Phenolic Compounds from Stem Bark of *Acanthopanax senticosus* and Their Pharmacological Effect in Chronic Swimming Stressed Rats

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Ten phenolic compounds, isofraxidin (1), (+)-syringaresinol-di-*O*-*β*-D-glucoside (2), syringin (3), chlorogenic acid (4), isofraxidin-7-*O*-*β*-D-glucoside (5), 2,6-dimethoxy-*p*-benzoquinone (6), (+)-pinoresinol-*O*-*β*-D-glucoside (7), (+)-syringaresinol-*O*-*β*-D-glucoside (8), (+)-pinoresinol-di-*O*-*β*-D-glucoside (9) and (+)-medioresinol-di-*O*-*β*-D-glucoside (10), were isolated from the stem bark of *Acanthopanax senticosus* HARMS and identified, respectively.

The aqueous extract of the stem bark exhibited a prolonging effect on the exercise time to exhaustion in chronic swimming stressed rats. The effect on the exercise time in the chronic swimming stressed rats was respectively tested for compounds 2 and 4, which are major constituents of the stem bark. As a result, it was indicated that compound 2 is the compound responsible for part of the pharmacological effect which the aqueous extract of the stem bark showed.

Keywords *Acanthopanax senticosus*; stem bark; phenolic compound; (+)-syringaresinol-di-*O*-*β*-D-glucoside; chlorogenic acid; aqueous extract; pharmacological effect; chronic swimming stressed rat; exhaustion exercise time

The crude drug "Shigoka (Siberian Ginseng)," prepared from the root bark of the Siberian plant, *Acanthopanax senticosus* HARMS (*Eleutherococcus senticosus* MAXIM.) (Araliaceae), has long been used in empirical oriental medicine for the nonspecific enhancement of resistance in humans and animals.[1)]

In Hokkaido, Japan, in order to keep natural resources from exhaustion, the stem bark of *A. senticosus* (Japanese name: ezoukogi) has been used as a restorative tonic in place of the root bark. We observed that the aqueous extract from the stem bark exhibited a prolonging effect on the exercise time to exhaustion in chronic swimming stressed rats. The pharmacological effect of the stem bark has not been reported, although some research has been carried out on the pharmacological effects of the root bark "Shigoka."[2)]

Our interest has been directed to the investigation of the constituents of the stem bark, with the aim of isolating some biologically active compounds.

This paper describes the isolation of ten phenolic compounds; lignans **2**, **7**, **8**, **9** and **10**, coumarins **1** and **5**, and others **3**, **4** and **6**, and the pharmacological effect of major compounds **2** and **4** in chronic swimming stressed rats.

Results and Discussion

Isolation and Structures of Constituents The extraction and separation were carried out as described in Experimental.

Compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9** and **10** were identified as isofraxidin,[3)] (+)-syringaresinol-di-*O*-*β*-D-glucoside,[4)] syringin,[5)] chlorogenic acid,[6)] isofraxidin-7-*O*-*β*-D-glucoside (7-*O*-*β*-D-glucosylisofraxidin),[3)] 2,6-dimethoxy-*p*-benzoquinone,[7)] (+)-pinoresinol-*O*-*β*-D-glucoside,[8)] (+)-syringaresinol-*O*-*β*-D-glucoside,[9)] (+)-pinoresinol-di-*O*-*β*-D-glucoside[10)] and (+)-medioresinol-di-*O*-*β*-D-glucoside[4)] by direct comparison [ultra-violet (UV), infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, mass spectrum (MS), thin-layer chromatogram (TLC) and mixed melting point, *etc.*] with respective authentic sample.

This is the first report of the isolation of **6**, **7**, **8**, **9** and **10** from *A. senticosus*, although the occurrence of **1**, **2**, **3**, **4** and **5** is already known in the root bark of *A. senticosus*.[11)]

S. Nishibe, one of the authors, and T. Deyama have also isolated the lignans **2**, **7**, **8**, **9** and **10** from bark of *Eucommia ulmoides* OLIV. (Eucommiaceae) used as a tonic.[4,9)]

It is noteworthy from the medicinal viewpoint that both of the barks used as a restorative tonic contain similar lignans, a major compound of which is **2**.

Effect of Aqueous Extract, Compounds 2 and 4 on the Exercise Time to Exhaustion in Chronic Swimming Stressed Rats "Shigoka," preparations from root bark of *A. senticosus* have been used extensively in the Soviet Union as an adaptogen whose properties are the ability to increase nonspecific body resistance to stress, fatigue and disease.[1)]

Here we examined the effect of the aqueous extract of the stem bark on the exercise time to exhaustion in chronic swimming stressed rats.

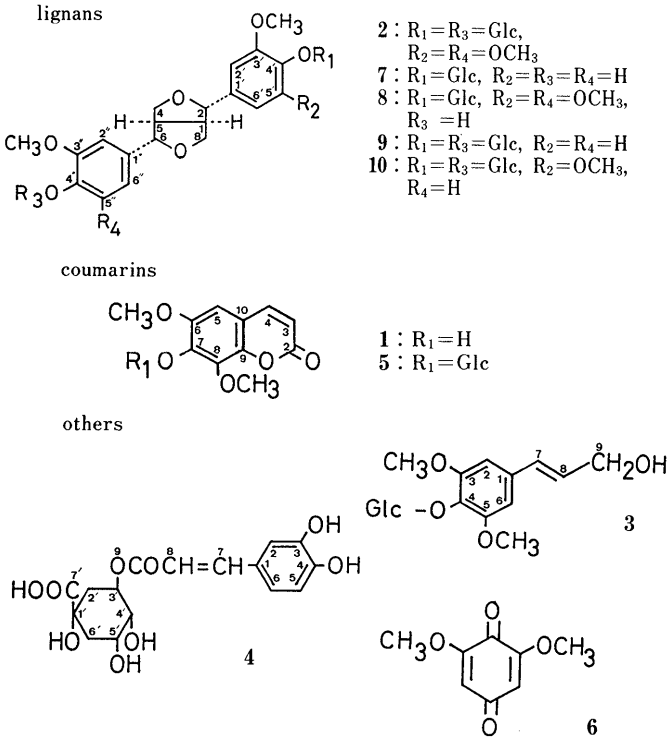

Chart 1

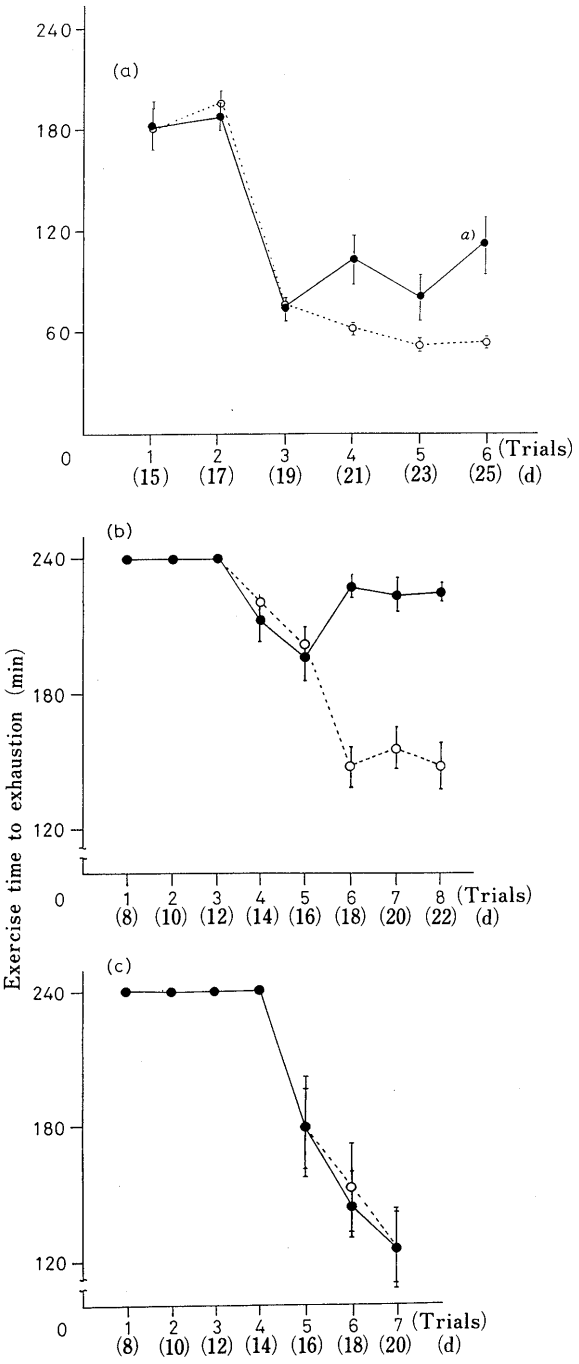

Fig. 1. Effect of Aqueous Extract of Stem Bark (a), (+)-Syringaresinol-di-*O*-*β*-D-glucoside (**2**) (b) and Chlorogenic Acid (**4**) (c) on Exercise Time to Exhaustion in Chronic Swimming Stressed Rats

Each point and bar represent the mean ± S.E. of 9—12 rats in one group. Figure in brackets indicates day after the first administration. (a) —●—, aq. extract, $n=9$. ---○---, control, $n=10$. *a*) $p<0.05$. (b) —●—, compound **2**, $n=12$. ---○---, control, $n=12$. $p<0.01$. (c) —●—, compound **4**, $n=12$. ---○---, control, $n=10$.

The aqueous extract was prepared as described in Experimental. For 25 d, the aqueous extract (500 mg/kg/d)[12] was orally administrated every day at the same hour of the day. From the 15th day after the first administration, the swimming stress was given to the rats under the same conditions at the same hour of the day and continued for 6 trial times every other day. Figure 1a shows the effect of the aqueous extract on the exercise time to exhaustion in the chronic swimming stressed rats. A significant difference was observed between the control group and the aqueous extract-treated group on and after the 4th trial time (the 21st day after the first administration). This result indicates that the aqueous extract of stem bark may protect rats from fatigue induced by chronic swimming stress.

Quantitative determination of the constituents in the aqueous extract by high-performance liquid chromatography (HPLC) showed that major constituents were compounds **2** and **4** in common with those of the methanol extract.[13]

Therefore, the effect of **2** and **4** on the exercise time to exhaustion in chronic swimming stressed rats was tested, respectively.

For 22 d, compound **2** (50 mg/kg/d)[12] was orally administrated every day at the same hour of the day. From the 8th day after the first administration, the swimming stress was given to the rats and continued for 8 trial times every other day. A significant difference was observed between the control group and the compound **2**-treated group on and after the 6th trial time (the 18th day after the first administration) as shown in Fig. 1b.

For 22 d, compound **4** (50 mg/kg/d)[12] was orally administrated every day at the same hour of the day. From the 8th day after the first administration, the swimming stress was given to the rats and continued for 7 trial times every other day. No significant difference was observed between the control group and the compound **4**-treated group as shown in Fig. 1c.

This result indicates that compound **2** is the compound responsible for part of the pharmacological effect which the aqueous extract of the stem bark showed.

More detailed pharmacological studies on the stem bark will be reported in another paper.[14]

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected.

The following instruments were used: optical rotation, JASCO-J 20C and JASCO-DIP-360; EI-MS, Shimadzu LKB-9000; fast atom bombardment-MS (FAB-MS), JEOL-JMS-DX 303.

Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

Isolation Dry powdered stem bark of *A. senticosus* (3 kg) collected in December 1982 at Rikubetsu, Hokkaido, Japan, was extracted four times with hot MeOH. The MeOH solution was evaporated to a small volume under reduced pressure, diluted with water and filtered. The filtrate was extracted successively with ether, $CHCl_3$ and *n*-BuOH. The ether layer was concentrated to dryness. The ether extract (6.1 g) was subjected to column chromatography, eluting with a benzene–$CHCl_3$ solvent system with gradually increasing proportions of $CHCl_3$ to give **1** (65.0 mg).

The $CHCl_3$ extract (5.9 g) was subjected to column chromatography, eluting with a $CHCl_3$–EtOH solvent system with gradually increasing proportions of EtOH to give **6** (16.5 mg).

The *n*-BuOH extract (34.8 g) was subjected to column chromatography, eluting with a $CHCl_3$–EtOH solvent system with gradually increasing proportions of EtOH to give **2** (180.0 mg), **3** (72.0 mg), **4** (231.5 mg), **7** (24.0 mg), **8** (26.4 mg), **9** (7.0 mg) and **10** (3.7 mg), respectively.

The aqueous residue extract (47.5 g) was chromatographed on Sephadex LH-20 with H_2O. The compound obtained was purified by preparative HPLC (conditions: column, ERC-ODS-2122 8 mm × 250 mm 5 μm; eluent, CH_3CN–H_2O (15 : 85); flow rate, 3.0 ml/min; detection, UV at 270 nm) to give **5** (70.0 mg).

Isofraxidin (1) Pale yellow amorphous powder from benzene, mp 147—148 °C. EI-MS *m/z*: 222 (M^+, $C_{11}H_{10}O_5$).

Compound **1** was identical with an authentic sample.[3]

(+)-Syringaresinol-di-*O*-*β*-D-glucoside (2) Colorless needles from

EtOH, mp 257—258 °C, $[\alpha]_D^{20} \pm 0°$ ($c=0.1$, pyridine). FAB-MS m/z: 765 $[M(C_{34}H_{46}O_{18})+Na]^+$.

Compound **2** was hydrolyzed with emulsin in the usual way to give (+)-syringaresinol and D-glucose.

Compound **2** was identical with an authentic sample.[4]

Syringin (3) Colorless crystalline powder from EtOH, mp 186—187 °C. FAB-MS m/z: 395 $[M(C_{17}H_{24}O_9)+Na]^+$.

Compound **3** was identical with an authentic sample.[5]

Chlorogenic Acid (4) Colorless crystalline powder from EtOH, mp 199—200.5 °C. EI-MS m/z: 354 (M^+, $C_{16}H_{18}O_9$).

Compound **4** was identical with an authentic sample.[6]

Isofraxidin-7-*O*-β-D-glucoside (5) Colorless crystalline powder from EtOH, mp 209—210 °C. FAB-MS m/z: 407 $[M(C_{17}H_{20}O_{10})+Na]^+$.

A solution of **5** in 10% H_2SO_4 soln. was heated over a boiling water bath for 1 h in the usual way to give **1** and D-glucose.

Compound **5** was identical with an authentic sample.[3]

2,6-Dimethoxy-*p*-benzoquinone (6) Pale yellow needles from MeOH–$CHCl_3$, mp 179—182 °C. EI-MS m/z (%): 168 (63) (M^+, $C_8H_6O_4$), 80 (40), 69 (100).

Compound **6** was identical with an authentic sample.[7]

(+)-Pinoresinol-*O*-β-D-glucoside (7) Amorphous powder from EtOH, mp 107—109 °C, $[\alpha]_D^{22}$ +8° ($c=0,1$ MeOH). FAB-MS m/z: 543 $[M(C_{26}H_{32}O_{11})+Na]^+$.

Compound **7** was hydrolyzed with emulsin in the usual way to give (+)-pinoresinol and D-glucose.

Compound **7** was identical with an authentic sample.[8]

(+)-Syringaresinol-*O*-β-D-glucoside (8) Colorless crystalline powder from EtOH, mp 187—188 °C, $[\alpha]_D^{22}$ −5.1° ($c=3.9$, MeOH). FAB-MS m/z: 603 $[M(C_{28}H_{36}O_{13})+Na]^+$.

Compound **8** was hydrolyzed with emulsin in the usual way to give (+)-syringaresinol and D-glucose.

Compound **8** was identical with an authentic sample.[9]

(+)-Pinoresinol-di-*O*-β-D-glucoside (9) Colorless crystalline powder from EtOH, mp 141—142 °C, $[\alpha]_D^{22}$ −24.1° ($c=0.1$, MeOH). FAB-MS m/z: 705 $[M(C_{32}H_{42}O_{16})+Na]^+$.

Compound **9** was hydrolyzed with emulsin in the usual way to give (+)-pinoresinol and D-glucose.

Compound **9** was identical with an authentic sample.[10]

(+)-Medioresinol-di-*O*-β-D-glucoside (10) Colorless needles from EtOH, mp 222—223 °C, $[\alpha]_D^{23}$ −9.1° ($c=0.1$, pyridine). FAB-MS m/z: 735 $[M(C_{33}H_{44}O_{17})+Na]^+$.

Compound **10** was hydrolyzed with emulsin in the usual way to give (+)-medioresinol and D-glucose.

Compound **10** was identical with an authentic sample.[4]

Preparation of Aqueous Extract for Pharmacological Experiment The aqueous extract for the pharmacological experiment was prepared by Yakuhan Pharmaceutical Co., Ltd., Kita-hiroshima, Hokkaido, Japan. The powdered stem bark of *A. senticosus* was extracted with hot water at 90 °C. The aqueous solution was concentrated to a syrup *in vacuo* at 65±2 °C. The syrup was spray-dried to a powder.

Methods of Swimming Stress 4 week old male rats of SD-strain weighing 100—120 g were caged in threes and fed a regular diet (Oriental-Kobo, Japan). The animal room was maintained at a temperature of 23±1 °C and kept on a 12 h light/dark cycle. The rats were divided randomly into two groups. 1 ml of the aqueous solution of the aqueous extract (500 mg/kg) or compound (50 mg/kg) was orally given to rats of one group and 1 ml of water was given to rats of the control group every day at the same hour of the day. During the test experiment, the aqueous extract or compound was given to the rats 1 h before the swimming stress. The rats were trained for 1 h 1 d before the experiment in the water-pool (the depth of water, 50 cm; 32±1 °C). The rats swam individually during both the training and test experiments. During the test, a weight equal to 5.0—6.0% of the rat's body weight was attached to the tail. Exercise time to exhaustion was established when the rat swam backwards, descended to the bottom, did not return to the surface within 12 s, and had no power to continue swimming.

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13) Quantitative determination of the constituents in the aqueous extract by HPLC [condition: apparatus, ERC-8710 (Erma); column, ERC-ODS (3 μ) 6 mm × 100 mm, eluent, CH_3CN–H_2O–HCOOH (15 : 85 : 1); flow rate, 1.0 ml/min; detection, UV at 270 and 345 nm; room temperature] was carried out by Yakuhan Pharmaceutical Co., Ltd. The results obtained (mg/100 g) are as follows: isofraxidin (**1**), 55; (+)-syringaresinol-di-*O*-β-D-glucoside (**2**), 1156; syringin (**3**), 454; chlorogenic acid (**4**), 1473; isofraxidin-7-*O*-β-D-glucoside (**5**), 109.
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