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Phytochemical Constituents from the Fruits of *Acanthopanax sessiliflorus*

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Six compounds were isolated from the fruits of *Acanthopanax sessiliflorus*. Their structures were elucidated as (-)-sesamin, scoparone, protocatechuic acid, ursolic acid, hyperin and 5-hydroxymethylfurfural by physicochemical and spectroscopic analysis. Among them, scoparone, ursolic acid and 5-hydroxymethylfurfural were isolated for the first time from *Acanthopanax* species.

Key words: *Acanthopanax sessiliflorus*, Araliaceae, (-)-Sesamin, Scoparone, Protocatechuic acid, Ursolic acid, Hyperin, 5-Hydroxymethylfurfural.

INTRODUCTION

Acanthopanax species are herbaceous genus of the family Araliaceae. They are distributed in Korea, Japan and China. They have traditionally been used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry, 1980). Among fifteen species of *Acanthopanax* growing in the Korean peninsula, *A. sessiliflorus* is know to be one of the most abundant species.

The compounds caffeic acid, caffeic acid ethyl ester, coniferyl aldehyde, sesamin, β-sitosterol, daucosterol, isofraxidin, hederasaponin B and syringin isolated from *Acanthopanax* species have been shown to have various levels of activity as anti-bacterial, anti-cancer, anti-inflammatory, anti-gout, anti-hepatitis, anti-hyperglycemic, anti-leishmanicidic, anti-oxidant, anti-pyretic, anti-xanthine oxidase, choleretic, radioprotectant, hemostatic, immuno-stimulatory and hypocholesterolemic effects (Davydov and Krikorian, 2000).

To date, investigations on the compounds have revealed the presence of liriodendrin (lirioresinol β-diglucoside) from the cortex of *A. sessiliflorus* forma *chungbunensis* (Ro *et al.*, 1977), and stigmasterol, β-sitosterol, campesterol and (+)-sesamin from the root bark of *A. sessiliflorus* (Yook *et al.*, 1977). The presence of Chiisanoside and eleutherosides B and E from *A. sessiliflorus* were previously identified by HPLC and LC/MS(Lee *et al.*, 2001) Only a few compounds including saturated and unsaturated fatty acids have been isolated from the seeds of *A. sessiliflorus* (Kim and Kim, 1987). Hydroxy fatly acids were isolated from the fruit of *A. sessiliflorus* (Asilbekova *et al.*, 1991)

The chromatographic separation of each fraction from this plant led to the isolation of (-)-sesamin, scoparone, protocatechuic acid, ursolic acid, hyperin and 5-hydroxymethylfurfural. These compounds were isolated for the first time from this plant part. Among them, scoparone, ursolic acid and 5-hydroxymethylfurfural were isolated for the first time from *Acanthopanax* species. This paper describes the isolation and structural determination of these compounds.

MATERIALS AND METHOD

Instruments and reagents

Melting points were determined with Mitamura Riken apparatus and were uncorrected. IR spectra were recorded with Jasco FT/IR-300E instrument on KBr disc. ^{1}H- and ^{13}C-NMR spectra were recorded with Bruker AVANCE 400 NMR spectrometer in DMSO, $CDCl_3$ and C_6D_5N using TMS as internal standard. MS spectra were measured with Jeol JMS-AX505WA mass spectrometer

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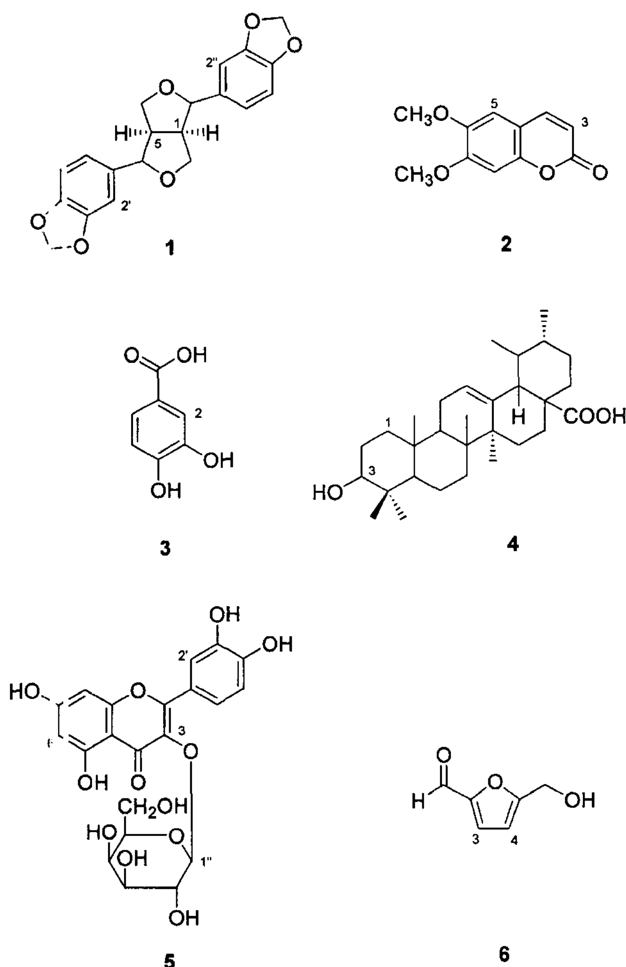

with a direct inlet. Silica gel 60 (Merck Co., 0.063-0.200 mm) was used for open column chromatography. Silica gel plates (Merck Co., Kieselgel 60 F_{254}) were used for TLC. Spots were detected by spraying with 20% H_2SO_4 in MeOH and heating.

Plant materials

The fruits of *Acanthopanax sessiliflorus* Nakai were collected at Kong Ju, Korea in October 1999 and authenticated by Prof. Seon Haeng Cho, Kong Ju University of Education, Korea. A voucher specimen (No. Shin 9910-1) of this plant was deposited at the Herbarium of Natural Products Research Institute (NPRI), Seoul National University, Korea.

Extraction and isolation

The air-dried powdered fruits (2 kg) were extracted three times with hot MeOH under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 672 g of the residue. This MeOH extract was suspended in water, and then fractionated successively with equal volumes of *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH, leaving residual water-soluble fraction. Each fraction was evaporated *in vaccuo* to yield the residues of *n*-hexane soluble fraction (52 g), $CHCl_3$ soluble fraction (49 g), EtOAc soluble fraction (31 g) and *n*-BuOH soluble fraction (75 g).

The *n*-hexane and $CHCl_3$ fraction (20 g) was chromatographed on a silica gel column (7 × 60 cm) using stepwise gradient eluting with a gradient of *n*-hexane-EtOAc to afford (-)-sesamin (5 mg, 90:10) and scoparone (11 mg, 80:20). The EtOAc fraction (20 g) was chromatographed on silica gel eluting with a gradient of *n*-hexane-EtOAc to afford protocatechuic acid (12 mg, 70:30) and ursolic acid (21 mg, 20:80), and with a gradient of EtOAc-MeOH to afford hyperin (153 mg, 90:10). The *n*-BuOH fraction (20 g) was chromatographed on silica gel eluting with a gradient of $CHCl_3$-MeOH to afford 5-hydroxymethylfurfural (15 mg, 97:3).

Compound 1 [(-)-sesamin]; mp: 122-124°; $[\alpha]_D$: -87.2 (*c* 1.08, $CHCl_3$); EIMS *m/z* (rel. int., %): 354 $[M]^+$ (100), 323 (12.6), 219 (7.5), 203 (34.7), 161 (64.8), 149 (90.6), 135 (53.8), 103 (8.3); IR ν_{max} (KBr) cm^{-1}: 1033; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 6.87 (2H, d, *J* = 1.2 Hz, H-2',2"), 6.82 (2H, dd, *J* = 8.0, 1.2 Hz, H-6',6"), 6.79 (2H, d, *J* = 8.0 Hz, H-5',5"), 5.96 (2X-OCH_2O-), 4.74 (2H, d, *J* = 4.2 Hz, H-2,6), 4.25 (2H, dd, *J* = 9.1, 6.8 Hz, H-4_e,8_e), 3.89 (2H, dd, *J* = 9.1, 3.5 Hz, H-4_a,8_a), 3.07 (2H, m, H-1,5); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_c (ppm): 147.9 (C-3',3"), 147.1 (C-4',4"), 135.0 (C-1',1"), 119.3 (C-6',6"), 108.1 (C-5',5"), 106.5 (C-2',2"), 101.0 (2X-OCH_2O-), 85.7 (C-2,6), 71.7 (C-4,8), 54.3 (C-1,5).

Compound 2 [scoparone]; mp: 144°; EIMS *m/z* (rel. int., %): 206 $[M]^+$ (100), 191 (39.7), 178 (17.9), 163 (28.9), 149 (6.7), 135 (17.2), 107 (12.4), 79 (11.3); IR ν_{max} (KBr) cm^{-1}: 1716, 1636, 1558, 1463; ^{1}H-NMR (400 MHz, DMSO) δ_H (ppm): 7.96 (1H, d, *J* = 9.4 Hz, H-4), 7.26 (1H, s, H-5), 7.08 (1H, s, H-8), 6.30 (1H, d, *J* = 9.4 Hz, H-3), 3.87 (-OCH_3), 3.81 (-OCH_3); ^{13}C-NMR (100 MHz, DMSO) δ_c (ppm): 161.0 (*C*=O), 153.0 (C-9), 149.8 (C-7), 146.3 (C-6), 144.7 (C-4), 113.1 (C-5), 111.6 (C-10), 109.4 (C-3), 100.5 (C-8), 56.6 (-OCH_3), 56.3 (-OCH_3).

Compound 3 [protocatechuic acid]; mp: 192-193°; EIMS *m/z* (rel. int., %): 154 $[M]^+$ (100), 137 (98.0), 109 (36.6), 81 (11.4), 63 (9.5); IR ν_{max} (KBr) cm^{-1}: 3444, 1635; ^{1}H-NMR (400 MHz, DMSO) δ_H (ppm): 12.31 (1H, s, -COO*H*), 7.33 (1H, d, *J* = 1.8 Hz, H-2), 7.28 (1H, dd, *J* = 8.2, 1.8 Hz, H-6), 6.78 (1H, d, *J* = 8.2 Hz, H-5); ^{13}C-NMR (100 MHz, DMSO) δ_c (ppm): 167.7 (-*C*OOH), 150.4 (C-4), 145.3 (C-3), 122.3 (C-6), 122.0 (C-1), 116.9 (C-2), 115.5 (C-5).

Compound 4 [ursolic acid]; mp: 280°; EIMS *m/z* (rel. int., %): 456 $[M]^+$ (4.8), 438 (6.4), 248 (100), 203 (52.2), 189 (12.5), 133 (8.4), 119 (7.1); IR ν_{max} (KBr) cm^{-1}: 3432, 1730, 1636, 1450; ^{1}H-NMR (400 MHz, C_6D_5N) δ_H (ppm): 5.44 (1H, t, *J* = 3.5 Hz, H-12), 3.34 (1H, dd, *J* = 4.1, 10.3 Hz, H-3α), 2.47 (1H, d, *J* = 3.5 Hz, H-18), 1.30 (3H, s, H-23), 1.25 (3H, s, H-27), 1.04 (3H, s, H-26), 0.99 (3H, s, H-24), 0.95 (3H, d, *J* = 6.6 Hz, H-30), 0.85 (3H, d, *J* = 6.3 Hz, H-29), 0.78 (3H, s, H-25); ^{13}C-NMR (100 MHz, C_6D_5N) δ_c (ppm): 179.9 (*C*OOH), 134.7 (C-13), 125.2 (C-12), 77.9 (C-3), 55.4 (C-18), 52.7 (C-5), 47.7 (C-17), 42.7 (C-14), 39.4 (C-4), 39.2 (C-8), 38.3 (C-1), 36.9 (C-22), 36.7 (C-10), 33.9 (C-7), 30.8 (C-19), 30.7 (C-20), 28.1 (C-15), 27.9 (C-21), 25.8 (C-27), 23.7 (C-11), 23.5 (C-2), 23.4 (C-30, 23), 23.3 (C-16), 21.1 (C-29), 17.1 (C-6), 16.7 (C-24, 25), 15.1 (C-26).

Compound 5 [hyperin]; mp: 253-254°; EIMS *m/z*: 302 $[quercetin]^+$; FABMS *m/z*: 465 $[M+H]^+$; IR ν_{max} (KBr) cm^{-1}: 3316, 2900, 1655, 1607, 1060; ^{1}H-NMR (400 MHz, DMSO) δ_H (ppm): 12.64 (1H, s, 5-O*H*), 7.67 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 7.53 (1H, d, *J* = 2.0 Hz, H-2'), 6.82 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, d, *J* = 1.9 Hz, H-8), 6.21 (1H, d, *J* = 1.9 Hz, H-6), 5.38 (1H, d, *J* = 7.8 Hz, galactosyl H-1''); ^{13}C-NMR (100 MHz, DMSO) δ_c (ppm): 177.9 (*C*=O), 164.5 (C-7), 161.6 (C-5), 156.6 (C-2, C-9), 148.9 (C-4'), 145.2 (C-3'), 133.9 (C-3), 122.4 (C-6'), 121.5 (C-1'), 116.3 (C-5'), 115.6 (C-2'), 104.3 (C-10), 102.2 (C-1''), 99.1 (C-6), 93.9 (C-8), 76.2 (C-5''), 73.6 (C-3''), 71.6 (C-2''), 68.3 (C-4''), 60.5 (C-6'').

Compound 6 [5-hydroxymethylfurfural]; mp: 35°; EIMS *m/z* (rel. int., %): 126 $[M]^+$ (74.7), 125 (16.1), 109 (16.4), 97 (100), 84 (34.1), 83 (56.1), 69 (38.8), 54 (31.5); IR ν_{max} (KBr) cm^{-1}: 3390, 2932, 2871, 1680, 1023; ^{1}H-NMR (400 MHz, DMSO) δ_H (ppm): 9.55 (1H, s, -C*H*O), 7.49 (1H, d, *J* = 3.4 Hz, H-3), 6.61 (1H, d, *J* = 3.4 Hz, H-4), 5.56 (1H, 6-O*H*), 4.51 (2H, d, *J* = 5.4 Hz, H-6); ^{13}C-NMR (100 MHz, DMSO) δ_c (ppm): 178.4 (-*C*HO), 162.6 (C-5), 152.1 (C-2), 124.8 (C-3), 110.1 (C-4), 56.3 (C-6).

RESULTS AND DISCUSSION

The chromatographic separation of each fraction from *A. sessiliflorus* led to the isolation of (-)-sesamin (**1**), scoparone (**2**), protocatechuic acid (**3**), ursolic acid (**4**), hyperin (**5**) and 5-hydroxymethylfurfural (**6**).

Compound 1 was obtained as needles from MeOH. The IR spectrum of **1** showed absorption bands for C-O at 1035 cm^{-1}. The ^{1}H-NMR spectrum of **1** showed ABX splitting proton signals at δ 6.87 (d, *J* = 1.2 Hz), δ 6.82 (dd, *J* = 8.0, 1.2 Hz) and δ 6.79 (d, *J* = 8.0 Hz). Furthermore, the singlet at δ 5.96 showed the methylenedioxy signal in its structure. The ^{13}C-NMR spectrum of **1** showed methylenedioxy signal at δ 101.0. The EIMS of **1** showed an $[M]^+$ ion at *m/z* 354 as a base peak. The molecular formula of **1** was determined to be $C_{20}H_{18}O_6$. Accordingly, the structure of **1** was elucidated as (-)-sesamin (3',4':3'',4''-bis(methylenedioxy)-2,8:4,6-diepoxylignan). Pelter *et al.* (1976) reported the general methods for the assignment of stereochemistry to 2,6-diaryl-3, 7-dioxa-bicyclo[3.3.0]octane lignans. Yook *et al.* (1977) reported the isolation of (+)-type of this compound from the root bark of *Acanthopanax sessiliflorus*. (-)-Sesamin decreased fatty acid synthesis in rat liver accompanying the down-regulation of sterol regulatory element binding protein-1 (Ida *et al.*, 2001), and exhibited significant anti-feedant activity and moderate growth inhibition towards 4th instar larvae of *Spilarctia oblique* (Srivastava *et al.*, 2001).

Compound 2 was obtained as needles from MeOH. The IR spectrum of **2** showed absorption bands for α,β-unsaturated C=O at 1716 cm^{-1}. In the ^{1}H-NMR spectrum of **2**, aromatic H-5 and H-8 at δ 7.26 (s) and δ 7.08 (s), H-3 and H-4 at δ 6.30 (d, *J* = 9.4 Hz) and δ 7.96 (d, *J* = 9.4 Hz) were observed, respectively. The singlet signals at δ 3.87 and δ 3.81 indicated two methoxy protons. The ^{13}C-NMR spectrum of **2** showed C=O signal at δ 161.0 and two methoxy signals at δ 56.6 and δ 56.3. The EIMS of **2** showed an $[M]^+$ ion at *m/z* 206 as a base peak. The molecular formula of **2** was determined to be $C_{11}H_{10}O_4$. Accordingly, the structure of **2** was elucidated as scoparone (6,7-dimethoxycoumarin). Joseph-Nathan *et al.* (1984) and Günther and Prestien (1975) reported the NMR data of scoparone. Overdoses of this compound can disturb the detoxification of other electrophilic toxicants including ultimate carcinogens (Nakamura *et al.*, 2001). After treatment with this compound, hepatic microsomal UDP glucuronyltransferase activity (Huh *et al.*, 1987) and hepatic cytosolic sulfotransferase activity (Huh *et al.*, 1990) were increased with dose-dependent manner. This compound from *Artemisia capillaris* can reduce cold ischemic injury in liver transplantation (Cho *et al.*, 2000).

Compound 3 was obtained as needles from MeOH. The IR spectrum of **3** showed absorption bands for OH at 3444 cm^{-1} and acid group at 1635 cm^{-1}. The ^{1}H-NMR spectrum of **3** showed ABX splitting proton signals at δ 7.33 (d, *J* = 1.8 Hz), δ 7.28 (dd, *J* = 8.2, 1.8 Hz) and δ 6.78 (d, *J* = 8.2 Hz). Furthermore, the singlet at δ 12.31 showed the carboxylic acid in its structure. The ^{13}C-NMR spectrum of **3** showed carboxylic acid signal at δ 167.7. The EIMS of **3** showed an $[M]^+$ ion at *m/z* 154 as a base peak. The molecular formula of **3** was determined to be

$C_7H_6O_4$. Accordingly, the structure of **3** was elucidated as pro-tocatechuic acid (3,4-dihydroxybenzoic acid). Park *et al.* (1993) reported the isolation of protocatechuic acid from *Acalypha australis*. This compound protects against *t*-BHP-induced hepatotoxicity by its anti-oxidant and anti-inflammatory characteristics accompanied by blocking of stress signal transduction (Liu *et al.*, 2002).

Compound 4 was obtained as crystals from MeOH. This compound showed positive at Libermann-Burchard test. The IR spectrum of **4** showed absorption bands for OH at 3432 cm^{-1} and acid group at 1730 cm^{-1}. The ^1H-NMR spectrum of **4** showed five tertiary methyl groups at δ 0.78-1.30 together with two secondary methyl groups at δ 0.95 (d, $J=6.3$ Hz) and δ 0.85 (d, $J=6.3$ Hz). Furthermore, the doublet at δ 2.47 and the olefinic proton at δ 5.44 showed the urs-12-en type in its structure. The doublet of doublets ($J=10.3$, 4.1 Hz) of proton with oxygenated carbon at δ 3.34 showed a 3β-hydroxyl functionality. The ^{13}C-NMR spectrum of **4** showed two olefinic signals at δ 125.2 and δ 134.7, one acid signal at δ 179.9, and oxygenated carbon of C-3 at δ 77.9. The EIMS of **4** showed an $[M]^+$ ion at *m/z* 456. The characteristic fragment ion peaks at *m/z* 208 and 262 showed the *retro* Diels Alder fragmentation commonly found in the spectra of olean-12-en or urs-12-en derivatives possessing a hydroxyl group in rings A/B and a carboxyl group in rings D/E (Nakatani *et al.*, 1989). The molecular formula of **4** was determined to be $C_{30}H_{48}O_3$. Accordingly, the structure of **4** was elucidated as ursolic acid (3-hydroxy-urs-12-en-28-oic acid) Choi *et al.* from *Rubus parvifolius* (1991) and Youn and Cho from *Rhododendron brachycarpum* (1991) reported the isolation of ursolic acid. This compound showed anti-tumor effects and chemopreventive properties in normal cells (Novotný *et al.*, 2001) and changes in tumor growth, O_2 consumption, and tumor interstitial fluid pressure (Lee *et al.*, 2001). Ursolic acid stmulates NO and TNF-α release and is able to up regulate iNOS and TNF-α expression through NF-κB transactivation in the resting macrophages (You *et al.*, 2001).

Compound 5 was obtained as yellow crystals from MeOH. This compound showed positive at Shinoda and Molisch test. The IR spectrum of **5** showed absorption bands for hydroxyl at 3316 cm^{-1} and C-O at 1060 cm^{-1}. In the ^1H-NMR spectrum of **5**, the typical flavonoid signals were observed. The singlet at δ 12.64 showed aromatic 5-OH. The proton signals at δ 7.67 (dd, $J=8.5$, 2.0 Hz), δ 7.53 (d, $J=2.0$ Hz) and δ 6.82 (d, $J=8.5$ Hz) showed ABX splitting pattern of B ring. The proton signals at δ 3.00-5.00 showed galactose. Aglycone of **5** was quercetin by acid hydrolysis. Due to the chemical shift of C-3 of quercetin changed from δ 136.5 to δ 133.9 and the anomeric proton signal of galactose at δ 5.38 (d, $J=7.8$ Hz), galactose was β-linkage at C-3 of aglycone. The ^{13}C-NMR spectrum of **5** showed C=O at δ 177.9. The carbon signal at δ 102.2 showed galactosyl C-1″ . In the EIMS of **5**, aglycone peak showed at *m/z* 302. The characteristic fragment ion peaks at *m/z* 153 and 121 showed the *retro* Diels Alder fragmentation of flavonoid (Markham, 1982). The FABMS of **5** showed $[M + H]^+$ peak at *m/z* 465. The molecular formula of **5** was determined to be $C_{21}H_{20}O_{12}$. Accordingly, the structure of **5** was elucidated as hyperin (quercetin 3-*O*-β-D-galactopyranoside). Choi *et al.* (1986) reported the isolation of hyperin from the leaves of *Rhododendron brachycarpum* and Jung *et al.* (1992) isolated from the leaves of *Kalopanax pictum*. This compound possessed inhibitory effects on influx of Ca^{2+} in the neonatal rat brain cells (Chen and Ma, 1999).

Compound 6 was obtained as yellow oil. The IR spectrum of **6** showed absorption bands for OH at 3390 cm^{-1} and C=O at 1680 cm^{-1}. The typical furan ring protons were observed at δ 6.61 (d, $J=3.4$ Hz) and 7.49 (d, $J=3.4$ Hz), together with an aldehyde at δ 9.55. The ^{13}C-NMR spectrum of **6** showed signals for the carbons of an aldehyde at δ 178.4. The EIMS of **6** showed an $[M]^+$ ion at *m/z* 126. The molecular formula of **6** was determined to be $C_6H_6O_3$. Accordingly, the structure of **6** was elucidated as 5-hydroxymethylfurfural (5-hydroxymethyl-2-furancarboxaldehyde). Shimizu *et al.* (1993) reported the isolation of this compound having aldose reductase activity. This compound does not pose a serious health risk (Janzowski *et al.*, 2000) and the physiological effects of this compound on *Saccharomyces cerevisiae* were studied (Taherzadeh *et al.*, 2000).

(-)-Sesamin, scoparone, protocatechuic acid, ursolic acid, hyperin and 5-hydroxymethylfurfural were isolated for the first time from this plant part. Especially, scoparone, ursolic acid and 5-hydroxymethylfurfural were isolated for the first time from *Acanthopanax* species.

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