Vasorelaxant Effects of *Acer nikoense* Extract and Isolated Coumarinolignans on Rat Aortic Rings

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The organic extract of the heartwood of *Acer nikoense* MAXIM. (Aceraceae) showed vasorelaxant activity on rat aorta with or without endothelium. Coumarin [scopoletin (1)] and coumarinolignans [cleomiscosin A (2) and aquillochin (3)] were isolated as major constituents from the organic extract of the heartwood of *A. nikoense*. Compounds 1—3 exhibited moderate vasorelaxant effects on rat aorta, while 2 and 3 showed vasorelaxant effects in the norepinephrine-stimulated and also in high K⁺-depolarized preparations.

Key words *Acer nikoense*; coumarinolignan; cleomiscosin A; aquillochin; vasorelaxant effect; aorta

We previously detected compounds with vasorelaxant effects, such as methyl brevifolin-carboxylate, from the leaves of *Phyllanthus niruri,*2) ebricoic acid from the extract of *Phellinus girus,*3) and forsythiaside from the fruit of *Forsythia suspensa.*4) In our attempts to search for compounds with vasorelaxant effects from various plants, in the present study we isolated three constituents from the heartwood of *Acer nikoense* (Aceraceae): *A. nikoense* is a Japanese folk medicine used as a remedy primarily for hepatic disorders. Constituents previously isolated from the bark and leaves are diarylheptanoids, phenolic compounds, and tannin,5) and their bioactivities such as antiinflammatory effects6) and protective effects against hepatic injury7) were also reported.

First, we found that the organic extract from the heartwood of *A. nikoense* showed vasorelaxant effects on rat aorta with or without endothelium. Second, scopoletin (1), cleomiscosin A (2), and aquillochin (3) were isolated as major constituents using SiO2 chromatography.8) Moreover, the results of our literature search revealed that 1 had vasorelaxant effects by inhibiting intracellular calcium mobilization from norepinephrine (NE)-sensitive stores.9) Consequently, we focused on the vasorelaxant effects of 2 and 3 isolated from the organic extract of the heartwood of *A. nikoense* and investigated their effects in comparison with that of 1, which was used as a positive control in the present study.

MATERIALS AND METHODS

Plant Material Dried *A. nikoense* heartwood was obtained from the Medicinal Plant Garden of Hoshi University, and identified by one of the authors (S.N.).

Chemicals NE, nicardipine, ethyleneglycol-bis-(β-aminoethyl ether)-tetraacetic acid (EGTA), and acetylcholine chloride (Ach) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Authentic samples of 1—3 were obtained from one of the authors (S.N.) (Fig. 1).

Extraction and Isolation Dried *A. nikoense* heartwood (62.5 g) was extracted with MeOH, and the extract solution was evaporated in vacuo. The residual extract (5.0 g) was further extracted successively with diethyl ether, ethyl acetate, and water. Then the extracts were evaporated in vacuo to obtain extracts of 0.51 g, 0.82 g, and 2.07 g from the successive solvents. Among the extracts tested for vasorelaxant effects on rat aorta, the ether extract exhibited the most potent relaxant effects on NE-induced aortic contraction. Compounds 1, 2, and 3 of *A. nikoense* were found in the diethyl ether fraction.

Assay for Vasorelaxation Experimental studies using animals were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture and Technology of Japan.

Isolation of Rat Aortic Strips The preparation of aortic rings and measurement of tension were performed as previously described.2—4) In brief, male Wistar rats weighing 240—340 g were killed by exsanguination from the carotid arteries under anesthesia. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs–Henseleit solution (KHS: NaCl 118.0 mM, KCl 4.7 mM, NaHCO3 25.0 mM, CaCl2 1.8 mM, NaH 2PO4 1.2 mM, MgSO4 1.2 mM, and glucose 11.0 mM). The aorta was cleaned by removing loosely attached fat and connective tissue and cut into 3-mm lengths. The tissue was placed in a well-oxygenated (95% O2, 5% CO2) bath of KHS 10 ml at 37°C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time, the KHS in the tissue bath was replaced every 20 min.

Experimental Protocol After equilibration, each aortic ring was contracted by treatment with NE 3×10⁻⁵ M. The presence of functional endothelial cells was confirmed by demonstrating relaxation with response to Ach 10⁻⁵ M, and
aortic rings in which 80% relaxation occurred was regard as tissue with endothelium. The removal of endothelial cells by rubbing was confirmed by the loss of ACh-induced relaxation. When the NE-induced contractions reached a plateau, samples were added.

For examination of Ca\(^{2+}\)-induced contraction in the presence of NE, the aortic rings were exposed to 1—3 (10\(^{-4}\) M) in Ca\(^{2+}\)-free KHS containing EGTA 0.01 mM for 1 h, followed by the addition of NE 10\(^{-6}\) M and nicardipine 10\(^{-6}\) M. Next, Ca\(^{2+}\) (10\(^{-5}\) to 10\(^{-3}\) M) was added cumulatively to the bath. Under these conditions, NE produced phasic contractions followed by decreases in tone to the resting level; Ca\(^{2+}\) (10\(^{-5}\) to 10\(^{-3}\) M) was then added cumulatively to the bath. For examination of Ca\(^{2+}\)-induced contractions in depolarized muscle, the aortic rings were exposed to Ca\(^{2+}\)-free KHS containing EGTA 0.01 mM and depolarized with isotonic K\(^+\) (60 mM). The aortic rings were exposed to 1—3 (10\(^{-4}\) M) for 1 h, after which Ca\(^{2+}\) (10\(^{-5}\) to 10\(^{-3}\) M) was cumulatively applied to the depolarized aorta in Ca\(^{2+}\)-free KHS.

**Statistical Analysis** The results are expressed as mean±S.E. Statistical evaluation of the data was performed using the Bonferroni-type multiple t-test.\(^{10}\) p values of less than 5% were considered to represent a statistically significant difference.

**RESULTS AND DISCUSSION**

The ether extract (1 mg/ml) of *A. nikoense* showed slow vasorelaxant effects on NE 3×10\(^{-7}\) M-precontracted rat aortic ring with endothelium (Fig. 2), while the same relaxant action was noted in the sample of aortic rings without endothelium (data not shown). Thus the findings suggest that the vasorelaxant effects of the extract on aortic rings are not dependent on the presence of endothelium.

Compounds, 1, 2, and 3 were isolated from the bioactive extracts as major constituents, which exhibited slow vasorelaxant effects on aortic rings with endothelium (Fig. 2). Furthermore, the same relaxant actions were observed in the sample of aortic rings without endothelium (data not shown).

When NE 10\(^{-6}\) M was added to the organ bath in Ca\(^{2+}\)-free KHS, the aortic rings showed transient phasic contractions. The transient phasic contractions were significant and markedly reduced after treatment with 1—3 (Fig. 3). Thus 1—3 may inhibit NE-induced Ca\(^{2+}\) release from the sarcoplasmic reticulum, and 1 showed vasorelaxant effects by inhibiting intracellular calcium mobilization.\(^{9}\) Therefore we hypothesized that 2 and 3 might also have the same action as 1 as the positive control.

We then found that 2 and 3 had moderate inhibitory effects on the contractions induced by cumulatively applied Ca\(^{2+}\) in aortic rings preincubated with NE 10\(^{-6}\) M and nicardipine 10\(^{-6}\) M in the Ca\(^{2+}\)-free medium, although the aortic rings were not significantly affected by treatment with 1 (Fig. 4). Furthermore, the Ca\(^{2+}\)-induced vasoconstriction of high K\(^+\) (60 mM)-depolarized rat aorta was also attenuated by treatment with 2 and 3, but not by 1 (Fig. 5).

The results indicate that the vasorelaxant effects of 2 and 3 may be attributed to the inhibition of both the nicardipine-sensitive and -insensitive Ca\(^{2+}\) channels. Although some of the coumarinolignans were reported to be antimalarial\(^{11}\) and anticancer agents,\(^{12,13}\) to our knowledge this was the first study to evaluate coumarinolignans for vasorelaxant effects on aortic rings. It would also be interesting to investigate coumarinolignans for their antiplatelet effects, since our previous study of methyl brevifolincarboxylate isolated from the leaves of *P. niruri* revealed that the constituent had both ef-
effects, suggesting the presence of a similar pharmacologic action.2,14)

In conclusion, the organic extract of *A. nikoense* and 1—3 as major constituents of the extract relaxed NE-precontracted rat aortic rings. The extract showed vasorelaxant effects on aortic rings with or without endothelium. Furthermore, 2 and 3 demonstrated vasorelaxant effects in the NE-stimulated and also in high K+

REFERENCES AND NOTES

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