Chemical Constituents of the Root of *Dystaenia takeshimana* and Their Anti-Inflammatory Activity

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In our ongoing search for bioactive compounds originating from the endemic species in Korea, we found that the hexane and EtOAc fractions of the MeOH extract from the root of *Dystaenia takeshimana* (Nakai) Kitagawa (Umbelliferae) showed cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) dual inhibitory activity by assessing their effects on the production of prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4) in mouse bone marrow-derived mast cells. By activity-guided fractionation, five coumarins, viz. psoralen (**2**), xanthotoxin (**3**), scopoletin (**4**), umbelliferone (**5**), and (+)-marmesin (**6**), together with β-sitosterol (**1**), were isolated from the hexane fraction, and two phenethyl alcohol derivatives, viz. 2-methoxy-2-(4'-hydroxyphe-nyl)ethanol (**7**) and 2-hydroxy-2-(4'-hydroxyphenyl)ethanol (**8**), three flavonoids, viz. apigenin (**9**), luteolin (**10**), and cynaroside (**11**), as well as daucosterol (**12**) were isolated from the EtOAc fraction using silica gel column chromatography. In addition, D-mannitol (**13**) was isolated from the BuOH fraction by recrystallization. Two of the coumarins, scopoletin (**4**) and (+)-marmesin (**6**), the two phenethyl alcohol derivatives (**7**, **8**) and the three flavonoids (**9–11**) were isolated for the first time from this plant. Among the compounds isolated from this plant, the five coumarins as well as the three flavonoids showed COX-2/5-LOX dual inhibitory activity. These results suggest that the anti-inflammatory activity of *D. takeshimana* might in part occur *via* the inhibition of the generation of eicosanoids.

Key words: *Dystaenia takeshimana*, Umbelliferae, Chemical constituents, Cyclooxygenase-2, 5-Lipoxygenase, Anti-inflammatory activity

INTRODUCTION

Dystaenia takeshimana (Nakai) Kitagawa, which belongs to the family Umbelliferae, is a 1.5~2 meter tall perennial herb distributed in Ulreung island as an endemic species in Korea. The root of this plant has long been used as pig feed and is sometimes called pig herb (Lee, 1996). Previous phytochemical investigations carried out on the roots of this plant resulted in the isolation of various coumarins and sterols, as well as mannitol (Kwon *et al.*,

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1992; Kim *et al.*, 1993). A preliminary biological assay on *D. takeshimana* indicated that the MeOH extracts of the roots and herbs exhibited potent inhibitory activity against NO production in LPS-activated murine macrophage-like RAW 264.7 cells (Kim *et al.*, 2004). During the course of our studies on the bioactive constituents derived from the endemic species in Korea (Park *et al.*, 2002; Jung *et al.*, 2002; Thuong *et al.*, 2005), we found that the hexane and EtOAc fractions of the MeOH extract showed potent inhibitory effects in the COX-2 and 5-LOX assays. However, the bioactive constituents of the roots of *D. takeshimana* have not yet been characterized. This paper describes the isolation, identification and biological activity of the compounds from the bioactive fractions, along with the inhibitory effects of the isolates on COX-2 and 5-LOX. Therefore, the development of dual inhibitors, which can simultaneously inhibit COX-2/5-LOX, might enhance their

individual anti-inflammatory effects and reduce the undesirable side effects associated with nonsteroidal anti-inflammatory drugs (NSAIDs).

MATERIALS AND METHODS

Plant material

The roots of *Dystaenia takeshimana* were collected in August 2002 at Ullung island in Gyungsangbukdo and identified by one of the authors (K. Bae). A voucher specimen (CNU 1539) was deposited at the herbarium in the College of Pharmacy, Chungnam National University.

Instruments and reagents

The melting points were measured using an Electrothermal melting point apparatus and are uncorrected. The UV and IR spectra were recorded with a Beckman Du-650 UV-VIS recording spectrophotometer and a JASCO Report-100 infrared spectrometer, respectively. The NMR spectra were recorded on a JEOL 300 FT-NMR spectrometer (^{1}H, 300 MHz, ^{13}C, 75 MHz) with tetramethylsilane (TMS) as the internal standard. The EIMS data were obtained using a Hewlett-Packard 5989B spectrometer. Column chromatography was carried out with silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck). Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} (0.25 mm, Merck), visualized with a UV lamp, and also by spraying with a 20% H_2SO_4 solution and heating.

Extraction and isolation

The roots of *D. takeshimana* (3.5 kg) were extracted with MeOH seven times under reflux for 3 h, then filtered and concentrated to yield the MeOH extract (640 g). The MeOH extract was suspended in H_2O and extracted with hexane, EtOAc and then BuOH to give the hexane soluble fraction (286 g), EtOAc soluble fraction (6 g) and BuOH soluble fraction (78 g), respectively. The hexane soluble fraction was subjected to column chromatography with silica gel and eluted using a stepwise gradient of hexane and EtOAc (50:1 → 1:1) to afford 130 subfractions. Fractions 41 and 55 were chromatographed on a silica gel column using hexane-EtOAc (gradient) as solvents, giving compounds 1 (30 mg), 2 (18 mg) and 3 (18 mg). Fractions 119 and 126 were further purified over a silica gel column, with hexane-EtOAc (gradient) as the solvent system, resulting in compounds 4 (2 mg) from fraction 119, and 5 (1 mg) and 6 (3 mg) from fraction 126. The EtOAc soluble fraction was chromatographed on silica gel using an eluting mixture of CH_2Cl_2-MeOH-H_2O (7:0.5:1), which afforded 70 subfractions. Fraction 4 was further purified by silica gel column chromatography with CH_2Cl_2-EtOAc and then RP_{18} using MeOH-H_2O (1 : 1) to afford

compound 7 (12 mg). Fraction 18 was chromatographed on a silica gel column using hexane-EtOAc as solvents, giving compound 8 (4 mg). Fractions 54 and 67 were rechromatographed by silica gel column chromatography (CH_2Cl_2–MeOH), which yielded 5 mg of compound 9 and 10 mg of compound 10 from fraction 54, and 11 mg of compound 11 and 20 mg of compound 12 from fraction 67. The BuOH soluble fraction was recrystallized from MeOH to yield compound 13 (8.3 g).

β-Sitosterol (1)

Whitish amorphous powder, IR v_{max} (KBr) cm^{-1}: 3400, 2920, 1680, 1450, 1055; ^{1}H-NMR (300 MHz, CDCl$_3$) δ: 5.35 (1H, d, J = 5.2 Hz, H-6), 3.53 (1H, m, H-3), 1.01 (3H, s, CH$_3$-19), 0.97 (3H, d, J = 6.5 Hz, CH$_3$-21), 0.92 (3H, d, J = 6.5 Hz, CH$_3$-26), 0.81 (3H, d, J = 6.6 Hz, CH$_3$-27), 0.84 (3H, t, J = 7.5 Hz, CH$_3$-29)0.69 (3H, s, CH$_3$-18); ^{13}C-NMR (75 MHz, CDCl$_3$) δ: 37.2 (C-1), 31.9 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.7 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.7 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 12.0 (C-18), 19.0 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.4 (C-26), 19.8 (C-27), 23.1 (C-28), 11.9 (C-29).

Psolalen (2)

Colorless needles, m.p. 163-164°C, UV λ_{max} (MeOH) nm: 242, 247, 291, 330; IR v_{max} (KBr) cm^{-1}: 1720, 1710, 1630, 1575; ^{1}H-NMR (300 MHz, CDCl$_3$) δ: 6.36 (1H, d, J = 9.6 Hz, H-3), 6.82 (1H, dd, J = 0.9, 2.4 Hz, H-3'), 7.44 (1H, br s, H-8), 7.67 (1H, s, H-5), 7.68 (1H, d, J = 2.4 Hz, H-2'), 7.78 (1H, d, J = 9.6 Hz, H-4); ^{13}C-NMR (75 MHz, CDCl$_3$) δ: 160.9 (C-2), 114.6 (C-3), 144.0 (C-4), 119.8 (C-5), 124.8 (C-6), 156.3 (C-7), 99.8 (C-8), 152.0 (C-9), 115.4 (C-10), 146.9 (C-2'), 106.3 (C-3'); EIMS m/z 186 [M]$^+$ (100), 158 [M – CO]$^+$ (88), 130 [M – 2CO]$^+$ (24), 102 [M – 3CO]$^+$ (40), 76 (16).

Xanthotoxin (3)

Colorless needles, m.p. 147-148°C, UV λ_{max} (MeOH) nm: 249, 262, 299; IR v_{max} (KBr) cm^{-1}: 1706, 1677, 1616, 1601, 1585; ^{1}H-NMR (300 MHz, CDCl$_3$) δ: 4.28 (3H, s, OCH$_3$-8), 6.35 (1H, d, J = 9.6 Hz, H-3), 6.81 (1H, d, J = 2.4 Hz, H-3'), 7.68 (1H, d, J = 2.4 Hz, H-2'), 7.34 (1H, s, H-5), 7.75 (1H, d, J = 9.6 Hz, H-4); ^{13}C-NMR (75 MHz, CDCl$_3$) δ: 160.4 (C-2), 114.7 (C-3), 144.3 (C-4), 112.9 (C-5), 126.1 (C-6), 147.6 (C-7), 133.7 (C-8), 142.9 (C-9), 116.4 (C-10), 146.6 (C-2'), 106.7 (C-3'), 61.3 (OCH$_3$-8); EIMS m/z 216 [M]$^+$ (100), 201 [M – CH$_3$]$^+$ (29), 188 [M – CO]$^+$ (10), 173 [M – (CO + CH$_3$)]$^+$ (45), 145 [M – (2CO + CH$_3$)]$^+$ (18), 117 [M – (3CO + CH$_3$)]$^+$ (3), 89 [M – (4CO + CH$_3$)]$^+$ (26), 63 (16).

Scopoletin (4)

Whitish amorphous powder, UV λ_{max} (MeOH) nm (log ε): 228 (4.06), 252 (3.63), 260 (sh, 3.59), 297 (3.63), 345 (4.00); λ_{max} (NaOAc) nm: 221 (4.21), 274 (sh, 3.53), 392 (4.15); IR ν_{max} (KBr) cm^{-1}: 1706, 1677, 1616, 1601, 1585; ^{1}H-NMR (300 MHz, CDCl$_3$) δ: 3.96 (3H, s, OCH$_3$-6), 6.27 (1H, d, J = 9.3 Hz, H-3), 6.85 (1H, s, H-8), 6.92 (1H, s, H-5), 7.60 (1H, d, J = 9.3 Hz, H-4); ^{13}C-NMR (75 MHz, CDCl$_3$) δ: 161.4 (C-2), 113.4 (C-3), 143.3 (C-4), 107.5 (C-5), 150.2 (C-6), 144.0 (C-7), 103.2 (C-8), 149.7 (C-9), 111.5 (C-10), 56.4 (OCH$_3$-6); EIMS m/z 192 [M]$^+$ (100), 177 [M − CH$_3$]$^+$ (47), 164 [M − CO]$^+$ (25), 149 [M − (CO + CH$_3$)]$^+$ (48), 121 [M − (2CO + CH$_3$)]$^+$ (22), 105 (22), 79 [M − (4CO + CH$_3$)]$^+$ (24), 69 (49).

Umbelliferone (5)

Whitish amorphous powder, UV λ_{max} (MeOH) nm: 216, 246, 254, 325; IR ν_{max} (KBr) cm^{-1}: 3150, 1720, 1688, 1619, 1574, 1460, 1238, 1130, 840; ^{1}H-NMR (300 MHz, CD$_3$OD + CDCl$_3$) δ: 6.16 (1H, d, J = 9.3 Hz, H-3), 6.71 (1H, d, J = 2.1 Hz, H-8), 6.77 (1H, dd, J = 2.1, 8.4 Hz, H-6), 7.39 (1H, d, J = 8.4 Hz, H-5), 7.77 (1H, d, J = 9.3 Hz, H-4); ^{13}C-NMR (75 MHz, CD$_3$OD + CDCl$_3$) δ: 162.9 (C-2), 113.7 (C-3), 145.0 (C-4), 129.6 (C-5), 113.7 (C-6), 162.0 (C-7), 102.8 (C-8), 156.2 (C-9), 112.2 (C-10); EIMS m/z 162 [M]$^+$ (71), 134 [M − CO]$^+$ (100), 105 [M − (CO + CHO)]$^+$ (34), 78 [M − 3CO]$^+$ (53), 69 (32), 63 (57), 51 (68).

(+)-Marmesin (6)

Amorphous powder, $[\alpha]_D^{22}$ = +24.4° (c 0.15, CHCl$_3$), ^{1}H-NMR (300 MHz, CDCl$_3$) δ: 1.24, 1.37 (3H each, s, 2 × CH$_3$-5'), 3.18 (1H, ddd, J = 0.9, 9.3, 15.9 Hz, H-3'a), 3.25 (1H, ddd, J = 1.5, 8.4, 15.9 Hz, H-3'b), 4.74 (1H, dd, J = 8.4, 9.3 Hz, H-2'), 6.21 (1H, d, J = 9.6 Hz, H-3), 6.74 (1H, s, H-8), 7.22 (1H, t, J = 1.2 Hz, H-5), 7.59 (1H, d, J = 9.6 Hz, H-4); ^{13}C-NMR (75 MHz, CDCl$_3$) δ: 161.4 (C-2), 112.2 (C-3), 143.7 (C-4), 123.4 (C-5), 125.1 (C-6), 163.1 (C-7), 97.9 (C-8), 155.7 (C-9), 112.7 (C-10), 91.1 (C-2'), 29.4 (C-3'), 71.6 (C-4'), 24.3, 26.0 (2 × CH$_3$-5'); EIMS m/z 246 [M]$^+$ (50), 228 [M − H$_2$O]$^+$ (4), 213 [M − (H$_2$O + CH$_3$)]$^+$ (24), 187 (100), 175 (12), 160 (29), 131 (20), 59 (60).

2-Methoxy-2-(4'-hydroxyphenyl)ethanol (7)

Amorphous powder, $[\alpha]_D^{22}$ = ±0° (c 0.11, MeOH), ^{1}H-NMR (300 MHz, CD$_3$OD) δ: 3.22 (3H, s, OCH$_3$), 3.48 (1H, br dd, J = 3.9, 11.4 Hz, H-1a), 3.61 (1H, dd, J = 8.1, 11.4 Hz, H-1b), 4.15 (1H, dd, J = 3.9. 8.1 Hz, H-2), 6.76 (2H, td, J = 2.1, 8.7 Hz, H-3', 5'), 7.11 (2H, td, J = 2.1, 8.7 Hz, H-2', 6'); ^{13}C-NMR (75 MHz, CD$_3$OD) δ: 56.8 (OCH$_3$), 67.8 (C-1), 86.0 (C-2), 116.2 (C-3', 5'), 129.3 (C-2', 6'), 130.9 (C-1'), 158.4 (C-4'); EIMS m/z 168 [M]$^+$ (3), 137 (100), 121 (16), 107 (7), 91 (11), 77 (6).

2-Hydroxy-2-(4'-hydroxyphenyl)ethanol (8)

Amorphous powder, $[\alpha]_D^{22}$ = +19.2° (c 0.1, MeOH), ^{1}H-NMR (300 MHz, CD$_3$OD) δ: 3.56 (2H, br d, J = 6.3 Hz, H-1), 4.58 (1H, t, J = 6.0 Hz, H-2), 6.74 (2H, td, J = 2.1, 8.7 Hz, H-3', 5'), 7.17 (2H, td, J = 2.1, 8.7 Hz, H-2', 6'); ^{13}C-NMR (75 MHz, CD$_3$OD) δ: 68.7 (C-1), 75.7 (C-2), 116.0 (C-3', 5'), 128.7 (C-2', 6'), 134.1 (C-1'), 158.0 (C-4'); EIMS m/z 154 [M]$^+$ (11), 123 (100), 107 (6), 95 (42), 77 (50).

Apigenin (9)

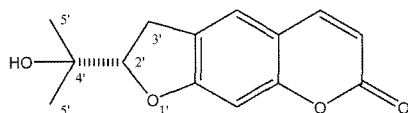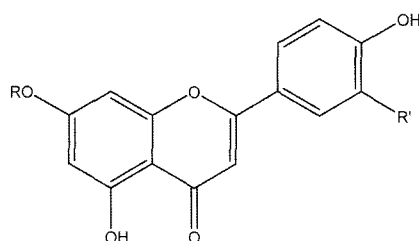Yellowish amorphous powder, UV λ_{max} (MeOH) nm: 267, 296 (sh), 336; IR ν_{max} (KBr) cm^{-1}: 3400, 1650, 1600, 1495; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 6.14 (1H, d, J = 2.1 Hz, H-6), 6.44 (1H, d, J = 2.1 Hz, H-8), 6.61 (1H, s, H-3), 6.88 (2H, d, J = 8.7 Hz, H-3', 5'), 7.81 (2H, d, J = 8.7 Hz, H-2', 6'); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 164.8 (C-2), 103.4 (C-3), 182.6 (C-4), 161.7 (C-5), 99.7 (C-6), 164.8 (C-7), 94.9 (C-8), 158.1 (C-9), 104.4 (C-10), 121.9 (C-1'), 129.3 (C-2', 6'), 161.9 (C-4'), 116.9 (C-3', 5').

Luteolin (10)
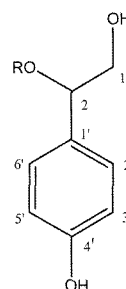
Yellowish amorphous powder, UV λ_{max} (MeOH) nm: 253, 267, 291 (sh), 349; IR ν_{max} (KBr) cm^{-1}: 3400, 1600, 1605, 1498; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 6.19 (1H, d, J = 2.1 Hz, H-6), 6.44 (1H, d, J = 2.1 Hz, H-8), 6.65 (1H, s, H-3), 6.89 (1H, d, J = 8.4 Hz, H-5'), 7.39 (1H, s, H-2'), 7.42 (H, dd, J = 2.1, 8.4 Hz, H-6'); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 164.8 (C-2), 103.8 (C-3), 182.5 (C-4), 162.4 (C-5), 99.7 (C-6), 164.9 (C-7), 94.7 (C-8), 146.6 (C-9), 104.6 (C-10), 122.4 (C-1'), 114.3 (C-2'), 158.1 (C-3'), 150.6 (C-4'), 116.9 (C-5'), 119.8 (C-6').

Cynaroside (11)

Yellowish amorphous powder, UV λ_{max} (MeOH) nm (log ε): 254 (4.21), 260 (sh, 4.17), 346 (4.13); λ_{max} (MeONa) nm: 262 (4.25), 390 (4.17); λ_{max} (NaOAc) nm: 256 (4.22), 260 (4.11), 402 (4.11); λ_{max} (NaOAc + H$_3$BO$_3$) nm: 256 (4.29), 369 (4.15); λ_{max} (AlCl$_3$) nm: 273 (4.20), 328 (3.72), 429 (4.21); λ_{max} (AlCl$_3$ + HCl) nm: 261 (4.19), 273 (sh, 4.16), 295 (sh, 3.96), 362 (sh, 4.06), 390 (4.10); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 4.19 (1H, m, Glc H-5"), 4.32–4.44 (4H, m, Glc H-2", 3", 4", 6"a), 4.56 (1H, dd, J = 1.8, 12.0 Hz, Glc H-6"b), 5.80 (1H, d, J = 7.2 Hz, Glc H-1"), 6.83 (1H, d, J = 2.1 Hz, H-6), 6.91 (1H, s, H-3), 6.98 (1H, d, J = 2.1 Hz, H-8), 7.26 (1H, d, J = 8.4 Hz, H-5'), 7.50 (H, dd, J = 2.4, 8.4 Hz, H-6'), 7.88 (1H, d, J = 2.4 Hz, H-2'), 13.61 (1H, br s, 5-OH); ^{13}C-NMR (75 MHz, pyridine-d_5) δ: 163.9 (C-2), 104.1 (C-3), 182.8 (C-4), 162.5 (C-5), 100.6 (C-6), 165.3 (C-7), 95.3 (C-8), 157.8 (C-9), 106.5 (C-10), 122.6 (C-1'), 114.6 (C-2'), 147.8 (C-3'), 151.9 (C-4'), 116.8 (C-5'), 119.6 (C-6'), 101.7 (C-1"), 74.8 (C-2"), 78.4 (C-3"), 71.1 (C-4"), 79.2 (C-5"), 62.3 (C-6").
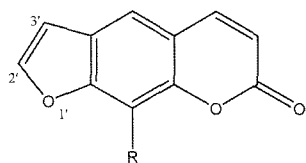
2 Psoralen   R= H
3 Xanthotoxin   R= OCH₃
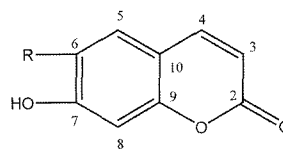
4 Scopoletin   R= OCH₃
5 Umbelliferone R= H

6 (+)-Marmesin

7 2-Methoxy-2-(4'-hydroxyphenyl)ethanol
R= CH₃

8 2-Hydroxy-2-(4'-hydroxyphenyl)ethanol
R= H

9 Apigenin   R= R'= H
10 Luteolin   R= H   R'= OH
11 Cynaroside R= Glucose R'= OH
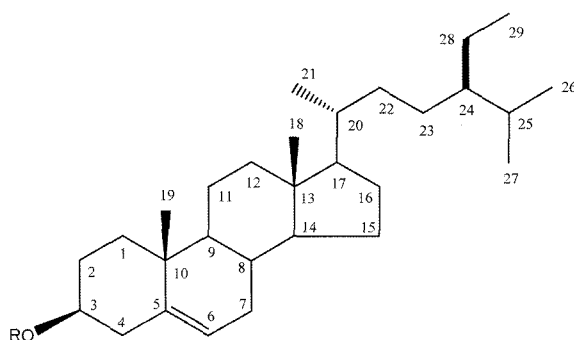
1 β–Sitosterol R= H
12 Daucosterol R= Glucose
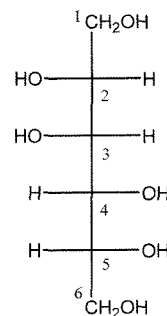
13 D-Mannitol

Daucosterol (12)

Amorphous powder, IR ν_{max}(KBr) cm⁻¹: 3420, 1640, 1450, 1380, 1110, 1080, 1020; ¹H-NMR (300 MHz, pyridine-d_5) δ: 5.35 (1H, br d, J = 5.1 Hz, H-6), 5.03 (1H, d, J = 7.6 Hz, H-1'), 1.02 (3H, s, CH₃-19), 0.66 (3H, s, CH₃-18); ¹³C-NMR (75 MHz, pyridine-d_5) δ: 37.6 (C-1), 30.4 (C-2), 78.7 (C-3), 39.6 (C-4), 40.2 (C-5), 122.0 (C-6), 32.4 (C-7), 32.3 (C-8), 50.6 (C-9), 37.1 (C-10), 21.5 (C-11), 40.1 (C-12), 42.7 (C-13), 57.0 (C-14), 24.7 (C-15), 28.7 (C-16), 56.5 (C-17), 12.1 (C-18), 19.4 (C-19), 36.5 (C-20), 19.2 (C-21), 34.4 (C-22), 26.7 (C-23), 46.3 (C-24), 29.7 (C-25), 19.5 (C-26), 20.1 (C-27), 23.6 (C-28), 12.3 (C-29), 102.7 (C-1'), 75.5 (C-2'), 78.6 (C-3'), 71.7 (C-4'), 78.4 (C-5'), 63.1 (C-6').

D-Mannitol (13)

Amorphous powder, ¹H-NMR (300 MHz, CD₃OD) δ: 3.66 (2H, dd, J = 5.7, 11.4 Hz, H-1a, 6a), 3.71–3.77 (2H, m, H-3, 4), 3.78 (2H, t, J = 8.4 Hz, H-2, 5), 3.86 (2H, dd, J = 2.4, 11.4 Hz, H-1b, 6b); ¹³C-NMR (75 MHz, CD₃OD) δ: 65.0 (C-1, 6), 71.0 (C-3, 4), 72.6 (C-2, 5).

Preparation and activation of bone marrow-derived mast cells (BMMCs)

Bone marrow cells from male Balb/cJ mice were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics and 10% fetal calf serum) and 50%

WEHI-3 cell conditioned medium as a source of IL-3. After 3 weeks more than 98% of the cells were found to be BMMCs when checked by the previously described procedure (Murakami *et al.*, 1995).

Determination of prostaglandin D_2 (PGD_2)

In order to measure the inhibitory activity on COX-2 by samples, the cells were suspended in enriched medium at a cell density of 5×10^5 cells/mL and preincubated with aspirin (10 μg/mL) for 2 h to irreversibly inactivate any preexisting COX-1. After washing, the BMMC were activated with *c-kit* ligand (KL, 100 ng/mL), IL-10 (100 U/mL) and LPS (100 ng/mL) at 37°C for 8 h in the presence or absence of the samples previously dissolved in dimethylsulfoxide (DMSO). All reactions were quenched by centrifugation at 120 g at 4°C for 5 min. The supernant and cell pellets were frozen immediately in liquid N_2 and stored at -80°C until needed for further analysis. Under these conditions, the COX-2-dependent phases of PGD_2 generation reached 1.6 ng/10^6 cells. The data is reported as the arithmetic mean of triplicate determinations.

Determination of leukotriene C_4 (LTC_4)

The BMMC suspended in enriched medium at a density of 1×10^6 cells/mL were pretreated with the samples for 15 min at 37°C and stimulated with KL (KL; 100 ng/mL). After 20 min stimulation, the supernatants were isolated and analyzed by EIA. The LTC_4 level was determined using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, U.S.A.) according to the manufacture's instructions. Under these conditions, the LTC_4 reached up to 500 pg/10^6 cells. The data is reported as the arithmetic mean of triplicate determinations.

RESULTS AND DISCUSSTION

During our search for biologically active compounds derived from the endemic species in Korea, the hexane and EtOAc fractions of the MeOH extract of the roots of *Dystaenia takeshimana* were shown to possess cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) dual inhibitory activities by assessing their effects on the production of the prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4) in mouse bone marrow-derived mast cells. Using bioassay-guided fractionation, thirteen compounds were isolated, among which five coumarins, viz. psoralen (2) (Kwon *et al.*, 1992; Sasaki *et al.*, 1980), xanthotoxin (3) (Kwon *et al.*, 1992; Sasaki *et al.*, 1980), scopoletin (4) (Kayser and Kolodziej, 1995; Sasaki *et al.*, 1980), umbelliferone (5) (Kim *et al.*, 1993), and (+)-marmesin (6) (Sasaki *et al.*, 1980), together with β-sitosterol (1) (Nes *et al.*, 1992; Chang *et al.*, 1981), were isolated from the hexane fraction, and two phenethyl alcohol derivatives,

viz. 2-methoxy-2-(4'-hydroxyphenyl) ethanol (7) (Matsumura *et al.*, 2002; Taniguchi *et al.*, 1996) and 2-hydroxy-2-(4'-hydroxyphenyl)ethanol (8) (Hisamoto *et al.*, 2004; Taniguchi *et al.*, 1996), three flavonoids, viz. apigenin (9) (Shin *et al.*, 1995), luteolin (10) (Shin *et al.*, 1995), and cynaroside (11) (Kim, 2001), as well as daucosterol (12) (Chang *et al.*, 1981) were isolated from the EtOAc fraction using silica gel column chromatography. The BuOH soluble fraction was recrystallized from MeOH to yield the compound, D-mannitol (13) (Hagiwara *et al.*, 2005). All of the compounds were identified by physicochemical and spectrometric methods and by comparing the data with the published values. As described in the reference (Matsumura *et al.*, 2002), compounds 7 and 8 were considered to be epimeric mixtures of 2-methoxy-2-(4'-hydroxyphenyl)ethanol and 2-hydroxy-2-(4'-hydroxyphenyl)ethanol, respectively. A simple coumarin, scopoletin (4), a dihydrofuranocoumarin, (+)-marmesin (6), two phenethyl alcohol derivatives (7 and 8), and three flavone type flavonoids (9-11) were isolated for the first time from this plant.

Murakami *et al.* (1995) reported that BMMCs exhibit biphasic PGD_2 biosynthetic responses over time, in addition to COX-1-dependent immediate and COX-2-dependent delayed responses. The immediate generation of PGD_2 that occurs within 2 h of culturing is associated with the coupling of COX-1, while the delayed generation of PGD_2, which occurs after several hours of culturing (2-10 h), is associated with the de novo induction and function of COX-2 after the stimulation with particular cytokines and LPS combinations (Murakami *et al.*, 1994). This cell model also appears to be suitable for assessing the effect of 5-LOX inhibitors, since the immediate generation of LTC_4 elicited by the IgE-dependent or cytokine-initiated stimulus occurs in BMMCs through 5-LOX (Murakami *et al.*, 1995). Therefore, the BMMC system is useful for screening selective COX-1/COX-2 or 5-LOX and COX-2/5-LOX dual inhibitors from various sources (Lee *et al.*, 2004). Prostagladins (PGs) elicit a variety of important biological responses. Among thees properties are their ability to induce pain, fever and the symptoms associated with various inflammatory responses. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and inflammatory swelling by blocking PGs synthesis at the COX stage. Arachidonic acid can also be converted to leukotrienes (LTs) by the action of 5-lipoxygenase (5-LOX). The development of dual inhibitors that can simultaneously inhibit COX-2 and 5-LOX might enhance their individual anti-inflammatory effects and reduce the undesirable side effects that are associated with nonsteroidal anti-inflammatory drugs (NSAIDs) (Fiorucci *et al.*, 2001; Lin *et al.*, 2004). Therefore, the inhibitory activity of each fraction or of the isolated compounds on the generation of both PGD_2 and LTC_4 in the BMMC was examined. When the

BMMCs were activated with a combination of KL, IL-10 and LPS in the presence or absence of each fraction or of the isolated compounds, the generation of PGD$_2$ in the COX-2-dependent phase and the generation of LTC$_4$ in the 5-LOX dependent phase were strongly inhibited by both hexane and EtOAc fractions derived from the MeOH extract (Table I). The inhibition values of the hexane fraction were 47.7 and 86.9% in the COX-2 and 5-LOX assays, respectively, while the corresponding values for the EtOAc fraction were 79.8 and 90.2%. The butanol and water fractions showed weak or no activity against the tested enzymes. In addition, the isolated compounds were also examined for their anti-inflammatory activity under the same conditions. Table II shows that the five coumarins exhibited dual COX-2 and 5-LOX inhibitory activity. In addition, three flavonoids also showed strong COX-2/5-LOX dual inhibitory activity. This suggests that the anti-inflammatory activity of *D. takeshimana* might occur in part *via* the inhibition of eicosanoid generation by both coumarins and flavonoids. However, further study will be needed to clarify the mechanisms for the action of

Table I. Anti-inflammatory activity of MeOH extract and fractions

Extract and fractions	COX-2 inhibition (%) (12.5 μg/mL)	5-LO inhibition (%) (25 μg/mL)
MeOH	0	-60.0
Hexane	47.7	86.9
EtOAc	79.8	90.2
BuOH	7.2	-11.4
H$_2$O	20.1	-73.5

Table II. Anti-inflammatory activity of isolated compounds

Compounds	COX-2 inhibition (%) (12.5 μg/mL)	5-LO inhibition (%) (25 μg/mL)
β-Sitosterol (1)	98.2	77.3
Psoralen (2)	88.2	84.1
Xanthotoxin (3)	60.9	94.1
Scopoletin (4)	62.1	70.0
Umbelliferone (5)	61.5	80.6
(+)-Marmesin (6)	61.2	56.7
2-Methoxy-2-(4'-hydroxyphenyl)ethanol (7)	13.0	-8.40
2-Hydroxy-2-(4'-hydroxyphenyl)ethanol (8)	44.0	57.6
Apigenin (9)	89.2	88.3
Luteolin (10)	92.5	91.3
Cynaroside (11)	83.3	97.6
Daucosterol (12)	2.20	19.3
Mannitol (13)	23.2	0.00
AA861		100
NS398	100	

the compounds isolated in this study.

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