# Enhancement of Contraction of Rat Mesenteric Artery by Acteoside: Role of Endothelial Nitric Oxide

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The present study describes the role of endothelium in the vascular response to purified acteoside from *Ligustrum purpurascens* in rat mesenteric arteries. In endothelium-intact rings, acteoside (3−50 μmol/L) enhanced phenylephrine-induced contraction without affecting the maximum response. This enhancement was absent in endothelium-denuded rings. Pretreatment with nitric oxide synthase (NOS) inhibitors, $N^G$-nitro-L-arginine (L-NNA, 100 μmol/L) and $N^G$-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L), or a selective guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,2-α]quinoxalin-1-one (ODQ, 10 μmol/L), increased both the sensitivity of vasoconstriction to phenylephrine and the maximal response. The enhancing effect of acteoside (30 μmol/L) was abolished in the presence of L-NAME, L-NNA, or ODQ. Tetraethylammonium ($TEA^+$, 3 mmol/L), a putative $K^+$ channel blocker, also abolished the effect of acteoside. $CaCl_2$ (0.01−10 mmol/L) induced contractions in 50 mmol/L $K^+$-containing Krebs solution. Neither acteoside nor $TEA^+$ affected $CaCl_2$-induced contraction in elevated $K^+$ solution. Acteoside (30 μmol/L) attenuated acetylcholine-induced endothelium-dependent relaxation. Acteoside did not influence relaxation induced by exogenous NO donors, hydroxylamine or sodium nitroprusside, in endothelium-denuded rings. Acteoside did not alter endothelium-independent relaxation induced by forskolin or NS 1619. The present results indicate that acteoside enhanced the evoked vasoconstriction, mainly through inhibition of endothelial NO production/release and inhibition of NO-mediated $TEA^+$-sensitive activation of $K^+$ channels.

Phenylethanoid glycosides, a class of water-soluble polyphenolic compounds, are widely distributed in many medicinal plants including *Ligustrum purpurascens*,[1] *Globularia trichosantha*,[2,3] *Verbascum spinosum*,[4] *Globularia trichosantha*,[5] *Euphrasia pectinata*,[6] and *Scrophularia ningpoensis*.[7] Some of these phenylethanoids possess various pharmacological activities. In southwestern China, the species *L purpurascens* is used to brew bitter tea; the leaves of *L. purpurascens* have been traditionally claimed to serve as a stimulant to the central nervous system, a diuretic, and a relief of high blood pressure.[8,9] An aqueous fraction extracted from the leaves of *Eremophila alternifolia* was reported to increase heart rate, cardiac contractility, and coronary perfusion rate in the Langendorff rat heart, and acteoside is the principal bioactive ingredient of the extract.[10] This cardiotonic effect may be in part caused by acteoside-stimulated liberation of prostacyclin,[11] which increases cardiac cAMP levels,[12] resulting in an increased cardiac activity. We have recently shown that acteoside relaxed rat aortas preconstricted by a thromboxane $A_2$ analogue (U46619) and probably acts as a pharmacological antagonist against vasoconstriction.[13] Four acteoside-related phenylethanoids isolated from *Brandisia hancei* inhibited proliferation of arterial smooth muscle cells stimulated by fetal bovine serum.[14] Acteoside was a strong protector against oxidation of human low-density lipoprotein (LDL) and also effective in preventing the peroxyl free radical-induced oxidation of α-tocophenol in human LDL.[15] Acteoside exhibited an inhibitory effect on protein kinase C activity[16,17] and reduced protein kinase C-mediated vascular contraction.[13] These reported pharmacological properties indicate that acteoside may have potentially beneficial effects in the vascular system. However, very few studies have examined the vascular action of phenylethanoid glycosides. The present study was therefore designed to evaluate the role of endothelium/NO in the vascular response to purified acteoside from *L. purpurascens* in the isolated rat mesenteric arteries. Since NO was found to exert a direct inhibitory action on $Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels in vascular smooth muscle cells, we also examined the effect of acteoside on relaxation induced by NS 1619, a putative $K_{Ca}$ channel activator.

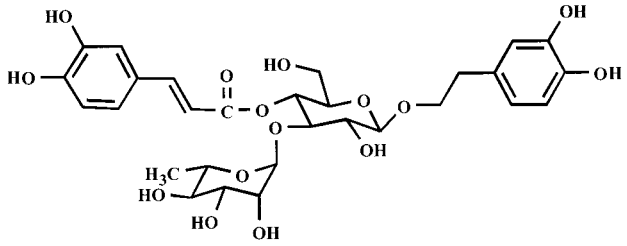

**Figure 1.** Chemical structure of acteoside.

## Results and Discussion

**Enhancing Effect on Phenylephrine-Induced Contraction.** In the endothelium-intact rings phenylephrine, an $\alpha_1$-adrenoceptor agonist, produced concentration-dependent contractions with $pEC_{50}$ values of 6.07 ± 0.11 and 5.98 ± 0.14, respectively, for the first and second vehicle-control concentration−response curves ($P > 0.05$, $n = 7$, Figure 2a). Removal of the endothelium enhanced the constricting potency of phenylephrine ($pEC_{50}$: 7.19 ± 0.07 and 7.04 ± 0.25, respectively, for the first and second vehicle-control concentration−response curves, $n = 7$, $P < 0.05$ compared with those obtained in endothelium-intact

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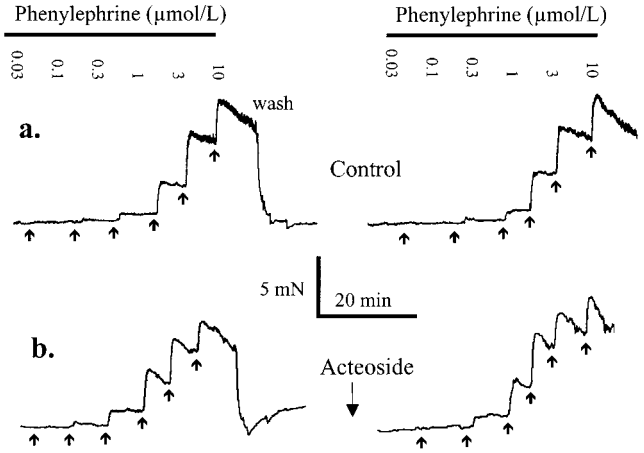


**Figure 2.** Representative traces showing two consecutive concentration−contraction relationships for phenylephrine in control (a) and in the presence of 30 $\mu$mol/L acteoside (b) before repeating the second concentration−response curve. This set of experiments was conducted in the endothelium-intact rat mesenteric artery rings. Scale bars apply to all traces.

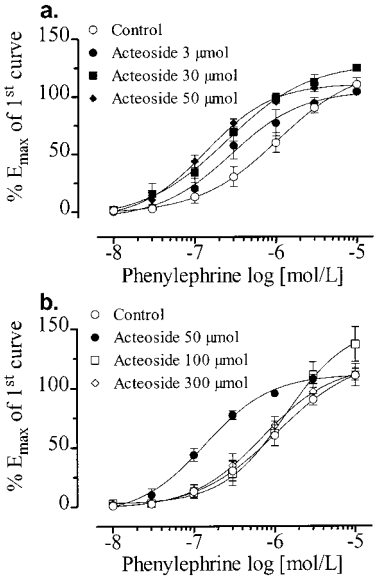


**Figure 3.** Concentration−contraction curves for phenylephrine in the control (○) and in the presence of acteoside (●, 3 $\mu$mol/L; ■, 30 $\mu$mol/L; ◆, 50 $\mu$mol/L) (a) or in the presence of acteoside (●, 50 $\mu$mol/L; □, 100 $\mu$mol/L; ◇, 300 $\mu$mol/L) (b). Acteoside was applied 30 min prior to repeating the second concentration−contraction curve. Data are means ± SEM of 5−7 experiments.

rings). Traces in Figure 2b show that pretreatment of endothelium-intact rings with 30 $\mu$mol/L acteoside potentiated the phenylephrine-induced contractile response. Pretreatment with acteoside (3−50 $\mu$mol/L) shifted the phenylephrine concentration−response curve to the right with little effect on the maximal contraction ($pEC_{50}$: 6.56 ± 0.13, 6.61 ± 0.12, 6.38 ± 0.07, respectively, for 3, 30, and 50 $\mu$mol/L acteoside, $P < 0.05$ compared with the control value of 5.98 ± 0.14, $n = 6-8$, Figure 3a), whereas acteoside at higher concentrations (100−300 $\mu$mol/L) did not affect the phenylephrine-induced contraction ($pEC_{50}$: 5.87 ± 0.15 and 6.15 ± 0.12, $n = 6$, for 100 and 300 $\mu$mol/L acteoside, respectively, $P > 0.05$ as compared with the control, Figure 3b). It is possible that higher concentrations of acteoside may have a direct endothelium-independent vasorelaxing action, which counteracts the enhancing effect on phenylephrine-induced tone. Acteoside (3−300 $\mu$mol/L) did not affect baseline tone ($n = 6-8$, data not shown).

**Table 1.** Phenylephrine-Induced Contraction in the Endothelium-Intact Rat Mesenteric Arteries[a]

| | $pEC_{50}$ | $E_{max}$(%) | $n$ |
|---|---|---|---|
| control | 5.98 ± 0.14 | 110.3 ± 3.3 | 7 |
| 30 $\mu$mol/L acteoside | 6.61 ± 0.12[b] | 120.1 ± 5.1 | 7 |
| 100 $\mu$mol/L L-NAME | 6.89 ± 0.20[b] | 147.9 ± 7.6[b] | 5 |
| acteoside + L-NAME | 6.98 ± 0.37[b] | 157.1 ± 13.3[b] | 5 |
| 100 $\mu$mol/L L-NNA | 6.89 ± 0.38[b] | 180.3 ± 24.1[b] | 5 |
| acteoside + L-NNA | 6.87 ± 0.34[b] | 172.5 ± 18.7[b] | 5 |
| 10 $\mu$mol/L ODQ | 7.28 ± 0.29[b] | 131.0 ± 10.9[b] | 5 |
| acteoside + ODQ | 7.17 ± 0.24[b] | 158.4 ± 14.7[b] | 5 |
| 3 mmol/L $TEA^+$ | 7.03 ± 0.16[b] | 136.9 ± 10.8[b] | 5 |
| acteoside + $TEA^+$ | 7.13 ± 0.22[b] | 135.9 ± 3.4[b] | 5 |
| 3 $\mu$mol/L indomethacin | 6.21 ± 0.25 | 99.5 ± 9.0 | 5 |
| acteoside + indomethacin | 6.52 ± 0.11[b] | 112.7 ± 4.8 | 5 |

[a] The $pEC_{50}$ values and the maximum contraction ($E_{max}$) induced by phenylephrine in the presence of various inhibitors with or without acteoside. Data are means ± SEM of $n$ experiments. [b] Significant differences ($P < 0.05$) are indicated between the control and treatment groups.

**Influence of Inhibitors of Nitric Oxide on the Acteoside-Induced Effect.** Acteoside (30 $\mu$mol/L) did not influence contraction induced by phenylephrine in endothelium-denuded rings ($pEC_{50}$: 7.04 ± 0.25 in control and 7.27 ± 0.30 in acteoside, $n = 6-7$, $P > 0.05$, Figure 4a), indicating that the observed potentiating effect might be caused by an action on the endothelium. Inhibition of NOS by 100 $\mu$mol/L L-NAME or 100 $\mu$mol/L L-NNA significantly potentiated the contractile response to phenylephrine (Figure 4b,c, Table 1). Concomitant treatment of endothelium-intact rings with 30 $\mu$mol/L acteoside plus L-NAME or L-NNA did not further increase the contractile sensitivity to phenylephrine (Figure 4b,c, Table 1). Pretreatment with 10 $\mu$mol/L ODQ significantly enhanced phenylephrine-induced contraction, while co-treatment with ODQ and 30 $\mu$mol/L acteoside did not cause further enhancement (Figure 4d, Table 1).

**Effect of Indomethacin and Tetraethylammonium.** Figure 5a shows that pretreatment with 3 $\mu$mol/L indomethacin affected neither phenylephrine-induced contraction nor acteoside (30 $\mu$mol/L)-induced enhancement in vessel tension ($n = 5$). $TEA^+$ at 3 mmol/L significantly increased the phenylephrine-induced contraction; co-treatment with $TEA^+$ and acteoside (30 $\mu$mol/L) did not cause further increase over that with $TEA^+$ or acteoside alone in vessel tension ($n = 5-7$, Figure 5b, Table 1). In a solution containing zero $Ca^{2+}$ and 50 mmol/L $K^+$, $CaCl_2$ induced concentration-dependent contractions. Neither 3 mmol/L $TEA^+$ nor 30 $\mu$mol/L acteoside altered $CaCl_2$-induced contraction in elevated $K^+$ solution ($n = 5$, Figure 5c).

**Effect on Acetylcholine-Induced Endothelium-Dependent Relaxation.** Acetylcholine induced concentration-dependent relaxation with a $pD_2$ of 7.87 ± 0.05 ($n = 6$), and 100 $\mu$mol/L L-NAME markedly inhibited and removal of endothelium abolished acetylcholine-induced relaxation ($n = 5$, Figure 6). Pretreatment of endothelium-intact rings with 30 $\mu$mol/L acteoside attenuated endothelial NO-mediated relaxation induced by acetylcholine ($pD_2$: 7.87 ± 0.05 in control and 7.60 ± 0.13 in acteoside, $n = 6-8$, $P < 0.05$, Figure 6).

**Effect on Endothelium-Independent Relaxation.** To examine whether acteoside may have an inhibitory effect on NO-induced relaxation by acting on cGMP formation in vascular smooth muscle cells, the relaxing effects of NO donors, hydroxylamine and sodium nitroprusside, were tested in endothelium-denuded rings in the absence or presence of acteoside. Both NO donors induced concentration-dependent relaxations; pretreatment with 30 $\mu$mol/L

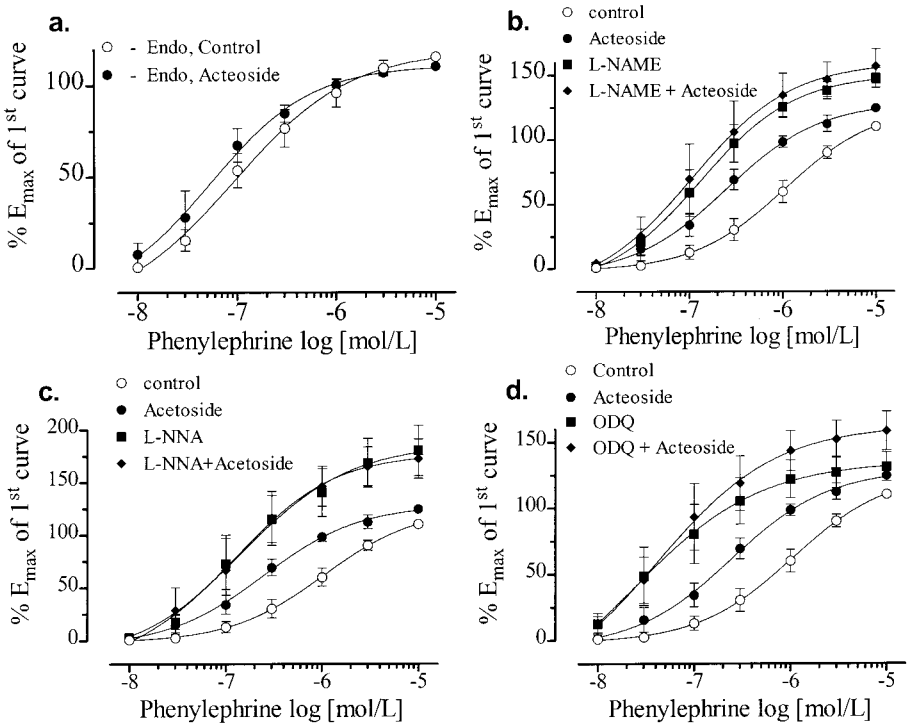


**Figure 4.** (a) Concentration−contraction curves for phenylephrine in endothelium-denuded rings (○, in control; ●, in 30 μmol/L acteoside). (b) Concentration−contraction curves for phenylephrine in endothelium-intact rings (○, in control; ●, in 30 μmol/L acteoside; ■, in 100 μmol/L L-NAME; ◆, in L-NAME plus acteoside). (c) Concentration−contraction curves for phenylephrine (○, in control; ●, in 30 μmol/L acteoside; ■, in 100 μmol/L L-NNA; ◆, in L-NNA plus acteoside). (d) Concentration−contraction curves for phenylephrine (○, in control; ●, in 30 μmol/L acteoside; ■, in 10 μmol/L ODQ; ◆, in ODQ plus acteoside). Data are means ± SEM of 5−7 experiments.

acteoside did not alter this relaxation ($pD_2$ for hydroxylamine: 6.80 ± 0.11 in control and 6.61 ± 0.10 in acteoside, $n = 5-6$; $pD_2$ for nitroprusside: 8.00 ± 0.16 in control and 7.75 ± 0.29 in acteoside, $n = 6$, $P > 0.05$ compared with respective control values, Figure 7a,b). ODQ at 10 μmol/L abolished the relaxation induced by both NO donors ($n = 4$, Figure 7a,b). Acteoside (30 μmol/L) did not influence the endothelium-independent relaxation induced by forskolin ($pD_2$: 7.74 ± 0.19 in control; 7.87 ± 0.08 in acteoside, $n = 5$, $P > 0,05$, Figure 7c) or by NS 1619 ($pD_2$: 4.72 ± 0.07 in control; 4.84 ± 0.04 in acteoside, $n = 5$, $P > 0.05$, Figure 7d).

We have recently isolated several phenylethanoid glycosides from *L. purpurascens* and *B. hancei* and reported their vasorelaxant, antiproliferative, and antioxidative properties.[13−15] In the present study, we observed a novel vascular action for acteoside purified from *L. purpurascens*. In isolated rat mesenteric arteries, acteoside enhanced the contractile response to phenylephrine in a concentration-dependent manner (3−50 μmol/L). This effect was abolished in the endothelium-denuded rings, suggesting that endothelium is likely a target for acteoside-induced action. Higher concentrations of acteoside (100−300 μmol/L) did not affect the phenylephrine-induced contraction. This may be caused by a direct relaxing effect of acteoside at higher concentrations, which counteracts its effect on endothelium. We showed previously that acteoside concentration-dependently relaxed the U46619-preconstricted rat aortic rings, and this effect was enhanced in the absence of functional endothelium.[13] It is possible that acteoside may inhibit release and/or formation of endothelium-derived relaxing factors such as NO at concentrations less than 100 μmol/L. Indeed, pretreatment of endothelium-intact rings with acteoside significantly attenuated relaxation induced by acetylcholine, an endothelial NO-dependent dilator. This effect may have been overridden by a direct relaxing effect of acteoside on vascular smooth muscle cells when the concentration of acteoside is increased above 100 μmol/L.

In response to neural or hormonal stimuli, endothelium liberates several vasorelaxing molecules (NO, hyperpolarizing factor, and prostacyclin) that jointly regulate the contractility of the adjacent vascular myocytes. Inhibition of production and/or release of any of these factors would potentiate the evoked vessel tension. Pretreatment of endothelium-intact rings with inhibitors of NO-mediated vasodilation, such as L-NAME, L-NNA, and ODQ, profoundly potentiated phenylephrine-induced response and increased the maximal contraction. Under this condition acteoside failed to cause further potentiation. These data indicate that acteoside is likely to inhibit NO production/release by acting on endothelium. This notion is supported by the inhibitory effect of acteoside on acetylcholine-induced endothelial NO-mediated relaxation. By comparing the enhancing effect of acteoside and NO inhibitors, acteoside did not affect the maximal contraction to phenylephrine, while L-NAME, L-NNA, or ODQ did. Similarly, L-NAME caused a greater inhibition of acetylcholine-induced relaxation than acteoside. The exact mechanisms responsible for this discrepancy between acteoside and NO inhibitors remain to be further investigated. Pretreatment with indomethacin did not influence acteoside-induced enhancing effect on phenylephrine-induced contraction, suggesting that prostacyclin is not involved.

A recent study shows that phenylethanoids including acteoside possessed NO radical-scavenging properties without affecting expression of inducible NOS in activated macrophages,[18] which possibly contribute in part to their antiinflammatory effects against arachidonic acid-induced ear edema in mouse[19] since NO production in the activated macrophage was stimulated by products of the lipoxygenase-dependent pathway of the arachidonic acid metabolism.[20] NO has been implicated in the increased vascular

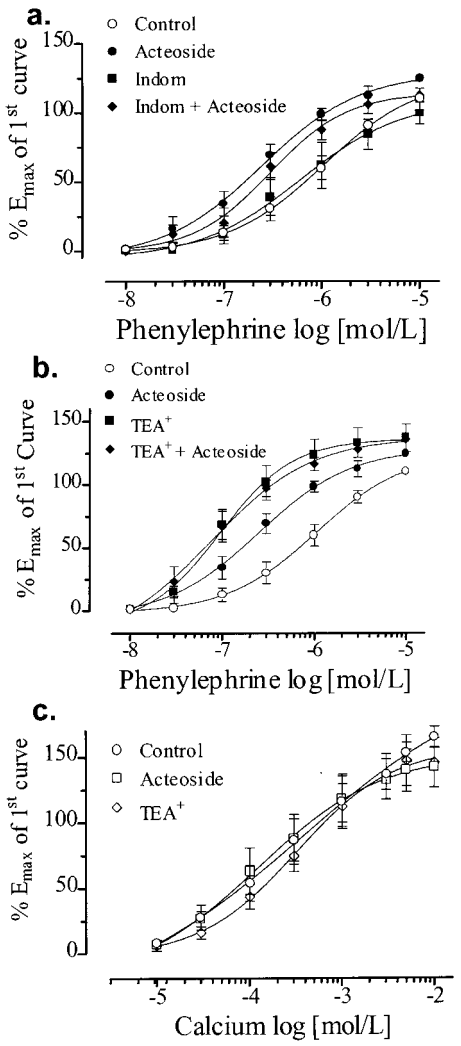


**Figure 5.** (a) Concentration−contraction curves for phenylephrine in endothelium-intact rings (○, in control; ●, in 30 μmol/L acteoside; ■, in 3 μmol/L indomethacin; ◆, in indomethacin plus acteoside). (b) Concentration−contraction curves for phenylephrine (○, in control; ●, in 30 μmol/L acteoside; ■, in 3 mmol/L $TEA^+$; ◆, in $TEA^+$ plus acteoside). (c) Lack of effects of acteoside or $TEA^+$ on $CaCl_2$-induced contraction in 50 mmol/L $K^+$-containing solution. Data are means ± SEM of 5−7 experiments.

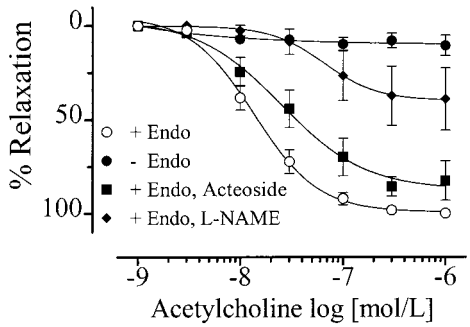


**Figure 6.** Acetylcholine-induced relaxant effect (○, with endothelium; ●, without endothelium; ■, in 30 μmol/L acteoside with endothelium; and ◆, in 100 μmol/L L-NAME with endothelium). Data are means ± SEM of 5−7 experiments.

permeability which is associated with acute immune complex inflammation.[21] If acteoside proves to inhibit the activity of endothelial NOS in the endothelium-intact blood vessels, this effect would play a role in the claimed antiinflammatory action of acteoside against acute edema.

NO directly inhibited $K_{Ca}$ channels in vascular smooth muscle cells.[22] In rat aortic arteries, relaxation induced by NO released from hydroxylamine or sodium nitroprusside was inhibited by putative $K_{Ca}$ channel blockers such as charybdotoxin, $TEA^+$, and tetrapentylammonium ions.[23] Pretreatment with $TEA^+$ enhanced the phenylephrine-induced contraction to the same extent as NO inhibitors, indicating that NO may activate $TEA^+$-sensitive $K^+$ channels in smooth muscle cells of the rat mesenteric arteries. In the presence of $TEA^+$, acteoside failed to influence the contractile response to phenylephrine. Neither $TEA^+$ nor acteoside, at concentrations that enhanced phenylephrine-induced contraction, affected $CaCl_2$-induced contraction in elevated $K^+$-containing solution. One major consequence of raising extracellular $K^+$ concentration is to diminish the electrochemical gradient for $K^+$ efflux, hence minimizing the effect of $K^+$ channel activation. In addition, acteoside did not influence the relaxant effect of NS 1619, a putative $K_{Ca}$ channel activator in smooth muscle.[24,25] These results suggest that the acteoside-induced enhancement of phenylephrine-induced vasoconstriction may be mediated through inhibition of NO-dependent, $TEA^+$-sensitive cellular pathways and that acteoside may not directly inhibit vascular $K_{Ca}$ channels.

Diffusion of endothelial NO into adjacent vascular smooth muscle cells normally activates guanylyl cyclase, the first step leading to vasodilation. It is possible that acteoside may also inhibit the activity of guanylyl cyclase. However, acteoside did not affect vasorelaxation induced by two NO donors, hydroxylamine and nitroprusside, in the endothelium-denuded rings. ODQ eliminated the relaxant responses to both NO donors, suggesting that acteoside is probably not acting as an inhibitor of guanylyl cyclase as does ODQ. The lack of effect on relaxation induced by forskolin may rule out the possible inhibition by acteoside on production of cAMP in vascular smooth muscle.

Our study has provided evidence for a novel vascular action for purified acteoside from *L. purpurascens* in isolated rat mesenteric arteries. Acteoside enhanced the phenylephrine-mediated contraction, and this effect was dependent upon the presence of functional endothelium. Inhibitory effect of acteoside on the $TEA^+$-sensitive NO-dependent pathway, but not on the activity of guanylyl cyclase or adenylyl cyclase, may be responsible for the acteoside-induced effect. If acteoside's inhibitory effect on NO-mediated relaxation occurs in vivo, this may contribute to the reported antiinflammatory action of acteoside against acute edema.

## Experimental Section

**Tissue Preparation.** Male Sprague−Dawley rats (~250−300 g) were killed by cervical dislocation and bled. The superior branch of the mesenteric artery was dissected out, and the surrounding connective tissue was carefully trimmed off. The artery from each rat was cut into three ring segments, approximately 3 mm in length. Each ring was mounted between two stainless steel hooks in Krebs solution-filled organ baths (10 mL). The Krebs solution contained (mmol/L) NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 25, $KH_2PO_4$ 1.2, and D-glucose 11.1. The bath solution was continuously bubbled with a mixture of 95% $O_2$ and 5% $CO_2$ and maintained at 37 °C and pH 7.4. During an equilibrium period of about 90 min, baseline tone of the vessel was kept at 5 mN (0.5 g). The isometric contraction was measured with a force-displacement transducer (Grass Instruments Corp.). In some experiments, the endothelial layer was disrupted mechanically by rubbing the lumen with fine plastic tubing. The functional removal of the endothelium was verified by the absence of a relaxant response (>90%) to 3 μmol/L acetylcholine at the beginning of each experiment. A total of 55 rats were used in

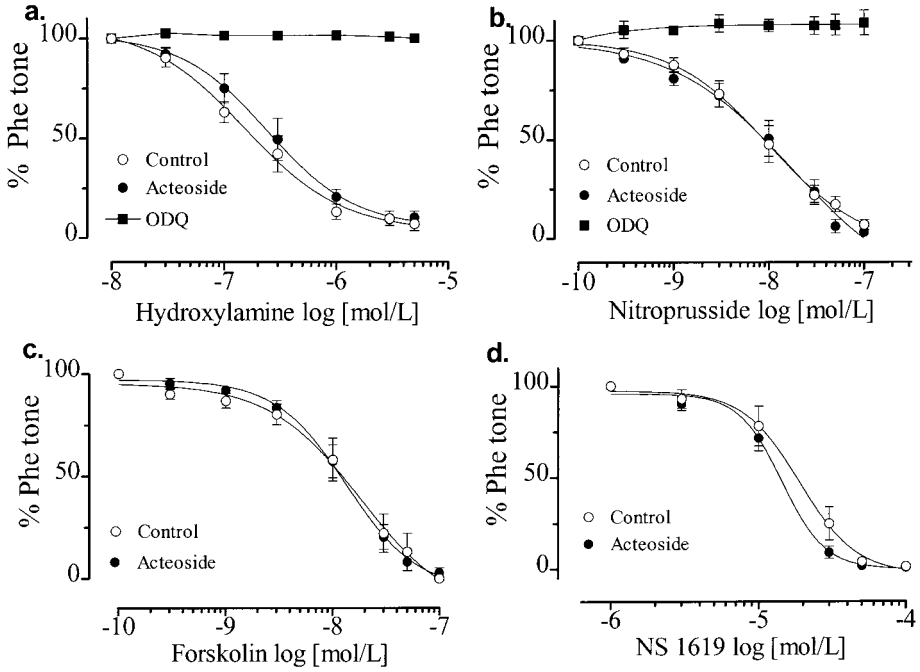


**Figure 7.** Lack of effect of 30 $\mu$mol/L acteoside on relaxation induced by hydroxylamine (a) or sodium nitroprusside (b) (○, in control; ●, in 30 $\mu$mol/L acteoside) and inhibition by 10 $\mu$mol/L ODQ (■). Lack of effect of 30 $\mu$mol/L acteoside on relaxation induced by forskolin (c) or NS 1619 (d) (○, in control; ●, in 30 $\mu$mol/L acteoside). Data are means ± SEM of 5−6 experiments.

this study after approval of animal use had been obtained from the Animal Research Ethics Committee, Chinese University of Hong Kong. Each experiment was carried out on rings prepared from different rats.

**Protocol 1.** Thirty minutes after being set up in organ baths, the contraction of each ring was initially induced by 1 $\mu$mol/L phenylephrine to test the contractile response followed by 3 $\mu$mol/L acetylcholine to evaluate the endothelial integrity. The ring was then rinsed three times in prewarmed, oxygenated Krebs solution to restore tension to baseline. The ring was contracted with phenylephrine applied cumulatively (1 nmol/L to 30 $\mu$mol/L) to obtain the first concentration−contraction curve. Once the maximal response had been obtained, the ring was washed with Krebs solution every 20 min until baseline tone returned, then incubated for 30 min with vehicle (0.2% DMSO) or with different concentrations of acteoside, and another concentration−contraction curve to phenylephrine was repeated. The effect of acteoside was also examined in endothelium-denuded rings.

**Protocol 2.** After the first concentration−response curve was obtained, the endothelium-intact rings were incubated for 30 min with individual inhibitors of the NO-mediated relaxation (100 $\mu$mol/L L-NAME, 100 $\mu$mol/L L-NNA, or 10 $\mu$mol/L ODQ) or with each inhibitor plus 30 $\mu$mol/L acteoside. The second concentration−response curve for phenylephrine was repeated. The effects of 3 mmol/L $TEA^+$ or 3 $\mu$mol/L indomethacin (30 min incubation time) were also tested on phenylephrine-induced contraction or on acteoside (30 $\mu$mol/L)-induced increase in the phenylephrine-induced response. In some experiments, the two consecutive contractile responses of endothelium-intact rings were induced by cumulative addition of $CaCl_2$ (0.1−10 mmol/L) in $Ca^{2+}$-free Krebs solution containing 50 mmol/L $K^+$. The rings were exposed to 3 mmol/L $TEA^+$ or 30 $\mu$mol/L acteoside for 30 min before repeating the second concentration−response curve for $CaCl_2$. In preparing the high $K^+$-containing solution, an equimolar concentration of $Na^+$ was replaced by $K^+$ to maintain the same ion strength.

**Protocol 3.** In the last group of experiments, the effect of acteoside was examined on the endothelium-dependent relaxation induced by acetylcholine in endothelium-intact rings and on the endothelium-independent relaxation induced by hydroxylamine, nitroprusside, forskolin, or NS 1619 in the endothelium-denuded rings.

**Chemicals.** Phenylephrine, acetylcholine, $N^G$-nitro-L-arginine methyl ester, $N^G$-nitro-L-arginine, hydroxylamine, sodium nitroprusside, tetraethylammonium chloride, indomethacin, forskolin (Sigma, St. Louis, MO), NS 1619 (RBI, MA), and 1H-[1,2,4]oxadiazolo[4,2-α]quinoxalin-1-one (Tocris, UK) were purchased. Acteoside was isolated and purified from dried leaves of *L. purpurascens* that was purchased from Zhao-tong, Yunnan Province, China, in 1998. Professor P. Y. Bai at Kunming Institute of Botany, Academia Sinica, identified the species of this plant. The voucher specimen (registration number H.P. Tsai 52669) is deposited in the Herbarium of Kunming Institute of Botany, Yunnan, China. Acteoside used in this study was prepared from the same batch of the dried leaves.[15] The chemical structure of acteoside was presented in Figure 1. Acteoside, forskolin, indomethacin, ODQ, and NS 1619 were dissolved in dimethyl sulfoxide (DMSO), and further dilution was made in fresh Krebs solution. DMSO at 0.2% in organ baths did not affect baseline tone or the phenylephrine-induced tone.

**Statistical Analysis.** To study the effect of acteoside on the agonist-induced contractile response, the values of $pEC_{50}$ were calculated as the negative log of the agonist concentration that caused 50% of the maximal contraction ($E_{max}$ %). These values were compared in the absence and presence of acteoside. $pD_2$ value was the negative log of the dilator concentration that caused 50% of the maximal relaxation. Data were presented as means ± SEM of $n$ experiments. Statistical significance was analyzed by Student's t test or by one-way ANOVA followed by Student-Newman-Keuls test when more than two treatments were compared. A $P$ value less than 0.05 was considered significant.

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