Arch Pharm Res Vol 26, No 1, 40-42, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Antibacterial Compounds from the Leaves of *Acanthopanax senticosus*

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(Received October 1, 2002)

Chiisanogenin (**1**), hyperin (**2**) and chiisanoside (**3**) were isolated from the leaves of *Acanthopanax senticosus*, and were tested for their inhibitory activities against 6 strains of bacteria. Among them, chiisanogenin (**1**) revealed broad but moderate antibacterial activities against G (+) and G (-) bacteria, the minimum inhibitory concentration (MIC) being in the range of 50-100 μg/ml.

Key words: *Acanthopanax senticosus*, Araliaceae, Chiisanogenin, Chiisanoside, Hyperin, Antibacterial activity

INTRODUCTION

Acanthopanax senticosus, which is distributed in northern Asia, has traditionally been used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry, 1980; Yook, 1990).

It had been studied extensively and shown to exhibit a variety of activities such as antibacterial, anticancer, anti-inflammatory, antigout, antihepatitis, antihyperglycemic, anti-leishmanicidic, antioxidant, antipyretic, antixanthine oxidase, choleretic, hemostatic, immunostimulatory, hypocholesterolemic and radioprotectant effects (Davydov and Krikorian, 2000). Investigations on the compounds from *A. senticosus* have revealed the presence of phenolic compounds such as isofraxidin, eleutherosides B and E, *etc.* 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), *etc.*

In the course of a series of studies for the purpose of evaluating naturally occurring antibacterial compounds, *A. senticosus* has been found to contain several compounds with relatively high antibacterial activity. We thus attempted to isolate and characterize active principles from the plant.

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MATERIALS AND METHODS

Instruments

Melting points were determined with Mitamura Riken apparatus and were uncorrected. MS spectra were measured with Jeol JMS-AX505WA mass spectrometer. 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 or pyridine using TMS as internal standard. Microorganisms were purchased from Korea Research Institute of Bioscience and Biotechnology. Other reagents were commercial grade without purification.

Plant materials

The leaves of *Acanthopanax senticosus* Harms were collected at Kong Ju Province, Korea in Oct. 2000, and verified by Prof. S. H. Cho, Kong Ju University of Education, Korea. A voucher specimen of this plant was deposited at the Herbarium of Natural Products Research Institute, Seoul National University, Korea.

Extraction and isolation

The air-dried powdered leaves of *A. senticosus* were extracted three times with MeOH under reflux. The MeOH extract was suspended in water, and then fractionated successively with equal volumes of CH_2Cl_2, EtOAc and *n*-BuOH, leaving residual water-soluble fraction. The CH_2Cl_2

fraction was chromatographed on a silica gel column eluting with a gradient of *n*-hexane-EtOAc to afford compound **1**. The EtOAc fraction was chromatographed on silica gel eluting with a gradient of $CHCl_3$-MeOH to afford compound **2**. The *n*-BuOH fraction was chromatographed on silica gel eluting with a gradient of $CHCl_3$-MeOH to afford compound **3**.

Chiisanogenin (**1**): Mp 232-234°C. EI-MS (70 eV) *m/z* (%): 484 (23.1) $[M]^+$, 396 (100), 368 (21.0), 161 (52.4). IR ν_{max} (KBr) cm^{-1}: 3447, 1718. ^{1}H-NMR δ_H (400 MHz, Pyridine): 5.10 (1H, s, H-23b), 5.00 (1H, s, H-23a), 4.91 (1H, d, *J* = 2.0 Hz, H-29b), 4.62 (1H, s, H-29a), 4.58 (1H, ddd, *J* = 9.4, 9.4, 9.4 Hz, H-11), 3.70 (1H, d, *J* = 8.0 Hz, H-1), 3.47 (1H, ddd, *J* = 4.6, 10.8, 10.8 Hz, H-19), 3.09 (1H, d, *J* = 14.4 Hz, H-2β), 2.89 (1H, dd, *J* = 8.0, 14.4 Hz, H-2α), 2.72 (1H, d, *J* = 9.4 Hz, H-9), 2.55 (1H, H-16β), 2.22 (1H, H-21α), 2.17 (1H, H-22α), 1.85 (3H, s, H-24), 1.76 (1H, H-15β), 1.68 (3H, s, H-30), 1.66 (1H, dd, *J* = 10.8, 10.8 Hz, H-18), 1.50 (1H, H-22β), 1.47 (1H, H-16α), 1.42 (1H, H-21β), 1.14 (1H, H-15α), 1.05 (3H, s, H-27), 0.98 (3H, s, H-26), 0.87 (3H, s, H-25). ^{13}C-NMR δ_C (100 MHz, Pyridine): 179.3 (C-28), 173.9 (C-3), 151.4 (C-20), 148.6 (C-4), 114.7 (C-23), 111.5 (C-29), 76.2 (C-11), 71.3 (C-1), 57.2 (C-17), 50.5 (C-5), 50.4 (C-18), 48.7 (C-19), 44.9 (C-9), 44.8 (C-10), 43.1 (C-14), 42.5 (C-8), 39.6 (C-2), 38.2 (C-22), 36.2 (C-13), 34.4 (C-17), 33.5 (C-7), 33.3 (C-12), 31.9 (C-21), 30.5 (C-15), 26.0 (C-6), 23.5 (C-24), 19.8 (C-25), 19.7 (C-30), 18.7 (C-26), 14.6 (C-27).

Hyperin (**2**): Mp 253-254°C. FABMS *m/z*: 465 $[M + H]^+$. EIMS (70 eV) *m/z* (%): 302 (100) $[M\text{-Gal}]^+$, 273 (7.3), 245 (4.2) 207 (11.1), 153 (6.1), 137 (7.4), 128 (7.1). IR ν_{max} (KBr) cm^{-1} 3316, 2900, 1655, 1607, 1060. ^{1}H-NMR δ_H (400 MHz, DMSO): 12.64 (1H, s, 5-OH), 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 δ_C (100 MHz, DMSO): 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").

Chiisanoside (**3**): Mp 228°C. FAB-MS *m/z*: 955 $[M + H]^+$. EI-MS (70 eV) *m/z* (%): 484 (23.3) $[M\text{-(Rha+Glc+Glc)}]^+$, 396 (100), 368 (22.0), 161 (57.4). IR ν_{max} (KBr) cm^{-1}: 3432, 1718, 1637, 1068. ^{1}H-NMR δ_H (400 MHz, Pyridine): 6.31 (1H, d, *J* = 8.2 Hz, inner Glc H-1), 5.81 (1H, s, Rha H-1), 5.12 (1H, s, H-23b), 5.00 (1H, s, H-23a), 4.91 (1H, d, *J* = 7.6 Hz, outer Glc H-1), 4.83 (1H, s, H-29b), 4.64 (1H, s, H-29a), 4.52 (1H, ddd, *J* = 9.0, 9.0, 9.0 Hz, H-11), 3.65 (1H, d, *J* = 7.9 Hz, H-1), 3.35 (1H, ddd, *J* = 4.6, 10.7, 10.7 Hz, H-19), 3.04 (1H, d, *J* = 14.6 Hz, H-2), 2.67 (1H, d, *J* = 9.0 Hz, H-9), 1.87 (3H, s, H-24), 1.67 (3H, d, *J* = 5.8 Hz, Rha H-6), 1.62 (3H, s, H-30), 1.08 (3H, s, H-26), 0.99 (6H, s, H-27 and H-25). ^{13}C-NMR δ_C (100 MHz, Pyridine): 174.8 (C-28), 172.8 (C-3), 149.8 (C-20), 147.4 (C-4), 113.6 (C-23), 110.4 (C-29), 104.8 (Glc-1), 102.5 (Rha-1), 95.1 (Glc-1), 78.4 (Glc-4), 78.0 (Glc-3), 77.7 (Glc-5), 76.8 (Glc-5), 76.2 (Glc-3), 75.0 (C-11), 74.9 (Glc-2), 73.8 (Rha-4), 73.7 (Glc-2), 72.4 (Rha-3), 72.3 (Rha-2), 70.5 (Glc-4), 70.2 (C-1, Rha-5), 70.0 (Glc-6), 61.0 (Glc-6), 56.5 (C-17), 49.4 (C-5), 49.3 (C-18), 47.3 (C-19), 43.8 (C-10), 43.7 (C-9), 41.9 (C-14), 41.4 (C-8), 38.5 (C-2), 36.7 (C-22), 34.9 (C-13), 33.2 (C-12), 32.0 (C-7), 31.9 (C-16), 30.7 (C-21), 29.3 (C-15), 25.1 (C-6), 23.4 (C-24), 18.9 (C-25), 18.6 (C-30), 18.2 (Rha-6), 17.7 (C-26), 13.5 (C-27).

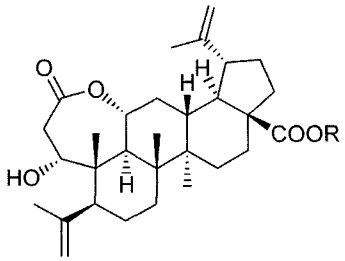
Antibacterial activity

Escherichia coli ATCC 35218, *Proteus vulgaris* ATCC 3851, *Salmonella typhimurium* ATCC 14028, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermis* ATCC 12228 and *Staphylococcus aureus* ATCC 65389 were used for the antibacterial activity. The effect of isolated compounds on the bacterial growth was determined by the 2-fold microtiter broth dilution method (Wu and Hancocks, 1999). Bacteria were grown overnight into m-plate count broth (Difco), harvested and then washed twice with sterile distilled water. Stock solutions of test compounds were prepared in 100 % dimethyl sulfoxide (DMSO) and stored at -20°C. Each stock solution was diluted with broth medium to prepare serial 2-fold dilutions in the range of 200 to 0.62 μg/mL before use. One hundred μL of the broth containing about 10^5 CFU/mL of test bacteria was added to each well of a 96-well microtiter plate and the minimum inhibitory concentration (MIC) was determined after overnight incubation at 37°C. The MIC was taken as the concentration at which no growth was observable.

RESULTS AND DISCUSSION

Three major compounds were isolated from the leaves of *A. senticosus* and their chemical structures were elucidated as chiisanogenin (**1**), hyperin (**2**) and chiisanoside (**3**) by spectral analysis and the comparison with the published data (Hahn *et al.*, 1984; Kasai *et al.*, 1986; Wald *et al.*, 1986). The structures of these compounds were shown at Fig. 1.

Hyperin (**2**) has inhibitory effects on influx of Ca^{2+} in the neonatal rat brain cells (Chen and Ma, 1999) and chiisanoside (**3**) has anti-cancer activity (Yook *et al.*, 1996), but none of the literature of chiisanogenin (**1**), hyperin (**2**) and chiisanoside (**3**) has remarked on any antimicrobial activity. The *in vitro* antibacterial activities of these compounds



1: R = H
3: R = Rha-Glc-Glc

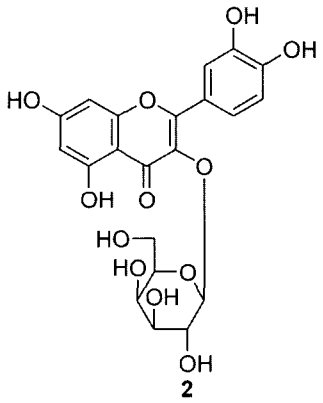


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Fig. 1. Structures of compounds isolated from *A. senticosus* leaves

Table I. Antibacterial activities of compounds isolated from *A. senticosus* leaves

	Organisms	MIC (μg/ml)		
		1	**2**	**3**
Gram (+)	*Bacillus subtilis* ATCC 6633	50	>200	>200
	Staphylococcus epidermis ATCC 12228	100	>200	>200
	Staphylococcus aureus ATCC 65389	100	>200	>200
Gram (-)	*Escherichia coli* ATCC 35218	>200	>200	>200
	Proteus vulgaris ATCC 3851	100	>200	>200
	Salmonella typhimurium ATCC 14028	100	>200	>200

against various microorganisms tested by the microtiter broth dilution method were therefore evaluated and summarized in Table I. Chiisanogenin (**1**) revealed broad but moderate antibacterial activities, whereas hyperin (**2**) and chiisanoside (**3**) did not display any inhibitory effects upon bacterial cell growth at the highest concentration tested (200 μg/mL). The MIC of chiisanogenin (**1**) was in the range of 50-100 μg/mL.

These biological results along with comparison of NMR spectrum of chiisanogenin (**1**) with that of chiisanoside (**3**) let us confirm that the inhibitory potency of these compounds are sensitively dependent upon the glycoside side chain moiety.

Currently we cannot fully understand this result and are not in a position to elucidate the structure-activity relationship of chiisanogenin (**1**) and chiisanoside (**3**), but these phenomena might be due to the differences in the binding affinities of both compounds on the active sites of the enzymes or receptors from the differences in the position of the side chain moiety. Further studies on the growth of the inhibitory activity of chiisanogenin (**1**) on bacteria seem desirable.

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