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Isolation of β-sitosterol, Phytol and Zingerone 4-O-β-D-glucopyranoside from *Chrysanthemum Boreale* Makino

Dong-Hyun Kim*, Myun-Ho Bang, Myoung-Chong Song*, Soo-Un Kim***, Young Jin Chang***, and Nam In Baek*†**

**Graduate School of Biotechnology & Plant Metabolism Research Center, Kyung Hee University, Suwon 449-701, Korea.*
***Ganghwa Agricultural R & D Center, Inchon 417-833, Korea.*
****Program in Applied Life Chemistry, School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea.*

ABSTRACT : The flowers of *Chrysanthemum boreale* Makino were extracted with 80% aqueous MeOH, and the concentrated extract was partitioned with *n*-hexane, EtOAc, *n*-BuOH and H_2O. Two compounds from the *n*-hexane fraction and one glucoside from the *n*-BuOH fraction were isolated through the repeated silica gel and ODS column chromatographies. From the result of physico-chemical data including NMR, MS and IR, the chemical structures of the compounds were determined as β-sitosterol (1), phytol (2) and zingerone 4-O-β-D-glucopyranoside (3). Compounds 2 and 3 were isolated for the first time from this plant.

Key words **:** *Chrysanthemum boreale*; Compositae; β-sitosterol; phytol; zingerone 4-O-β-D-glucopyranoside;

INTRODUCTION

Chrysanthemum boreale Makino is a perennial herb which is an aromatic shrub with yellow flowers and grows to 30-120 ㎝ tall (Jung *et al*, 1990). Among the Compositae family *C. boreale* and other related species such as *C. indicum* and *C. lavandulaefolium* are known as important medicinal plants and have been used for the treatment of several infectious diseases such as pneumonia, colitis, stomatitis, carbuncle, fever, and sore, and used to treat vertigo and hypertensive symptoms (Jang *et al*, 1998; Kim *et al*, 2003). Flowers of *C. boreale* are also a common folk liquor in Korea. The constituents of *C. boreale* have been studied by a number of researchers, that is, guaianolide sesquiterpene lactones, flavonoids and polyacetylenes (Lee *et al*, 2001; Lee *et al*, 2003). Here, we report on the isolation and identification of three compounds from the flowers of *C. boreale*.

MATERIALS AND METHODS

Plant material

The flower of *Chrysanthemum boreale* Makino was supplied from Gwang Rok botanical garden (2003, Bundang) and identified by Dr. Dae Keun Kim, Woosuk University, Jeonju, Korea. A voucher specimen (KHU031031) was reserved at the Laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea.

Instrumentation

Uncorrected melting point was determined on a Fisher-John apparatus. Optical rotations were measured on a P-1010 digital polarimeter (JASCO, Japan). EI-MS and FAB-MS were recorded on a JMSAX 505-WA (JEOL, Japan). IR spectra were run on a Spectrum One FT-IR spectrometer Perkin Elmer, USA). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer Varian, USA).

Isolation of the compounds from the flowers of *Chrysanthemum boreale* Makino

The dried flowers of *C. boreale* (2.5 ㎏) were extracted three times at room temperature with 80% aqueous MeOH (10 L×3). The extracts were partitioned with water (1 L), *n*-hexane(1 L×3), EtOAc (1 L×3) and *n*-BuOH (1 L×3), successively. The *n*-hexane extract (5.9 g) was applied to the silica gel column chromatography (c.c.) (5×20 ㎝) and eluted with *n*-hexane : EtOAc (12 : 1 → 7 : 1 → 5 : 1 → 3 : 1, each 1 L) and monitored by thin layer chromatography (TLC) to produce

† Corresponding author: (Phone) +82-31-201-2661 (E-mail) nibaek@khu.ac.kr
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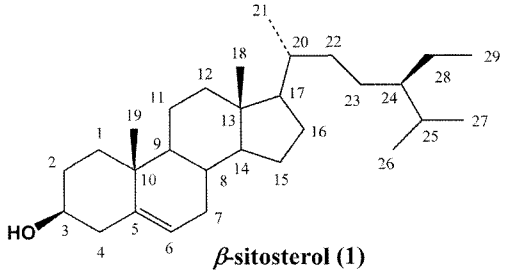
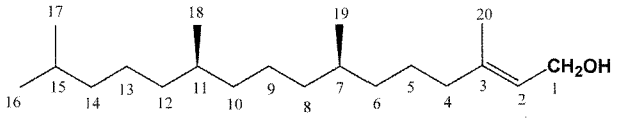
26 fractions (CBH1 to CBH26). CBH9 (124 mg, the fraction eluted with *n*-hexane : EtOAc = 7 : 1) was eluted with *n*-hexane : EtOAc (10 : 1, 1 L) from the silica gel c.c. (3×12 cm) to produce 5 fractions (CBH9-1 to CBH9-5). CTE11-5 (171 mg) was purified by ODS c.c. (3×8 cm) using acetone : acetonitrile (1 : 1, 1 L) as eluent to ultimately produce compound **1**{19 mg, Rf: 0.26 on ODS TLC in acetone : acetonitrile (2 : 1)}. Also, CBH10 (354 mg, the fraction eluted with *n*-hexane : EtOAc = 5 : 1) was purified by ODS c.c. (3×9 cm) using acetone : acetonitrile (1 : 2, 2 L) as eluent to yield compound **2** {15 mg, Rf: 0.75 on ODS TLC in acetone : acetonitrile (1 : 1)}. The *n*-BuOH extract (44 g) was fractionated by silica gel column chromatography (c.c.) and eluted with *n*-hexane : EtOAc (9 : 1 → 7 : 1 → 5 : 1 → 3 : 1 → 1 : 1, each 2 L) and analyzed by TLC to produce 32 fractions (CBB1 to CBB32). CBB19 (1.8 g, the fraction eluted with *n*-hexane : EtOAc = 3 : 1) was fractionated by silica gel c.c., and eluted with $CHCl_3$-MeOH (10 : 1, 2 L) to produce 8 fractions (CBB19-1 to CBB19-8). CBB19-6 (136 mg) was purified by ODS c.c using MeOH-H_2O (1 : 3, 1 L) as the eluent to yield compound **3** {15 mg, Rf: 0.42 on ODS TLC in MeOH : H_2O (1 : 1)}.

β-sitosterol (**1**): white powder (*n*-hexane-$CHCl_3$); m.p. 140 ℃; $[\alpha]_D$ = –37° (*c* = 0.2, $CHCl_3$); EI/MS *m/z*: 414 $[M]^+$; IR_υ ($CHCl_3$, cm^{-1}) 3400, 1640, 1050, 802, 845, 830; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 5.31 (1H, d, *J* = 5.2 Hz, H-6), 3.49 (1H, m, H-3), 0.97 (3H, s, H-19), 0.88 (3H, d, *J* = 6.4 Hz, H-21), 0.81 (3H, t, *J* = 7.2 Hz, H-29), 0.80 (3H, d, *J* = 7.2 Hz, H-26), 0.77 (3H, d, *J* = 6.8 Hz, H-27), 0.64 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C) 140.88 (C-5), 121.88 (C-6), 72.04 (C-3), 57.02 (C-14), 56.30 (C-17), 50.38 (C-9), 46.10 (C-24), 42.58 (C-4), 42.58 (C-13), 40.05 (C-12), 37.53 (C-1), 36.79 (C-10), 36.43 (C-20), 34.22 (C-22), 32.19 (C-7), 32.19 (C-8), 31.95 (C-2), 29.43 (C-25), 28.54 (C-16), 26.35 (C-23), 24.60 (C-15), 23.36 (C-28), 21.38 (C-11), 20.13 (C-26), 19.71 (C-19), 19.34 (C-27), 19.09 (C-21), 12.30 (C-29), 12.18 (C-18).

phytol (**2**): colorless oil; $[\alpha]_D$ = +0.2° (*c* = 0.2, $CHCl_3$); EI/MS *m/z*: 296 $[M]^+$; IR ($CHCl_3$, cm^{-1}) 3334, 2954, 2923, 2868, 1669; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 5.38 (1H, tq, *J* = 6.8, 1.6 Hz, H-2), 4.13 (2H, d, *J* = 6.8 Hz, H-1), 1.96 (2H, t, *J* = 8.0 Hz), 1.64 (3H, br. s, H-20), 1.53-0.99 (methine&methylene), 0.84 (6H, d, *J* = 6.4 Hz, H-16, 17), 0.82 (3H, d, *J* = 6.4 Hz, H-18), 0.82 (3H, d, *J* = 6.8 Hz, H-19); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C) 140.19 (C-3), 122.95 (C-2), 59.42 (C-1), 39.91 (C-4), 39.40 (C-5), 37.46 (C-9), 37.39 (C-6), 37.32 (C-8), 36.65 (C-10), 32.76 (C-11), 32.73 (C-7), 28.03 (C-15), 25.18 (C-12), 24.86 (C-13), 24.53 (C-14), 22.79 (C-19), 22.69 (C-20), 19.82 (C-18), 19.79 (C-16), 16.26 (C-17).

zingerone 4-O-β-D-glucopyranoside (**3**): amorphous powder ($CHCl_3$-MeOH); $[\alpha]_D$ = +31.4° (*c* = 0.2, MeOH); pos. FAB/MS





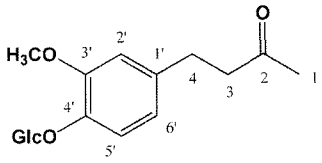


Fig. 1. Chemical structures of compounds **1-3** isolated from the flowers of *Chrysanthemum boreale* Makino.

m/z: 357 $[M+H]^+$; IR_υ (MeOH, cm^{-1}) 3400, 1710; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 7.50 (1H, d, *J* = 8.4 Hz, H-5'), 6.91 (1H, br. s, H-2'), 6.76 (1H, br. d, *J* = 8.4 Hz, H-6'), 5.66 (1H, d, *J* = 6.4 Hz, anomeric-H), 3.71 (3H, s, OMe), 2.86 (2H, t, *J* = 7.6 Hz, H-4), 2.68 (2H, t, *J* = 7.6 Hz, H-3), 2.03 (3H, s, H-1); ^{13}C-NMR (100 MHz, pyridine-d_5, δ_C) 207.20 (C-2), 150.05 (C-4'), 146.20 (C-3'), 135.66 (C-1'), 120.80 (C-6'), 116.40 (C-5'), 113.34 (C-2'), 102.33 (anomeric-C), 78.88 (C-3), 78.59 (C-5), 74.95 (C-2), 71.26 (C-4), 62.38 (C-6), 55.95 (OMe), 45.32 (C-3), 29.88 (C-4), 29.75 (C-1).

RESULTS AND DISCUSSION

The MeOH extract obtained from the flowers of *C. boreale* was successively fractionated into H_2O, *n*-hexane, EtOAc, and *n*-BuOH layers. The *n*-hexane and *n*-BuOH fraction were subjected to repetitive chromatography on silica gel and ODS column chromatographies. Three known compounds **1-3** were recovered from these fractions.

Compound **1**, white powder, showed absorbance bands resulting from the hydroxyl (3400 cm^{-1}) and olefine (1640 cm^{-1}) in the IR spectrum and a molecular ion peak $[M]^+$ at *m/z* 414 in the EI/MS. In the ^{1}H-NMR spectrum, an olefinic methine (δ_H 5.31) and an oxygenated methine (δ_H 3.49) signals were observed. Also, a number of methylene and methine signals

(δ_H 2.29-1.00) were observed. The two singlet methyl (δ_H 0.97, 0.64), three doublet methyl (δ_H 0.88, 0.80, 0.77) and a triplet methyl (δ_H 0.81) signals were observed. In the ^{13}C-NMR spectrum 29 signals consisting of a quaternary (δ_C 140.88), an olefinic methine (δ_C 121.88), an oxygenated methine (δ_C 72.04) and six methyl (δ_C 20.13, 19.71, 19.34, 19.09, 12.30, 12.18) signals were observed. Thus, we concluded that compound **1** is a sterol compound composed of one double bond and one hydroxyl group. Compound **1** was eventually identified as stigmast-5-en-3-β-ol (β-sitosterol) through the comparison of different physical and spectral data with literature data (Kim *et al*, 2004).

Compound **2**, colorless oil, showed absorbance bands due to the hydroxyl (3334 cm^{-1}) and olefine (1669 cm^{-1}) in the IR spectrum and a molecular ion peak $[M]^+$ at *m/z* 296 in the EI/MS. The ^{1}H-NMR spectrum revealed an olefinic methine (δ_H 5.38), an oxygenated methylene (δ_H 4.13) and an allyl methylene (δ_H 1.96). Also, in the high magnet field region, an allyl singlet methyl (δ_H 1.64) and four doublet methyl (δ_H 0.842, 0.82, 0.82) including several methine or methylene signals were observed indicating compound **2** to be an aliphatic alcohol with one double bond. In the ^{13}C-NMR spectrum 20 signals consisting of an olefinic quaternary (δ_C 140.19) and a methine (δ_C 122.95), an oxygenated methylene (δ_C 59.42), five methyl (δ_C 22.79, 22.69, 19.82, 19.79, 16.29), nine methylene and three methine signals were observed. Thus, we concluded that compound **2** is an acyclic aliphatic diterpenoid. Compound **2** was ultimately identified as phytol by comparing different physical and spectral data with literature data (Bang *et al*, 2002).

Comound **3**, amorphous powder, showed the absorbance bands due to the hydroxyl (3400 cm^{-1}) and olefine (1710 cm^{-1}) in the IR spectrum and molecular ion peak $[M+H]^+$ at *m/z* 357 in the positive FAB/MS. In the ^{1}H-NMR spectrum, three olefinic methine (δ_H 7.50, d, J = 8.4 Hz; 6.91, br. s; 6.76, br. d, J = 8.4 Hz) resulting from a 1,3,4-trisubstituted benzene ring, a methoxy group (δ_H 3.71), two methylene (δ_H 2.86, 2.68) and a methyl (δ_H 2.03) signals were observed. Also, the oxygenated methine signal (δ_H 5.66) of an anomeric proton and a number of oxygenated methine and methylene signals proved the existence of a sugar molecule. In the ^{13}C-NMR spectrum 17 signals consisting of one ketone (δ_C 207.20), two olefinic oxygenated quaternary and an olefinic quaternary (δ_C 150.05, 146.20, 135.66) and three olefinic methine (δ_C 120.80, 116.40, 113.34) signals were observed. In the high magnet field region, a methoxy group (δ_C 55.95), two methylene (δ_C 45.32, 29.88) and a methyl (δ_C 29.75) signals were observed. These results led to the conclusion for compound **3** to have a benzene ring and a butane including a ketone. Also, the comparison between the chemical shifts of this sugar and those reported in the literature (Seo *et al*, 1978) resulted in identification of the sugar as a β-D-glucopyranose. Compound **3** was finally identified as zingerone 4-O-β-D-glucopyranoside through the comparison of different physical and spectral data with literature data (Fan *et al*, 2001; Higuchi *et al*, 1977). Of the compounds isolated in this study, phytol and zingerone 4-O-β-D-glucopyranoside is the first to be isolated from *C. boreale*. Also, it was reported that β-sitosterol had uterotrophic effect as accelerating an acid phosphate activity, antivirus, antiinflammatory and antifebrile activity (Hyun *et al*, 1996). Phytol was already known as SSADH and ACAT inhibitory diterpenoid related to arteriosclerosis and hypertension and having antimutagenic and anticancer effects (Bang *et al*, 2002; Jang *et al*, 2003; Kim *et al*, 1993; Lawson *et al*, 1989). And zingerone, which is an aglycone of zingerome 4-O-β-D-glucopyranoside, have been known as antioxidant (Kabuto *et al*, 2005). We are going to perform various activity assays for the compounds isolated in this study.

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