong Hwan Sohn[c]; Young Ho Kim[a]
[a] College of Pharmacy, Chungnam National University, Daejeon, South Korea [b] RNL BIO Co., Ltd, Seoul, South Korea [c] Department of Pharmacy, Institute of Pharmaceutical Research Development, Wonkwang University, Iksan, South Korea

Online publication date: 28 September 2010

To cite this Article Tung, Nguyen Huu , Ra, Jeong Chan , Sohn, Dong Hwan and Kim, Young Ho(2010) 'A new diarylheptanoid from the bark of *Alnus japonica*', Journal of Asian Natural Products Research, 12: 10, 921 — 924
To link to this Article: DOI: 10.1080/10286020.2010.507196
URL: http://dx.doi.org/10.1080/10286020.2010.507196

Journal of Asian Natural Products Research
Vol. 12, No. 10, October 2010, 921–924

NOTE

A new diarylheptanoid from the bark of *Alnus japonica*

Nguyen Huu Tung[a], Jeong Chan Ra[b], Dong Hwan Sohn[c] and Young Ho Kim[a]*

[a]*College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea;* [b]*RNL BIO Co., Ltd, 1596-7 Nakseongdae-Dong, Gwanak-Gu, Seoul, South Korea;* [c]*Department of Pharmacy, Institute of Pharmaceutical Research Development, Wonkwang University, Iksan 570-749, South Korea*

(*Received 28 May 2010; final version received 6 July 2010*)

A new diarylheptanoid, epihirsutanonol (**1**), was isolated from the bark of *Alnus japonica*, along with two known ones (**2** and **3**). Their structures were elucidated on the basis of extensive spectroscopic evidence. The new compound **1** showed significant hepatoprotective activity on the basis of *t*-butylhydroperoxide-induced hepatocyte injury *in vitro* assay.

Keywords: *Alnus japonica*; diarylheptanoid; epihirsutanonol; hepatoprotective activity

1. Introduction

Alnus japonica has been used in folk oriental medicine as remedies for fever, hemorrhage, diarrhea, and alcoholism [1]. Chemical constituents of *A. japonica* have been reported including numerous diarylheptanoids, along with several triterpenoids, and flavonoids [2–5]. Our previous study also led to the identification of many diarylheptanoids from the bark of *A. japonica*, and some of which exhibited antioxidant and antiviral activities [6,7]. In our ongoing study on phytochemical components from this plant, three diarylheptanoids (**1**–**3**), including a new one, namely epihirsutanonol, were isolated from the bark (Figure 1). This paper deals with the structural elucidation of the new compound **1** and the evaluation of hepatoprotective activity of all three diarylheptanoids on the basis of *t*-butylhydroperoxide (*t*-BH)-induced hepatocyte injury *in vitro* assay.

2. Results and discussion

Compound **1**, a colorless syrup, has molecular formula $C_{19}H_{22}O_6$ deduced by HR-EI-MS at *m/z* 346.1417 $[M]^+$. The IR spectrum of **1** showed absorption bands at ν_{max} 3396, 1644, 1435, 1042 cm^{-1} corresponding to hydroxyl, ketone, and aromatic bands. ^1H and ^{13}C NMR spectra of **1** revealed two sets of similar benzene rings and seven carbons of a heptane chain (δ 209.3, 58.2, 52.2, 46.3, 41.3, 33.0, and 30.1) with a ketone carbon at δ 209.3 (C-3) and an oxymethine carbon at δ 58.2 (C-5). The 3,4-dihydroxy substitution pattern of two benzene rings was defined from the ^{13}C NMR signals at δ 144.7 and 146.4 for two adjacent hydroxy-bearing aromatic carbons (C-3′,4′,3″,4″) and the ^1H NMR signals [δ 6.68 (d, $J = 8.0$ Hz), 6.65 (d, $J = 8.0$ Hz), 6.63 (d, $J = 2.0$ Hz), 6.61 (d, $J = 2.0$ Hz), 6.50 (dd, $J = 8.0, 2.0$ Hz), and 6.48 (dd, $J = 8.0, 2.0$ Hz)] characteristic

*Corresponding author. Email: yhk@cnu.ac.kr

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.507196
http://www.informaworld.com

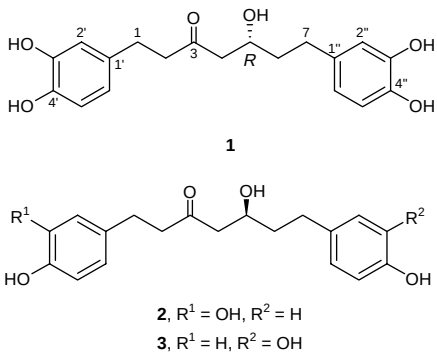

Figure 1. Structures of compounds **1**–**3**.

for two 1,3,4-trisubstituted aromatic rings, respectively. The linkage position between the heptane chain and the two aromatic rings (at C-1 and C-7) and the location of the ketone function (at C-3) and the hydroxyl group (at C-5) were unambiguously indicated with assignments from H–H COSY and HMBC experiments (Figure 2). Hence, the planar structure of **1** was proposed as 1,7-bis(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-one as same as hirsutanonol, which has been isolated from *A. japonica* [3] and other *Alnus* species [8] with 5*S* stereochemistry and $[\alpha]_D^{20}$ −17 ($c = 0.12$, MeOH). Accordingly, the stereochemistry of C-5 of **1** was inferred to be *R* from its positive optical rotation, $[\alpha]_D^{20}$ + 17 ($c = 0.12$, MeOH) and spectral evidence. In the ^{13}C NMR spectrum, the carbon signals C-3 and C-5 shifted upfield at δ 209.3 and 58.2; and in the ^{1}H NMR spectrum, the proton signal H-5 moved downfield at δ 4.26 as compared with those of hirsutanonol [3,8]. The absolute stereochemistry of C-5 was further concluded as 5*R* on the basis of the positive Cotton effect associated with the carbonyl $n \rightarrow \pi^*$ transition at 306 nm ($\Delta\varepsilon$ + 9.45) in the circular dichroism (CD) spectrum of **1** consistent with those of similar compounds [9,10]. Based on the above evidence, compound **1** was determined to be 5*R*-1,7-bis(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-one, namely epihirsutanonol.

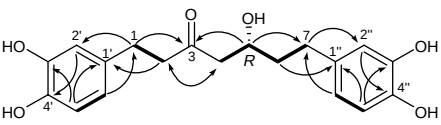

Figure 2. Important H–H COSY and HMBC correlations of **1**.

Compounds **2** and **3** were identified to be 5*S*-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-5-hydroxyheptane-3-one (**2**) and 5*S*-1-(4-dihydroxyphenyl)-7-(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-one (**3**), respectively, by their NMR and MS data, which agrees with those reported in the literature [11].

For hepatoprotective testing, all the isolates were checked for their protective effects on primary rat hepatocytes pharmacologically induced into necrosis using a 1.2 mM *t*-BH treatment for 50 min. *t*-BH is metabolized to free radical intermediates, which can subsequently initiate lipid peroxidation, affect cell integrity, trigger lactate dehydrogenase (LDH) leakage released into the cell culture medium, and form covalent bonds with cellular molecules to cause cell injury [12]. The LDH value of the vehicle and *t*-BH-treated cells was increased to 3.24 ± 0.14-fold that of the vehicle-treated control cells. At sample-treated cells, compounds **1**–**3** inhibited LDH release in a dose-dependent manner (Figure 3). Particularly, new diarylheptanoid **1** was effectively stronger than silibinin (Sigma Chemical Co., St Louis, MO, USA), a known approved hepatoprotective agent.

Consequently, the present work contributed in part the hepatoprotective evidence of diarylheptanoids from *A. japonica*. Especially, one new diarylheptanoid, epihirsutanonol (**1**), was investigated and found to show good hepatoprotective effect.

3. Experimental

3.1 General experimental procedures

The following instruments were used to obtain physical data: optical rotations, JASCO DIP-360 digital polarimeter; UV

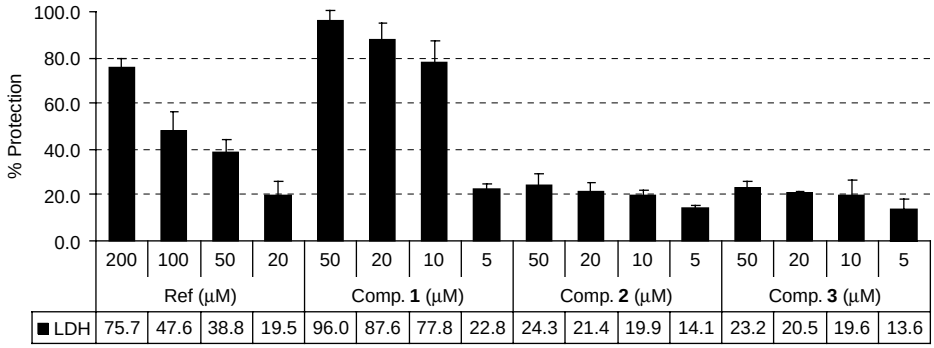

Figure 3. Effect of the isolated compounds **1**–**3** on *t*-BH-induced necrosis in primary cultured rat hepatocytes.

spectra, Beckman Du-650 UV–vis recording spectrometer; CD spectrum, JASCO J-700 spectropolarimeter; IR spectra, Perkin-Elmer 577 spectrometer; NMR spectra, Bruker DRX 500 NMR, and Jeol ECA 400 NMR spectrometers; HR-EI-MS, JEOL JMS-GCMATE mass spectrometer.

For column chromatography, silica gel (70–230 and 230–400 mesh, Merck, Darmstadt, Germany) and YMC C-18 resins (30–50 μm, Fuji Silysia Chemical Ltd, Aichi, Japan) were used. For TLC, Kieselgel 60 F_{254} (1.05715; Merck) and RP-18 F_{254s} (Merck) plates were used; and spots were visualized by spraying with 10% aqueous H_2SO_4 solution, followed by heating.

3.2 Plant material

The bark of *A. japonica* was collected in the northeast of China in September 2006, and was taxonomically identified by one of the authors (Y.H. Kim). A voucher specimen (CNU 08102) has been deposited at the College of Pharmacy, Chungnam National University, South Korea.

3.3 Extraction and isolation

Air-dried *A. japonica* bark (1.0 kg) was extracted with 3× 3 liters of 95% EtOH at room temperature. The combined extracts were concentrated *in vacuo* to dryness. The EtOH residue (308 g) was suspended in 2.0 liters of water and successively partitioned with CH_2Cl_2, EtOAc, and *n*-BuOH (each 2.0 liters ×3) to obtain soluble fractions of CH_2Cl_2 (39 g), EtOAc (83 g), and *n*-BuOH (15 g). The EtOAc-soluble fraction was fractionated over a silica gel column using a gradient of $CHCl_3$–MeOH (15:1–0:1, v/v) to give six fractions (1.1–1.6). Next, fraction 1.4 (5.1 g) was further chromatographed over a reversed-phase (RP) column using MeOH–H_2O (5:6, v/v) as eluent to five subfractions (2.1–2.5). Subfraction 2.1 (250 mg) was chromatographed on a RP column with MeOH–H_2O (5:6, v/v) and then on a silica gel column with $CHCl_3$–MeOH–H_2O (75:10:1, v/v/v) to obtain **1** (5.2 mg). Finally, subfraction 2.3 (580 mg) was rechromatographed over a RP column using MeOH–H_2O (5:3, v/v), followed by a silica gel column with $CHCl_3$–MeOH–H_2O (50:10:1, v/v/v) to obtain **2** (13.6 mg) and **3** (18.1 mg).

3.3.1 *Epihirsutanonol (**1**)*

Colorless syrup; $[\alpha]_D^{20} + 17$ ($c = 0.12$, MeOH); UV λ_{max}^{MeOH} (nm) ($\log \varepsilon$): 282 (3.04), 219 (3.49); IR (film) ν_{max}: 3396, 1644, 1435, 1042 cm^{-1}; CD (MeOH) $\Delta\varepsilon_{306nm}$ +9.45; ^1H NMR (CD_3OD, 500 MHz) δ: 6.68 (1H, d, $J = 8.0$ Hz, H-5′), 6.65 (1H, d, $J = 8.0$ Hz, H-5″), 6.63

(1H, d, $J = 2.0$ Hz, H-2″), 6.61 (1H, d, $J = 2.0$ Hz, H-2′), 6.50 (1H, dd, $J = 8.0$, 2.0 Hz, H-6″), 6.48 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 4.26 (1H, m, H-5), 2.94 (1H, dd, $J = 17.0$, 8.5 Hz, H-4a), 2.80 (1H, dd, $J = 17.0$, 5.0 Hz, H-4b), 2.70 (4H, s, H-1, H-2), 2.66 (1H, m, H-7a), 2.58 (1H, m, H-7b), 1.95 (1H, m, H-6a), 1.88 (1H, m, H-6b); ^{13}C NMR (CD_3OD, 125 MHz) δ: 30.1 (C-1), 46.3 (C-2), 209.3 (C-3), 52.2 (C-4), 58.2 (C-5), 41.3 (C-6), 33.0 (C-7), 134.0 (C-1′), 116.6 (C-2′), 146.4 (C-3′), 144.7 (C-4′), 116.5 (C-5′), 120.7 (C-6′), 133.9 (C-1″), 116.8 (C-2″), 146.4 (C-3″), 144.7 (C-4″), 116.5 (C-5″), 120.9 (C-6″); HR-EI-MS *m/z*: 346.1417 $[M]^+$ (calcd for $C_{19}H_{22}O_6$, 346.1416).

3.4 t-BH-induced hepatocyte injury in vitro *assay*

Primary rat hepatocytes were isolated and hepatocyte necrosis was induced by a 50-min treatment with 1.2 mM *t*-BH. Hepatocytes were pretreated with the compounds of interest 10 min prior to the addition of *t*-BH. Control experiments indicated that the vehicles (DMSO) used in this study had no influence on cellular damage. Hepatotoxicity was assessed by LDH leakage that was quantified by the Autodry Chemistry Analyzer (SPOTCHEM™ SP4410, Arkray, Kyoto, Japan). Silibinin (purity > 98%, Sigma Chemical Co.), a known hepatoprotective agent, was used as the positive control [13].

3.5 Statistical analysis

Data are expressed as mean ± SE of three independent experiments in triplicate. The calculations were performed using GraphPad Prism program ver. 4.0 (2004) (GraphPad Software, Inc., San Diego, CA, USA).

Acknowledgements

This study was supported by the Priority Research Center Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815), Republic of Korea.

References

[1] S.J. Lee, *Korea Folk Medicine* (Seoul National University Publishing Center Press, Seoul, Korea, 1996), p. 40.
[2] H.J. Kim, S.H. Yeom, M.K. Kim, J.G. Shim, I.N. Paek, and M.W. Lee, *Arch. Pharm. Res.* **28**, 177 (2005).
[3] M. Kuroyanagi, M. Shimomae, Y. Nagashima, N. Muto, T. Okuda, N. Kawahara, T. Nakane, and T. Sano, *Chem. Pharm. Bull.* **53**, 1519 (2005).
[4] M.W. Lee, T. Tanaka, G.I. Nonaka, and I. Nishioka, *Phytochemistry* **31**, 2835 (1992).
[5] M. Nomura, T. Tokoroyama, and T. Kubota, *Phytochemistry* **20**, 1097 (1981).
[6] N.H. Tung, S.K. Kim, J.C. Ra, Y.H. Zhao, D.H. Sohn, and Y.H. Kim, *Planta Med.* **76**, 626 (2010).
[7] N.H. Tung, H.J. Kwon, J.H. Kim, J.C. Ra, Y. Ding, J.A. Kim, and Y.H. Kim, *Bioorg. Med. Chem. Lett.* **20**, 1000 (2010).
[8] M.W. Lee, M.S. Park, D.W. Jeong, K.H. Kim, H.H. Kim, and S.H. Toh, *Arch. Pharm. Res.* **23**, 50 (2000).
[9] P.M. Giang, P.T. Son, K. Matsunami, and H. Otsuka, *Chem. Pharm. Bull.* **54**, 139 (2006).
[10] H. Itokawa, H. Morita, I. Midorikawa, R. Aiyama, and M. Morita, *Chem. Pharm. Bull.* **33**, 4889 (1985).
[11] T. Aoki, S. Ohta, and T. Suga, *Phytochemistry* **29**, 3611 (1990).
[12] H.W. Chen, T. Chiang, W.C. Yang, and C.K Lii, *Food Chem. Toxicol.* **38**, 1089 (2000).
[13] E.J. Park, Y.Z. Zhao, Y.C. Kim, and D.H. Sohn, *Planta Med.* **71**, 508 (2005).