Comparative Study on the Vasodilatory Effects of Three Quinazoline Alkaloids Isolated from *Evodia rutaecarpa*

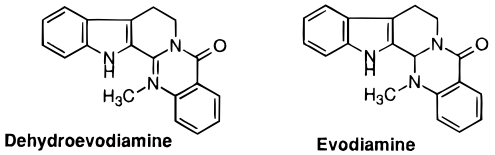
Wen-Fei Chiou,[†,‡] Jyh-Fei Liao,[‡] and Chieh-Fu Chen*[,†,‡]

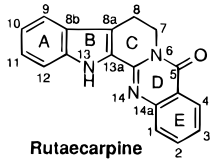
National Research Institute of Chinese Medicine, Taipei Hsien, Taiwan, and Department and Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan

Received August 4, 1995[⊗]

The vasoreactivity of dehydroevodiamine (**1**), evodiamine (**2**), and rutaecarpine (**3**), quinazoline alkaloids isolated from *Evodia rutaecarpa*, to aorta smooth muscle demonstrated that they produce a vasodilatory effect on endothelium-intact rat aorta with equal potency. Compound **3** produced a full (100%) nitric oxide-dependent vasodilatation, whereas **2** and **1** produced a partially endothelium-dependent effect, 50% and 10%, respectively. At the same time, **1** and **2** may also act by other mechanisms, including probably an α_1-adrenoceptor blocking action and a 5-HT antagonizing action, respectively.

The dried, unripe fruit of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae, popularly known in China as "Wu-Chu-Yu", has been prescribed for the treatment of gastrointestinal disorders (abdominal pain, dysentery), headache, postpartum hemorrhage, and amenorrhea,[1] according to traditional Chinese medical practice. It also has been reported to possess CNS stimulating,[2] transient hypertensive,[2,3] and positive inotropic and chronotropic effects.[4] A number of quinazolinocarboline alkaloids have been isolated from Wu-Chu-Yu, including dehydroevodiamine (**1**), evodiamine (**2**), rutaecarpine (**3**), rutaevine, wuchuyine, and rhetsinine.[5,6] Recently, we reported that the nitric oxide (NO) system is involved in the vasodilator effects of **1**, **2**, and **3** in rat-isolated mesenteric arteries[7,8] on the basis of circumstantial evidence that L-N^G-nitroarginine, a nitric oxide synthase inhibitor, and methylene blue, a guanylyl cyclase inhibitor, can both attenuate their effects. It was shown that these structurally similar alkaloids relax endothelium-intact preparations with equal potency, but the extent of NO involvement in their vasodilating action of rat mesenteric artery was different. Therefore, other mechanisms of action must be involved in their vasodilatory effects.





In the present study, the pharmacological actions of **1**, **2**, and **3** were evaluated in rat thoracic aorta. An attempt to elucidate the underlying mechanisms of action, including endothelium dependency and the possible involvements of mechanisms such as α_1-adrenergic, serotonergic receptor, and Ca^{2+}-channel blocking activities, has been carried out.

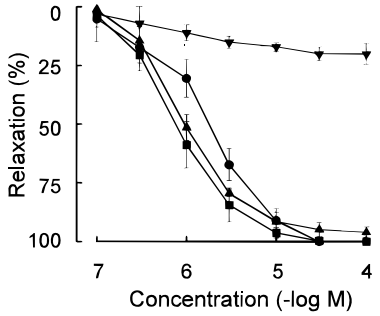


Figure 1. Comparison of the relaxant potencies of dehydroevodiamine (**1**, ●)-, evodiamine (**2**, ■)-, rutaecarpine (**3**, ▲)-, and/or DMSO (▼)-induced vasodilatation in endothelium-intact aorta. The response is expressed as the percentage of relaxation of the phenylephrine-induced contraction (100% represents complete relaxation). Each point and vertical bar represents the mean ± SE (N = 10−13 in each group).

Results and Discussion

The alkaloids **1**, **2**, and **3** produced concentration-dependent (10^{-7}−10^{-4} M) relaxation in thoracic aortic rings contracted by 3×10^{-7} M phenylephrine (PE) (Figure 1). The relaxation pattern of all these alkaloids was almost the same, with respect to the onset of effect as well as the duration of effect. The results showed that **1** and **2** produced almost complete relaxation, while **3** produced a 92% relaxation at the maximum-effect concentration (10^{-4} M). The concentration required to elicit a response equal to 50% of the maximum response (EC_{50}) for **1**, **2**, and **3** was 1.64, 0.93, and 1.05 μM, respectively (Table 1). In general, the results indicated that **1**, **2**, and **3** elicited concentration-related vasodilatation in endothelium-intact aortic rings with equal potency.

To determine whether the relaxation effects of these alkaloids were endothelium dependent, endothelium-intact and -denuded tissues were contracted with 0.3 or 0.1 μM PE respectively, to equal contractile magnitude. The results indicated that **1** relaxed rat aorta in a concentration-dependent manner irrespective of the presence or absence of endothelium (Figure 2A), whereas

* To whom correspondence should be addressed: Institute of Pharmacology, National Yang-Ming University, Shih-Pai, Taiwan, R.O.C. Phone: (02) 821-3874, 822-5592. FAX: (02) 820-1856.
† National Research Institute of Chinese Medicine.
‡ Department and Institute of Pharmacology.
⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1996.

0163-3864/96/3259-0374$12.00/0

Table 1. Relaxant Potencies of Dehydroevodiamine (**1**), Evodiamine (**2**), and Rutaecarpine (**3**) on Phenylephrine-Precontracted Endothelium-Intact (+EC) and Endothelium-Denuded (−EC) Aortic Rings[a,b]

	1	**2**	**3**
(+EC) (n = 9−13)			
EC_{50} (μM)	1.64 ± 0.23	0.93 ± .07	1.05 ± 0.06
E_{max} (%)	100.0 ± 0.0	100.0 ± 0.0	96.0 ± 2.3
(−EC) (n = 9−12)			
EC_{50} (μM)	7.78 ± 0.12	8.14 ± 0.09	1.75 ± 0.06
E_{max} (%)	100.0 ± 0.0	49.8 ± 6.4	23.0 ± 5.4

[a] Results are indicated as mean ± SE. [b] Potencies are expressed as EC_{50} (μM) and E_{max} (maximal relaxant effect, %).

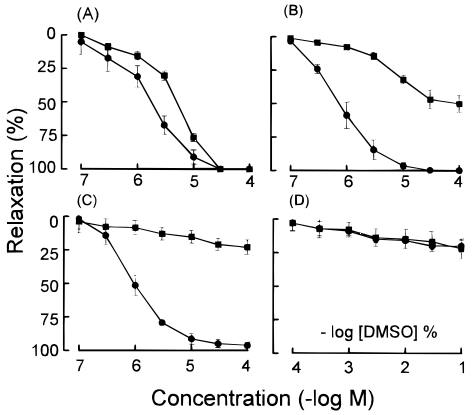


Figure 2. Effects of endothelium removal on dehydroevodiamine (**1**) (A; 0.1−100 μM)-, evodiamine (**2**) (B; 0.1−100 μM)-, rutaecarpine (**3**) (C; 0.1−100 μM)-, and vehicle DMSO (D; 0.001−0.1%)-evoked relaxation in aortic rings. The response is expressed as the percentage of relaxation of the phenylephrine-induced contraction (100% represents complete relaxation). Each point and vertical bar represents the mean ± SE, endothelium-intact (●) and -denuded (■) (n = 8−10 in each group).

the concentration-relaxation curves for **2** and **3** were markedly altered in endothelium-denuded preparations (Figure 2B, 2C). In endothelium-denuded tissues for compound **2** the concentration-response curve was shifted to the right, and the maximum response was depressed to 49.8 ± 6.4%. Dramatically, **3**-induced relaxation almost disappeared after removal of the endothelium, and the rest of **3**-evoked responses in endothelium-denuded preparations appeared to be caused by the vehicle, DMSO (Figure 2D). The order of potency in endothelium-denuded preparations in terms of EC_{50} value and maximal response was **1** > **2** > **3** (Table 1).

In conclusion, our results indicated that the extent of endothelium involvement in the vasodilatory effect of **1**, **2** and **3** were quite different as determined by endothelium removal. The vasodilatory effect of **3** was totally dependent on the presence of endothelium. In contrast, the effect of **1** was only slightly endothelium-dependent, and the effect of **2** was partially endothelium-dependent.

The release of endothelium-derived mediators stimulated by a drug may play an important role in its vasodilatory effect. The relaxing substances include NO, endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin (PGI_2).[9−11] In an attempt to elucidate the possible mechanisms involved in the vasodilatory effects of **1**, **2**, and **3**, the effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME), tetraethylammonium chloride (TEA), and indomethacin were examined on the relaxation produced by three compounds. The cumulative concentration-response curves for **1**, **2**, and **3** in intact aorta before and after treatment with L-NAME, TEA, or indomethacin are illustrated in Figure 3 (left panels). L-NAME, TEA, or indomethacin had no significant effect on the basal tension (data not shown) and the tension caused by 3×10^{-7} M PE (Figure 3, right panels).

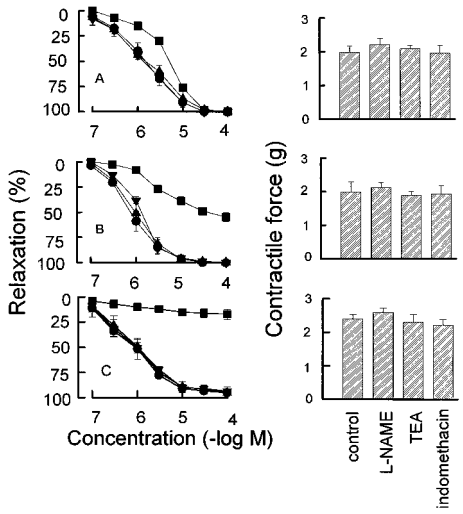


Figure 3. Left panels: vasodilator effects of (A) dehydroevodiamine (**1**), (B) evodiamine (**2**), and (C) rutaecarpine (**3**) on phenylephrine-precontracted endothelium-denuded aortic rings before (●) and after incubation with L-NAME (■), indomethacin (▲), and TEA (▼). The response is expressed as the percentage of relaxation of the phenylephrine-induced contraction (100% represents complete relaxation). Each point and vertical bar represents the mean ± SE. Right panels: contractions produced by phenylephrine in the absence and presence of L-NAME, indomethacin, and TEA. Results are mean ± SE of the relative contractile forces (g), (n = 9−13 in each group) (L-NAME: N^{ω}-nitro-L-arginine methyl ester, TEA: tetraethylammonium chloride).

The relaxant effect of **3** was almost abolished by L-NAME treatment (Figure 3C), which resembles that observed in endothelium-denuded preparations. L-NAME treatment also modestly influences the concentration-relaxation response to **2** (Figure 3B). The EC_{50} value for **2** in the presence of L-NAME was 2.92 ± 0.11 μM, significantly greater than that for the control (0.78 ± 0.91 μM). In contrast, L-NAME treatment had much less effect on **1**-induced vasodilatation (EC_{50}: 1.60 ± 0.91 vs. 5.83 ± 0.64 μM in the absence and presence of L-NAME, respectively). The vasodilatory effects of **1**, **2**, or **3** on PE-precontracted preparations were not significantly affected by indomethacin incubation for 45 min. Similarly, in the presence of TEA, relaxations to **1**, **2**, and **3** still remained.

TEA is known to inhibit the effect of EDHF,[12] and indomethacin is known to inhibit the synthesis of PGI_2.[13] Secondly, L-NAME, a NO synthase inhibitor, significantly inhibited the vasodilatory effects of these alkaloids with results identical to those obtained from endothelium removal. Finally, methylene blue, a guanylyl-cyclase inhibitor,[14] with identical results to L-NAME treatment, was found to affect relaxant responses to all these agents (data not shown). Thus, only NO (but neither EDHF nor PGI_2) was involved in the relaxing effects of **3**, **2**, and **1**.

Because the data indicated that **1** and **2** still produced relaxation in endothelium-denuded rings, experiments were subsequently carried out to determine whether

Table 2. Potencies of Dehydroevodiamine (**1**) and Evodiamine (**2**) Against Contractions to (A) Phenylephrine (10^{-9}−10^{-5} M) and (B) 5-Hydroxytryptamine (10^{-7}−10^{-4} M) in Endothelium-Denuded Preparations[a,b]

	1				**2**			
	vehicle	0.1 μM	1.0 μM	10.0 μM	vehicle	0.1 μM	1.0 μM	10.0 μM
				(A)				
EC_{50} (M)	1.18×10^{-8}	2.23×10^{-8} [d]	6.01×10^{-8} [d]	3.55×10^{-7} [d]	1.01×10^{-8}	1.49×10^{-8}	1.98×10^{-8}	2.67×10^{-8}
E_{max} (g)	2.22 ± 0.20	2.10 ± 0.17	2.05 ± 0.09	2.00 ± 0.06	2.36 ± 0.11	1.96 ± 0.09[c]	1.85 ± 0.09[d]	1.64 ± 0.11[d]
pA_2		6.89 ± 0.01				–		
				(B)				
EC_{50} (M)	4.15×10^{-7}	5.02×10^{-7}	5.23×10^{-8}	5.77×10^{-7}	7.21×10^{-7}	1.08×10^{-6} [c]	2.39×10^{-6} [c]	5.64×10^{-6} [d]
E_{max} (g)	2.61 ± 0.05	2.57 ± 0.23	2.41 ± 0.12	2.32 ± 0.09	2.50 ± 0.11	2.43 ± 0.15	2.35 ± 0.15	2.20 ± 0.18
pA_2		–				6.25 ± 0.01		

[a] Results are indicated as mean ± SE. [b] Potencies are expressed as EC_{50} (M) and E_{max} (maximal contractile force, g). [c] $p < 0.01$. [d] $p \leq 0.001$ indicate statistical significance of the difference between vehicle and **1** (or **2**) treatment; pA_2 value is calculated by using Schild analysis.

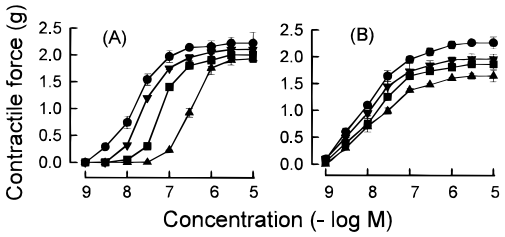


Figure 4. Concentration-response curves in rat thoracic aorta for phenylephrine (1 nm −10 μM) in the presence of 0.1% DMSO (control group; ●) or vessels pretreated with 0.1 (▼), 1.0 (■), and 10.0 μM (▲) of (A) dehydroevodiamine (**1**) and (B) evodiamine (**2**). Results are mean ± SE of the relative contractile forces (g), (n = 9−13 in each group).

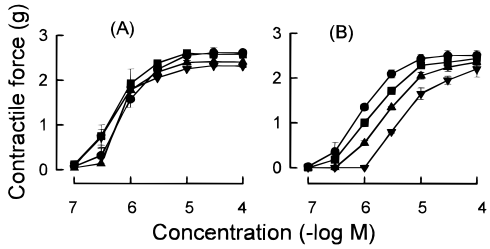


Figure 5. Concentration-response curves in rat thoracic aorta for 5-HT (0.1−100 μM) in the presence of 0.1% DMSO (control group; ●) or vessels pretreated with 0.1 (■), 1.0 (]tus), and 10.0 μM (▼) of (A) dehydroevodiamine (**1**) and (B) evodiamine (**2**). Results are mean ± SE of the relative contractile forces (g), (n = 8−11 in each group).

these two compounds could also act on the α-adrenoceptors of vascular smooth muscle. The concentration-response curve (CRC) of PE in the endothelium-denuded preparations was assessed in the presence of different concentrations of **1** or **2**. Results showed that PE (1 nM to 10 μM) produced a concentration-dependent contraction with a EC_{50} of 11.8 ± 0.7 nM and a maximal contraction of 2.22 ± 0.20 g (N = 14). Exposure to **1** (0.1, 1, and 10 μM) for 10 min produced parallel shifts of the PE's CRC to the right without supressing the maximal response, consistent with competitive antagonism (Figure 4A). The pA_2 values of **1** was 6.89 ± 0.01 (Table 2).

Compound **2** also caused a concentration-dependent shift of PE's CRC to the right (Figure 4B). However, **2** caused a concentration-dependent depression in maximal response of the CRC for PE. Results showed that a 10-min incubation with **2** at concentrations of 0.1, 1, and 10 μM, reduced maximal contractile response to PE (10^{-5} M) by 83.1 ± 3.8, 78.4 ± 3.8, and 69.5 ± 4.7% of the control (vehicle group) values, respectively. The EC_{50} values of PE in the presence of vehicle (0.1% DMS)), 0.1, 1, and 10 μM of **2** were 10.1, 14.9, 19.8, and 26.7 nM, respectively (Table 2).

To study further whether **1** or **2** has an antagonizing effect on other receptors, 5-HT-induced contraction was evaluated. The EC_{50} of 5-HT-induced contraction in endothelium-denuded preparation was around $4-7 \times 10^{-7}$ M (Table 2). As shown in Figure 5A, all concentrations of **1** (0.1−10 μM) had no significant effect on 5-HT-induced contraction, whereas **2** (0.1−10 μM) tended to produce parallel rightward shifts in the CRC of 5-HT (Figure 5B) with a pA_2 value of 6.25 ± 0.01 μM (Table 2).

Our results demonstrated that **1** competitively antagonized PE-induced contractions with a pA_2 value of 6.89. Using a radioligand receptor binding assay, we found that **1** competed with the [^{3}H]prazosin for binding to the α_1-adrenoceptors in rat-heart-membrane preparation with a K_i value of 2.4 μM (Liao and Chen, unpublished data). Compound **2** also shifted the CRC of PE, but suppressed the maximal response induced by PE in a concentration-dependent manner. This result indicated that **2**, unlike **1**, is not a competitive antagonist of α_1-adrenoceptors. Receptor binding assay studies also indicated that **2** had no significant effect on [^{3}H]prazosin binding in rat-heart-membrane preparations (Liao and Chen, unpublished data). Therefore, other mechanisms of action must be involved in the vasodilatory effect of **2**. It has been reported that receptor agonists (like PE), although mainly acting on the receptors, may depolarize smooth muscle at high concentrations.[15−17] Because **2** also inhibits high KCl-induced contraction (data not shown), the inhibitory effect of **2** on PE-induced contraction may be partially due to a blocking action on voltage-dependent Ca^{2+} channels. In another experiment, it was demonstrated that **2** was effective in antagonizing the contractile responses to 5-HT, although some depression on the maximal response of 5-HT was observed. Whether **2** interacts with the vascular 5-HT receptors remains to be determined by receptor binding assays. Similarly, whether **2** also interacts with the L-type Ca^{2+} channels should be determined. Recently, we evaluated the interaction with Ca^{2+} channels using radioligand binding assay study.[18] Results showed that none of these pure compounds (except **1** at a concentration higher than 100 μM) was directly acting on the 1,4-dihydropyridine (DHP) binding site of Ca^{2+} channels.[18] Therefore, it provided evidence to conclude that the Ca^{2+} channels' inhibitory effect of **1**, **2**, or **3** as reported in the literature[19] or in our previous studies[7,8,20,21] was not

due to an action on the DHP binding site of L-type Ca^{2+} channels. However, the interaction of **1**, **2**, or **3** on other ligand (nifedipine and diltiazem) binding sites of L-type Ca^{2+} channels or other types of Ca^{2+} channels remains to be further determined.

The order of endothelium dependency of tested alkaloids was **3** ≫ **2** > **1** in rat aorta, while it was **3** > **2** = **1** in mesenteric artery.[7,8] In brief, the vasodilatory effect of **3** was totally (100%) dependent on the presence of endothelium in aorta but only 65−70% dependent in mesenteric artery. Compound **2** exerts a partial (about 50%) endothelium-dependent relaxation in aorta but only a slight (about 20%) relaxation in mesenteric artery. Compound **1**, in contrast to the partial (about 20%) endothelium-dependent effect observed in mesenteric artery, was less endothelium-dependent in aorta. The difference between aorta and mesenteric artery appears to be related to functional properties of the endothelium. Considerable heterogeneity of endothelium-dependent responses among different vascular beds has been observed.[22]

In conclusion, the present study demonstrated that **1**, **2**, and **3** produce a vasodilatory effect on endothelium-intact aorta with equal potency. Compound **3**, however, produced a fully (100%) NO-dependent vasodilatation in rat aorta, whereas **2** and **1** relaxed it in a partially endothelium-dependent manner at approximately 50% and 10%. Meanwhile, **1** and **2** also relaxed it by probably other different mechanisms, including probably an α_1-adrenoceptor blocking or a 5-HT antagonizing actions, respectively. Differences in chemistry of these three alkaloids may account for the appearance of variation in action mechanisms.

Experimental Section

Isolated Rat Aorta. The experiments were carried out on thoracic aortas from male Sprague-Dawley rats weighting 230−280 g. Animals were killed by stunning and exsanguination. The thoracic aorta was removed and immersed in cold gassed (95% O_2 + 5% CO_2) Krebs solution of the following composition (mM): NaCl (118), KCl (4.8), $CaCl_2$ (2.5), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $NaHCO_3$ (24), and glucose (11). Aortic rings were prepared according to Chiou *et al*.[7,8] In brief, the vessels were cleaned of fat and connective tissues. The aorta was then cut into rings (5 mm in length) and placed in a 5-mL isolated organ bath filled with gassed (95% O_2 − 5% CO_2) Krebs solution maintained at 37 °C. Two fine stainless steel wires were inserted through the lumen of the segment; one was anchored to a stationary support and the other was connected to an isometric transducer (Grass FT-03C). Changes in vessel tone were recorded on a polygraph (Gould RS-3400). The initial tension was adjusted to 1.4−1.6 g, followed by equilibration for more than 90 min, washing at 20-min intervals. Cumulative concentration-response curves, with 0.3 log unit concentration intervals, were used to quantitate the sensitivity of the tissue to drugs.

General Experimental Procedures. In some experiments, endothelial cells were removed by rubbing the intimal surface with a moistened cotton swab. Such preparations failed to relax in response to a maximal concentration of acetylcholine (3 μM) but responded normally to a maximal concentration of sodium nitroprusside (0.1 μM). For measurement of relaxation, aorta was precontracted with PE at a concentration inducing approximately 80% of the maximal contraction (EC_{80}: 0.3 μM for the preparations with endothelium and 0.1 μM for preparations without endothelium). After washout and 30 min of equilibration, the effects of cumulative concentrations (0.1−100 μM) of test substances on PE-induced contractions were studied in preparations with and without endothelium. After reaching the plateau of contraction, test substances were added in a cumulative manner. The results are expressed as the geometric mean ± SE for N-separate experiments, and the relaxant responses are indicated as percentages of maximal relaxation. Statistical evaluation was made using unpaired Student's *t*-test, $P < 0.05$ being considered significant. The concentration of agents that produced 50% of the maximal relaxation or contraction (EC_{50}) was estimated from the log concentration-effect curves in each tissue.

To assess the involvement of endothelium-derived relaxing substances, some potential inhibitors such as N^{ω}-nitro-L-arginine methyl ester (a nitric oxide synthase inhibitor),[23] tetraethylammonium chloride (an inhibitor of endothelium-derived hyperpolarizing factor,[12] and indomethacin (an inhibitor of vascular prostacyclin biosynthesis),[13] were preincubated in the organ bath for appropriate concentrations (3×10^{-4} M, 10^{-2} M, 3×10^{-5} M, respectively) and incubation times (10 min, 1 h, and 45 min, respectively) before construction of a second cumulative CRC to test substances.

To determine the involvement of α_1-adrenergic, serotonergic-, or Ca^{2+} channel-blockade in the vasodilating effects of these quinazoline alkaloids, various concentrations of alkaloids were added and incubated for 15 min before construction of a second cumulative CRC with PE and 5-HT. Results are expressed as actual contractile tension to PE, 5-HT, or KCl before and after alkaloid treatment. We calculated the pA_2 values for each concentration of test alkaloids according to the following formula: $pA_2 = -\log([\text{antagonist}]/[\text{dose ratio} - 1])$.[24]

Chemicals. Phenylephrine HCl, acetylcholine Cl, N^{ω}-nitro-L-arginine methyl estger, tetraethylammonium Cl, indomethacin, 5-HT, and DMSO were purchased from Sigma Co. (St. Louids, MO). Phenylephrine HCl was dissolved in distilled H_2O containing 0.1% ascorbic acid and stored in a freezer (−20 °C). On the day of the experiment, final dilutions of PE were made with Krebs solution. All other drugs were dissolved in Krebs solution except the test alkaloids, which were dissolved in DMSO resulting a stable stock solution of 0.1 M. Series dilutions were prepared fresh and protected from light.

Isolation of Alkaloids. **1**, **2**, and **3** were isolated and identified as previously described from the dried, unripened fruit of *Evodia rutaecarpa* in our Institute.[6]

Acknowledgment. This work was supported to grants-in-aid to Prof. C. F. Chen from the National Science Council, Taipei, Taiwan, R.O.C. (NSC 84-2331-B-077-005-M04), for which the authors are deeply grateful.

References and Notes

(1) Li, S. C. *Pen-tsao Kang Mu* (Republished by National Research Institute of Chinese Medicinee, 1976, Taipei); 1596. Chapter 32, p 1064.
(2) Liao, J. F.; Chen, C. F.; Chow, S. Y. *J. Formosan Med. Assoc.* **1981**, *79*, 30.

(3) Chow, S. Y.; Chen, S. M.; Yang, C. M. *J. Formosan Med. Assoc.* **1976**, *215*, 277.
(4) Chen, C. F.; Chen, S. M.; Chow, S. Y. *Am. J. Chin. Med.* **1981**, *1*, 39 (1981).
(5) Chang, H. M.; But, P. P. H. *Pharmacology and Applications of Chinese Materia Medica*, World Scientific Publishing: Singapore, 1987; Vol. I, p 605.
(6) Lin, L. C.; Chou, C. J.; Chen, K. T.; Chen, C. F. *J. Chin. Med.* **1991**, *2*, 94.
(7) Chiou, W. F., Chou, C. J.; Shum, A. Y. C.; Chen, C. F. *Eur. J. Pharmacol.* **1992**, *215*, 277.
(8) Chiou, W. F.; Chou, C. J.; Liao, J. F.; Shum, A. Y. C.; Chen, C. F. *Eur. J. Pharmacol.* **1994**, *257*, 59.
(9) Vanhoutte, P. M.; Rubanyi, G. M.; Miller, V. M.; Houston, D. S. *Ann. Rev. Physiol.* **1986**, *48*, 307.
(10) Chen, G.; Suzuki, H.; Weston, A. H. *Br. J. Pharmacol.* **1988**, *95*, 1165.
(11) Chen, G.; Suzuki, H. *J. Physiol. (London)* **1989**, *410*, 91.
(12) Hasunuma, K.; Yamaguchi, T.; Rodman, D. M.; O'Brien, R. F.; McMurtry, I. F. *J. Physiol.* **1991**, *4*, L97.
(13) Gryglewski, R. J.; Moncada, S.; Palmer, R. M. J. *Br. J. Pharmacol.* **1986**, *87*, 685.
(14) Martin, W.; Villani, G. M.; Jothianandan, D.; Furchgott, R. F. *J. Pharmacol. Exp. Ther.* **1984**, *232*, 708.
(15) Peiper, U.; Griebel, L.; Wende, W. *Pflugers Arch. ges. Physiol.* **1971**, *330*, 74.
(16) Casteels, R.; Kitamura, K.; Kuriyama, H.; Suzuki, H. *J. Physiol.* **1977**, *271*, 41.
(17) Bolton, T. B. *Physiol. Rev.* **1979**, *59*, 606.
(18) Yu, L. L.; Liao, J. F.; Chou, C. J.; Chen, C. F. *J. Chin. Med.* **1995**, *6*, 149.
(19) Loh, S. H.; Lee, A. R.; Huang, W. H.; Lin, C. I. *Br. J. Pharmacol.* **1992**, *106*, 517.
(20) Lin, C. I.; Loh, S. H.; Luk, H. N.; Lue, W. M.; Chen, C. F. *J. Chin. Med.* **1990**, *1*, 84.
(21) Yang, H. Y.; Li, S. Y.; Chen, C. F. *Asia Pacific J. Pharmacol.* **1988**, *3*, 191.
(22) Luscher, T. F.; Cooke, J. P.; Houston, D. S.; Neves, R.; Vanhoutte, P. M. *Mayo Clin. Proc.* **1987**, *62*, 601.
(23) Gardineer, S. M.; Compton, A. M.; Bennett, T.; Palmer, R. M. J.; Moncada, S. *Br. J. Pharmacol.* **1990**, *101*, 10.
(24) Mackay, D. *J. Pharm. Pharmacol.* **1978**, *30*, 312.

NP960161+