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Phytochemical Constituents of *Acanthopanax senticosus* (Rupr. & Maxim.) Harms Stem

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ABSTRACT : Five constituents were isolated from the stem of *Acanthopanax senticosus*. Their structures were elucidated as (-)-sesamin (1), *iso*-fraxidin (2), 5-hydroxymethylfurfural (3), syringin (4) and acanthoside D (5) by spectral analysis. Among these compounds, 5-hydroxymethylfurfural (3) was isolated for the first time from this plant.

Key words : *Acanthopanax senticosus*, Araliaceae, acanthoside D, 5-hydroxymethylfrufural, *iso*-fraxidin, (-)-sesamin, syringin

INTRODUCTION

Approximately fifteen species of the genus *Acanthopanax* are known to be self-grown in the Korean peninsula. *A. senticosus*, which is distributed in northern Asia, has been traditionally used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry, 1980; Yook, 1990). Many studies have shown that this herb exhibits a variety of pharmacological activities such as anti-bacterial, anti-cancer, anti-inflammatory, anti-gout, anti-hepatitis, anti-hyperglycemic, anti-leishmanicidic, anti-oxidant, anti-pyretic, anti-xanthine oxidase, choleretic, hemostatic, immunostimulatory, hypocholesterolemic and radioprotectant effects (Davydov & Krikorian, 2000). Recently we reported the inhibitory effect of the water extract from the stem bark of *A. senticosus* on hyperlipidemia in rats (Lee *et al.*, 2001). Investigations on the compounds from *A. senticosus* have revealed the presence of phenolic compounds from the stem barks (Nishibe *et al.*, 1990), eleutheroside E_2 and isomaltol 3-O-α-D-glucopyranoside from the roots (Li *et al.*, 2001), and chiisanoside, chiisanogenin and hyperin from the leaves (Lee *et al.*, 2003), *etc.* But there is no report on furanaldehyde-type compounds from this plant.

In this study, we elucidated the structures of constituents from *A. senticosus* stem.

MATERIALS AND METHODS

Plant material

The stem of *Acanthopanax senticosus* (Rupr. & Maxim.) Harms was collected at Jilin Province, China in Oct. 2002, and verified by Prof. S. H. Cho, Kongju National University of Education, Korea. A voucher specimen of this plant was deposited at the R & D Center for Functional Foods, Institute of Food and Culture, Pulmuone Co. Ltd., Korea.

Instruments and reagents

MS spectra were measured with Jeol JMS-AX505WA mass spectrometer. ^1H- and ^{13}C-NMR spectra were recorded with Bruker AVANCE 500

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NMR spectrometer in $CDCl_3$, C_6D_5N or DMSO using TMS as internal standard. All other chemicals and reagents were analytical grade.

Extraction and Isolation

The air-dried powdered stem of *A. senticosus* was extracted with H_2O under reflux. The resultant extract was combined and lyophilized to afford the residue. The H_2O extract was re-suspended in H_2O and then extracted successively with equal volumes of $CHCl_3$, EtOAc, and *n*-BuOH. Each fraction was evaporated *in vacuo* to obtain $CHCl_3$ (14.82 g), EtOAc (23.55 g), *n*-BuOH (48.62 g), and H_2O (394.6 g) fractions.

A portion of the $CHCl_3$ fraction (4 g) was chromatographed on silica gel column (7 × 60 ㎝, No. 7734) eluting with a gradient of $CHCl_3$-MeOH to afford 20 sub-fractions. No. 6 sub-fraction (ASC-33, 100:0.5) was done over preparative TLC using $CHCl_3$-Me_2CO (8:2) to give compounds 1 (3 ㎎, R_f 0.95) and 2 (5 ㎎, R_f 0.55). A portion of the EtOAc fraction (6 g) was done over silica gel column eluting with a gradient of $CHCl_3$-MeOH to afford 25 sub-fractions. No. 5 sub-fraction (ASE-25, 100:0.5) was done over silica gel column (No. 7729) eluting with a gradient of $CHCl_3$-Me_2CO to give compound 3 (4 ㎎, 8:2). A portion of the *n*-BuOH fraction (10 g) was done on silica gel column eluting with a gradient of $CHCl_3$-MeOH to afford compounds 4 (326 ㎎, 95:5) and 5 (697 ㎎, 9:1).

Compound 1; EI-MS (70 eV, rel. int., %): *m/z* 354 $[M]^+$ (100), 323 (12.6), 219 (7.5), 203 (34.7), 161 (64.8), 149 (90.6), 135 (53.8), 103 (8.3); ^{1}H-NMR (500 MHz, $CDCl_3$-*d*): δ 6.85 (2H, d, J = 1.3 Hz, H-2'), 6.80 (2H, dd, J = 1.3, 8.0 Hz, H-6'), 6.77 (2H, d, J = 8.0 Hz, H-5'), 5.95 (2H, $-OCH_2O-$), 4.74 (2H, d, J = 4.2 Hz, H-2), 4.23 (2H, dd, J = 6.7, 9.6 Hz, H-4_{eq}), 3.87 (2H, dd, J = 3.0, 9.6 Hz, H-4_{ax}), 3.05 (2H, m, H-1); ^{13}C-NMR (125 MHz, $CDCl_3$-*d*): δ 148.2 (C-3'), 147,3 (C-4'), 135.2 (C-1'), 119.6 (C-6'), 108.9 (C-5'), 106.7 (C-2'), 101.3 (2X $-OCH_2O-$), 85.9 (C-2), 71.9 (C-4), 54.5 (C-1).

Compound 2; EI-MS (70 eV, rel. int., %): *m/z* 222 $[M]^+$ (12.6), 221 (100), 207 (3.5), 206 (28.1), 194 (1.6), 193 (13.9), 179 (1.6), 178 (14.3); ^{1}H-NMR (500 MHz, $CDCl_3$-*d*): δ 7.60 (1H, d, J = 9.5 Hz, H-4), 6.66 (1H, s, H-5), 6.28 (1H, d, J = 9.5 Hz, H-3), 6.11 (1H, s, 7-OH), 4.10 (3H, s, 8-OCH3), 3.94 (3H, s, 6-OCH_3); ^{13}C-NMR (125 MHz, $CDCl_3$-*d*): δ 160.8 (C-2), 144.9 (C-6), 144.0 (C-4), 143.3 (C-8), 142.7 (C-9), 134.7 (C-7), 113.8 (C-5), 111.5 (C-10), 103.4 (C-3), 61.9 (8-OCH3), 56.7 (6-OCH_3).

Compound 3; EI-MS (70 eV, rel. int., %): *m/z* 126 $[M]^+$ (7.0), 125.9 (90.9), 97 (100), 84 (89.8), 69 (22.8); ^{1}H-NMR (500 MHz, C_6D_5N-d_5): δ 9.72 (1H, s, -CHO), 7.32 (1H, d, J = 3.5 Hz, H-3), 6.45 (1H, d, J = 3.5 Hz, H-4), 4.89 (2H, s, H-6); ^{13}C-NMR (125 MHz, C_6D_5N-d_5): δ 178.2 (-CHO), 163.7 (C-5), 153.3 (C-2), 124.7 (C-3), 110.1 (C-4), 57.6 (C-6).

Compound 4; FAB-MS: *m/z* 373 $[M + H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): 6.73 (2H, s, H-2,6), 6.46 (1H, d, J = 15.9 Hz, H-7), 6.33 (1H, dt, J = 5.1, 15.9 Hz, H-8), 4.84 (1H, d, J = 7.5 Hz, glycosyl H-1'), 4.10 (2H, td, J = 1.4, 5.1 Hz, H-9), 3.77 (6H, s, 2 OMe); ^{13}C-NMR (125 MHz, DMSO-d_6): 152.7 (C-3,5), 133.0 (C-4), 131.0 (C-7), 129.0 (C-8), 128.1 (C-1), 104.5 (C-2,6), 103.1 (Glc C-1'), 77.4 (Glc C-5'), 76.5 (Glc C-3'), 74.9 (Glc C-2'), 71.0 (Glc C-4'), 62.0 (C-9), 60.5 (Glc C-6'), 56.3 (OMe).

Compound 5; FAB-MS: *m/z* 419 $[M + H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): 6.67 (4H, s, H-2',6'), 4.88 (2H, d, J = 7.3 Hz, glycosyl H-1"), 4.67 (2H, d, J = 3.6 Hz, H-2), 4.28 (2H, t, J = 5.5 Hz, H-4_{eq}), 4.20 (2H, t, J = 7.5 Hz, H-4_{ax}), 3.76 (12H, s, 4 OMe), 3.19 (2H, m, H-1); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 153.2 (C-3',5'), 138.1 (C-4'), 134.1 (C-1'), 104.6 (C-2',6'), 103.3 (Glc C-1"), 85.7 (C-2), 77.5 (Glc C-5"), 76.7 (Glc C-3"), 74.5 (Glc C-2"), 72.1 (C-4), 70.2 (Glc C-4"), 61.2 (Glc C-6"), 57.0 (OMe), 54.2 (C-1).

RESULTS AND DISCUSSION

Isolation of compounds from the stems of *A. senticosus* was conducted by open column chromatography. A chromatographic separation of the $CHCl_3$, EtOAc and *n*-BuOH fractions from *A. senticosus* stems led to the isolation of compounds 1,

2, 3, 4 and 5, respectively.

Compound 1 from the $CHCl_3$ fraction was obtained as needles from MeOH. The ^1H-NMR spectrum of 1 showed ABX splitting proton signals at δ 6.85 (d, J = 1.3 Hz), 6.80 (dd, J = 1.3, 8.0 Hz) and 6.77 (d, J = 8.0 Hz). Furthermore, the singlet at δ 5.95 showed the methylenedioxy signal in its structure. The ^{13}C-NMR spectrum of 1 showed a methylenedioxy signal at δ 101.3. The EI-MS 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 (= eleutheroside B_4) by comparing its spectral data in the literature (Lee *et al.*, 2002). It 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 from the $CHCl_3$ fraction was obtained as needles from MeOH. In the ^1H-NMR spectrum of 2, an aromatic H-5 at δ 6.66 (s), H-4 and -3 at δ 7.60 (d, J = 9.5 Hz) and 6.28 (d, J = 9.5 Hz) were observed, respectively. The singlet signals at δ 4.10 (s) and 3.94 (s) indicated two methoxy protons. The ^{13}C-NMR spectrum of 2 showed C=O signal at δ 160.8 and two methoxy signals at δ 61.9 and 56.7. The EI-MS of 2 showed an $[M]^+$ ion at *m/z* 222. The molecular formula of 2 was determined to be $C_{11}H_{10}O_5$. Accordingly, the structure of 2 was elucidated as *iso*-fraxidin (= 8-methoxyscopoletin) by comparing its spectral data in the literature (Nishibe *et al.*, 1990). It has been isolated from *Impatiens balsamina* root cultures (Pharkphoom *et al.*, 1995). This compound from *Micrandra elata* also showed cytotoxicity in lymphocytic leukemia in mice and stimulated bile as well (Borris *et al.*, 1980).

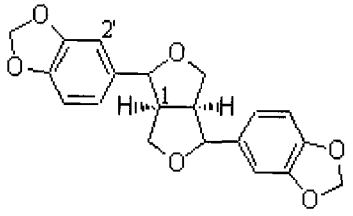
Compound 3 from the EtOAc fraction was obtained as yellow oil. The typical furan ring protons were observed at δ 7.32 (d, J = 3.5 Hz) and 6.45 (d, J = 3.5 Hz), together with an aldehyde at δ 9.72. The ^{13}C-NMR spectrum of 3 showed signals for the carbons of an aldehyde at δ 178.2. The EI-MS of 3 showed an $[M]^+$ ion at *m/z* 126. The molecular formula of 3 was determined to be $C_6H_6O_3$. Accordingly, the structure of 3 was elucidated as 5-hydroxymethylfurfural (= 5-hydroxymethylfuranaldehyde) by comparing its spectral data in the literature (Lee *et al.*, 2002). Shimizu *et al.* (1993) reported the isolation of this compound having aldose reductase activity. This compound dose 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).

Compound 4 from the *n*-BuOH fraction was obtained as needles in MeOH. In the ^1H-NMR spectrum of 4, an aromatic proton at δ 6.73 (s, H-2,6) and methylene protons at δ 6.46 (d, J = 15.9 Hz) and 6.33 (dt, J = 5.1, 15.9 Hz) were observed, respectively. The signal at δ 4.84 (d, J = 7.5 Hz) showed an anomeric proton. The ^{13}C-NMR spectrum of 4 showed signals of an anomeric carbon at δ 103.1 and methoxy carbons at δ 56.3, respectively. The FAB-MS of 4 showed an $[M + H]^+$ ion at m/z 373. The molecular formula of 4 was determined to be $C_{17}H_{24}O_9$. Accordingly, the structure of 4 was elucidated as syringin (= eleutheroside B) by comparing its spectral data in the literature (Nishibe *et al.*, 1990). It was found to possess immunomodulatory activity by which it inhibited the *in vitro* immunohaemolysis of antibody-coated sheep erythrocytes by guinea-pig serum through suppression of C3-convertase of the classical complement (Cho *et al.*, 2001).

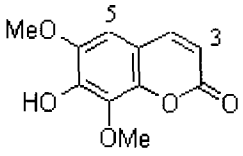
Compound 5 from the *n*-BuOH fraction was obtained as needles in MeOH. In the ^1H-NMR spectrum of 5, aromatic protons at δ 6.67 (s, H-2',6') and methoxy protons at δ 3.76 (s) were observed, respectively. The signals of oxymethine protons at δ 4.67 (d, J = 3.6 Hz, H-2) and methylene protons at δ 4.28 (t, J = 5.5 Hz, $H\text{-}4_{eq}$) and 4.20 (t, J = 7.5 Hz, $H\text{-}4_{ax}$) were elucidated lignan compounds. The signal at δ 4.88 (d, J = 7.3 Hz) showed an anomeric proton. The ^{13}C-NMR spectrum of 5 showed signals for the carbons of an anomeric carbon at δ 103.3. The FAB-MS of 5 showed an $[M + H]^+$ ion at *m/z* 419. The molecular formula of 5 was determined to be $C_{34}H_{46}O_{18}$. Accordingly, the structure of 5 was elucidated as acanthoside D (= eleutheroside E) by comparing its spectral data in the literature (Hong *et al.*, 2001). It

exhibited a prolonging effect on the exercise time to exhaustion in chronic swimming stressed rats (Nishibe *et al.*, 1990).

As shown in Fig. 1, five constituents which were elucidated as (−)−sesamin (1), *iso*−fraxidin (2), 5−hydroxymethylfurfural (3), syringin (4) and acanthoside D (5) were isolated from the stems of *Acanthopanax senticosus*. We already reported the isolation of 5−hydroxymethylfurfural from *A. sessiliflorus* fruit (Lee *et al.*, 2002). But there is no report on furanaldehyde−type compounds from *A. senticosus*. Among these isolated compounds, 5−hydroxymethylfurfural (3) was isolated for the first time from *A. senticosus* stem.



1



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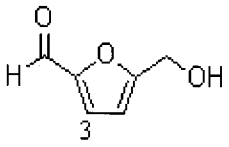

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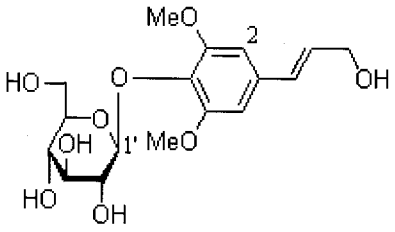
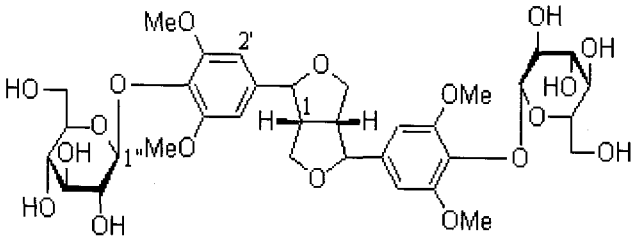


4



5

Fig. 1. Structures of compounds 1, 2, 3, 4 and 5 from *A. senticosus* stem.

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