Natural Product Sciences
9(1) : 28-30 (2003)

X-Ray Crystallography of a New Sesquiterpene Lactone isolated from *Inula britannica* var. *chinensis*

Ah-Reum Han[1], Woongchon Mar[2], and Eun-Kyoung Seo[1]*

[1]*College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea*
[2]*College of Pharmacy & Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea*

Abstract – A sesquiterpene lactone, 1-*O*-acetyl-4*R*,6*S*-britannilactone (**1**) which has a new stereochemistry was isolated from the flowers of *Inula britannica* L. var. *chinensis* (Rupr.) Reg. Its stereostructure was determined by a x-ray crystallography.
Keywords – *Inula britannica* L. var. *chinensis* (Rupr.) Reg, 1-*O*-acetyl-4*R*,6*S*-britannilactone, x-ray crystallography

Introduction

Inula britannica (Asteraceae) is a wild plant found in Eastern Asia, including Korea, Japan, and China. The flowers of *I. britannica* have been used for the treatment of digestive disorders, bronchitis, and inflammation in traditional medicine (Bensky *et al.*, 1993). Several biological activities such as cytotoxic activity against several human tumor cell lines (Park *et al.*, 1998), antioxidant activity (Park *et al.*, 2000), and hepatoprotective effects (Song *et al.*, 2000) have been reported previously. In addition, the iNOS inhibitory activity of some sesquiterpene lactones from this plant (Han *et al.*, 2001) has been published. In our previous study on *I. britannica* L. var. *chinensis* (Rupr.) Reg., the iNOS activity of sesquiterpene lactones including bigelovin, 2,3-dihydroaromaticin, and ergolide has been found (Lee *et al.*, 2002).

During our continuous study on *I. britannica* var. *chinensis* (Rupr.) Reg., a sesquiterpene lactone, 1-*O*-acetyl-britannilactone (**1**) with a new stereochemistry of 4*R*, 6*S* was isolated. Although its plain structure (Zhou *et al.*, 1993) and stereoisomer (Jeske *et al.*, 1993) was reported previously, the stereostructure of compound **1** is reported for the first time by x-ray crystallography in the present study. Its ^{1}H and ^{13}C NMR data supported the structure of **1**.

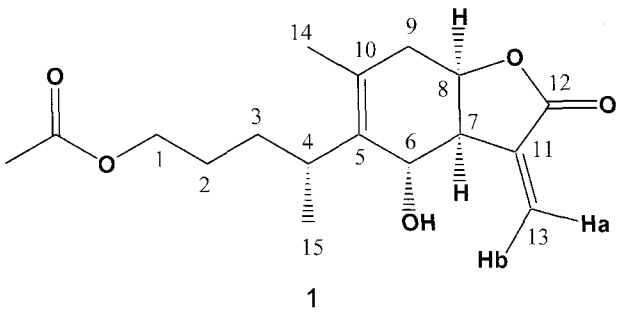
Experimental

General – The melting point were measured on a Mettler FP62 and are 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), respectively. ^{1}H, ^{13}C, DEPT, COSY, ROESY, HSQC, and HMBC NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA). TMS was used as internal standard. EIMS and EIHRMS 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, 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 1% sulfuric acid in EtOH.

Plant material – The flowers of *I. britannica* L. var. *chinensis* (Rupr.) Reg were purchased from an 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 (2×3 L), EtOAc (1.5 L×3), 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

*Author for correspondence
Fax: +82-2-3277-3051, E-mail: Yuny@ewha.ac.kr



1

using hexane-acetone (50:1→0:100) as a solvent system. Fractions eluted with hexane-acetone (8:15:1) were subjected to further Si gel column chromatography using Hexane-EtOAc (6:10:100). Fraction 4 which were further chromatographed on Si gel with Hexane-EtOAc (4:1), afforded crude precipitation of **1**. Compound **1** was recrystallized from EtOAc (565.1 mg).

Compound 1 (1-*O*-acetyl-4*R*,6*S*-britannilactone) – colorless cubic crystals; m.p. 126°C. $[\alpha]^{25}_{D}$: 102° (CH_2Cl_2, c 0.098). UV (MeOH): λ_{max} = 226 (3.8) nm. IR (film): ν_{max} = 3495, 2938, 1733, 1656, 1255, 1159, 955, 821, 772, 639^{-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): = 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), 1.30 (1H, m, H-3a), 1.22 (1H, m, H-2b), 1.07 (3H, d, *J* = 7.2 Hz, H-15), 0.99 (1H, m, H-3b). ^{13}C-NMR ($CDCl_3$, 100 MHz): = 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).

X-ray crystallography – The structure determinations by x-ray crystallography were undertaken in order to elucidate the nature of the compounds and their stereochemistry prior to further chemical studies.

Colorless cubic single crystals were obtained by recrystallization in ethyl acetate. Crystal diffraction data were collected from the well-shaped single crystal on a Simens P4 x-ray diffractometer with graphite monochromated Cu-K_α radiation. The structure was solved by direct methods in the orthorhombic space group $P2_12_12_1$, with Z = 4. In the present structure determination, hydrogen atoms were treated by a independent and constrained refinement. The non-hydrogen atoms were refined anisotropically using full-matrix least-squares on F^2. Crystal data, data collection procedures, structure determination methods and refinement results are summarized in Table 1.

Table 1. Crystal data, data collection details and structure refinement results

Empirical fomular	$C_{17}H_{24}O_5$
Formular weight	308.36
Temperature (K)	290(2)
Wavelength (Å)	0.71073
Crystal system	Orthorhombic
Space group	$P2_1P2_1P2_1$
Unit cell dimensions	
a (Å)	8.0033(12)
b (Å)	12.360(3)
c (Å)	16.881(3)
α (deg)	90
β (deg)	90
γ(deg)	90
Volume (Å^3)	1669.9(5)
Z	4
Density (calculated) (Mg m^{-3})	1.227
Absorption coefficient (mm^{-1})	0.089
F(000)	664
Crystal size (mm^3)	0.10×0.40×0.40
è range for data collection (deg)	2.04 to 25.99
Limiting indices	$-1 \le h \le 9$, $-1 \le k15$, $-20l1$
Reflections collected	2503
Independent reflections (R_{int} = 0.0183)	2325
Completeness to = 25.99 (%)	99.9
Absorption correction	none
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2325 / 0 / 200
Goodness-of-fit on F^2	1.073
Final *R* indices [*I* >2ó(*I*)]	R_1 = 0.0467, wR_2 = 0.0890
R indices (all data)	R_1 = 0.0804, wR_2 = 0.1001
Absolute structure parameter	1.0(18)
Extinction coefficient	0.0098(12)
Largest diff. Peak and hole (e$^-$Å^{-3})	0.151 and -0.137

Result and Discussion

The dried flowers of *I. britannica* var. *chinensis* were extracted with MeOH, which was further partitioned with hexane, EtOAc and BuOH, successively. The compound **1** was afforded by the repeated silica gel column chromatography of ethyl acetate fraction and recrystallized from EtOAc. The structure of compound **1** was identified by chemical and spectral data. The IR spectrum of compound **1** showed strong absorption bands at 3495 and 1733 cm^{-1} which indicated the presence of hydroxyl group and esters. The ^{1}H and ^{13}C and DEPT NMR spectra of compound **1** indicated the presence of three methyls including a carboxylic carbonyl group, five methylenes, four methines, and five quaternary carbons including a acetyl functionality. The stereochemistry of compound **1** was determined by x-ray crystallography as shown in Fig. 1. Although stereoisomers

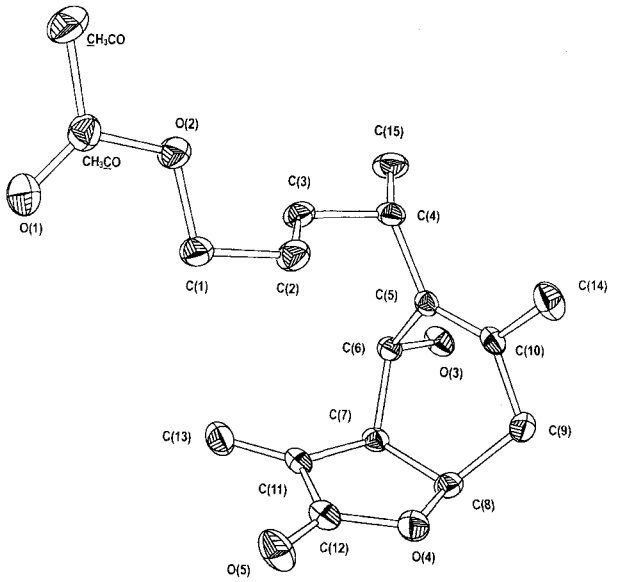


Fig. 1. X-Ray structure of compound **1**.

(Jeske *et al.*, 1993) and plain structure (Zhou *et al.*, 1993) of compound **1** were previously published, 1-*O*-acetyl-4*R*,6*S*-britannilactone (**1**) has never been published before.

Acknowledgement

This investigation was supported by a grant of the Korea Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6-01NA01-0002). We are grateful to Dr. Myung Hee Jung at KRICT, Korea, for the x-ray crystallography.

References

Bensky, D., Gamble, A., Kaptchuk, T. J., and Bensky, L. L. Chinese Herbal Medicine: Materia Medica. Revised edition. Eastland Press, Seattle, pp. 193-194 (1999).

Han, J. W., Lee, B. G., Kim, Y. K., Yoon, J. W., Jin, H. K., Hong, S., Lee, H. Y., Lee, K. R., and Lee, H. W. Ergolide, sesquiterpene lactone from *Inula britannica*, inhibits inducible nitric oxide synthase and cyclooxygenase-2 expression in RAW 264.7 macrophages through the inactivation of NF-kB. *Br. J. Pharmacol.*, **133**, 503-512 (2001).

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).

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).

(Accepted February 17, 2002)