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The Inhibitory Principle of Lipopolysaccharide-Induced Nitric Oxide Production from *Inula britannica* var. *chinensis*

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A sesquiterpene lactone, 1-*O*-acetyl-4*R*,6*S*-britannilactone (**1**) isolated from the flowers of *Inula britannica* L. var. *chinensis* (Rupr.) Reg. (Compositae), was found as an iNOS inhibitory constituent for the first time with an IC_{50} value of 22.1 μM which is more potent than the positive control, L-N^6-(1-iminoethyl)lysine (IC_{50} = 33.7 μM). Structure of compound **1** was identified by 1D and 2D NMR experiments and by comparison with the reference standard.

Key words: *Inula britannica* L. var. *chinensis* (Rupr.) Reg., Compositae, 1-*O*-acetyl-4*R*,6*S*-britannilactone, iNOS inhibitor

INTRODUCTION

Nitric oxide (NO) is a free radical molecule involved in a wide range of physiological and pathological mechanisms (Dawson *et al.*, 1994). And NO has been implicated as playing a role in many pathological conditions, including allergic airway diseases, pneumonitis, vasculitis, acute rejection of allograft, and toxic shock syndrome. Inducible nitric oxide synthase (iNOS) is an inflammation-induced enzyme that catalyzes the production of NO, a molecule that may lead to carcinogenesis. At inflammation sites such as the liver, tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and interferon-γ (IFNγ) induce the expression of iNOS (Nathan, 1997). Therefore, an inhibitor of iNOS may be effective as a therapeutic agent for the pathological conditions related to NO.

The flowers of *Inula britannica* have been used for the treatment of digestive disorders, bronchitis, and inflammation in traditional medicine (Bensky *et al.*, 1993). There have been several reports on biological activities of this plant including cytotoxicity (Park *et al.*, 1998), antioxidant activity (Park *et al.*, 2000), and hepatoprotective effects (Song *et al.*, 2000). We have reported several iNOS inhibitory constituents from *Inula britannica* L. var. *chinensis* (Rupr.) Reg. (Compositae) (Lee *et al.*, 2002) previously. Herein, we report the sesquiterpene lactone, 1-*O*-acetyl-4*R*,6*S*-britannilactone (**1**) as an additional possible iNOS inhibitory constituent for the first time.

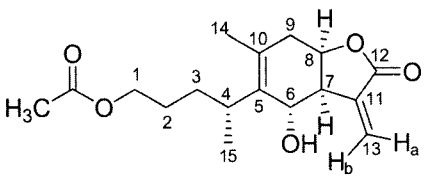

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Fig. 1. The structure of compound **1**

MATERIALS AND METHODS

General

The melting points were measured on a Mettler FP62 and were uncorrected. Optical rotation was measured with a P-1010 polarimeter (Jasco, Japan) at 25°C. UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA, U.S.A.), respectively. ^1H, ^{13}C, DEPT, COSY, ROESY, HSQC, and HMBC NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA, U.S.A.). TMS was used as internal standard. EIMS and HREIMS were recorded on a JMS 700 GC-mass spectrometer (JEOL, Japan). Flash column chromatography was carried out on Si gel 60 (230-400 mesh, Merck,

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Germany) with mild nitrogen pressure. Column chromatography was monitored by TLC (Si gel 60 F_{254} plates, 0.25 mm thickness) with visualization under UV light (254 and 365 nm) and 10% sulfuric acid in EtOH.

Plant materials

The flowers of *Inula britannica* L. var. *chinensis* (Rupr.) Reg. were purchased from a herb market in Seoul, Korea. A voucher specimen (No. NPRI-A124) has been deposited at the Natural Products Resource Depository of Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul, Korea.

Extraction and isolation

The dried flowers of *I. britannica* var. *chinensis* (3 kg) were ground and extracted with MeOH (6×6 L) for 24 h by percolation. The filtered MeOH solutions were evaporated under vacuum, and then water (2 L) was added. The aqueous MeOH extract was partitioned with hexane (2×2 L), EtOAc (3×1.5 L), and BuOH (2×2 L), successively. A Si gel column chromatography of the EtOAc extract (43.9 g) using a gradient solvent system of chloroform-MeOH (100:0→0:100), afforded 8 fractions. Fraction 2 eluted with chloroform-MeOH (99:1) from the first column chromatography was further fractionated using hexane-acetone (50:1→0:100) as a solvent system. Fractions eluted with hexane-acetone (8:1→5:1) were subjected to further Si gel column chromatography using hexane-EtOAc (6:10: 100). Fraction 4, which was further chromatographed on Si gel with hexane-EtOAc (4:1), afforded crude precipitation of **1**. Compound **1** was recrystallized from EtOAc [565.1 mg, yield 0.19 %(w/w)].

1-*O*-Acetyl-4*R*,6*S*-britannilactone (1)

Colorless cubic crystals; m.p. 126°C; $[\alpha]_D^{25}$: +102° (CH_2Cl_2, c 0.098); UV (MeOH) λ_{max} (log e) 226 (3.8) nm; IR (film) ν_{max} 3495, 2938, 1733, 1656, 1255, 1159, 955, 821, 772, 639 cm^{-1}; EIMS *m/z* (%) 308 (10) $[M]^+$, 285 (50), 268 (60), 248 (35), 189 (100), 143 (95), 91 (65), 55 (50); HREIMS *m/z* 308.1624 $[M]^+$ calcd. 308.3746 for $C_{17}H_{24}O_5$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.31 (1H, d, *J* = 2.4 Hz, H-13a), 5.72 (1H, d, *J* = 2.4 Hz, H-13b), 5.01 (1H, m, H-8), 4.18 (1H, s, H-6), 3.93 (2H, m, H-1), 3.53 (1H, m, H-7), 2.84 (1H, d-like, *J* = 16.2 Hz, H-9a), 2.69 (1H, m, H-4), 2.46 (1H, dd, *J* = 16.2, 2.2 Hz, H-9b), 2.04 (3H, s, 1-COMe), 1.76 (3H, s, H-14), 1.40 (1H, m, H-2a, interchangeable with H-2b), 1.30 (1H, m, H-3a, interchangeable with H-3b), 1.22 (1H, m, H-2b, interchangeable with H-2a), 1.07 (3H, d, *J* = 7.2 Hz, H-15), 0.99 (1H, m, H-3b, interchangeable with H-3a); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 171.5 (1-COMe), 170.1 (C-12), 137.2 (C-11), 136.8 (C-5), 131.3 (C-10), 124.0 (C-13), 76.1 (C-8), 68.5 (C-6), 64.5 (C-1), 45.3 (C-7), 34.6 (C-9), 33.1 (C-4), 31.4 (C-3), 26.7 (C-2), 21.2 (1-COMe), 20.5 (C-14), 19.5 (C-15); ^{1}H-^{1}H COSY correlations ($CDCl_3$, 400 MHz): H-1/H-2a, H-2b; H-4/H-3a, H-3b, H-15; H-7/H-6, H-8; H-9a/H-8, H-9; H-13a/H-13b; ^{1}H-^{1}H ROESY correlations ($CDCl_3$, 400 MHz): H-1/H-3a, H-3b, H-13b; H-2a/H-6, H-9a, H-14, H-15; H-2b/H-4, H-6, H-15; H-3a/H-15; H-3b/H-6, H-15; H-4/H-14; H-6/H-13b, H-15; H-7/H-13a, H-13b; H-14/H-9a, H-9b; ^{1}H-^{13}C HMBC correlations ($CDCl_3$, 400 MHz): see Fig. 2.

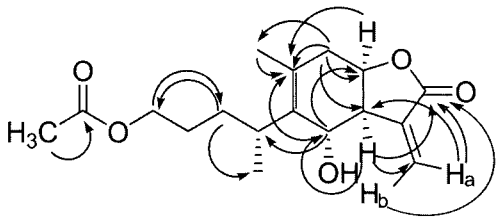

Fig. 2. HMBC NMR correlations of compound **1**

Assay methods

For the determination of iNOS activity, RAW 264.7 murine macrophage cells (ATCC TIB-71) were seeded in 96-well plates (2×10^5 cells/200 μL), cultured for 24 h and then incubated with lipopolysaccharide (LPS; 1 μg/mL) in the presence of various concentrations (using final 0.25% dimethyl sulfoxide as a vehicle solvent) for additional 24 h. Nitrite contents produced in the medium were determined by measuring the absorbance at 540 nm based on the Griess reaction (Green *et al.*, 1982). Cytotoxicity was measured by the mitochondrial-dependent reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to formazan (Mosmann, 1983) using the same treatment procedure as for the determination of the iNOS enzyme activity. Inhibitory effects are represented as the molar concentration (μM) giving 50% inhibition (IC_{50}) relative to the vehicle control.

RESULTS AND DISCUSSION

Compound **1** was identified as 1-*O*-acetylbritannilactone which belongs to the secoeudesmanolide type of sesquiterpene lactone, by comparison of all of the physical and spectral data of **1** with published values (Jeske *et al.*, 1993; Zhou *et al.*, 1993). The configuration at C-4 and C-6 of **1** was determined as *R* and *S*, respectively, by an x-ray crystallography in our previous study (Han *et al.*, 2003). Compound **1**, a new stereoisomer of 1-*O*-acetylbritannilactone, exhibited an inhibitory activity in LPS-induced NO production with an IC_{50} value of 22.1 mM which is more potent than the positive control, L-N_6-(1-iminoethyl)lysine (IC_{50} 33.7 mM). Therefore, **1** was considered as a possible potent iNOS inhibitory constituent from *I. britannica* var. *chinensis* for the first time in the present study.

During our continuous work on *I. britannica* var. *chinensis*, the pseudoguaianolide type of known sesquiterpenes, bigelovin, 2,3-dihydroaromaticin, and ergolide were previously reported as minor iNOS inhibitory constituents (Lee *et al.*, 2002). In the present investigation, the secoeudesmanolide sesquiterpene, compound **1** was found out to be the major iNOS inhibitory constituent of this plant. To the best of our knowledge, the iNOS inhibitory activity of any isomers of 1-*O*-acetylbritannilactone has never been reported previously. .

The ^{1}H- and ^{13}C-NMR assignments for **1** were completed by aids of DEPT, HSQC, HMBC, COSY, and ROESY NMR techniques as reported in the Experimental of this paper. Three methyl signals at δ_H 2.04, 1.76, and 1.07 were assigned to 1-COMe, H-14 and H-15, respectively, according to the following HMBC correlations: 1-COMe/CO; H_2-3/C-15; H-14/C-10, H_2-9/C-14. The proton signals at d_H 3.53 and 5.01 that were correlated to the carbon signals at δ_C 45.3 and 76.1, respectively, in the HSQC spectrum of **1**, were assigned to H-7 and H-8, respectively, from the HMBC correlations of H-7/C-13 and H-8/C-10. In addition, the ROESY correlations between H-7 and H-13a, 13b supported this assignment. The ROESY spectrum of **1** exhibited the correlation between H-6 and H-13b, whereas it did not show any correlation between H-6 and H-13a, providing the evidence for the positions of H-13b and H-13a. All other two- and three-bond connectivities in the HMBC data were consistent with the determined structure of **1**.

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REFERENCES

Bensky, D. and Gamble, A., Chinese Herbal Medicine: Materia Medica. Revised edition. Eastland Press, Seattle, pp. 193-194 (1993).

Dawson, T. M. and Snyder, S. H., Gases as biological messengers: nitric oxide and carbon monoxide in the brain. *J. Neurosci.*, 14, 5147-5159 (1994).

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R., Analysis of nitrate, nitrite, and [^{15}N]-nitrite in biological fluids. *Anal. Biochem.*, 126, 131-138 (1982).

Han, A. R., Mar, W., and Seo, E. K., X-Ray Crystallography of a New Sesquiterpene Lactone isolated from *Inula britannica* var. *chinensis*. *Nat. Prod. Sci.*, 9, 28-30 (2003).

Jeske, F., Huneck, S., and Jakupovic, J., Secoeudesmanolides from *Inula japonica*. *Phytochemistry*, 34, 1647-1649 (1993).

Lee, H. T., Yang, S. W., Kim, K. H., Seo, E. K., and Mar, W., Pseudoguaianolide isolated from *Inula britannica* var. *chinensis* as inhibitory constituents against inducible nitric oxide synthase. *Arch. Pharm. Res.*, 25, 151-153 (2002).

Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 55-63 (1983).

Nathan, C., Inducible nitric oxide synthase: What difference does it make? *J. Clin. Invest.*, 100, 2417-2423 (1997).

Park, E. J. and Kim, J. Cytotoxic sesquiterpene lactones from *Inula britannica*. *Planta Med.*, 64, 752-754 (1998).

Park, E. J., Kim, Y., and Kim, J., Acylated Flavonol Glycosides from the Flower of *Inula britannica*. *J. Nat. Prod.*, 63, 34-36 (2000).

Song, Q. H., Kobayashi, T., Iijima, K., Hong, T., and Cyong, J. C., Hepatoprotective effects of *Inula britannica*. *Phytother. Res.*, 14, 180-186 (2000).

Zhou, B. N., Bai, N. S., Lin, L. Z., and Cordell, G. A., Sesquiterpene lactones from *Inula britannica*. *Phytochemistry*, 34, 249-252 (1993).