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Jacaranone and Related Compounds from the Fresh Fruits of *Ternstroemia japonica* and their Antioxidative Activity

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Jacaranone and related compounds (**1-3**) were isolated, along with three triterpenes (**4-6**), from the fresh fruits of *Ternstroemia japonica*. The compounds were identified as jacaranone (**1**), 3-hydroxy-2,3-dihydrojacaranone (**2**), 3-methoxy-2,3-dihydrojacaranone (**3**), 3-*O*-acetyl-oleanolic acid (**4**), 3-*O*-acetylursolic acid (**5**), and ursolic acid (**6**). Jacaranone and its derivatives were isolated for the first time from Theaceae. Of the isolated compounds, compound **3** is a new compound. Jacaranone (**1**) exhibited weak antioxidative effect on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical.

Key words: *Ternstroemia japonica*, Theaceae, Jacaranone, Triterpene, Antioxidative activity, DPPH radical

INTRODUCTION

Ternstroemia japonica Thumb. (Theaceae) is widely distributed in Korea, Japan, Taiwan, and China. The tree is a useful source of lumber, dye, and horticulture (Kim, 1996). Its fruits have been used for the treatment of chest pain and numbness in traditional Chinese medicine (Akamatsu, 1970).

Oleanolic acid, primulagenin A, dihydropriverogenin A, and A_1-barrigenol were previously isolated from the leaves of *T. japonica* (Yoshioka *et al.*, 1972), and ternstroemiaxanthin, an aldehydic C_{40}-phytocarotenoid, was isolated from the seeds of the same plant (Kikuchi and Yamaguchi, 1974). In our previous study on this plant, six new saponins, ternstroemiasides A-F, were isolated, along with two known ones from the *n*-BuOH soluble fraction of the fresh fruits (Shin *et al.*, 2003).

In our continuous search for further antioxidative constituents from the same plant, we isolated additional compounds from the EtOAc fraction of the fresh fruits. This report describes the isolation and structure elucidation of these compounds: three jacaranone derivatives (**1-3**) and three triterpenes (**4-6**). Their characterization was made by spectroscopic methods including 2D-NMR techniques, while their antioxidative activities were evaluated by measuring scavenging effect on DPPH radical.

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MATERIALS AND METHODS

Plant materials

The fruits of *Ternstroemia japonica* were collected in October 1999, in Busan, Korea. The plant was identified by K. S. Im, Pusan National University, and the voucher specimen (No. 991005L) is deposited in the Natural Product Chemistry Laboratory of Pusan National University.

Instruments

Column chromatography was carried out on Kieselgel 60 (Merck, 63-200 mm). HPLC was performed on LKB Bromma 2248 HPLC Pump with YMC ODS-H80 (250×10 mm I.D., 4 μm, 8 nm) and YMC-Pack SIL (250×10 mm I.D., 5 μm, 12 nm), using JASCO RI-1530 detector. ^{1}H- and ^{13}C-NMR spectra were measured on Varian UNITY INOVA 500 and Bruker AC 200 spectrometers. LRFABMS spectra were recorded on JEOL JMS 110/110 instrument. IR spectra were recorded on JASCO FT/IR-410 in KBr disc method. Melting points were obtained by Fisher micro melting point apparatus (hot-stage type). JASCO DIP-370 digital polarimeter was used for measuring the optical rotation. DPPH was purchased from Sigma Chemical Co., and absorbance was measured on Shimadzu UVmini 1240 UV-VIS spectrophotometer.

Extraction and isolation

The fresh fruits (3.5 kg) of *T. japonica* were ground and extracted three times with MeOH at room temperature. The solvent was evaporated under reduced pressure and the resulting residue was partitioned between EtOAc and water. A portion of the EtOAc soluble fraction (10 g) was subjected to silica gel column chromatography, eluting with solvent system of $CHCl_3$-MeOH mixture (20:1→5:1) to give ten fractions (Fr.1~10).

Fr.3 was chromatographed on silica gel with a solvent system of increasing polarity (*n*-hexane-EtOAc and $CHCl_3$-MeOH) to give five subfractions (Fr.3-1~3-5), and Fr. 3-3 was subjected to silica gel HPLC eluting with *n*-hexane-EtOAc (6:1) to give compounds **4** (7.4 mg) and **5** (48.6 mg). Fr.4 was subjected to silica gel column chromatography, eluting with $CHCl_3$-MeOH (15:1), and purified by silica gel column chromatography, eluting with *n*-hexane-EtOAc (4:1) to yield compound **6** (46.0 mg). Fr.7 was chromatographed on silica gel column with eluants of increasing polarity (*n*-hexane-EtOAc and $CHCl_3$-MeOH) to give nine subfractions (Fr.7-1~7-9). Fr.7-2 was separated by reversed-phase HPLC using MeOH-water (1:1 and 7:3) to give compound **1** (25.0 mg) and compound **2** (2.2 mg). Fr.7-3 was separated by reversed-phase HPLC, eluting with MeOH-water (6:4 and 1:1), to afford compound **3** (5.1 mg).

Jacaranone (1)

Yellow amorphous powder; m.p. 77°C; LRFABMS *m/z* 205 $[M + Na]^+$ (calcd for $C_9H_{10}O_4Na$, 205); IR ν_{max} (KBr, cm^{-1}): 3390 (OH), 1720 (-$COCH_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.97 (2H, d, *J* = 10 Hz, H-3, 5), 6.19 (2H, d, *J* = 10 Hz, H-2, 6), 3.75 (3H, s, H-9), 2.71 (2H, s, H-7); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 186.0 (C-1), 171.0 (C-8), 149.0 (C-3, 5), 128.2 (C-2, 6), 67.2 (C-4), 52.2 (C-9), 43.4 (C-7).

3-Hydroxy-2,3-dihydrojacaranone (2)

Yellow amorphous solid; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.00 (1H, d, *J* = 10 Hz, H-5), 5.59 (1H, d, *J* = 10 Hz, H-6), 4.08 (1H, dd, *J* = 8.5, 4.5 Hz, H-3), 3.68 (3H, s, H-9), 2.85 (1H, d, *J* = 15 Hz, H-7a), 2.80 (1H, d, *J* = 15 Hz, H-7b), 2.70 (1H, dd, *J* = 17, 8.5 Hz, H-2a), 2.63 (1H, dd, *J* = 17, 4.5 Hz, H-2b); ^{13}C-NMR (50 MHz, CD_3OD): δ 200.2 (C-1), 172.4 (C-8), 152.2 (C-5), 129.9 (C-6), 71.6 (C-3), 71.2 (C-4), 52.2 (C-9), 43.4 (C-7), 42.9 (C-2).

3-Methoxy-2,3-dihydrojacaranone (3)

Yellow amorphous solid; HRFABMS *m/z* 237.0740 $[M + Na]^+$ (calcd for $C_{10}H_{14}O_5Na$, 237.0739); ^{1}H-NMR (500 MHz, CD_3OD): δ 6.95 (1H, d, *J* = 10 Hz, H-5), 5.93 (1H, d, *J* = 10 Hz, H-6), 3.78 (1H, dd, *J* = 9.0, 5.0 Hz, H-3), 3.68 (3H, s, H-9), 3.41 (3H, s, 3-OMe), 2.85 (1H, d, *J* = 14.5 Hz, H-7a), 2.81 (1H, d, *J* = 14.5 Hz, H-7b), 2.72 (1H, m, H-2a), 2.72 (1H, m, *J* = 17, 5.0 Hz, H-2b); ^{13}C-NMR (50 MHz, CD_3OD): δ 199.5 (C-1), 172.1 (C-8), 152.5 (C-5), 129.8 (C-6), 81.5 (C-3), 71.5 (C-4), 57.9 (3-OMe), 52.2 (C-9), 43.3 (C-7), 39.6 (C-2).

3-*O*-Acetyloleanolic acid (4)

Colorless crystal; m.p. 268°C; $[\alpha]_D$ +74.5° (*c* 0.6, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.28 (1H, m, H-12), 4.49 (1H, dd, H-3), 2.82 (1H, dd, *J* = 13.5, 4.5 Hz, H-18), 2.05 (3H, s, 3-OAc), 0.75, 0.85, 0.87, 0.91, 0.93, 0.94, 1.13 (3H each, s, CH_3×7); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 183.3 (C-28), 171.0 (3-OAc), 143.6 (C-13), 122.6 (C-12), 81.0 (C-3), 55.3 (C-5), 47.6 (C-9), 46.6 (C-17), 45.9 (C-19), 41.6 (C-14), 41.0 (C-18), 39.3 (C-8), 38.1 (C-1), 37.7 (C-4), 37.0 (C-10), 33.8 (C-21), 33.1 (C-29), 32.6 (C-7), 32.5 (C-22), 30.7 (C-20), 28.1 (C-23), 27.7 (C-15), 25.9 (C-27), 23.6 (C-30), 23.5 (C-2), 23.4 (C-16), 22.9 (C-11), 21.3 (3-OAc), 18.2 (C-6), 17.2 (C-26), 16.7 (C-25), 15.4 (C-24).

3-*O*-Acetylursolic acid (5)

Colorless oil; m.p. 289-290°C; $[\alpha]_D$ +62.3° (*c* 1.15, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.24 (1H, m, H-12), 4.50 (1H, dd, H-3), 2.18 (1H, d, *J* = 11.5 Hz, H-18), 2.04 (3H, s, 3-OAc), 1.06 (3H, s, Me-27), 0.92 (3H, s, Me-25), 0.91 (3H, d, *J* = 6.5 Hz, Me-30), 0.88 (3H, d, *J* = 6.0 Hz, Me-29), 0.86 (3H, s, Me-24), 0.80 (3H, s, Me-23), 0.73 (3H, s, Me-26); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 183.2 (C-28), 171.0 (3-OAc), 138.0 (C-13), 125.8 (C-12), 80.9 (C-3), 55.3 (C-5), 52.6 (C-18), 48.0 (C-17), 47.5 (C-9), 41.9 (C-14), 39.5 (C-8), 39.0 (C-19), 38.8 (C-20), 38.3 (C-1), 37.7 (C-4), 36.9 (C-10), 36.7 (C-22), 32.9 (C-7), 30.6 (C-21), 28.2 (C-15), 28.0 (C-23), 24.1 (C-16), 23.6 (C-2), 23.6 (C-27), 23.3 (C-11), 21.3 (3-OAc), 21.2 (C-30), 18.2 (C-6), 17.1 (C-29), 17.0 (C-26), 16.7 (C-24), 15.5 (C-25).

Ursolic acid (6)

White powder; m.p. 285-288°C; $[\alpha]_D$ +67.5° (*c* 1.01, MeOH); ^{1}H-NMR (500 MHz, pyridine-d_5): δ 1.05 (3H, s, Me-27), 0.91 (3H, s, Me-25), 0.90 (3H, d, *J* = 6.5 Hz, Me-30), 0.86 (3H, d, *J* = 6.0 Hz, Me-29), 0.85 (3H, s, Me-24), 0.78 (3H, s, Me-23), 0.71 (3H, s, Me-26); ^{13}C-NMR (125 MHz, pyridine-d_5): δ 179.9 (C-28), 139.3 (C-13), 125.7 (C-12), 78.2 (C-3), 55.9 (C-5), 53.6 (C-18), 48.1 (C-17), 48.1 (C-9), 42.6 (C-14), 40.0 (C-8), 39.5 (C-19), 39.5 (C-20), 39.4 (C-4), 39.1 (C-1), 37.5 (C-10), 37.3 (C-22), 33.6 (C-7), 31.1 (C-21), 28.8 (C-15), 28.7 (C-23), 25.0 (C-2), 24.0 (C-16), 23.7 (C-27), 23.0 (C-29), 21.4 (C-30), 18.8 (C-6), 17.6 (C-11), 17.5 (C-26), 16.6 (C-24), 15.7 (C-25).

Antioxidative activity

The free radical scavenging activity was evaluated by measuring the decrease in absorbance of DPPH due to the chemical trapping of the unpaired electron (Mukoda *et*

al., 2001). MeOH solutions (4 mL) of samples at various concentrations were added to a solution of DPPH in MeOH (1.5×10^{-4} M, 1 mL), and the reaction mixtures (total volume, 5 mL) were shaken vigorously. After storing these mixtures in open air for 30 minutes, the remaining amounts of DPPH were determined by colorimetry at 520 nm (Yoshida *et al.*, 1989). The radical scavenging activity was expressed by the ratio of the decreased absorbance of DPPH by sample, relative to the absorbance (100%) of the control DPPH solution. The mean values were obtained from triplicate experiments. Quercetin was employed as a positive control.

RESULTS AND DISCUSSION

The IR spectrum of compound **1** showed a carbonyl absorption band at 1720 cm^{-1} and a hydroxyl band at 3390 cm^{-1}. The LRFABMS (positive ion mode) of compound **1** displayed a quasi-molecular ion peak at *m/z* 205 $[M + Na]^+$. In the ^{1}H-NMR spectrum, the two doublet resonances at δ 6.19 (J = 10 Hz) and δ 6.97 (J = 10 Hz) suggested the presence of a *para*-disubstituted cyclohexadiene ring. This was also supported by the increased intensity of vinyl carbon signals (δ 149.0 and 128.2). The correlations between the vinyl protons (δ 6.97 and 6.19) and carbonyl carbon (δ 186.0) in HMBC spectrum revealed a carbonyl group as one of the substituents on the ring. The peak at δ 67.2 (C-4) in ^{13}C-NMR spectrum, which was correlated with the vinyl protons in HMBC spectrum, suggested another substituent is a hydroxyl group. Additionally, the correlation between another carbonyl carbon (δ 171.0) and methoxy protons (δ 3.75) in HMBC spectrum indicated the presence of a methyl ester moiety. The location of the methyl ester group was determined by the correlation between carbonyl carbon (δ 171.0) and methylene protons (δ 2.71, s), and between methylene protons (δ 2.71, s) and quaternary carbon (δ 67.2) in HMBC spectrum. These observations led to the identification of **1** as jacaranone.

Jacaranone was previously isolated from plants of *Jacaranda* sp. (Ogura *et al.*, 1976), *Senecio* sp. (Bohlmann *et al.*, 1981; Mericli *et al.*, 1989), *Packera* sp. (Perez-Castorena *et al.*, 2001), and *Ajuga* sp. (Muhammad *et al.*, 1999). It was reported to show antitumor (Ogura *et al.*, 1976), antibacterial (Cabezas *et al.*, 1991), and antiviral (Tan *et al.*, 1992) activities.

The ^{1}H-NMR spectrum of compound **2** displayed two vinyl protons at δ 7.00 (d, J = 10 Hz) and 5.59 (d, J = 10 Hz). Compound **2** was supposed to be 2,3-dihydro-3-hydroxy derivative of jacaranone due to the halved intensity of the vinyl proton signals compared to compound **1**, and the presence of additional peaks at δ 2.70 (H-2a), 2.63 (H-2b), and 4.08 (H-3) in ^{1}H-NMR spectrum. The downfield shifted carbonyl peak (δ 186.0→200.2) supported this skeleton. Moreover, methylene proton peaks at δ 2.70 (H-2a) and δ 2.63 (H-2b) showed correlations with the peaks at δ 200.2 (C-1) and δ 71.6 (C-4) in HMBC spectrum. The signal at δ 71.6 (C-4) in ^{13}C-NMR spectrum was assignable to a quaternary oxygenated carbon as in compound **1**. The location of methyl ester moiety was same with that of compound **1**. Compound **2** was thus identified as 3-hydroxy-2,3-dihydrojacaranone. This compound was previously isolated from *Senecio confusus* Britt. (Mericli *et al.*, 1989) and *Pseudogynoxys cunninghammii* (Jakupovic *et al.*, 1986), but its stereochemistry has not been determined so far.

Compound **3** is a methoxy derivative of compound **2**. The ^{13}C-NMR data were similar to those of compound **2**, except for the presence of an additional methoxy carbon peak at δ 57.9. The correlation between methoxy protons and C-3 in the HMBC spectrum indicated that the methoxyl group is attached to C-3 of the ring. The key HMBC correlations are shown in Fig. 1. Compound **3** is a new compound in nature.

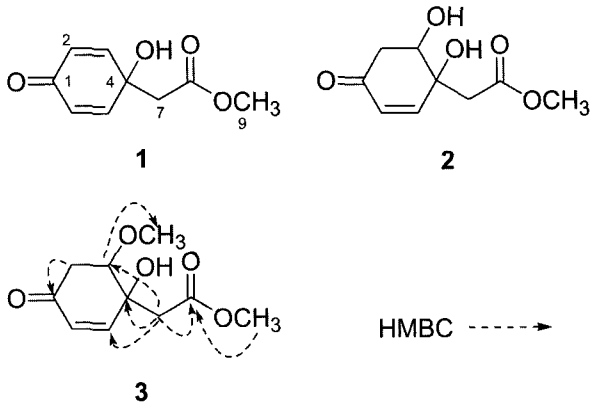

Fig. 1. Structures of **1-3** and key HMBC correlations of **3**

Triterpenes (**4-6**) were identified as 3-*O*-acetyloleanolic acid, 3-*O*-acetylursolic acid, and ursolic acid, respectively, on comparison of ^{1}H-, and ^{13}C-NMR data with those in the literature (Santos *et al.*, 1997; Fujita *et al.*, 2000; Olafsdottir *et al.*, 2001).

To the best of our knowledge, this is the first report of jacaranone derivatives (**1-3**) from Theaceae. Compounds were assayed for antioxidative activity. Of the compounds tested, **1** showed weak scavenging activity on DPPH radical (EC_{50}, 86 μg/mL) and other compounds (**2-6**) were virtually inactive (EC_{50}, >300 μg/mL), while the EC_{50} value of quercetin, a positive control, was 4.5 μg/mL.

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REFERENCES

Akamatsu, K., Wakanyaku. Ishiyaku Shuppan, Tokyo, p.234 (1970).

Bohlmann, F., Zdero, C., King, R. M., and Robinson, H., The first acetylenic monoterpene and other constituents from *Senecio clevelandii*. *Phytochemistry*, 20, 2425-2427 (1981).

Cabezas, J. A., Ciccio, J. F., Hidalgo, G., and Echandi, G., Synthesis and antibacterial activity of jacaranone and ester side chain analogs. *Rev. Latinoam. Quim.*, 22, 49-52 (1991).

Fujita, R., Duan, H., and Takaishi, Y., Terpenoid from *Tripterigyum hypoglaucum*. *Phytochemistry*, 53, 715-722 (2000).

Jakupovic, J., Chau-Thi, T. V., and Castro, V., Cyclohexene derivatives from *Pseudogynoxys cunninghammii*. *Fitoterapia*, 58, 187-188 (1987).

Kikuchi, K. and Yamaguchi, M., Structure of ternstroemiaxanthin, a new aldehydic C_{40}-carotenoid. *Bull. Chem. Soc. Jpn.*, 47, 885-887 (1974).

Kim, T. J., *Korean Resources Plants* Vol. 3, Seoul Nat'l Univ. Press, Seoul, p. 107, 1996.

Mericli, A. H., Mericli, F., Jakupovic, J., Bohlmann, F., Dominguez, X. A., Vega, and H. S., Eremophilane derivatives and other constituents from Mexican *Senecio* species. *Phytochemistry*, 28, 1149-1153 (1989).

Muhammad, P., Ahmad, S., Nawaz, H. R., Ullah, N., and Malik, A., New acetylated quinols from *Ajuga parviflora*. *Fitoterapia*, 70, 229-232 (1999).

Mukoda, T., Sun, B., and Ishiguro, A., Antioxidant activities of buckwheat hull extract toward various oxidative stress *in vitro* and *in vivo*. *Biol. Pharm. Bull.*, 24, 209-213 (2001).

Ogura, M., Cordell, G. A., and Farnsworth, N. R., Potential anticancer agents III. Jacaranone, a novel phytoquinoid from *Jacaranda caucana*. *Lloydia*, 39, 255-257 (1976).

Olafsdottir, E. S., Omarsdottir, S., and Jaroszewski, J. W., Constituents of three Icelandic *Alchemilla species*. *Biochemical Systematics and Ecology*, 29, 959-962 (2001).

Perez-Castorena, A. L., Arciniegas, A., Martinez, F., Marquez, C., and Villasenor, J. L., Chemical constituents of *Packera coahuilensis* and *Packera bellidifolia*. *Biochemical Systematics and Ecology*, 29, 203-206 (2001).

Santos, G. G., Alves, J. C. N., Rodilla, J. M. L., Duarte, A. P., Lithgow, A. M., and Urones, J. G., Terpenoids and other constituents of *Eucalyptus globulus*. *Phytochemistry*, 44, 1309-1312 (1997).

Shin, M. H., Wang, W., Nam, K. I., Jo, Y., Jung, J. H., and Im, K. S., Triterpenoid saponins from the fruits of *Ternstroemia japonica*. *J. Nat. Prod.*, 66, 1351-1355 (2003).

Tan, G. T., Miller, J. F., Kinghorn, A. D., Hughes, S. H., and Pezzuto, J. M., HIV-1 and HIV-2 reverse transcriptases: a comparative study of sensitivity to inhibition by selected natural products. *Biochem. Biophys. Res. Commun.*, 185, 370-378 (1992).

Yoshida, T., Mori, K., Hatano, T., Okumura, T., Uehara, I., Komagoe, K., and Okuda, T., Studies on inhibition mechanism of autooxidation by tannins and flavonoids. *Chem. Pharm. Bull.*, 37, 1919-1921 (1989).

Yoshioka, I., Takeda, R., Matsuda, A., and Kitagawa, I., Saponin and sapogenol. V. Sapogenol constituents of seeds of *Camellia sasanqua* and leaves of *Ternstroemia japonica*. *Chem. Pharm. Bull.*, 20, 1237-1242 (1972).