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Phytomedicine 13 (2006) 442–445
Phytomedicine
www.elsevier.de/phymed

Vascular effects of 7-epiclusianone, a prenylated benzophenone from *Rheedia gardneriana*, on the rat aorta

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Abstract

The vascular effects of 7-epiclusianone on the rat aorta were investigated. In the rat aortic rings with functional endothelia, 7-epiclusianone up to 10 μM induced a concentration-dependent vasodilatation of the sustained contractions induced by phenylephrine (0.3 μM). At concentrations higher than 10 μM, 7-epiclusianone induced a concentration-dependent contraction in the aortic rings. The vasodilator effect of 7-epiclusianone was drastically decreased with L-NAME (100 μM) as well as in endothelium-denuded aortic rings. Moreover, indomethacin (10 μM) induced a significant shift to the left in the vasodilator but did not modify the vasoconstrictor effect of 7-epiclusianone. In arteries without pre-contraction, 7-epiclusianone (3–100 μM) induced concentration-dependent contraction only in endothelium-intact and in the presence of L-NAME (100 μM). This effect was inhibited by indomethacin (10 μM) and ZM230487 (1 μM), selective inhibitors of cyclooxygenase and of 5-lipoxygenase, respectively. We can conclude that at low concentrations 7-epiclusianone induces an endothelium-dependent vasodilator effect in rat aortic rings. At higher concentrations and in conditions where NO synthase was inhibited, 7-epiclusianone induces a vasocontractile effect. Nitric oxide seems to participate in the vasodilatation, while endothelial cyclooxygenase- and 5-lipoxygenase-derived products play a role in the vasoconstrictor effect.

Keywords: *Rheedia gardneriana*; Benzophenone; Endothelium; Nitric oxide; Lipoxygenase; Cyclooxygenase

Introduction

Polyprenylated benzophenones have been described as antioxidant, free radical scavengers; as antiulcer agents; as inhibitors of iNOS and COX-2 expression in colon carcinoma; and as apoptosis inducers (Pan et al., 2001; Tanaka et al., 2000; Yamaguchi et al., 2000a, b), demonstrating a broad spectrum of biological activities for this class of compounds. The association of antioxidant and anti-inflammatory effect with vasodilatation would be relevant for the treatment of cardiovascular illnesses such as atherosclerosis and ischemia.

7-epiclusianone (Fig. 1) is a prenylated benzophenone isolated as the main constituent from *Rheedia gardneriana* Miers ex Planch & Triana (Santos et al., 1998). The biological activities of this substance remain poorly investigated. There is only one previous report demonstrating in vitro activity of 7-epiclusianone against trypomastigotes of *Trypanosoma cruzi* (Alves et al., 1999). In the present work, we began the investigation

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doi:10.1016/j.phymed.2005.01.014

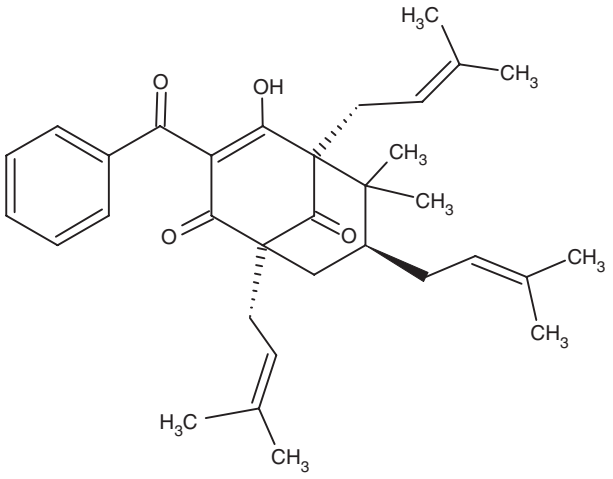

Fig. 1. Chemical structure of 7-epiclusianone.

of the effects of 7-epiclusianone on the vascular system.

Materials and methods

Experimental procedure

Animal experiments were performed according to the recommendations of the Brazilian Council for Animal Care and to the directives of the Ethics Committee of the Universidade Federal de Minas Gerais. Male Wistar rats (200–250 g each) were killed by cervical dislocation and exsanguinated. The aortic rings were mounted and maintained in Krebs-Henseleit solution of the following composition (mM): NaCl 110.8, KCl 5.9, $NaHCO_3$ 25.0, $MgSO_4$ 1.07, $CaCl_2$ 2.49, NaH_2PO_4 2.33 and glucose 11.51, as described by Guedes et al. (2002). 7-epiclusianone was added cumulatively during the tonic contractions induced by phenylephrine (0.3 μM) or in aortic rings without pre-contraction. The results obtained with the vasodilator effect induced by 7-epiclusianone were expressed as a percentage of the decrease in maximal contraction induced by phenylephrine, taking as 100% relaxation the point when the basal line was reached. For the contractile effects of 7-epiclusianone the results were expressed as mN/mm. Fifty-percent inhibitory concentrations (IC_{50} values) were calculated graphically from concentration–response curves.

Drugs

Acetylcholine chloride, L-NAME, indomethacin and Phenylephrine chloride were purchased from Sigma (USA). ZM230487 was a gift from Zeneca Pharmaceuticals. Pure 7-epiclusianone (99.82% by analytical calculation) was obtained as described previously (Santos et al., 1998). It was solubilized in a mixture of distilled water/cremophor as a 10^{-2} M solution and diluted to the desired concentration with distilled water just before use. The final concentration of cremophor never exceeded 0.1%, which was without effect when exposed to control preparations. Indomethacin was dissolved in 0.5%w/v sodium bicarbonate. The other compounds were freely dissolved in water.

Statistics

Results are expressed as the mean ± s.e.m of five experiments. Student's *t*-test (for IC_{50} values) and One-Way ANOVA followed by Bonferroni's Multiple Comparison Test (for concentration-response curves) were used to analyze the results and statistical significance was determined at $p<0.05$.

Results and discussion

In endothelium-intact aortic rings, 7-epiclusianone induced concentration-dependent relaxation with a maximal effect (E_{max}) of 61.7 ± 3.3% and an IC_{50} value of 8.1 ± 1.5 μM (Fig. 2) in rat aortic rings pre-contracted with phenylephrine (0.3 μM). This vasodilator effect was dramatically reduced ($E_{max} = 25.7 \pm 7.9\%$) in endothelium-denuded arteries (Fig. 2A). Acetylcholine, a well-known endothelium-dependent vasodilator, also induced a concentration-dependent vasodilator effect with an E_{max} of 70.6 ± 6.2% and an IC_{50} value of 0.15 ± 0.05 μM ($n = 5$), approximately 50 times more potent than 7-epiclusianone (not shown). In addition, pre-treatment of the aortic rings with L-NAME (100 μM), a selective inhibitor of NO synthase at a concentration that completely inhibited the vasodilator effect of acetylcholine (Rees et al., 1990), induced a significant reduction in the vasodilator effect induced by 7-epiclusianone (Fig. 2B). However, a significant shift to the left (IC_{50} value = 2.5 ± 0.9 μM; $p<0.05$) without modification of E_{max} was observed in aortic rings pre-treated with indomethacin (10 μM; Fig. 2B), a selective inhibitor of cyclooxygenase at a concentration that completely inhibited the production of cylcooxygenase-derived factors (Cole et al., 1986). The above results demonstrate that 7-epiclusianone at low concentrations induces a vasodilator effect via a mechanism dependent on the presence of a functional endothelium and on the production of NO. They also suggest the participation of cyclooxygenase-derived contractile factor(s).

7-epiclusianone at concentrations higher than 10 μM induced concentration-dependent contractions in aortic rings pre-contracted with phenylephrine (0.3 μM). In an attempt to clarify this effect, the contractile effect of 7-epiclusianone was investigated in aortic rings without pre-contraction. We observed that 7-epiclusianone was

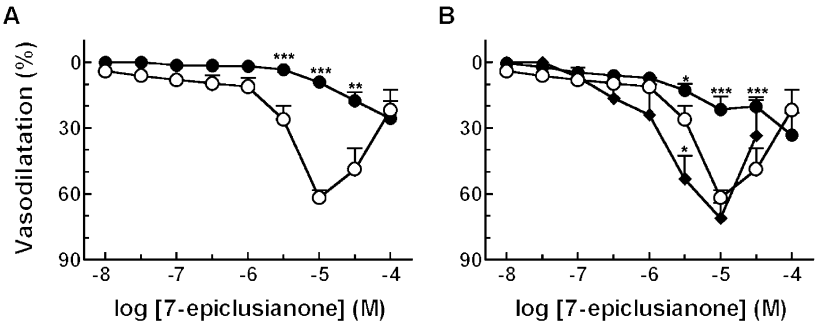

Fig. 2. Relaxant effect induced by 7-epiclusianone in rat aortic rings pre-contracted with phenylephrine (0.3 μM). (A) Effect of 7-epiclusianone in aortic rings in the presence (control; ○) and in the absence of a functional endothelium (●). (B) Effect of 7-epiclusianone in rat aortic rings with a functional endothelium in the absence (control; ○) and in the presence of L-NAME (100 μM; ●) and indomethacin (10 μM; ◆). Results are expressed as mean ± s.e.m. of five experiments. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared to control curves by One-Way ANOVA.

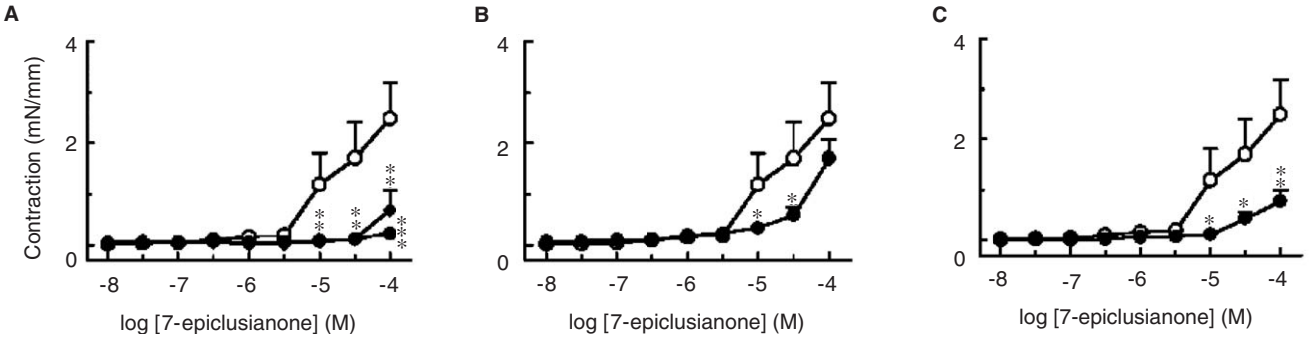

Fig. 3. Contractile effect induced by 7-epiclusianone in rat aortic rings. (A) Effect of 7-epiclusianone in rings with (●) and without (◆) a functional endothelium, and in rings with endothelium treated with L-NAME (100 μM; control; ○). (B) Effect of 7-epiclusianone in aortic rings with endothelium and L-NAME (100 μM; control; ○) in the presence (●) of indomethacin (10 μM). (C) Effect of 7-epiclusianone in aortic rings with endothelium and L-NAME (100 μM; control; ○) in the presence (●) of ZM230487 (1 μM). Results are expressed as mean ± s.e.m. of five experiments. *$p<0.05$ and **$p<0.01$ compared to control curves by One-Way ANOVA.

able to induce concentration-dependent contractions at concentrations higher than 3 μM, in rings with a functional endothelium and in the presence of L-NAME (Fig. 3A). No significant contraction was observed in endothelium-denuded rings (Fig. 3A), suggesting that the contractile-effect induced by 7-epiclusianone was dependent on the production of endothelium-derived contractile factors, and that this effect was masked by the production of NO. Products derived from arachidonic acid metabolism are well-known to induce endothelium-dependent contraction in the vasculature (Cole et al., 1986; Walch et al., 2000), for this reason we investigated the participation of cyclooxygenase- and lipoxygenase-derived contractile factors in the vasoconstriction induced by 7-epiclusianone. We observed that indomethacin (10 μM) induced a significant reduction of the contractile curve of 7-epiclusianone (Fig. 3B). In the presence of a selective inhibitor of 5-lipoxygenase (Negro et al., 1997), ZM230487 (1 μM), the contractile effect of 7-epiclusianone was also significantly reduced (Fig. 3C). The above results are indicative of the participation of cyclooxygenase- and 5-lipoxygenase-derived contractile factors in the vasoconstriction induced by 7-epiclusianone.

We can conclude that low concentrations of 7-epiclusianone induce an endothelium- and NO-dependent vasodilator effect in rat aortic rings. At higher concentrations, 7-epiclusianone induces a contractile effect where cyclooxygenase and 5-lipoxygenase-derived products are involved. The vasodilator effect of 7-epiclusianone could be of interest for the treatment of cardiovascular illnesses. However, care should be taken due to the vasoconstrictor effect observed at higher concentrations.

Acknowledgements

To the National Research Council (CNPq; Brazil) for research fellowships (A.J.C., V.S.L., M.H.S., T.J.N.,

S.F.C.) and Research foundation from Minas Gerais (FAPEMIG; Brazil) for financial support.

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