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Triterpenic Compounds from "Orujo" Olive Oil Elicit Vasorelaxation in Aorta from Spontaneously Hypertensive Rats

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There is currently a considerable amount of interest in the benefits of certain dietary elements, and in particular of olive oil, in endothelial function and thus in hypertension. "Orujo" or pomace olive oil is obtained from the residues of the olive by a novel centrifugation process, and it is a good dietary source of triterpenic compounds such as oleanolic and maslinic acid, erythrodiol, and uvaol. Until now, there was no information available regarding the properties of these triterpenoids on the vasculature of hypertensive animals. However, in this in vitro study, we have analyzed the vasorelaxation induced by these triterpenoids in isolated aorta from spontaneously hypertensive rats (SHR). The triterpenoids tested induced concentration-dependent vasorelaxation, mostly involving nitric oxide (NO). Indeed, the responses were attenuated by removal of the endothelium or following pretreatment with the NO synthase inhibitor L-NAME. Furthermore, the differences that were observed in the potency of relaxation, the selectivity, and the dependence on the endothelium were attributed to structural features of the triterpenoids. In conclusion, triterpenic components in pomace olive oil induce vasorelaxation of the aorta from SHR, and this effect generally involves endothelial NO.

KEYWORDS: Spontaneously hypertensive rat; aorta; "orujo" or pomace olive oil; triterpenoids

INTRODUCTION

"Orujo" olive oil, as it is called in Spain, is obtained from the pomace that remains after the mechanical extraction of virgin olive oil. The oil that can be extracted from this "orujo" using a novel centrifugation process (*1*) is a rich source of some biologically active components from the skin and leaves of the olive. However, these compounds are only found in low concentrations (trace concentrations) in virgin olive oil (*2*). Among these compounds, we can find the potentially beneficial triterpenoids, including oleanolic and maslinic acids, as well as the alcohols erythrodiol and uvaol (**Figure 1**).

The triterpenoid oleanolic and maslinic acids are found widely distributed throughout the vegetable kingdom. Oleanolic acid has been isolated from more than 120 vegetable species either in its free form or as a glycone of triterpenoid saponins, and, as such, it has been extensively studied (*3*, *4*). Many pharmacological properties of this triterpenoid have been reported, and it has been attributed with antiinflammatory (*5*−*7*), hepatoprotective (*8*, *9*), and antitumoral activities (*10*, *11*). Oleanolic acid has also been shown to display a protective effect against low-density lipoprotein (LDL) oxidation (*12*). Indeed, ingestion of pomace olive oil rich in oleanolic acid and erythrodiol has recently been reported to decrease microsomal lipid peroxidation in rats (*13*). Despite the numerous pharmacological activities identified for oleanolic acid, there is little data available regarding its effect, as well as that of the rest of the triterpenoids, on parameters related to cardiovascular disease. Nevertheless, recent "in vivo" studies have demonstrated the benefits of

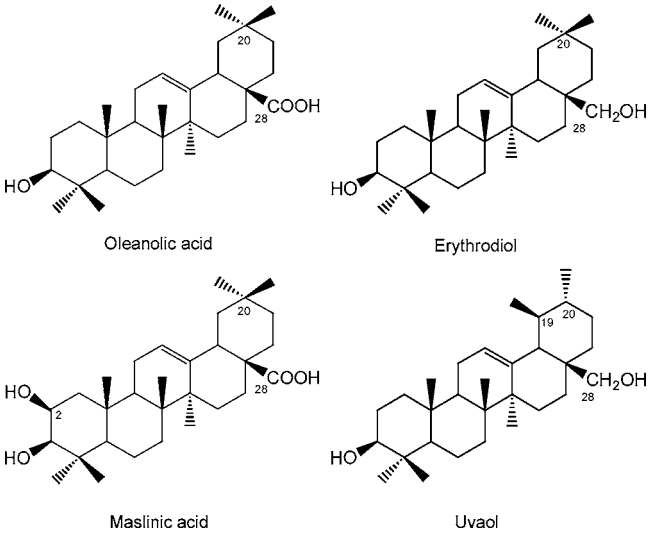

Figure 1. Chemical structures of triterpenic compounds contained in "orujo" olive oil.

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oleanolic acid in preventing hypertension (*14*). This activity could be attributed to a potent diuretic−natriuretic activity, and an antioxidant and antihyperlipidemic effect. Accordingly, oleanolic acid has also been shown to possess vasodepressor, cardiotonic, and antidysrhythmic properties (*15*). Likewise, uvaol and the maslinic acid derivative methyl maslinate also display antidysrhythmic properties. Hence, it has been proposed that these triterpenoids might represent a useful additional plant-derived therapy for hypertension.

It is well known that many cardiovascular diseases such as hypertension, heart failure, and diabetes are associated with impaired endothelial-dependent relaxation (*16*). This endothelial dysfunction is defined by imbalances in the endothelial production of vasodilating and vasoconstricting mediators, as manifested in the aorta of hypertensive rats (*17*, *18*). Therapeutic strategies to combat the injury to the vascular endothelium have been described, generally aimed at readjusting the molecular and biochemical mechanisms underlying this dysfunction. One approach to treat the affected endothelium involves improving endothelium-dependent vasodilatation, which is mediated by augmenting the influence endothelial protective factors (e.g., prostacyclin, NO). Alternatively, this can be attempted by inhibiting the synthesis/release of pathogenic factors (e.g., Ang II, endothelin, prothrombotic factors) (*19*).

Nutritional supplements to treat hypertension are becoming ever more relevant. Indeed, the consumption of virgin olive oil has recently been shown to reduce blood pressure in healthy and hypertensive patients (*20*, *21*), as well as reduce cholesterol ester content. Furthermore, dietary virgin olive oil helps to normalize the lipid composition of the aorta from SHR (*22*, *23*). Despite the considerable amount of research dealing with the effects of olive oil and its components in cardiovascular diseases, little attention has been paid to the effects on hypertension of pomace olive oil and its pentacyclic triterpenoids. Indeed, the molecular mechanisms underlying the effects of pentacyclic triterpenoids on hypertension remain unknown. We previously reported that oleanolic acid and erythrodiol display vasodilator effects on the isolated aorta from normotensive rats (*24*). Accordingly, the purpose of this study was to determine the activity of the triterpenoids found in pomace olive oil, oleanolic acid, maslinic acid, erythrodiol, and uvaol, on vascular reactivity in the thoracic aorta from spontaneously hypertensive rats (SHR). In addition, we set out to elucidate the endothelium-derived factors involved in the relaxation produced by these triterpenoids. The pharmacological activity of these triterpenic compounds, as well as their beneficial effects in an animal model of hypertension, could improve the commercial value of pomace olive oil as a functional food product.

MATERIALS AND METHODS

Animals. Male spontaneously hypertensive rats (SHR), 12-week-old, were purchased from Charles River Laboratories Spain SA (Barcelona, Spain). Rats were given free access to a diet of standard chow and water. Systolic blood pressure and heart rate were indirectly measured in the conscious rats by tail-cuff method with Digital Pressure Meter Letica 5,000 (Barcelona, Spain). SHR with systolic pressure higher than 170 mmHg were used in the experiments. The protocol for animal handling and experimentation was approved by the Institutional Committee on Investigation in Animals (Universidad de Sevilla, Seville, Spain).

Isolated Vascular Preparations. The rats were killed by cervical dislocation, and the thoracic aorta was rapidly dissected. The artery was immediately placed in cold modified Krebs−Henseleit bicarbonate solution (composition in mM: NaCl 118, KCl 4.75, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.2, and glucose 11) and carefully cleaned by removing fat and adhering connective tissue. Next, artery segments (2−3 mm length) were suspended in organ baths containing 10 mL of Krebs−Henseleit solution at 37 °C and continuously bubbled with a 95% O_2−5% CO_2 gas mixture. Two tungsten wires were inserted into the lumen, and the preparations were mounted with one wire in the organ bath and the other attached to a force-displacement transducer (Pioden UF-1) coupled to a Powerlab data acquisition system (AD-Instruments, Australia), so that the mechanical activity could be measured isometrically, as previously described (*25*). Each ring was stretched to a resting tension of 2 g and allowed to equilibrate for 60 min. During this period, tissues were restretched and washed every 30 min with warm Krebs solution.

After equilibration, aortic rings were challenged with 10^{-6} M phenylephrine to test their maximal contractile capacity, and the presence of endothelium was assessed by the ability of 10^{-6} M acetylcholine to induce more than 50% relaxation. In some experiments, the endothelium was mechanically removed by gently rubbing the intimal surface.

Experimental Protocols. Arteries with and without functional endothelium were precontracted at 80% of their maximal contraction with phenylephrine (10^{-6} M). In some experiments, single concentrations of KCl (25 or 80 mM) were used to precontract the aortic rings. Afterward, to test their vasorelaxant effects, cumulative concentration−response curves for oleanolic acid, erythrodiol, maslinic acid, and uvaol were performed (10^{-7}−10^{-4} M) in the aortic segments. To analyze the influence of endothelium-derived factors in the vasorelaxations, concentration−response curves were carried out in the presence of N^{ω}-nitro-L-arginine (L-NAME; 3×10^{-4} M, an NO synthase inhibitor), indomethacin (INDO; 10^{-5} M, to inhibit factors derived from cyclooxygenase), ICI 192,605 (10^{-5} M, a thromboxane A_2 receptor antagonist), or the combination of the superoxide anion (O_2^-) scavenger, superoxide dismutase (SOD; 150 U/mL) plus the H_2O_2 inactivator catalase (Cat; 1000 U/mL). All inhibitors were incubated with the intact aortic segments for 20 min before the precontraction with phenylephrine (10^{-6} M), except for ICI 192,605 and SOD plus Cat, added 30 and 15 min, respectively, prior to the contraction. Sarco/endoplasmic reticulum Ca^{2+}-ATPase involvement was investigated by using cyclopiazonic acid (CPA; 3×10^{-5} M), added to the bath at the same time as phenylephrine.

Chemicals. Oleanolic acid, erythrodiol, and uvaol were provided by Extrasynthese (Genay, France). Maslinic acid was extracted from olive leaves, as previously described (*26*). ICI 192,605 and cyclopiazonic acid was purchased from Tocris (Biogen Cientifica SL, Barcelona, Spain), and all others chemicals were obtained from Sigma Chemical Co (St Louis, MO). All reactants were dissolved in distilled water except oleanolic acid, erythrodiol, maslinic acid, uvaol, indomethacin, and ICI 192,605, which were dissolved in dimethylsulfoxide (DMSO). The final DMSO concentration (<0.1%) in the tissue bath did not significantly affect the vascular responses.

Statistical Analysis. Relaxation induced by the testing triterpenoids was expressed as a percentage of the maximal contraction previously obtained with the agonist, phenylephrine or KCl. The maximum relaxation (E_{max} values) and the concentration of the triterpenoids producing 50% of maximum vasoconstrictor response (IC_{50} values) were calculated by nonlinear regression analysis of each individual concentration−response curve using GraphPad Prism Software (San Diego, CA). Also, the area under curve (AUC) was calculated for some experiments to obtain a measure of the cumulative drug effect. Results were expressed as mean ± SEM of the number of rats indicated in each case. Data were analyzed using one-way analysis of variance (ANOVA), followed by Fisher's multiple comparison test, as appropriate. Differences were considered significant when $P < 0.05$.

RESULTS

Relaxant Effects of Triterpenoid Compounds on Phenylephrine-Evoked Contractions. The tested triterpenoids induced significant concentration-dependent relaxation of intact vessels precontracted with phenylephrine (**Figure 2**). The vasodilator effects of the testing compounds were also analyzed in endot-

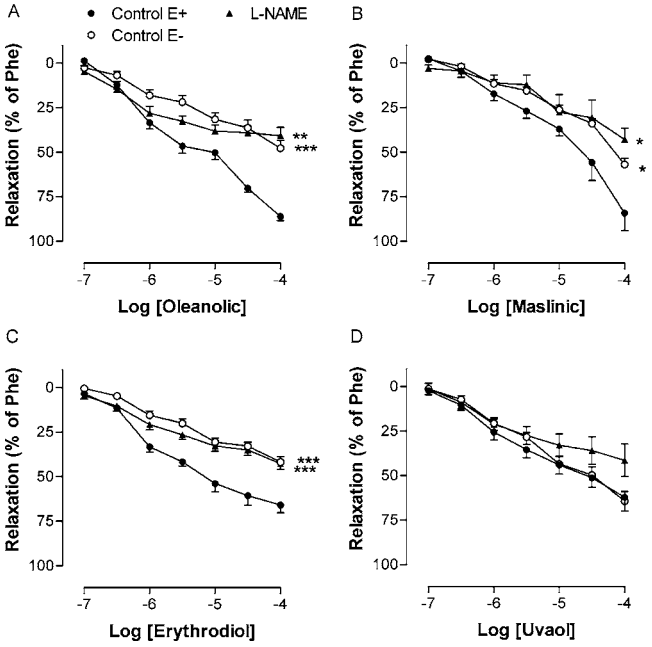

Figure 2. Effect of oleanolic acid (**A**), maslinic acid (**B**), erythrodiol (**C**), and uvaol (**D**) in phenylephrine precontracted rat thoracic aortic rings with (E+) or without functional endothelium (E−), or with functional endothelium in the presence of N$^{\omega}$-nitro-L-arginine (L-NAME; 3×10^{-4} M). Data are expressed as mean ± SEM of 6–7 experiments. Concentration–response curves constructed in the absence of inhibitors were considered as control curves. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus control curves (E+).

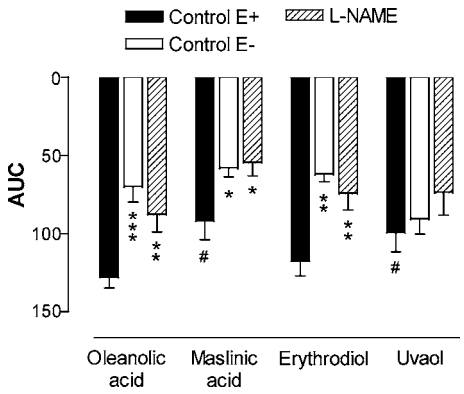

Figure 3. The effect of endothelium removal or the presence of the inhibitor, N$^{\omega}$-nitro-L-arginine (L-NAME; 3×10^{-4} M) on concentration–response curves for triterpenic compounds in isolated aorta precontracted by phenylephrine given as area under curve (AUC). Data are expressed as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus control curves. #$P < 0.01$ versus oleanolic acid response in E+ segments.

helium-denuded aorta from SHR rats (control E−). The concentration−response relaxations induced by the two triterpenoid acids and erythrodiol were significantly attenuated by endothelial removal (**Figure 2**). The area under the concentration−response curves was significantly decreased in E− preparations (**Figure 3**). However, the vasodilator effect of uvaol was similar in intact and endothelium-denuded aortic rings (**Figure 2D**).

In aortic rings with functional endothelium, the order of maximal relaxation was oleanolic acid ≅ maslinic acid > erythrodiol ≅ uvaol (**Table 1**). Oleanolic and maslinic acids, despite inducing similar maximal relaxation in intact arteries, showed significantly different IC_{50} values (2.88 ± 0.60 and 14.10 ± 0.55 μM, respectively; $P < 0.01$), indicating the highest relaxation potency for oleanolic acid. On the other hand, the

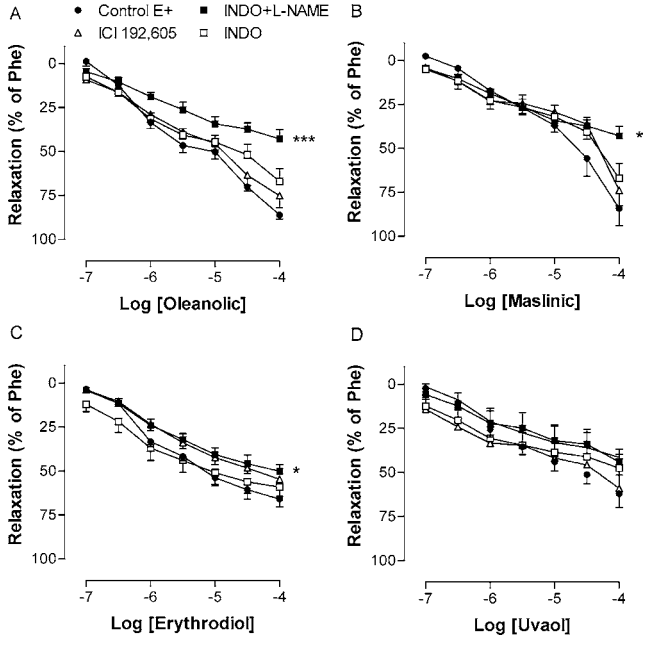

Figure 4. Effect of triterpenic compounds in phenylephrine precontracted rat thoracic aortic rings with functional endothelium in the absence (control curves) or in the presence of indomethacin (INDO; 10^{-5} M), INDO plus N$^{\omega}$-nitro-L-arginine (L-NAME; 3×10^{-4} M), or ICI 192,605 (10^{-5} M). Data are expressed as mean ± SEM of 5–8 experiments. *$P < 0.05$; ***$P < 0.001$ versus control curves.

alcohols erythrodiol and uvaol displayed less pronounced vasodilatation and higher IC_{50} values (9.33 ± 0.91 and 15.84 ± 0.77 μM, respectively), as compared to oleanolic acid ($P < 0.01$). These results were additionally estimated by AUC (**Figure 3**).

The inhibitory effect of the triterpenoids on the phenylephrine-induced contractile response in endothelium-intact aortic rings was investigated with regards to L-NAME or indomethacin and ICI 192,605 exposure. The basal vascular tone was not significantly affected by any of these blockers, while phenylephrine-induced contraction was only increased by L-NAME, which reflects the inhibitory effect of the eNOS blockers on basal NO release. Preincubation with the NO-synthase inhibitor, L-NAME, attenuated the concentration−response curves for oleanolic and maslinic acids and erythrodiol in intact endothelium preparations (E+) (**Figure 2**), and significantly lower AUCs were shown as compared to control (**Figure 3**). In contrast, the relaxant response of uvaol was not modified by pretreatment with L-NAME (**Figure 2D**).

The presence of the prostaglandin synthesis inhibitor, indomethacin, did not significantly affect either triterpenic acid- or alcohol-induced relaxation. Further ICI 192,605, which has been reported to block the thromboxane A_2 receptor, failed to modify the endothelium-dependent relaxation induced by the tested triterpenoids (**Figure 4**).

To facilitate the investigation of the endothelial relaxing factors involvement, it was necessary to eliminate the activity of the main vasoactive factors in the aorta, NO, and prostanoids derived from cyclooxygenase. Inhibition of these mediators was achieved using a classical combination of L-NAME and indomethacin. The presence of both inhibitors attenuated the oleanolic acid-induced relaxation (**Figure 4A**), and this attenuation of the relaxant response was almost similar to that observed in the presence of the NO synthase inhibitor alone (**Figure 2A**). Combination of L-NAME with indomethacin was also able to decrease the relaxation induced by maslinic acid and eryhtrodiol

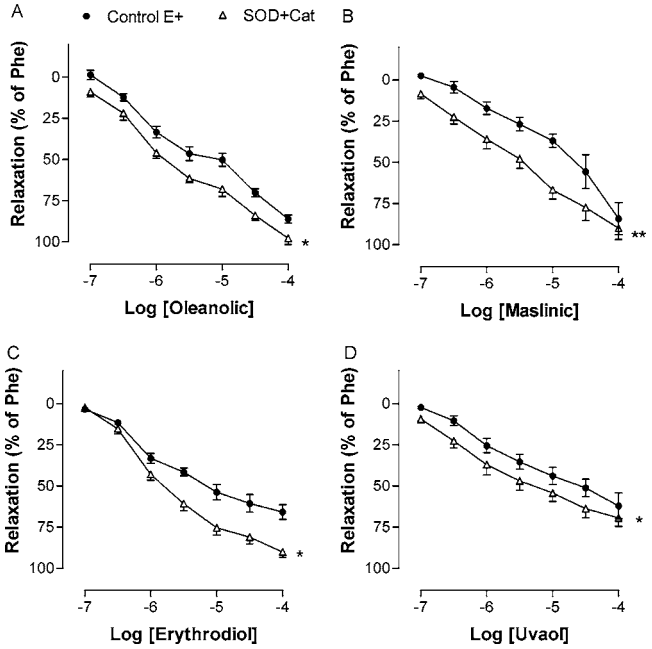

Figure 5. Effect of triterpenic compounds in phenylephrine precontracted rat thoracic aortic rings with functional endothelium in the absence (control curves) or in the presence of superoxide dismutase (SOD; 150 U/mL) plus Catalase (Cat; 1000 U/mL). Data are expressed as mean ± SEM of 5–8 experiments. *$P < 0.05$; **$P < 0.01$ versus control curves.

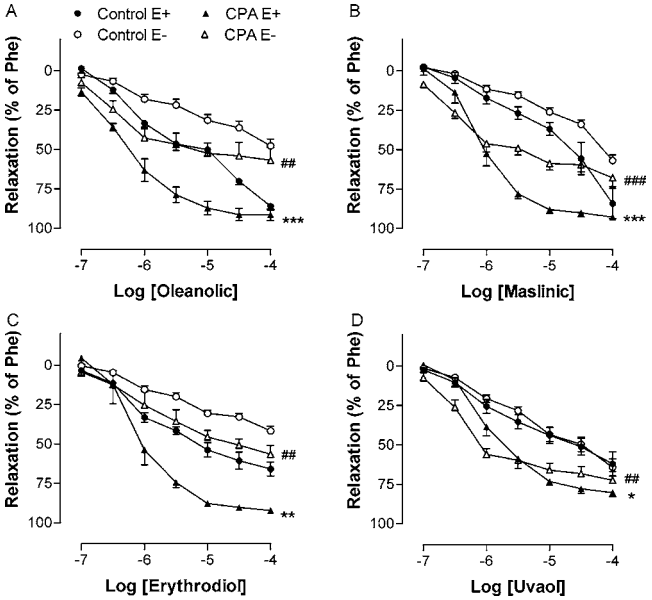

Figure 6. Effect of triterpenic compounds in phenylephrine precontracted rat thoracic aortic rings with or without functional endothelium in the absence (control curves) or in the presence of cyclopiazonic acid (CPA; 3×10^{-5} M). Data are expressed as mean ± SEM of 5–6 experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus control curves with functional endothelium. ##$P < 0.01$; ###$P < 0.001$ versus control without endothelium.

(**Figure 4B** and **C**, respectively). The concentration−response curve for uvaol was unaffected by that combination (**Figure 4D**).

To test whether an increase of oxidative stress status that enhances NO breakdown might be induced by these compounds in the aorta from hypertensive rats, the effect of SOD and catalase was studied. Under these conditions, these reactive oxygen species scavengers potentiated the endothelium-dependent relaxation to the four triterpenoids (**Figure 5**).

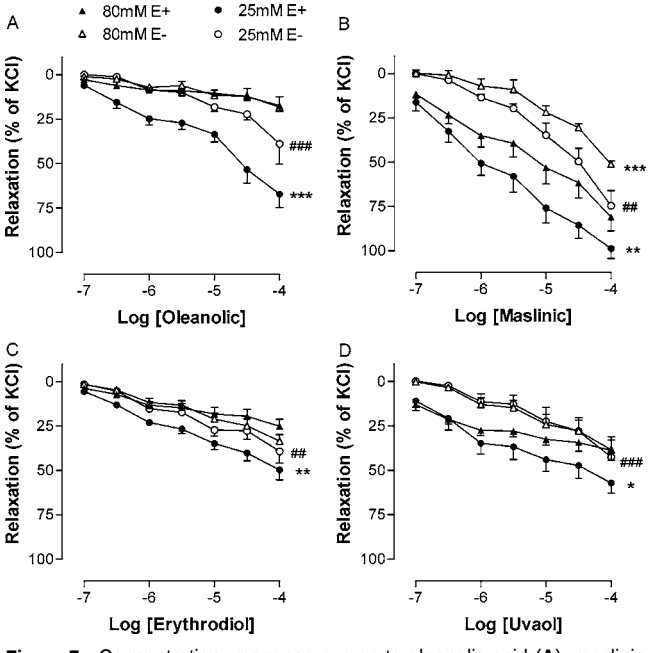

Figure 7. Concentration–response curves to oleanolic acid (**A**), maslinic acid (**B**), erythrodiol (**C**), and uvaol (**D**) in rat thoracic aortic rings precontracted by KCl (25 or 80 mM) with (E+) or without functional endothelium (E–). Data are expressed as mean ± SEM of 5–7 experiments. Mean values for 25 mM KCl contracted segments with functional endothelium were significantly different from 80 mM KCl with functional endothelium: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Mean values for 25 mM KCl contracted segments with functional endothelium were significantly different from 25 mM KCl contracted denuded arteries: ##$P < 0.01$; ###$P < 0.001$.

Table 1. Vasorelaxant Effect of the Triterpenoids (0.1 mM) against Phe (1 μM)- or KCl (25 or 80 mM)-Induced Contractions in Hypertensive Rat Aortic Rings[a]

	maximal relaxation (%)			ratio	
	Phe	KCl 25 mM	KCl 80 mM	Phe/K25	Phe/K80
oleanolic acid	86.12	67.22*	17.00***	1.28	4.95
maslinic acid	84.24	98.76	81.05	0.85	1.04
erythrodiol	65.94#	49.75	25.13***	1.32	2.62
uvaol	62.18#	57.19	38.79*	1.09	1.60

[a] The rings were either preconstricted with Phe or KCl. Cumulative concentrations of the triterpenoids were subsequently added to the bath. Data are expressed as mean ± SEM of 5–7 experiments. *$P < 0.05$; ***$P < 0.001$ versus vasorelaxation against Phe-evoked contractions. #$P < 0.01$ versus oleanolic acid response in E+ segments.

Endothelium-dependent relaxation induced by the triterpenic compounds was significantly increased in the presence of the sarco/endoplasmic Ca^{2+}-ATPase blocker CPA. This enhanced relaxation was also observed in endothelium-denuded aortic rings exposed to CPA (**Figure 6**). Concentration response curves for uvaol in the presence of the sarco/endoplasmic calcium pump inhibitor were independent of endothelium denudation, as expected.

Relaxant Effects of Triterpenoid Compounds on Depolarization-Evoked Contractions. Oleanolic acid had no relaxant effects on endothelium intact aortic rings contracted by 80 mM KCl, and the absence of relaxation was also observed in endothelial denuded vessels (**Figure 7A**). However, oleanolic acid was able to induce an endothelium-dependent relaxation in 25 mM KCl-contracted aortas, and this relaxant response was lower as compared to that observed on phenylephrine-contrac-

tions ($P < 0.05$) (IC_{50} values: 11.48 ± 0.64 μM for 25 mM KCl and 2.88 ± 0.60 μM for phenylephrine) (**Table 1**). Maslinic acid displayed vasorelaxant responses in high and low K^+ concentration contractions, and these effects were attenuated by endothelium removal (**Figure 7B**). Although there were no differences between concentration−response curves evoked by maslinic acid in 25 mM K^+ medium or in phenylephrine-contracted intact arteries, the potency of relaxation was higher in the former (IC_{50} values: 2.40 ± 0.53 μM for 25 mM KCl and 14.10 ± 0.55 μM for phenylephrine) (**Table 1**). Eryhtrodiol and uvaol displayed a slight relaxation on high K^+ (80 mM)-induced depolarization; nevertheless, the relaxant responses were more pronounced on 25 mM evoked contractions, as compared to 80 mM contractions (**Figure 7C** and **D**). The described vasodilatation induced by uvaol was significantly decreased in endothelium denuded aortic rings (**Figure 7D**).

DISCUSSION

There is now significant evidence that virgin olive oil and some of its components have beneficial effects in cardiovascular diseases (*27*−*29*). The "orujo" or olive pomace oil obtained from olive residues has traditionally been used by the Spanish population, although the possible beneficial health effects of this oil and its components have not been examined. Our study has focused on the triterpenic fraction from pomace olive oil, the concentrations of which are higher than in other olive oils. The main finding of this study was that oleanolic and maslinic acids, as well as the alcohols erythrodiol and uvaol, all triterpenic components of pomace olive oil, can dilate aortic rings from hypertensive animals. These four triterpenoids induced concentration-dependent relaxation in the aorta isolated from spontaneously hypertensive rats (SHR), albeit to a different extent. Furthermore, most of them exhibited activity mediated by the endothelium. These findings are in agreement with our previous data on the aorta of normotensive rats where, again, relaxation was found to be mainly dependent on the endothelium (*24*). The lack of information regarding the molecular mechanisms underlying the vascular and cardiovascular effects of these triterpenoids reinforces the interest of this research.

Hypertension is associated with marked changes in the cardiovascular system, especially in the structure and function of the arteries. The mechanisms that give rise to functional changes may involve alterations in the responsiveness of vascular smooth muscle and/or disturbances in the activity of the endothelium (*30*). With respect to hypertension, the alterations in vascular tone mediated by the endothelium are characterized by an imbalance in the activity of endothelium-dependent relaxing and contracting factors. Accordingly, the endothelium-dependent vasodilatation in response to acetylcholine is impaired in different models of hypertension such as SHR when compared to normotensive WKY rats (*31*, *32*). Indeed, we found that the endothelial dependence and the vasodilatation induced by these triterpenoids in the aorta from normotensive rats (*24*) were maintained in the aorta from SHR. Indeed, no significant differences in the rate of relaxation between normotensive and hypertensive rats were observed.

The studies carried out here establish that oleanolic and maslinic acid, as well as the alcohol erythrodiol, induce endothelium-dependent relaxation. Indeed, increasing the bioavailability of NO from the vascular endothelium seemed to be responsible for this relaxation because vasodilatation was significantly attenuated in the presence of L-NAME. This NO synthase inhibitor diminished relaxation, which reached the same level as that observed in aortic rings lacking endothelium. In contrast, the vasodilatation caused by uvaol was characterized as endothelium-independent but not dependent on the production/release of NO. This was witnessed by the fact that it was not affected by pretreatment with L-NAME and physical removal of the endothelium.

Most of the triterpenoids analyzed in the present study exhibited stronger relaxant effects against contractions induced by phenylephrine than against those induced by high K^+ (Phe/K80 ratio > 1.0). Oleanolic acid showed the highest selectivity against phenylephrine-induced contractions, followed by erythrodiol. Although oleanolic acid and maslinic acid share a similar structure, oleanolic acid was more effective in inhibiting the contractions induced by phenylephrine, whereas maslinic acid was equally effective against contractions induced by KCl (80 or 25 mM) and phenylephrine. These two triterpenoids differ in the addition of a −OH group at C-2, and it is likely that the presence of this substitution in maslinic acid decreases the selectivity of these pentacyclic triterpenoids against phenylephrine-induced contractions. Therefore, while these compounds are structurally similar, we could observe differences in the detailed mechanism through which they induced relaxation. In this regard, the importance of the functional group at C-28 should be taken into consideration, because oleanolic and maslinic acid (−COOH at C-28) are more potent relaxants than the triterpenoid alcohols erythrodiol and uvaol ($-CH_2OH$ at C-28). In addition, the dependence on the presence of the endothelium in vasorelaxation could be determined by the presence of two methyl groups at C-20. Indeed, in contrast to the rest of triterpenoids studied that displayed endothelium-dependent vasorelaxation, uvaol is monomethylated at C-20, and it induced relaxation independent of the endothelium.

It is noteworthy that the stimulation of prostanoid synthesis from cyclooxygenase does not appear to be involved in the vasorelaxation observed here. Indeed, as was previously observed in aortas from wistar rats, the inhibitor of these metabolites, indomethacin, did not reverse the triterpenoid-induced relaxation. However, in the aorta isolated from normotensive rats, it is believed that vasoconstrictors derived from cyclooxygenase are involved in the vasoactive effect of erythrodiol. Nevertheless, the relaxant response to the four triterpenoids examined here, including that to erythrodiol, was unaltered by exposure of the hypertensive vessels to the antagonist of the thromboxane A_2 receptor, ICI 192,605. Thus, in this way we ruled out the involvement of vasoconstrictors derived from cyclooxygenase, such as thromboxane A_2.

The destruction of NO is mediated by superoxide anions, which are in turn broken down by SOD (*33*). Moreover, superoxide anions are the source of secondary reactive oxygen species such as H_2O_2 and hydroxyl radicals (*34*). The enhanced formation of superoxide anions has been demonstrated in vessels from SHR, reducing the bioavailability of NO (*35*). Indeed, the relaxant response induced by oleanolic acid, maslinic acid, erythrodiol, and uvaol was significantly enhanced after exposure to the scavengers of radical oxygen species, SOD and catalase. This effect could be explained by the fact that endothelium-dependent relaxation in the aorta of hypertensive rats is intensified by SOD, possibly because it prevents the destruction of NO by superoxide anions (*36*).

In the aorta from normotensive rats, the endothelium-dependent relaxation induced by oleanolic acid and erythrodiol was reduced by the sarco/endoplasmic Ca^{2+}-ATPase blocker, CPA. These data suggest that the effects of these triterpenoids on the Ca^{2+} homeostasis of endothelial cells might contribute to vasodilatation in normotensive animals (*24*). In contrast, the endothelium-dependent vasodilatation induced by the triterpenic

compounds in the aorta of hypertensive rats was enhanced following blockage of the sarco/endoplasmic calcium pump with CPA. Moreover, a decrease in resting cytosolical calcium and alterations in intracellular calcium release has been reported in endothelial cells from SHR (*37*). Additionally, in SHR, the calcium pump displays greater sensitivity to CPA when compared to normotensive rats (*38*). Accordingly, the possible involvement of Ca^{2+} homeostasis on the vasodilatation induced by these triterpenic compounds may be related to the altered homeostasis of Ca^{2+} on endothelial and smooth muscle cells in SHR.

The results of this study suggest that triterpenic components of olive oil, predominantly from pomace olive oil, are able to induce vasodilatation of the aorta from SHR. Moreover, some structural features of these compounds appear to be prerequisites for the dependence of this effect on the endothelium. These data contribute to define the molecular pathways through which the vascular endothelium is involved in the vasodilatation induced by these triterpenic compounds (oleanolic and maslinic acids, erythrodiol, and uvaol) with respect to hypertension. Thus, this study identifies the potential nutritional properties of pomace olive oil, a dietary source of these vasodilator compounds.

ABBREVIATIONS USED

SHR, spontaneously hypertensive rat; NO, nitric oxide; eNOS, endothelial nitric oxide-synthase; L-NAME, N^{ω}-nitro-L-arginine; INDO, indomethacin; Phe, phenylephrine; SOD, superoxide dismutase; CPA, cyclopiazonic acid.

LITERATURE CITED

(1) di Giovacchino, L.; Mucciarella, M. R.; Costantini, N.; et al. Double oil extraction from olive paste and olive pomace by centrifugal decanter at 2-phases, type integral. *Riv. Ital. Sostanze Grasse* **2002**, *79*, 351−5.

(2) Perez-Camino, M. C.; Cert, A. Quantitative determination of hydroxy pentacyclic triterpene acids in vegetable oils. *J. Agric. Food Chem.* **1999**, *47*, 7, 1558−62.

(3) Liu, J. Oleanolic acid and ursolic acid: research perspectives. *J. Ethnopharmacol.* **2005**, *100*, 92−4.

(4) Herrera, M. D.; Rodríguez-Rodríguez, R.; Ruiz-Gutiérrez, V. Functional properties of pentacyclic triterpenes contained in "orujo" olive oil. *Curr. Nutr. Food Sci.* **2006**, *2*, 45−50.

(5) Honda, T.; Rounds, B. V.; Bore, L.; et al. Synthetic oleanane and ursane triterpenoids with modified rings A and C: a series of highly active inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **2000**, *43*, 4233−46.

(6) Lee, S. M.; Park, J. G.; Lee, Y. H.; Lee, C. G.; Min, B. S.; Kim, J. H.; Lee, H. K. Anti-complementary activity of triterpenoides from fruits of Zizyphus jujuba. *Biol. Pharm. Bull.* **2004**, *27*, 1883−6.

(7) Leu, Y. L.; Kuo, S. M.; Hwang, T. L.; Chiu, S. T. The inhibition of superoxide anion generation by neutrophils from Viscum articulactum. *Chem. Pharm. Bull.* **2004**, *52*, 858−60.

(8) Yim, T. K.; Wu, W. K.; Pak, W. F.; Ko, K. M. Hepatoprotective action of an oleanolic acid-enriched extract of Ligustrum lucidum fruits is mediated through an enhancement on hepatic glutathione regeneration capacity in mice. *Phytother. Res.* **2001**, *15*, 589−92.

(9) Kim, K. A.; Lee, J. S.; Park, H. J.; Kim, J. W.; Kim, C. J.; Shim, I. S.; Kim, N. J.; Han, S. M.; Lim, S. Inhibition of cytochrome P450 activities by oleanolic acid and ursolic acid in human liver microsomes. *Life Sci.* **2004**, *74*, 2769−79.

(10) Hsu, H. Y.; Yang, J. J.; Lin, C. C. Effects of oleanolic acid and ursolic acid on inhibiting tumor growth and enhancing the recovery of hematopoietic system postirradiation in mice. *Cancer Lett.* **1997**, *111*, 7−13.

(11) Choi, C. Y.; You, H. J.; Jeong, H. G. Nitric oxide and tumor necrosis factor-alpha production by oleanolic acid via nuclear factor-kappaB activation in macrophages. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 49−55.

(12) Andrikopoulos, N. K.; Kaliora, A. C.; Assimopoulou, A. N.; Papageorgiou, V. P. Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro low-density lipoprotein oxidation. *J. Med. Food* **2002**, *5*, 1−7.

(13) Perona, J. S.; Arcemis, C.; Ruiz-Gutierrez, V.; Catala, A. Effect of dietary high-oleic-acid oils that are rich in antioxidants on microsomal lipid peroxidation in rats. *J. Agric. Food Chem.* **2005**, *53*, 730−5.

(14) Somova, L. I.; Nadar, A.; Rammanan, P.; Shode, F. O. Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. *Phytomedicine* **2003**, *10*, 115−21.

(15) Somova, L. I.; Shode, F. O.; Mipando, M. Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. *Phytomedicine* **2004**, *11*, 121−9.

(16) Mombouli, J. V.; Vanhoutte, P. M. Endothelial dysfunction: from physiology to therapy. *J. Mol. Cell. Cardiol.* **1999**, *31*, 61−74.

(17) Boulanger, C. M. Secondary endothelial dysfunction: hypertension and heart failure. *J. Mol. Cell. Cardiol.* **1999**, *31*, 39−49.

(18) de Champlain, J. Do most antihypertensive agents have a sympatholytic action? *Curr. Hypertens. Rep.* **2001**, *3*, 305−13.

(19) John, S.; Schmieder, R. E. Impaired endothelial function in arterial hypertension and hypercholesterolemia: potential mechanisms and differences. *J. Hypertens.* **2000**, *18*, 363−74.

(20) Ferrara, L. A.; Raimondi, A. S.; d'Episcopo, L.; Guida, L.; Dello Russo, A.; Marotta, T. Olive oil and reduced need for antihypertensive medications. *Arch. Intern. Med.* **2000**, *160*, 837−42.

(21) Perona, J. S.; Ruiz-Gutierrez, V. Triacylglycerol molecular species are depleted to different extents in the myocardium of spontaneously hypertensive rats fed two oleic acid-rich oils. *Am. J. Hypertens.* **2005**, *18*, 72−80.

(22) Herrera, M. D.; Perez-Guerrero, C.; Marhuenda, E.; Ruiz-Gutierrez, V. Effects of dietary oleic-rich oils (virgin olive and high-oleic-acid sunflower) on vascular reactivity in Wistar-Kyoto and spontaneously hypertensive rats. *Br. J. Nutr.* **2001**, *86*, 349−57.

(23) Perona, J. S.; Rodriguez-Rodriguez, R.; Ruiz-Gutierrez, V. Effects of oleic acid rich oils on aorta lipids and lipoprotein lipase activity of spontaneously hypertensive rats. *J. Agric. Food Chem.* **2005**, *53*, 7330−6.

(24) Rodriguez-Rodriguez, R.; Herrera, M. D.; Perona, J. S.; Ruiz-Gutierrez, V. Potential vasorelaxant effects of oleanolic acid and erythrodiol, two triterpenoids contained in "orujo" olive oil, on rat aorta. *Br. J. Nutr.* **2004**, *92*, 635−42.

(25) Bueno, R.; Alvarez de Sotomayor, M.; Perez-Guerrero, C.; Gomez-Amores, L.; Vazquez, C. M.; Herrera, M. D. L-Carnitine and propionyl-L-carnitine improve endothelial dysfunction in spontaneously hypertensive rats: different participation of NO and COX-products. *Life Sci.* **2005**, *77*, 2082−97.

(26) García-Granados, A. Process for the industrial recovery of oleanolic and maslinic acids contained in the olive milling subproducts. Patent number 128: 179706, 1997.

(27) Kok, F. J.; Kromhout, D. Atherosclerosis−epidemiological studies on the health effects of a Mediterranean diet. *Eur. J. Nutr.* **2004**, *43*, Suppl 1:I/2−5.

(28) Perona, J. S.; Canizares, J.; Montero, E.; Sanchez-Dominguez, J. M.; Catala, A.; Ruiz-Gutierrez, V. Virgin olive oil reduces blood pressure in hypertensive elderly subjects. *Clin. Nutr.* **2004**, *23*, 1113−21.

(29) Visioli, F.; Bogani, P.; Grande, S.; Galli, C. Mediterranean food and health: building human evidence. *J. Physiol. Pharmacol.* **2005**, *56*, Suppl 1:37−49.

(30) Shimokawa, H. Endothelial dysfunction in hypertension. *J. Atheroscler. Thromb.* **1998**, *4*, 118−27.

(31) Luscher, T. F.; Vanhoutte, P. M. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* **1986**, *8*, 344−8.

(32) Callera, G. E.; Varanda, W. A.; Bendhack, L. M. Impaired relaxation to acetylcholine in 2K-1C hypertensive rat aortas involves changes in membrane hyperpolarization instead of an abnormal contribution of endothelial factors. *Gen. Pharmacol.* **2000**, *34*, 379−89.

(33) Laight, D. W.; Kaw, A. V.; Carrier, M. J.; Anggard, E. E. Interaction between superoxide anion and nitric oxide in the regulation of vascular endothelial function. *Br. J. Pharmacol.* **1998**, *124*, 238−44.
(34) Yang, D.; Feletou, M.; Boulanger, C. M.; Wu, H. F.; Levens, N.; Zhang, J. N.; Vanhoutte, P. M. Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats. *Br. J. Pharmacol.* **2002**, *136*, 104−10.
(35) de Champlain, J.; Wu, R.; Girouard, H.; Karas, M.; EL Midaoui, A.; Laplante, M. A.; Wu, L. Oxidative stress in hypertension. *Clin. Exp. Hypertens.* **2004**, *26*, 593−601.
(36) Sekiguchi, F.; Yanamoto, A.; Sunano, S. Superoxide dismutase reduces the impairment of endothelium-dependent relaxation in the spontaneously hypertensive rat aorta. *J. Smooth Muscle Res.* **2004**, *40*, 65−74.
(37) Wang, R.; Sauve, R.; de Champlain, J. Abnormal regulation of cytosolic free calcium in vascular endothelial cells from spontaneously hypertensive rats. *J. Hypertens.* **1995**, *13*, 993−1001.
(38) Zerrouk, A.; Auguet, M.; Dabire, H.; Brisac, A. M.; Safar, M.; Chabrier, P. E. Differential effects of tyrosine kinase inhibitors on contraction and relaxation of the aortas of normotensive and hypertensive rats. *Eur. J. Pharmacol.* **1999**, *374*, 49−58.

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