J. Pharm. Pharmacol. 1998, 50: 237–241
Received March 11, 1997
Accepted October 28, 1997

Comparative Analysis of the Vascular Actions of Diterpenes Isolated from *Euphorbia canariensis*

FRANCISCO J. MIRANDA, JOSÉ A. ALABADÍ, MARTA ORTÍ, JOSE M. CENTENO, MARTA PIÑÓN, ALBERTO YUSTE*, JUAN F. SANZ-CERVERA*, J. ALBERTO MARCO* AND ENRIQUE ALBORCH†

*Department of Physiology and *Organic Chemistry, University of Valencia and †Research Centre, Hospital La Fe, Valencia, Spain*

Abstract

We have analysed the effects of 2,3-di*epi*ingol 7,12-diacetate-8-isobutyrate (compound **1**), ingenol-3-angelate-17-benzoate (compound **2**), ingenol-3-angelate-17-benzoate-20-acetate (compound **3**) and 3,5,7,8,9,15-hexahydroxyjatropha-6(17),11-dien-14-one-5,8-bis(2-methylbutyrate)-7-(2-methylpropionate) (compound **4**), four diterpenes isolated from *E. canariensis*, on the isometric tension developed by isolated rabbit basilar and carotid arteries.

Concentration–response curves to these compounds were obtained cumulatively in both arteries at resting tension and active tone (KCl, 50 mM). At resting tension a concentration-dependent contraction was induced by the four compounds. In the basilar artery the order of potency was **3** = **1** > **2** = **4**, without significant differences between E_{max} values. In the carotid artery the order of potency was **3** > **2** = **1** = **4** and there were no significant differences between the E_{max} (maximum effect) values of compounds **1–3**, all of which were higher than that of compound **4**. In pre-contracted basilar artery compounds **1–3** induced concentration-dependent relaxation and compound **4** was almost ineffective; the order of potency was **3** > **2** = **1** without significant differences between E_{max} values. In the carotid artery with active tone the four compounds tested induced further contractions; the order of potency was **3** > **2** = **4** > **1** without significant differences between E_{max} values.

These results show that the four diterpenes are potent active substances in rabbit basilar and carotid arteries and that there are regional differences between their action. The four compounds tested contract basilar and carotid arteries at resting tension. Compounds **1–3** relax pre-contracted basilar artery but not carotid artery.

In recent years several studies have demonstrated the importance and variety of pharmacological actions of natural diterpenes; the wide range includes anti-tumour activity (Habtemariam 1995), therapeutic usefulness in the treatment of patients with gastric ulcers because of their ability to promote epithelial regeneration of gastric mucosa (Shirakabe et al 1995); immunosuppressive and anti-rheumatic activity (Gu et al 1995); and effectiveness in protecting biological systems against oxidative stress (Haraguchi et al 1995). Forskolin, a diterpene from *Coleus forskolii*, is a potent cAMP stimulator which relaxes different vascular beds and reduces whole-blood platelet aggregation (Lambert et al 1994; Christenson et al 1995).

Correspondence: F. J. Miranda, Departamento de Fisiología, Facultad de Farmacia, Universidad de Valencia, Avda Vicent Andrés Estellés s/n, E-46100 Burjassot, Valencia, Spain.

Diterpenes derived from Euphorbiaceae have been reported to have vascular effects. Phorbol esters can induce contraction of rabbit aorta (Bazan et al 1993) and cat (Salaíces et al 1990) and canine (Sugawa et al 1991) cerebral arteries. Jatrophone, a diterpene isolated from some *Jatropha spp.* (Euphorbiaceae), can induce relaxation of rat aorta (Duarte et al 1992) or portal vein (Silva et al 1995).

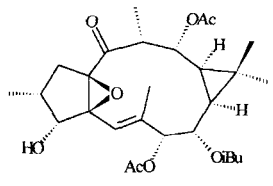
Our laboratory is interested in the vascular action of new diterpenes isolated from *Euphorbia canariensis*, the latex from which contains many compounds of great interest to chemists and pharmacologists. We have recently reported the vascular action of two of these compounds (Miranda et al 1997a, b) and shown they can induce contraction, and in some cases relaxation, of rabbit basilar and carotid arteries. The aim of the current investigation was to analyse the contractile or

relaxant response of isolated rabbit basilar and common carotid arteries to 2,3-di*epi*ingol 7,12-diacetate-8-isobutyrate (compound **1**), ingenol-3-angelate-17-benzoate (compound **2**), ingenol-3-angelate-17-benzoate-20-acetate (compound **3**) and 3,5,7,8,9,15-hexahydroxyjatropha-6(17),11-dien-14-one-5,8-bis(2-methylbutyrate)-7-(2-methylpropionate) (compound **4**) (Figure 1), four new diterpenes isolated from the latex of *Euphorbia canariensis*. The chemistry of compounds **1–3** has recently been described (Marco et al 1997).

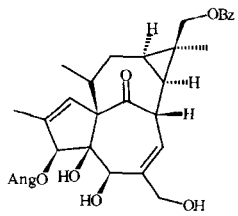
Materials and Methods

Drugs and solutions

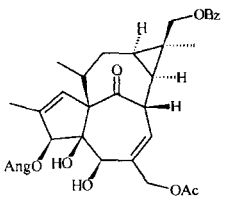
Diterpenes were isolated from the latex of *Euphorbia canariensis* L. (Marco et al 1997). Briefly, the latex (ca 90 g), collected in Malpais de Güimar, Tenerife, Canary Islands in May 1993 was dissolved in hot methanol (700 mL) and the solution was cooled to room temperature to furnish a voluminous, white precipitate of common triterpenes which were isolated by filtration. Evaporation of the solvent filtrate in-vacuo gave a whitish, oily material. This was dissolved in the minimum amount of methanol, reversed-phase silica gel RP-2 was added (3 g silica gel (g extract)$^{-1}$), and the solvent was then totally eliminated in-vacuo. The powdery material obtained was placed on the top of an open chromatographic column filled with silica gel RP-2 and eluted under a slight argon pressure (1·5–2 atm) first with water, then with 70 : 30 methanol–water and finally with methanol. Column chromatography of the methanol–water fraction (elution with a gradient from 10 : 1 hexane–Et_2O to 100% Et_2O), monitored when necessary by preparative thin-layer chromatography (TLC) or high-performance liquid chromatography of the intermediate fractions, enabled isolation of the compounds.



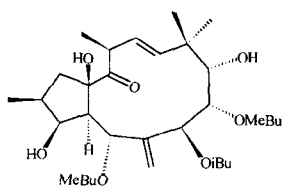
Compound **1**



Compound **2**



Compound **3**



Compound **4**

Figure 1. *The chemical structures of compounds* **1–4**.

Compounds **1–4** were dissolved in ethanol and diluted with twice-distilled water.

The composition of the modified Ringer-Locke solution was (mM): NaCl, 120; KCl, 5·0; $CaCl_2{\cdot}2H_2O$, 2·2; $MgCl_2{\cdot}7H_2O$, 1·0; $NaHCO_3$, 24; and glucose, 5·6. In KCl-depolarizing solution NaCl was replaced by an equimolar amount of KCl.

Recording of isometric tension

New Zealand white rabbits, 2·5–3·5 kg, anaesthetized by intravenous injection of 2% sodium thiopental (sodium pentothal, Abbott), were killed by intravenous injection of potassium chloride (10 mEq). The whole brain was rapidly removed and the basilar artery and one common carotid artery were dissected free and cut into cylindrical segments 3 and 4 mm long, respectively. Each segment was prepared for isometric tension recording in a 5-mL organ bath. The vascular preparations were maintained at 37 °C in modified Ringer-Locke solution oxygenated with 95% O_2 and 5% CO_2 at a pH of 7·3–7·4. A resting tension of 0·5 g (basilar artery) and 2 g (carotid artery) was applied to the arterial segments and they were left to equilibrate for 60–90 min. Tension was readjusted when necessary and the bath fluid was changed every 15 min. After this period of equilibration the reactivity of the arterial segments was checked by depolarization with KCl (50 mM); the arteries with a contraction of less than 500 mg (basilar) or 1500 mg (carotid) were discarded.

Concentration–response curves (10^{-8}–10^{-4} M) to compounds **1–4** were obtained cumulatively for basilar and common carotid arteries at both resting tension and active tone (KCl, 50 mM).

Statistical analysis

The contractile responses to the four compounds obtained in arteries at resting tension were expressed as a percentage of the previous contraction induced with 50 mM KCl. For pre-contracted arteries contractile and relaxant values were expressed as a percentage of the active tone. Only one concentration–response curve was obtained for each arterial segment. The maximum effect (E_{max}) and the concentration of the drug which produced half E_{max} (ED50) were calculated for each concentration–response curve. Mean ED50 and its confidence limits (95% interval) were calculated by obtaining the mean and the confidence limits of the pD_2 values ($-\log$ ED50), because they conformed to a normal distribution.

The mean, standard deviation and standard error of the mean (s.e.m.) were calculated from all the contraction values obtained in each experiment. The order of potency and the efficacy of the different diterpenes were determined by applying the Student-Newman-Keuls test to pD_2 and E_{max} values obtained from the concentration–response curves. Regional differences between the actions of the compounds were analysed by applying the Student *t*-test to the pD_2 and E_{max} values obtained from the appropriate concentration–response curves of the basilar and carotid arteries. A P value < 0.05 was considered to be indicative of significance.

Results

Cumulative addition of compounds **1–4** induced concentration-dependent contraction of basilar (Figure 2) and carotid (Figure 3) arteries at resting tension. In the basilar artery the order of potency was **3** = **1** > **2** = **4**, without significant differences between E_{max} values. In the carotid artery the order of potency was **3** > **2** = **1** = **4** and there were no significant differences between the E_{max} values of compounds **1–3**, all of which were higher than that of compound **4**. Comparison of the vascular beds showed that the maximum contractions induced by compounds **1–3** were significantly higher in the carotid artery than in the basilar artery. For compounds **1** and **3** the ED50 values for the carotid artery were also significantly higher than those for the basilar artery.

In pre-contracted basilar artery compounds **1–3** induced concentration-dependent relaxation and compound **4** was almost ineffective (Figure 4). The order of potency was **3** > **2** = **1**, without significant differences between E_{max} values.

In carotid artery with active tone (Figure 5), the four compounds tested induced further concentration-dependent contractions. The order of potency was **3** > **2** = **4** > **1** without significant differences between E_{max} values.

In all instances the arterial responses developed slowly and were sustained; the concentration–response curves took approximately 3–4 h to complete.

Table 1 summarizes the E_{max} and ED50 values of the concentration–response curves obtained for the

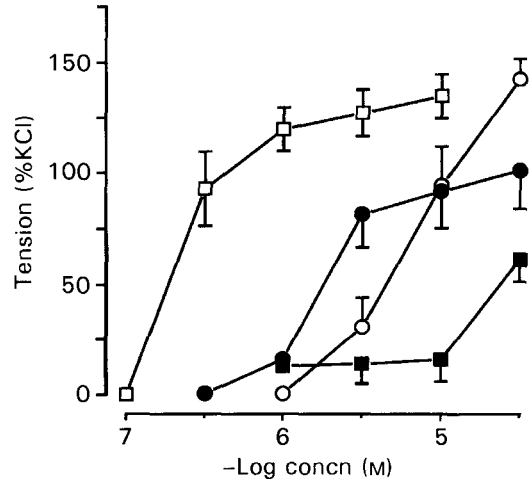


Figure 3. Concentration–response curves for the effects of compounds **1** (○), **2** (●), **3** (□) and **4** (■) on isolated rabbit common carotid artery. Tension values (mean ± s.e.m.) are expressed as percentages of the maximum effect achieved by previous depolarization with KCl (50 mM).

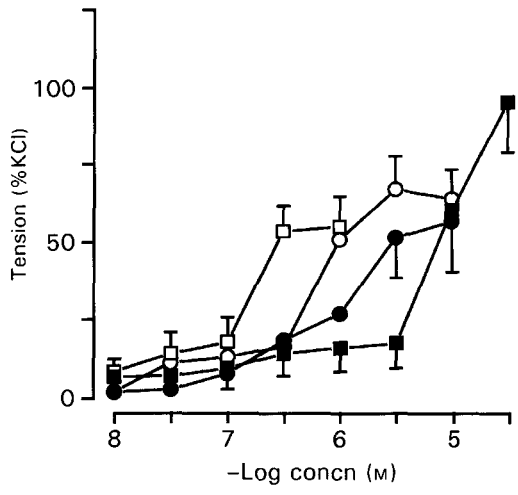


Figure 2. Concentration–response curves for the effects of compounds **1** (○), **2** (●), **3** (□) and **4** (■) on isolated rabbit basilar artery. Tension values (mean ± s.e.m.) are expressed as percentages of the maximum effect achieved by previous depolarization with KCl (50 mM).

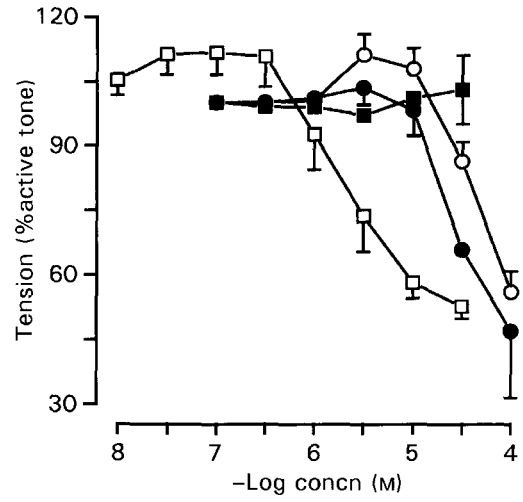


Figure 4. Concentration–response curves for the effects of compounds **1** (○), **2** (●), **3** (□) and **4** (■) on pre-contracted (KCl 50 mM) isolated rabbit basilar artery. Tension values (mean ± s.e.m.) are expressed as percentages of the active tone (= 100).

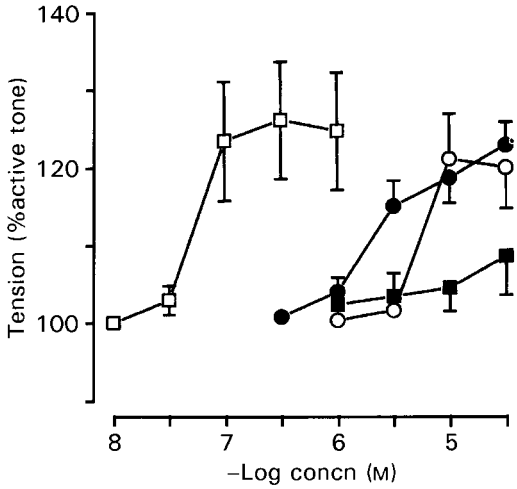


Figure 5. Concentration–response curves for the effects of compounds **1** (○), **2** (●), **3** (□) and **4** (■) on pre-contracted (KCl 50 mM) isolated rabbit common carotid artery. Tension values (mean ± s.e.m.) are expressed as percentages of the active tone (= 100).

four compounds under the different experimental conditions described above.

Discussion

The latex obtained from tens of species of several genera (e.g. Croton, Hippomane, Euphorbia, Hura, Jatropha, etc.) of the Euphorbiaceae family is rich in diterpenes related to tigliane (e.g. phorbol), daphnane (e.g. resiniferol), ingenane (e.g. ingenol) and jatrophane (e.g. jatrophone). These diterpenes not only have medicinal potential but are also proving to be useful pharmacological tools as activators of the phosphorylating enzyme PKC. Phorbol esters can induce slow-developing sustained contraction of cat (Salaíces et al 1990) and canine (Sugawa et al 1991) cerebral arteries by a mechanism that, at least partially, implies the activation of PKC. More recently we have demonstrated the contractile and relaxant activity of ingenol (Miranda et al 1997a) and ingol (Miranda et al 1997b) derivatives isolated from the latex of *E. canariensis*. The current results show that the ingol derivative compound **1**, the ingenol derivatives compounds **2** and **3** and the jatrophane derivative compound **4** strongly contract rabbit basilar and carotid arteries. The contractile efficacy was similar for the basilar artery, but compounds **3** and **2** had the highest potency. For the carotid artery, the efficacy of compound **4** was the lowest; those of the other three compounds were similar, compound **3** being the most potent; the potencies of compounds **1**, **2** and **4** were not significantly different. In both arteries, the contraction was slowly-developed and sustained, similar to the contractions induced by phorbol esters (Salaíces et al 1990; Sugawa et al 1991) and other *E. canariensis* derivatives (Miranda et al 1997a, b).

The possibility of arterial segments showing a relaxant response to these diterpenes, as has been reported for jatrophone in rat aorta (Duarte et al 1992) or portal vein (Silva et al 1995), was also investigated. Concentration–response curves to these compounds were obtained for basilar and carotid arteries pre-contracted with KCl. The response of the two arteries tested was completely different. Whereas compounds **1–3** induced concentration-related relaxation of the basilar artery, further contractions were induced in the carotid artery. Compound **3** was the most potent of the four

Table 1. Maximum effect (%) and concentration (M) of compounds **1–4** producing half the maximum effect (ED50) for isolated rabbit basilar and common carotid arteries.

	Basilar artery ED50	Maximum effect	n	Carotid artery ED50	Maximum effect	n
Resting tension						
Compound **1**	$2{\cdot}7\ (1{\cdot}6–4{\cdot}6) \times 10^{-7}$	66 ± 11	11	$6{\cdot}0\ (4{\cdot}7–7{\cdot}8) \times 10^{-6}$*	160 ± 11*	12
Compound **2**	$1{\cdot}3\ (0{\cdot}9–2{\cdot}0) \times 10^{-6}$	57 ± 13	9	$4{\cdot}8\ (3{\cdot}1–7{\cdot}4) \times 10^{-6}$	144 ± 8*	14
Compound **3**	$7{\cdot}1\ (4{\cdot}5–11{\cdot}2) \times 10^{-8}$	54 ± 10	8	$2{\cdot}4\ (2{\cdot}1–2{\cdot}8) \times 10^{-7}$*	127 ± 10*	10
Compound **4**	$4{\cdot}7\ (2{\cdot}9–9{\cdot}3) \times 10^{-6}$	93 ± 16	10	$9{\cdot}3\ (6{\cdot}2–14{\cdot}1) \times 10^{-6}$	61 ± 10	10
Active tone						
Compound **1**	$2{\cdot}9\ (2{\cdot}6–3{\cdot}3) \times 10^{-5}$	−44 ± 5	12	$2{\cdot}6\ (2{\cdot}3–2{\cdot}9) \times 10^{-5}$	21 ± 6*	11
Compound **2**	$2{\cdot}3\ (2{\cdot}1–2{\cdot}6) \times 10^{-5}$	−53 ± 4	13	$5{\cdot}1\ (3{\cdot}5–7{\cdot}4) \times 10^{-6}$*	30 ± 7*	15
Compound **3**	$1{\cdot}5\ (1{\cdot}1–1{\cdot}8) \times 10^{-6}$	−48 ± 3	10	$7{\cdot}1\ (5{\cdot}7–8{\cdot}7) \times 10^{-8}$*	26 ± 7*	10
Compound **4**	–	2·6 ± 8	10	$6{\cdot}6\ (5{\cdot}0–8{\cdot}7) \times 10^{-6}$	8 ± 5	10

Results are expressed as percentages of previous depolarization with 50 mM KCl (resting tension) or as percentages of 50 mM KCl-induced active tone. ED50 values are means and confidence limits. Maximum effect values are means ± s.e.m. *$P < 0{\cdot}01$, significantly different from corresponding values for basilar artery.

compounds tested. The opposite response to these compounds by pre-contracted basilar arteries (relaxations) and carotid arteries (contractions) is in agreement with data obtained by use of the same arterial beds with some related compounds (Miranda et al 1997a, b). Taking into account that cerebral vasospasm and consequent neuronal ischaemia are reported to be important factors in the pathogenesis of several cerebrovascular disorders such as subarachnoid haemorrhage (Findlay et al 1991) and eclampsia (Lewis et al 1988), vasodilator drugs are of the maximum usefulness in the treatment of ischaemic disorders and, therefore, these regional differences in the effect of vasoactive substances could be highly important.

In summary, compounds **1–4** have potent activity on rabbit basilar and common carotid arteries and there are important regional differences in their action. The four compounds contract basilar and carotid arteries at resting tension. Compounds **1–3** relax pre-contracted basilar but not carotid artery.

Acknowledgements

Marta Ortí and Jose M. Centeno hold a research fellowship from Conselleria de Educación y Ciencia de la Generalitat Valenciana and Ministerio de Educación y Ciencia (Spain), respectively.

References

Bazan, E., Campbell, A. K., Rapoport, R. M. (1993) Effects of phorbol esters on contraction and protein kinase C activation in rabbit aorta. Life Sci. 54: 29–33

Christenson, J. T., Thulesius, O., Nazzal, M. M. (1995) The effect of forskolin on blood flow, platelet metabolism, aggregation and ATP release. Vasa 24: 56–61

Duarte, D. F. P., Sant'Ana, A. E. G., Calixto, J. B. (1992) Analysis of the vasorelaxant action of jatrophone in the isolated aorta of the rat: influence of potassium-channel blockers. Eur. J. Pharmacol. 215: 75–81Findlay, J. M., Macdonald, R. L., Weir, B. K. A. (1991) Current concepts of pathophysiology and management of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Cerebrovasc. Brain Metab. Rev. 3: 336–361

Gu, W. Z., Chen, R., Brandwein, S., McAlpine, J., Burres, N. (1995) Isolation, purification, and characterization of immunosuppressive compounds from *Tripterygium*: triptolide and tripdiolide. Int. J. Immunopharmacol. 17: 351–356

Habtemariam, S. (1995) Cytotoxicity of diterpenes from *Premna schimperi* and *Premna oligotricha*. Planta Med. 61: 368–369

Haraguchi, H., Saito, T., Okamura, N., Yagi, A. (1995) Inhibition of lipid peroxidation and superoxide generation by diterpenoids from *Rosmarinus officinalis*. Planta Med. 61: 333–336

Lambert, T. L., Dev, V., Rechavia, E., Forrester, J. S., Litvack, F., Eigler, N. L. (1994) Localized arterial wall drug delivery from a polymer-coated removable metallic stent. Kinetics, distribution and bioactivity of forskolin. Circulation 90: 1003–1011

Lewis, L. K., Hinshaw Jr, D. B., Will, A. D., Nasso, A. N. (1988) CT and angiographic correlation of severe neurological disease in toxemia of pregnancy. Neuroradiology 30: 59–64

Marco, J. A., Sanz-Cervera, J. F., Yuste, A. (1997) Ingenane and lathyrane diterpenes from the latex of *Euphorbia canariensis*. Phytochemistry 45: 563–570

Miranda, F. J., Alabadí, J. A., Piñón, M., Ortí, M., Centeno, J. M., Yuste, A., Sanz-Cervera, J. F., Marco, J. A., Alborch, E. (1997a) Analysis of the vascular effects of an ingenol derivative isolated from *Euphorbia canariensis*. Pharm. Sci. 3: 113–116

Miranda, F. J., Alabadí, J. A., Pérez, P., Ortí, M., Centeno, J. M., Yuste, A., Sanz-Cervera, J. F., Marco, J. A., Alborch, E. (1997b) Analysis of the vascular responses to DBI, an ingol derivative isolated from *Euphorbia canariensis*. J. Pharm. Pharmacol. 49: 573–576

Salaíces, M., Balfagón, G., Arribas, S., De Sagarra, M. R., Marín, J. (1990) Effects of phorbol 12,13-dibutyrate on the vascular tone and on norepinephrine- and potassium-induced contractions of cat cerebral arteries. J. Pharmacol. Exp. Ther. 255: 66–73

Shirakabe, H., Takemoto, T., Kobayashi, K., Ogoshi, K., Kimura, K., Nakamura, K., Watanabe, H. (1995) Clinical evaluation of trepenone, a mucosal protective agent, in the treatment of patients with gastric ulcers: a nationwide, multicenter clinical study. Clin. Ther. 17: 924–935

Silva, A. M., Brum, R. L., Calixto, J. B. (1995) The relaxant action of jatrophone in rat portal vein. A comparison with protein kinase C inhibitors. Life Sci. 57: 863–871

Sugawa, M., Koide, T., Naitoh, S., Takato, M., Matsui, T., Asano, T. (1991) Phorbol 12,13-diacetate-induced contraction of the canine basilar artery: role of protein kinase C. J. Cereb. Blood Flow Metab. 11: 135–142