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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4609–4611

Vasorelaxant activity of cyclic peptide, cyclosquamosin B, from *Annona squamosa*

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Received 28 February 2006; revised 22 May 2006; accepted 5 June 2006
Available online 19 June 2006

Abstract—A cyclic octapeptide, cyclosquamosin B (**2**), isolated from the seeds of *Annona squamosa* showed a vasorelaxant effect on rat aorta. It showed a slow relaxation activity against norepinephrine (NE)-induced contractions of rat aorta with/without endothelium. It showed inhibition effect on vasocontraction of depolarized aorta with high concentration potassium, but moderately inhibition effect on NE-induced contraction in the presence of nicardipine. These results showed that the vasorelaxant effect by **2** might be attributed mainly to inhibition of calcium influx from extracellular space through voltage-dependent calcium channels.

The vasodilators are useful for treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation. Several endothelium-dependent vasodilators, such as bradykinin, acetylcholine, and histamine, have been reported to elevate Ca^{2+} levels in endothelial cells and activate NO release, leading to vasorelaxation.[1] On the other hand, contractile response in smooth muscle is caused by an influx of Ca^{2+} through voltage-dependent Ca^{2+}-channels (VDC) and/or receptor-operated Ca^{2+}-channels (ROC).[2] The endothelium-independent vasodilators, such as nicardipine, niphedipine, dirtiazeme, and verapamile, have been reported to inhibit VDC and led to an decrease in the intracellular Ca^{2+} concentration in smooth muscle, leading to vasorelaxation.[2]

Recently, we have reported that some cyclic peptides such as dichotomin J from *Stellaria dichotoma* var. *lanceolata*[3] showed a vasorelaxant effect on rat aorta. During our search for bioactive compounds targeting aortic smooth muscle from medicinal plants, we found that the extract from the seeds of *Annona squamosa* showed vasorelaxant effect on rat aorta. *A. squamosa* (Annonaceae) is a fruit tree and the seeds contain many acetogenins consisting of long-chain fatty acids.[4] Our efforts on identifying new vasodilators resulted in the isolation of seven known cyclic peptides, cyclosquamosins A–G (**1**–**7**), whose structures were established by spectroscopic data.[5] This paper describes vasodilator effects of cyclosquamosins A–G (**1**–**7**) on rat aorta as well as its action mode of cyclosquamosin B (**2**).

The seeds of *A. squamosa* were extracted with MeOH, and the MeOH extract was in turn partitioned with EtOAc, *n*-BuOH, and H_2O. Chromatographic purification of the *n*-BuOH-soluble fraction from the seeds of *A. squamosa* showing vasorelaxant activity on rat aorta resulted in the isolation of seven cyclic peptides, cyclosquamosins A–G (**1**–**7**).[5]

When NE 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response, we added cyclosquamosins A–G (**1**–**7**) at 10^{-4} M and observed slow vasorelaxant actions (Fig. 1). Cyclosquamosin B (**2**) showed the most potent vasorelaxant effect, whereas cyclosquamosin C (**3**) with methionine sulfoxide (Mso) was found to have no vasorelaxant effect, indicating that Met residue, was necessary for vasodilation. On

Keywords: Cyclicpeptide; *Annona squamosa*; Vasorelaxant effect.
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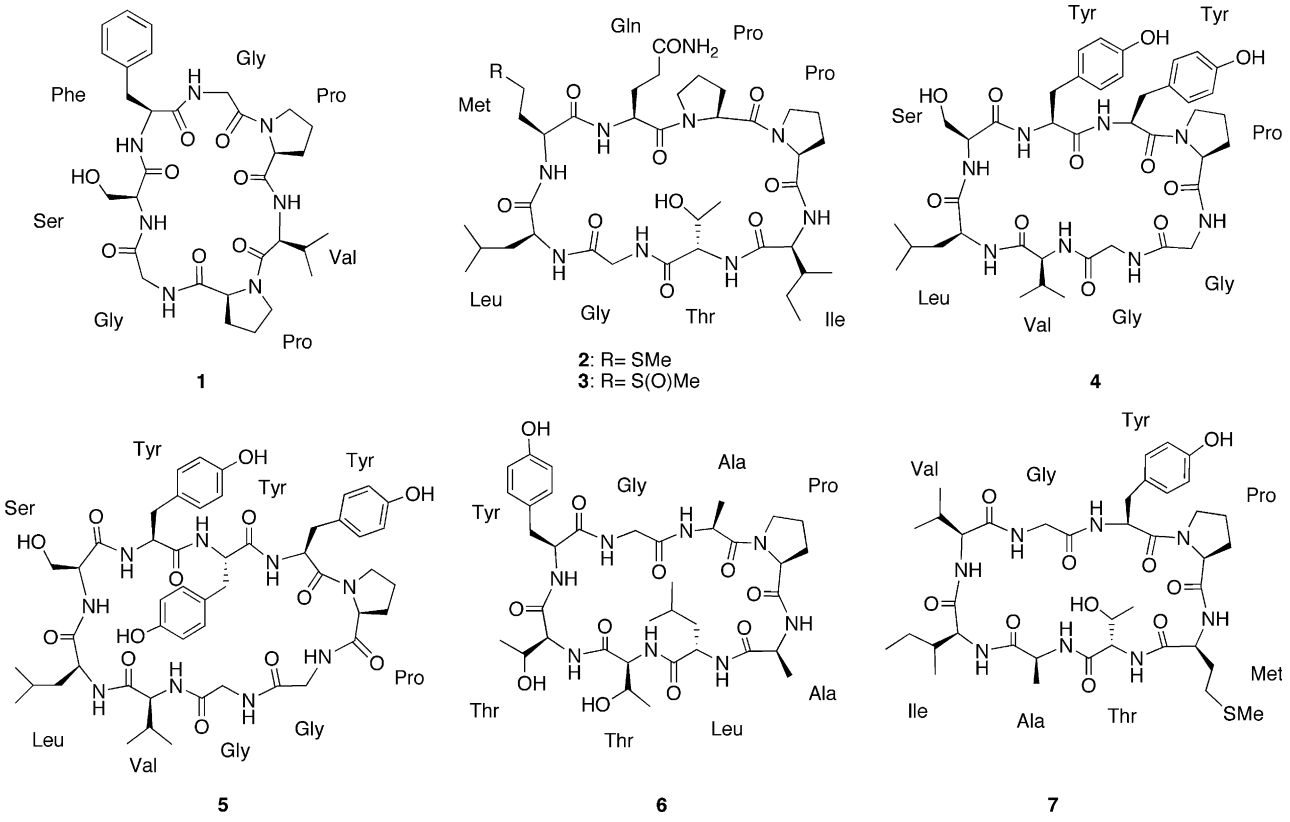
doi:10.1016/j.bmcl.2006.06.008



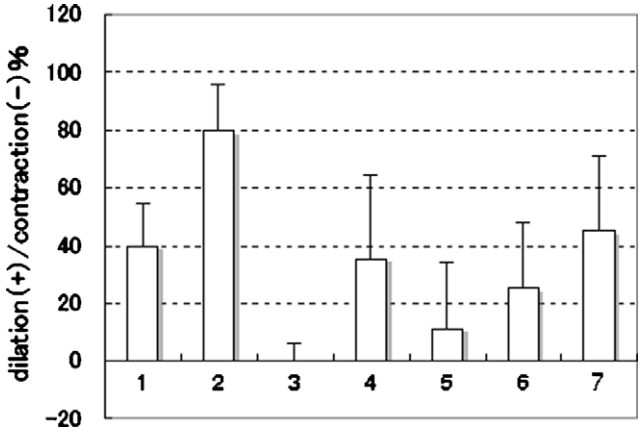


Figure 1. Effects of 10^{-4} M cyclosquamosins A–G (**1**–**7**) on aortic rings precontracted with 3×10^{-7} M norepinephrine (NE).

the other hand, cyclic octapeptide, cyclosquamosin G (**7**) with Met residue was found to be less potent than cyclosquamosin B (**2**), although cyclic octapeptides (**2** and **7**) possess common structural features as shown in Figure 1. Number of Pro residues was different from each other, indicating that cyclosquamosins B (**2**) and G (**7**) took a different conformational state in solution. The geometry between two Pro residues in cyclosquamosin B (**2**) was shown to be *cis* by the strong NOE correlation between two Hα in Pro residues, which was also supported by the ^{13}C chemical shifts (δ 31.6 and 24.9)[6] of β and γ positions in the second Pro residue and the occurrence of a doublet signal of Hα in the second Pro residue (δ 4.74, d, $J = 7.3$ Hz).[7] One or two Pro residues are contained in cyclosquamosins A–G (**1**–**7**). The presence of Pro residues would reduce the conformational space and the appropriate amide geometry may be important in the conformation of the whole molecule.

As shown in Figure 2, cyclosquamosin B (**2**) at the concentration of 10^{-4}–10^{-6} M decreased NE-induced vasocontractions in a concentration-dependent manner and the same relaxant action was seen in the sample of aortic rings without endothelium (data not shown). Thus, the findings suggest that the inhibitory effect of cyclosquamosin B (**2**) on aortic rings is not dependent on the presence of endothelium.

Ca^{2+} can contract aortic rings concentration dependently in Ca^{2+}-free KHS after depolarization with isotonic

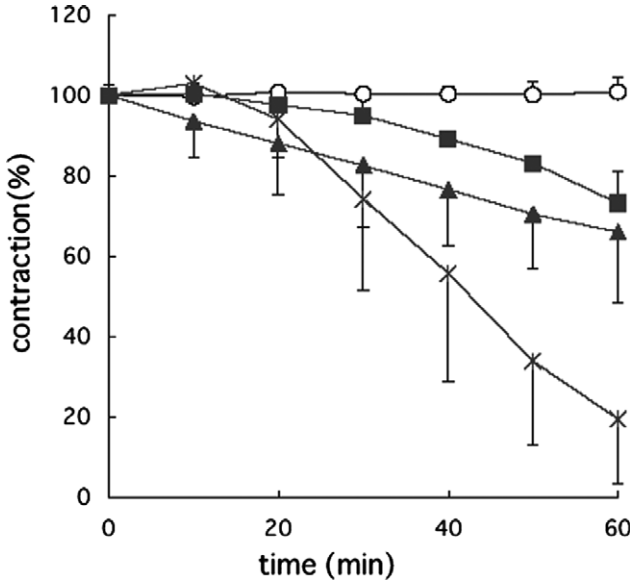


Figure 2. Relaxation responses induced by cyclosquamosin B (**2**) in aortic rings precontracted with 3×10^{-7} M Norepinephrine (NE); Symbols: –○–, control; –×–, **2** at 10^{-4} M; –▲–, **2** at 10^{-5} M; and –■–, **2** at 10^{-6}M.

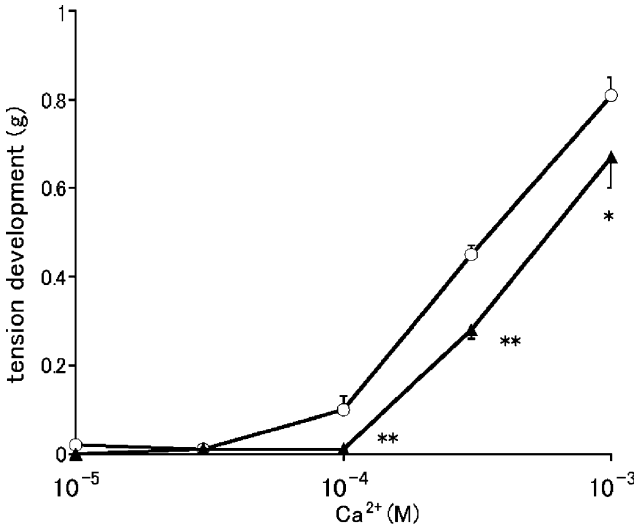


Figure 4. Concentration–response relationships for contractile responses of aortic rings to Ca^{2+} in a Ca^{2+}-free medium preincubated with NE (10^{-6} M) and Nicardipine (10^{-6} M); Symbols: –○–, control; –■–, cyclosquamosin B (**2**) at 10^{-5} M; and –▲–, **2** at 10^{-5} M. Values are means ± SE ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$.

high K^{+}(60 mM) by the influx via voltage-dependent Ca^{2+} channels (VDCs); this contraction was significantly inhibited by cyclosquamosin B (**2**) at the concentration of 10^{-5} M (Fig. 3).

In addition, the NE (10^{-6} M)-induced contractions of the aortic rings in the presence of nicardipine (10^{-6} M) in Ca^{2+}-free KHS occurred in a Ca^{2+}(10^{-5}–10^{-3} M) concentration-dependent manner, presumably due to $[Ca^{2+}]_i$ via receptor-operated Ca^{2+} channels (ROCs). Cyclosquamosin B (**2**) inhibited these contractions at the concentrations of 10^{-5} M moderately (Fig. 4), suggesting that cyclosquamosin B (**2**) exerts inhibitory effects on $[Ca^{2+}]_i$. Consequently, the vasorelaxant activity of cyclosquamosin B (**2**) may be attributed to the inhibition of VDC and partially dependent on ROC.

In this work, we found that a cyclic octapeptide, cyclosquamosin B (**2**) from the seeds of *A. squamosa*, which have been used as traditional medicine, showed hypotensive effect on rat aorta and mode of action was deduced to be inhibition of VDC.

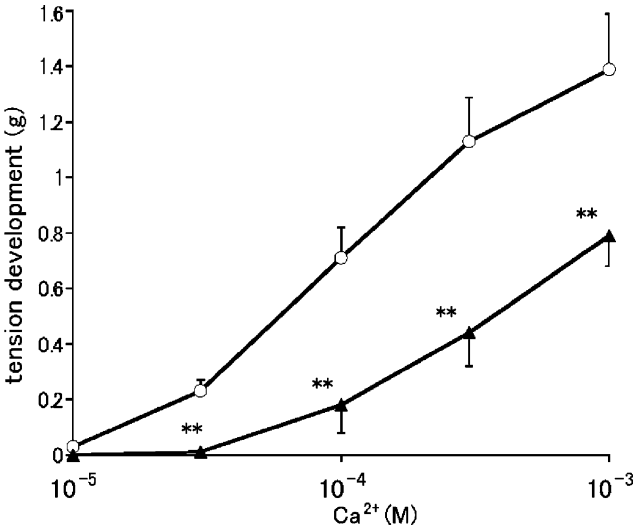


Figure 3. Concentration–response relationships for contractile responses of the aortic rings to Ca^{2+} in a Ca^{2+}-free medium preincubated with high potassium (60 mM); Symbols: –○–, control; –▲–, cyclosquamosin B (**2**) at 10^{-5} M, Values are means ± SE ($n = 4$). $^{**}P < 0.01$

Acknowledgments

This work was partly supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan, and a grant from Tokyo Crude Drugs Association.

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