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Review

Hypotensive action of naturally occurring diterpenes: A therapeutic promise for the treatment of hypertension

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ABSTRACT

Plants have always been an exemplary source of drugs and many of the currently available medicines have been directly or indirectly derived from them. For this reason, the research, development and use of natural products as therapeutic agents, especially those derived from plants, have been increasing in recent years. A great deal of attention has focused on the naturally occurring antispasmodic phytochemicals as potential drugs for the treatment of cardiovascular diseases. Arterial hypertension is a common and progressive disorder that poses a major risk for cardiovascular and renal diseases. Recent data have revealed that the global burden of hypertension is an important and increasing public health problem worldwide and that the level of awareness, treatment and control of hypertension varies considerably among countries. The research on naturally occurring blood pressure-lowering agents is rapidly expanding due to the high potential of such molecules as new antihypertensive drugs. Recently, a great number of plant-derived substances, such as diterpenoids, have been evaluated as possible antihypertensive agents. Naturally occurring diterpenes such as forskolin and stevioside, exhibit vasorelaxant action and inhibit vascular contractility by different mechanisms of action. In this review we will discuss the mechanisms underlying the hypotensive action displayed by diterpenes and their potential use in human hypertension. We will also discuss the use of these compounds in the treatment of glaucoma, which is characterized by increased intraocular pressure (IOP).

Contents

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1. Introduction

The research, development and use of natural products as therapeutic agents, especially those derived from higher plants, have been increasing in recent years [1]. The most commonly used drugs from modern medicine such as aspirin, anti-malarial, and anti-cancer drugs were originated from plant sources. However, despite the fact that plants provide a rich source of novel biologically active compounds, only a small percentage has been phytochemically investigated [2] and studied for their medical potential [3]. A multidisciplinary approach to drug discovery involving the generation of truly novel molecular diversity from natural product sources combined with total and combinatorial synthetic methodologies as well as computational methods provides the best solution to increase the productivity in drug discovery and development [4].

The treatment of arterial hypertension with plant-derived products is well documented. The Mediterranean areas have a significantly lower incidence of cardiovascular diseases when compared to other European countries. This phenomenon has been associated with the use of virgin olive oil, which is one of the main components of the Mediterranean diet [5]. Several studies have shown that high olive oil intake reduces blood pressure. The cardioprotective activity of olive oil is attributed to its minor components, such as α-tocopherol, polyphenols, and other phenolic compounds [6]. Mechanisms underlying the cardioprotective action of olive oil include an increased generation of vascular relaxing factors such as nitric oxide (NO) [7] and prostacyclin [8].

Hibiscus tea, an herbal drink consumed both hot and cold by people around the world, is the infusion made from the calyces (sepals) of the *Hibiscus sabdariffa* flower. Interestingly, *H. sabdariffa* extract, standardized on 9.6 mg of total anthocyanins, displays the same hypotensive action of captopril (50 mg/day), a well-known antihypertensive agent [9]. *H. sabdariffa* exerts important antihypertensive action with a wide margin of tolerability and safety. It significantly reduces plasma angiotensin-converting enzyme (ACE) activity and serum sodium concentrations without modifying potassium levels [10]. More recently, a study of 65 subjects showed that 3 cups of hibiscus tea daily for 6 weeks reduced systolic blood pressure in pre-hypertensive and mildly hypertensive subjects [11]. The antihypertensive action of hibiscus has been attributed to anthocyanins, which act e.g. as ACE-inhibitors [10,11].

Diterpenoids form a large class of plants-derived secondary metabolites that possess a wide spectrum of important biological activities. Many reports have extensively shown that several classes of diterpenoids exert significant cardiovascular effects [12–17]. These studies pointed out the diterpenoids as a promising source of new prototypes for the discovery and development of novel cardiovascular therapeutic agents. Moreover, several studies have shown that the diterpene forskolin reduces intraocular pressure (IOP) in animals [18–20] and humans [21–23] indicating that this compound is a therapeutic promise for the treatment of glaucoma [22,23]. The present review brings new perspectives to the study of the hypotensive property of some diterpene-type compounds and their use in the treatment of arterial hypertension and glaucoma. The purpose of this review is to provide insight into the role of diterpenes in the regulation of arterial blood pressure and IOP, and the possible mechanisms underlying their biological effects. For the present review, research about the effects of diterpenes on blood pressure and IOP was searched using the database PUBMED, selected and read. The search was carried out in English, using the following keywords: diterpenes, antihypertensive, hypertension, blood pressure, intraocular pressure, glaucoma, hypotension and calcium. Data from pharmacology and pharmacognosy textbooks were also surveyed. Information analysis started with the title, followed by the abstract and, finally, the complete report.

2. Diterpenes as main compounds in medicinal plants with antihypertensive action

The rational for the study of the cardiovascular actions of diterpenes is based on the fact that many medicinal plants contain diterpenoids and their cardiovascular activity can most likely be attributed to these compounds. The crude extract of *Marrubium vulgare* (Horehound, Lamiaceae) is widely used as antihypertensive treatment in traditional medicine and it has been shown to induce vascular relaxation and to decrease systolic blood pressure in spontaneously hypertensive rats [24]. Marrubenol (1,4-naphthalenediol, 1-[2-(3-furanyl)ethyl]decahydro-5-(hydroxymethyl)-2,5,8a-trimethyl,[1R-(1α, 2α, 4β, 4aα, 5β, 8aβ)] is a pure compound with vasorelaxant activity that was isolated, identified and characterized from the water extract of *M. vulgare*. The mechanism of its relaxant activity is attributed to its interaction with L-type Ca^{2+} channels [25].

Two diterpenes were isolated from the dichloromethane extract of *Croton zambesicus*, a plant that is widely used in African folk medicine for the treatment of hypertension [26]. Both diterpenes induced vascular relaxation via blockage of extracellular Ca^{2+} influx, an effect that would be crucial for the antihypertensive action displayed by *C. zambesicus*. The leaves of *Orthosiphon aristatus* (Lamiaceae) have been prescribed in traditional Indonesian medicine for the treatment of hypertension [27]. The chloroform-soluble portion of the water decoction showed an inhibitory effect on the contractile responses in rat thoracic aorta smooth muscle stimulated with K^+, an effect that is thought to be closely related to its antihypertensive activity [27]. Four isopimarane-type diterpenes were isolated from the leaves of *O. aristatus* [28]. These pimaranes exhibited relaxant activity in isolated aortas, a finding that might be related to the use of this plant for the treatment of hypertension in Javanese traditional medicine [28].

The extracts/infusions prepared from the stem bark of *Croton cajucara* Benth. (Euphorbiaceae) are used in folk medicine for the treatment of hypertension [29]. Chemical investigations on the bark led to the isolation of several diterpenes such as trans-dehydrocrotonin, a diterpene that displays vasorelaxant activity [30]. *Andrographis paniculata* (Burm. F.) Nees (Acanthaceae) has a medicinal reputation in Malaysia as a potent medicine in the treatment of arterial hypertension [31]. The aqueous extract of *A. paniculata* decreases blood pressure in rats [32], a response that is induced by 14-deoxyandrographolide, a diterpene isolated

from this plant, that was shown to reduce blood pressure and heart rate in anaesthetized rats [33].

Several medicinal plants with antihypertensive activity have been chemically investigated and diterpenoids are pointed as their major constituents. For this reason, a great number of studies have focused on the cardiovascular properties of these compounds. Substantial progress in analytical techniques as well as the knowledge of important biological processes associated with many cardiovascular diseases and novel bioassays methods has led to the development of a number of screening techniques, which are being used to study the cardiovascular actions of diterpenes. A consequence of this approach is that many diterpenes previously reported in the literature are being "rediscovered" and the mechanisms underlying their cardiovascular effects are being investigated.

3. Hypotensive action of naturally occurring diterpenes: experimental studies

Stevioside is a diterpenoid glycoside, comprising an aglycone (steviol) and three molecules of glucose. This substance is a sweet glycoside extracted from *Stevia rebaudiana* Bertoni. This plant is a small shrub originally grown in South America, particularly in Brazil and Paraguay where it is known as *Stevia* or honey leaf [34]. The major components of the leaf are stevioside (5–10% of total dry weight), rebaudioside A (2–4%), rebaudioside C (1–2%) and dulcoside A (0.4–0.7%) [35]. In addition to their sweetness, *Stevia* extract and stevioside have been used as a traditional medicine by local people in South America for hundreds of years [36]. Accordingly, the effects of stevioside and extracts prepared from the leaves of *Stevia* on cardiovascular parameters are well demonstrated. The first experiment aiming to investigate the cardiovascular effects of this compound in rats was conducted in 1977 [37]. It was found that stevioside induces diuresis and a marked decrease in mean arterial pressure and heart rate. In an initial attempt to investigate the mechanisms underlying the cardiovascular effects elicited by stevioside, it was found that intravenous infusion of this compound (8 and 16 mg/kg per hour) in normotensive rats produced a marked hypotensive effect in a dose-dependent manner, as well as diuresis and natriuresis [12]. The authors suggested that stevioside induced a decrease in mean arterial pressure and promoted renal vasodilatation by lowering renal vascular resistance. The vasodilator effect is likely to occur via blockage of Ca^{2+} channels since verapamil, a Ca^{2+} channel blocker, enhanced the systemic effect of stevioside, whereas $CaCl_2$ infusion reduced the vasodilator response of stevioside [12]. In isolated rat aortic rings, stevioside dose-dependently relaxed endothelium-intact and endothelium-denuded arteries contracted with vasopressin [14]. The relaxation induced by stevioside was not affected by methylene blue, a guanylate cyclase inhibitor, showing that the relaxation was not mediated by the cyclic guanosine monophosphate (cGMP)-NO pathway. Using cultured aortic smooth muscle cells (A7r5) the authors found that stevioside blocked Ca^{2+} influx but it was not effective in inhibiting intracellular Ca^{2+} release. Thus, these results indicated that the vasorelaxation induced by stevioside was mediated mainly through inhibition of extracellular Ca^{2+} influx. In addition to the in vitro effects of stevioside, it was also found that this compound induced hypotension in conscious spontaneously hypertensive rats [14]. The hypotensive effect was maximal 60 min after intraperitonial injection of 25 mg/kg stevioside. This finding confirmed previous observations where stevioside at 16 mg/kg per hour [38] or 100 and 200 mg/kg [39] were administered intravenously and induced hypotension in normotensive and hypertensive rats.

The precise antihypertensive mechanism of stevioside remains not completely understood. In fact, it is possible to propose that stevioside exerts its hypotensive effect by acting on multiple sites of action. Indomethacin, a non-selective cyclooxygenase inhibitor, is able to attenuate the hypotension induced by intravenous administration of stevioside (16 mg/kg) [13]. Therefore, it appears that vasodilator prostaglandin(s) play an appreciable role in mediating stevioside effects. However, the antihypertensive effect elicited by stevioside is not related to a decrease on plasma noradrenaline, adrenaline or dopamine levels [39]. Other diterpenes such as pimaradienoic acid, kaurenoic acid and jatrophone also induce their cardiovascular effects by acting on multiple sites of action [15,16,40].

Alterations on plasma volume could also account for the antihypertensive effects of stevioside and *Stevia* extract. Accordingly, the intravenous administration of stevioside in rats induces diuresis, natriuresis and increases renal plasma flow without affecting glomerular filtration rate [41]. The increase in renal plasma flow induced by stevioside is associated with vasodilatation of both afferent and efferent arterioles [13]. Decreased fluid and sodium reabsorption in the proximal tubule could also account for the increased diuresis or urine flow rate. This observation is supported by the fact that stevioside increases glucose clearance, further indicating that there is a drop in glucose reabsorption by proximal renal tubular cells [42]. Oral administration of the crude extract of *S. rebaudiana* also induces diuresis in rats [43,44]. Moreover, intravenous injection of steviol in rats induces diuresis and natriuresis with no significant changes in renal plasma flow and glomerular filtration rate [45]. Chronic oral intake as well as acute intravenous administration of stevioside and steviol produces diuresis and natriuresis leading to decreased plasma volume. However, these studies did not allow discrimination of the systemic effect from the direct effect on kidney function. The direct infusion of stevioside into rat renal artery induces diuresis. This response occurs as a consequence of decreased proximal tubular reabsorption as indicated by lithium clearance [46], suggesting that the target of stevioside is at the proximal tubule.

The mechanisms underlying the cardiovascular actions of stevioside are summarized in Fig. 1. Stevioside reduces blood pressure by affecting vascular resistance via inhibition of extracellular Ca^{2+} influx and the release of a vasodilator prostaglandin. Stevioside also produces diuresis and natriuresis resulting in reduction of extracellular fluid volume. It is important to note that due to its popular use as sweetener, toxicological properties of stevioside have been extensively studied. Investigation of possible toxicity of stevioside in rodents, showed that stevioside intake as high as 15 g/kg produced no acute toxicity [47,48]. These findings may provide important information about the potential use of stevioside in the treatment of arterial hypertension.

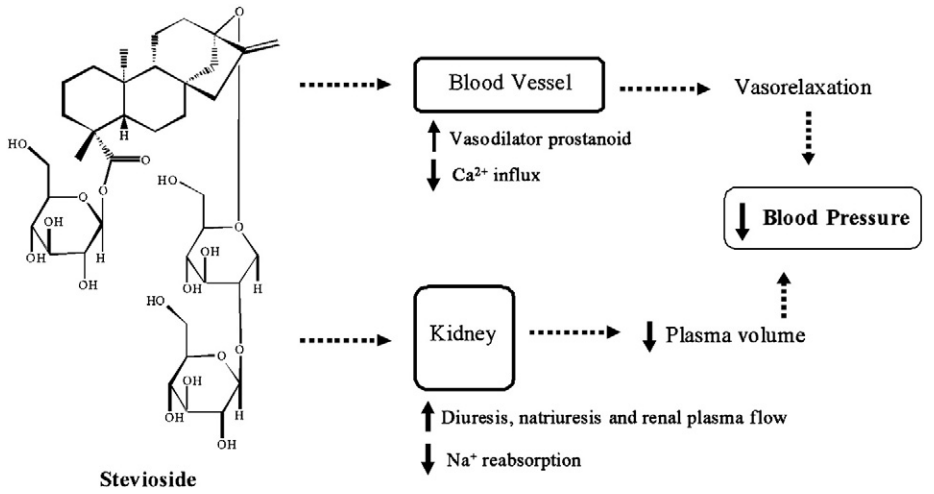


Fig. 1. Mechanisms underlying the cardiovascular actions of stevioside. Stevioside reduces blood pressure by decreasing the vascular resistance via inhibition of extracellular Ca^{2+} influx and by stimulating the release of a vasodilator prostaglandin. Stevioside also induces diuresis, natriuresis and reduction of Na^{+} reabsorption resulting in reduction of extracellular fluid volume.

In addition to stevioside, several diterpenoids are described to exert cardiovascular actions. Forskolin (7 beta-acetoxy-8, 13-epoxy-1 alpha,6 beta,9 alpha-trihydroxy-labd-14-ene-11-one) is the main active compound in the Ayurvedic herb *Coleus forskohlii*. The labdane-type diterpene forskolin is the primary constituent of clinical interest in *C. forskohlii*. The primary mode of action of forskolin is to increase cyclic adenosine monophosphate (cAMP) and cAMP-mediated functions, via activation of the enzyme adenylate cyclase [49]. For this reason, forskolin is commonly used to raise levels of cAMP in the study of cell physiology [50]. The physiological and biochemical effects of increased intracellular cAMP levels include: inhibition of platelet aggregation [51], relaxation of the arteries and lowering of blood pressure [52–54]. However, forskolin possesses additional mechanisms of action independent of its ability to directly stimulate adenylate cyclase and cAMP-dependent physiological responses. Forskolin has been shown to inhibit the binding of platelet-activating factor (PAF) [51] and inhibit a number of membrane transport proteins and channel proteins [55]. Several studies demonstrated that forskolin lowers blood pressure via relaxation of vascular smooth muscle [52,53,56,57]. In the vasculature, forskolin activates adenylate cyclase, producing an increase in cAMP, which in turn will activate cAMP-dependent protein kinase (PKA) and produce relaxation [58]. Moreover, the relaxation induced by forskolin also involves hyperpolarization of smooth muscle and Ca^{2+} extrusion across the plasma membrane [59] (Fig. 2). In humans, intravenous administration of forskolin (3 μg/kg/min) reduced diastolic blood pressure and improved left ventricular function in patients with cardiomyopathy [56]. In a similar study, it was demonstrated that when administered intravenously, forskolin (4 μg/kg/min) decreased vascular resistance, improved left ventricular contractility and induced a 20-percent reduction in arterial pressure [57]. Forskolin also exhibited a direct effect on cerebrovascular vasodilatation via cAMP activation. Intravenous administration of forskolin (10 μg/kg/min) increased blood flow to the brain in rabbits. This response was accompanied by a small decrease in blood pressure, although cerebral oxygen consumption remained stable, further indicating that forskolin may be useful in cases of cerebral vascular insufficiency and post-stroke [54]. The ability of forskolin to inhibit platelet aggregation is of additional benefit in cardiovascular disease [51].

Intravenous administration of the diterpene 14-deoxy-11,12-didehydroandrographolide (1.7 to 6.7 mmol/kg) in normotensive rats caused a significant fall in both blood pressure and heart rate in a dose-dependent manner [33]. The hypotensive action displayed by the diterpene involves blockade of the autonomic ganglia and the renin–angiotensin system as well as the inhibition of β-adrenoreceptors. The β_1-adrenoreceptor subtype is found in cardiac muscle and its activation by circulating catecholamines induces positive inotropic and chronotropic effects [60]. In isolated rat right atria, 14-deoxy-11,12-didehydroandrographolide antagonizes the positive chronotropic effect elicited by the β-adrenoreceptor agonist isoproterenol, which supports the idea that the bradycardic effect elicited by the diterpene in vivo is due to a direct β_1-adrenoreceptor blocking action.

The aqueous and ethanolic extracts of *Croton schiedeanus* Schlecht (Euphorbiaceae) can elicit vasodilator actions in isolated rat aortic rings and, after intravenous administration, decrease blood pressure in spontaneously hypertensive rats [61,62]. Infusions prepared from its stem bark and leaves are used in folk medicine to treat hypertension [29,63]. Phytochemical investigations on the bark have led to the isolation of clerodane-type diterpenes such as *trans*-dehydrocrotonin [64] (Table 1). The diterpene *trans*-dehydrocrotonin is the most active compound and studies carried out with this diterpene revealed its wide pharmacological profile that includes vasorelaxant actions. *Trans*-dehydrocrotonin (10^{-6}–10^{-3} mol/l) concentration-dependently relaxed phenylephrine- or KCl-contracted rat endothelium-intact aortic rings [30]. It is well established that contractions of rat aortic rings induced by KCl rely almost exclusively on Ca^{2+} influx through activation of voltage-sensitive channels [65], whereas contractions induced by phenylephrine are mediated by an increase in Ca^{2+} influx through both receptor-operated [66] and voltage-sensitive channels [67,68]. Since

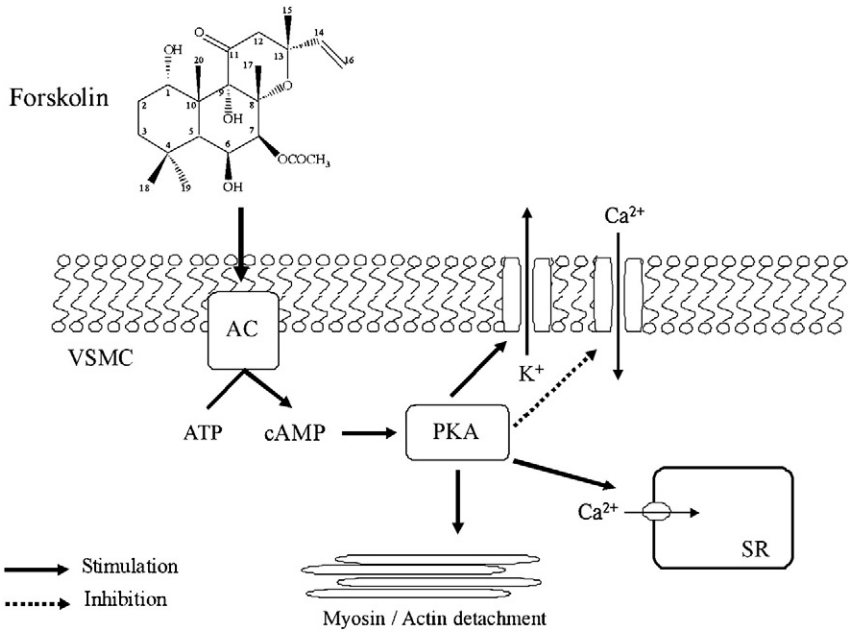
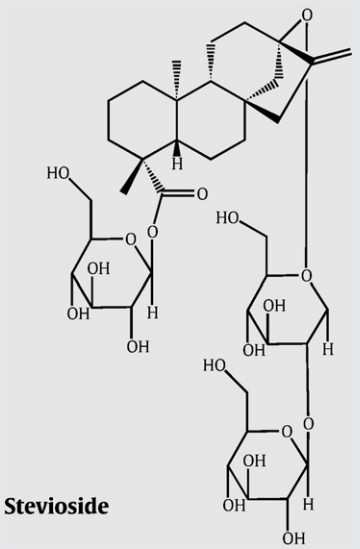
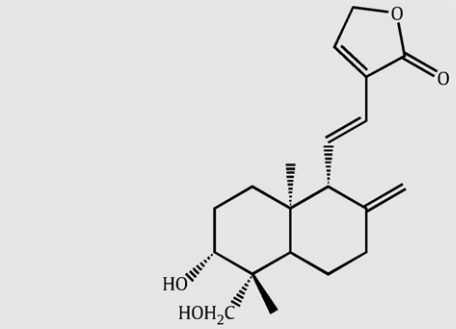
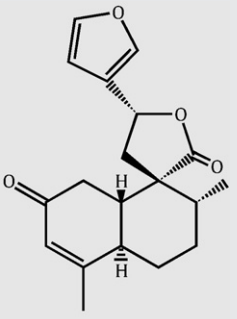


Fig. 2. Mechanisms underlying forskolin-induced vascular relaxation. Forskolin activates adenylate cyclase (AC) and enhances cAMP levels in vascular smooth muscle cells (VSMC). cAMP activates protein kinase A (PKA), which leads to the phosphorylation of the L-type Ca^{2+} channel, re-uptake of Ca^{2+} by the sarcoplasmic reticulum (SR), hyperpolarization by opening of K^+ channels and dephosphorylation of myosin light chain.

trans-dehydrocrotonin relaxed aortic rings pre-contracted with both contractile agents, it could be suggested that the diterpene blocks Ca^{2+} influx through interference with both voltage- and receptor-operated channels. Based on these in vitro findings, the potential antihypertensive activity of this diterpene was tested. Intravenous injection of *trans*-dehydrocrotonin at 10 and 15 mg/kg induced marked hypotension and bradycardia in normotensive rats [69]. The hypotension induced by *trans*-dehydrocrotonin does not involve cholinergic activation since atropine, a non-selective muscarinic receptor antagonist, and hexamethonium, a ganglionic blocker, did not affect this response. However, a possible role for NO in mediating such response was suggested since L-NAME, a NO synthase inhibitor, abolished *trans*-dehydrocrotonin-induced hypotension. In vitro experiments showed that the relaxation induced by *trans*-dehydrocrotonin in endothelium-intact preparations was greatly abolished after endothelial removal or incubation with L-NAME, pointing to the involvement of an endothelium-dependent component related to endothelial NO synthase activity in its vasorelaxant effect. However, at higher concentrations, the relaxant effect of *trans*-dehydrocrotonin persists even after endothelial denudation, indicating both endothelium-dependent and -independent mechanisms in the vasorelaxant effect of the diterpene, an observation that is in agreement with earlier reports on other diterpenes [15,16]. The cardiovascular effects of *trans*-dehydrocrotonin also involve its direct action in the heart. The diterpene induces a negative chronotropic effect in spontaneously beating rat right atria, which is consistent with the bradycardic effect seen in vivo. The negative inotropic effect of *trans*-dehydrocrotonin does not involve stimulation of muscarinic receptors or blockade of β-adrenoceptor, suggesting that other mechanisms are involved in the depressor action of *trans*-dehydrocrotonin in the right atria [69]. Thus, *trans*-dehydrocrotonin exerts its hypotensive effect by acting on multiple sites of action. The diterpene exerted hypotensive and bradycardic effects in vivo that were related to vasorelaxant effects and a direct negative chronotropic effect on the right atria of rats.

In a bioassay-directed phytochemical study, the cardiovascular effects of *ent*-kaur-16-en-19-oic acid and *ent*-kaur-16-en-15-one-19-oic acid, two kaurane-type diterpenes, were investigated. Intravenous administration of these two compounds at 10 mg/kg in normotensive rats produced an immediate decrease of systolic blood pressure (by 17 and 18%, respectively), no change of diastolic blood pressure and a significant decrease of heart rate by 20 and 55%, respectively. Similar results were obtained by our laboratory with *ent*-kaur-16-en-19-oic acid (15 mg/kg, i.v.), which was found to decrease mean arterial pressure of conscious normotensive rats [70]. The relaxant effect of these diterpenes on isolated rat aorta was independent of the integrity of the endothelium. Moreover, these diterpenes pronouncedly and time-dependently increased coronary flow. Finally, the diuretic and natriuretic activities of these diterpenes were very high, comparable to that of hydrochlorothiazide [71]. In summary, these kaurane-type diterpenes produced systemic hypotension and coronary vasodilation, and cardiac bradycardia. In addition to the direct cardiovascular effects, distinct diuretic and natriuretic effects may also account for the hypotensive action displayed by these compounds. In fact, a more detailed study on the vascular relaxation effects of *ent*-kaur-16-en-19-oic acid showed that this diterpene blocks extracellular Ca^{2+} influx by interacting with both voltage- and receptor-operated channels. In addition, its action also involves the stimulation of neuronal NO synthase and activation of the NO-cGMP pathway, which in turn activates opening of K^+ channels [15]. The diterpene also stimulates the production of NO from endothelial cells [15,72].

Table 1
Name, structure, dose/via of administration and the proposed mechanisms of action of diterpenes that exhibit hypotensive action in rats.

Name/structure	Dose/administration	Mechanism(s) of action proposed	Reference
 **Stevioside**	8 and 16 mg/kg intravenous (infusion)	Reduces vascular resistance via inhibition of extracellular Ca^{2+} influx and the release of a vasodilator prostaglandin.	Melis and Sainati [12]
	25 mg/kg intraperitonial	Induces diuresis and natriuresis resulting in reduction of extracellular fluid volume.	Lee et al. [14]
	100 and 200 mg/kg intravenous (bolus)		Chan et al. [39]
 **14-deoxy-11,12-didehydroandrographolide**	1.7 to 6.7 mmol/kg intravenous (bolus)	Activation of the autonomic ganglia, the renin–angiotensin system and cardiac β-adrenoreceptor. Reduces vascular resistance via release of no.	Zhang et al. [33]
 ***trans*-dehydrocrotonin**	10 and 15 mg/kg intravenous (bolus)	Direct negative chronotropic and inotropic effects in the heart by a non-adrenergic non-cholinergic mechanism.	Silva et al. [69]

(*continued on next page*)

Table 1 (*continued*)

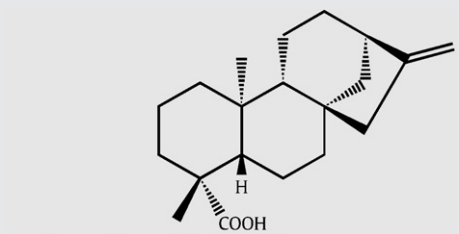
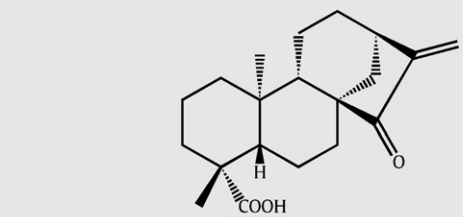
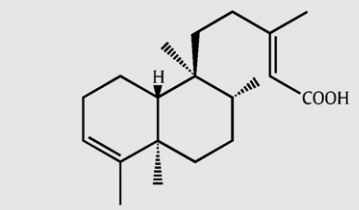
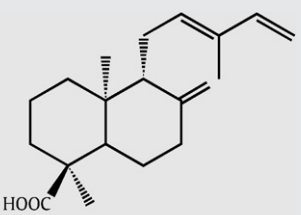
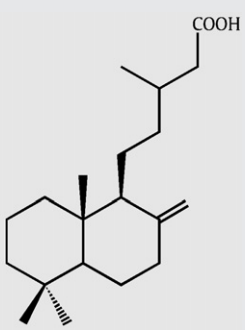
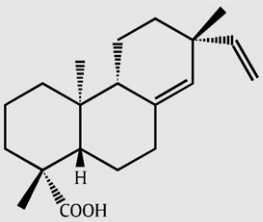
Name/structure	Dose/administration	Mechanism(s) of action proposed	Reference
 ***ent*-kaur-16-en-19-oic acid**	10 mg/kg intravenous (bolus)	Reduces vascular resistance via inhibition of extracellular Ca^{2+} influx. Induces diuresis and natriuresis resulting in reduction of extracellular fluid volume.	Somova et al. [71]
 ***ent*-kaur-16-en-15-one-19-oic acid**	10 mg/kg intravenous (bolus)	Reduces vascular resistance via inhibition of extracellular Ca^{2+} influx. Induces diuresis and natriuresis resulting in reduction of extracellular fluid volume.	Somova et al. [71]
 **Kolavenic acid**	30 mg/kg/ intravenous (bolus)	Muscarinic receptor activation.	Saleem et al. [73]
 **8-(17), 12*E*, 14-labdatrien-18-oic acid**	5–30 mg/kg intravenous (bolus)	Reduces vascular resistance via inhibition of extracellular Ca^{2+} influx and the release of NO and a vasodilator prostaglandin.	De Oliveira et al. [76]
 **Labd-8 (17)-en-15-oic acid**	1–10 mg/kg intravenous (bolus)	Reduces vascular resistance possibly via inhibition of extracellular Ca^{2+} influx. Activation of the autonomic ganglia	Lahlou et al. [77]

Table 1 (*continued*)

Name/structure	Dose/administration	Mechanism(s) of action proposed	Reference
 ***ent*-pimara-8(14),15-dien-19-oic acid**	1–15 mg/kg intravenous (bolus)	Reduces vascular resistance via inhibition of extracellular Ca^{2+} influx and the release of NO and a vasodilator prostaglandin.	Tirapelli et al. [17]

The diterpene kovalenic acid (Table 1) contributes to the hypotensive action displayed by the root bark extract of *Polyalthia longifolia* var. *pendula* [73]. Intravenous injection of kovalenic acid (30 mg/kg) induced a 22% decrease in blood pressure in normotensive rats and this response was completely abolished by atropine. This observation suggests a role for muscarinic receptor activation in kovalenic acid-induced hypotension. The muscarinic receptor mediating relaxation of vascular smooth muscle is the M_3 subtype, which is located in endothelial cells [74] and induces vasodilatation via NO release [75]. Thus, the hypotension elicited by kovalenic acid may be partially related to an active vascular relaxation mediated by an endothelial NO pathway through peripheral muscarinic receptor activation.

The cardiovascular effects of the diterpene 8 (17), 12*E*, 14-labdatrien-18-oic acid were demonstrated using a combined approach of in vivo and in vitro techniques. Intravenous administration of 8 (17), 12*E*, 14-labdatrien-18-oic acid at 5, 10, 20, and 30 mg/kg in normotensive rats induced a short-lasting hypotension that was significantly attenuated by L-NAME, further indicating that the diterpene causes hypotension through peripheral vasodilatation, mediated in part by NO. Experiments in rat isolated superior mesenteric arteries support a role for NO as well as the participation of vasodilator prostaglandin(s) in the relaxation induced by the diterpene. Moreover, 8 (17), 12*E*, 14-labdatrien-18-oic acid blocks Ca^{2+} entry through voltage-dependent Ca^{2+} channels, an observation that is supported by the findings that the diterpene inhibited Ca^{2+}-induced contraction in rat isolated superior mesenteric arteries and reduced L-type Ca^{2+} currents in isolated cells [76].

Labd-8 (17)-en-15-oic acid (Table 1) is another diterpene described to exert cardiovascular effects in rats. Intravenous injection of the diterpene (1–10 mg/kg) induces immediate and dose-dependent decreases in mean arterial blood pressure in normotensive rats. Labd-8 (17)-en-15-oic acid-induced hypotension is unlikely to occur through activation of β_2-adrenergic vascular receptors since it remained significantly unaffected by pretreatment with propranolol, a non-selective β-adrenergic receptors antagonist. Blockade of ganglionic neurotransmission with hexamethonium significantly reduced the hypotensive response to the diterpene further indicating that the hypotension is partially dependent on an operational central sympathetic neural drive to the vascular system. This sympathoinhibitory action of Labd-8 (17)-en-15-oic acid may be of a central origin and/or from a peripheral action at sympathetic ganglia and/or presynaptic sites. In vitro findings showed that the diterpene induces a concentration-dependent vasodilator effect in endothelium-intact aortic rings. Release of NO from vascular endothelial cells is not involved in this response [77].

More recently, data from our laboratory showed that bolus injection of the diterpene *ent*-pimara-8(14),15-dien-19-oic acid (1–15 mg/kg) (Table 1) produced a dose-dependent decrease in mean arterial pressure from conscious normotensive rats. Studies on isolated aorta and on isolated smooth muscle cells loaded with FURA-2 support the notion that hypotension induced by the diterpene could be mediated by the direct vasorelaxant action of this pimarane on the vascular smooth muscle, a response that involves the blockade of extracellular Ca^{2+} influx [17]. In fact, we showed previously that the endothelium-independent vascular effects of *ent*-pimara-8(14),15-dien-19-oic acid are partly dependent on the activation of the NO-cGMP pathway and the release of metabolites derived from the arachidonic acid pathway [16].

The pharmacological assays with isolated diterpenes indicate a therapeutic potential in several chronic cardiovascular diseases, such as arterial hypertension. It is interesting to note that the underlying mechanism of the hypotensive action of a single diterpene involves multiple actions in different targets. However, a comparison of the effects displayed by different diterpenes shows that they share common points. First, several diterpenes are described to induce hypotension due to direct vasorelaxation that involves Ca^{2+} blockade. Second, some diterpenes display a renal action that is related to increased natriuresis and diuresis. Structural differences of diterpenes could be a possible explanation for the diverse mechanisms of action displayed by these compounds since slight modifications on the chemical structure of these compounds can alter their vascular effects [78].

The arterial pressure is controlled by the peripheral resistance and cardiac output. An increase in peripheral resistance or cardiac output will increase arterial pressure, whereas a decrease in any of these parameters will decrease blood pressure. Arterial hypertension is a pathological state resulting from an inappropriate relationship between vascular capacity and blood volume. The diterpenes act, directly or indirectly, in these parameters to reduce blood pressure. Some of these compounds cause vascular dilation, and

thereby reduce arterial pressure due to a reduction in peripheral resistance. Water retention induces an increase in blood pressure. Pressure rises because fluid retention increases blood volume, which increases venous pressure, venous return, cardiac output, and ultimately arterial pressure. Some diterpenes are described to induce diuresis and natriuresis, which in turn would reduce water retention and blood pressure. Considering their cardiovascular effects in experimental studies, diterpenes may represent potential agents with antihypertensive actions in humans. In fact, some of these compounds were already tested for their hypotensive action in humans as discussed in the next section.

4. Hypotensive action of naturally occurring diterpenes: clinical studies

Epidemiological studies show that arterial hypertension is an important risk factor for cardiovascular mortality and morbidity [79]. Improvements in the pharmacological treatment of hypertension contribute to a reduction in the incidence of cardiovascular disease [80]. As mentioned before, many reports have extensively shown that several classes of diterpenoids exert significant cardiovascular effects in experimental studies. These studies pointed out the diterpenoids as a promising source of new prototypes for the discovery and development of novel cardiovascular therapeutic agents. Thus, diterpenes likely fulfill the definition of a pharmacological preconditioning class of compounds and may have therapeutic use in cardiovascular diseases. As mentioned above, stevioside is the major component of the leaf of *S. rebaudiana* Bertoni, which is effective in lowering blood pressure in rats [12,37]. The effects of extracts prepared from *Stevia* on cardiovascular parameters in humans showed that administration of tea prepared with *S. rebaudiana* leaves, for 30 days to 18 normotensive human subjects (ranging in age from 20 to 40 years), produced a discrete lowering of mean arterial pressure and bradycardia [81]. On the basis of these initial results it was suggested that both the stevioside and aqueous extract of *Stevia* may be useful hypotensive agents [81]. In a multicentre, randomized, double-blind, placebo-controlled study with 106 hypertensive subjects, administration of stevioside (250 mg thrice daily) produced a reduction in blood pressure [82]. Both the systolic and diastolic blood pressures significantly decreased in the stevioside group (systolic: from 166.0 ± 9.4 to 152.6 ± 6.8 mmHg; diastolic: from 104.7 ± 5.2 to 90.3 ± 3.6 mmHg). The blood pressure began to decrease after 7 days and persisted throughout the 12-month treatment period with stevioside capsules. One important finding of this study is that the toxicological profile showed that the treatment with stevioside did not alter the serum levels of creatinine, creatinine phosphokinase, aspartate aminotransferase or alanine aminotransferase, indicating no potential toxicity of stevioside at this dose. Moreover, the tolerability of stevioside was satisfactory since few patients reported minor side-effects such as dizziness or nausea.

Hypertensive patients taking capsules containing 500 mg stevioside powder, three times daily for 2 years, showed significant decreases in systolic and diastolic blood pressures compared with baseline (systolic: from 150 ± 7.3 to 140 ± 6.8 mmHg; diastolic: from 95 ± 4.2 to 89 ± 3.2 mmHg) [83]. The blood pressure began to decrease one week after taking stevioside capsules and the tolerability of stevioside was satisfactory. Thus, stevioside was found to be safe and effective in the treatment of hypertension although the amplitude of blood pressure lowering was slightly less than with other antihypertensive agents [82,83].

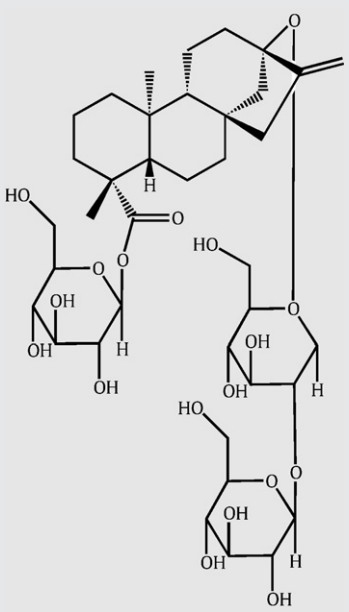
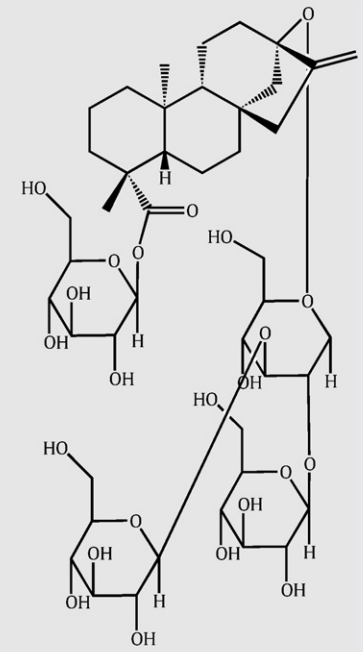
The antihypertensive potential of oral stevioside obtained from the leaves of *S. rebaudiana* was also evaluated in a prospective, randomized, double-blind, placebo-controlled, single center clinical trial in Brazil [84]. Stevioside administered orally (3.75, 7.5 or 15 mg/kg/day) twice a day reduced systolic and diastolic blood pressures. However, similar findings were observed in subjects from the placebo group. Thus, stevioside did not show antihypertensive effects but seemed to be safe and well tolerated. The lack of antihypertensive action contrasted with previous findings in two clinical trials [82,83], a fact that could be attributed to lower basal values of blood pressure, a small number of patients or the frequency of daily ingestion of the capsules [84].

Although results from some of the studies support a blood pressure-lowering effect of stevioside in individuals with elevated blood pressure [82,83], more recently conducted studies in normotensive individuals have failed to support these findings. In this line, it was reported that administration of stevioside (750 mg/day for 3 days) failed to significantly alter blood pressure in nine normotensive subjects [85]. However, we must be aware of the fact that the antihypertensive action of stevioside was described to occur one week after the beginning of the treatment [82,83]. Studies on the long-term treatment with stevioside in normotensive subjects showed that this diterpene lacks hypotensive action [86]. Thus, blood pressure lowering-effect of stevioside is only observed in hypertensive subjects and this diterpene does not appear to have any significant impact on blood pressure in humans with normal and low-normal resting blood pressure.

Stevioside is unlikely to be absorbed in the intestine since it is a hydrophilic diterpenoid glycoside with a high molecular weight. However, the bacterial intestinal flora of humans is able to convert stevioside into its aglycone, steviol [87]. The latter appears to be the major metabolite of stevioside appearing into the blood circulation following oral ingestion. As a consequence, much attention has been paid to the pharmacokinetics of stevioside. Studies in human volunteers receiving stevioside (750 mg/day) for 3 days showed no measurable amount of stevioside in the feces of all subjects, whereas free steviol was present [85]. Taken together, these data indicate that following oral ingestion of stevioside, it is steviol that is taken up by the intestine into the blood and it is probably responsible for the cardiovascular effects of stevioside.

In addition to stevioside, several other sweet compounds such as steviobioside, rebaudioside A, B, C, D, E and ducoside A were isolated from *S. rebaudiana* Bertoni leaves. All of these isolated diterpenoid glycosides have the same chemical backbone structure (steviol) but differ in the residues of carbohydrate at positions C13 and C19 [88]. Because of the similarities in their chemical structure, rebaudioside A (Table 2) and stevioside were expected to produce similar cardiovascular effects. However, studies addressing the effects of rebaudioside A on blood pressure are limited [89]. Eight weeks of daily ingestion of rebaudioside A had no effect on blood pressure in a rodent model of type 2 diabetes [90]. Similar results were found in humans, where consumption of

Table 2
Name, structure, dose, period of treatment and the cardiovascular action of diterpenes in humans.

Name/structure	Oral dose	Period of treatment	Cardiovascular action	Reference
 **Stevioside**	750 mg daily	12 months	Reduction in systolic and diastolic blood pressure	Chan et al. [82]
	1500 mg daily	24 months	Reduction in systolic and diastolic blood pressure	Hsieh et al. [83]
	3.75, 7.5 or 15 mg/kg/day	24 weeks	No effect	Ferri et al. [84]
	750 mg daily	3 days	No effect	Geuns et al. [85]
	250 mg daily	3 months	No effect	Barriocanal et al. [86]
 **rebaudioside A**	1000 mg/daily	16 weeks	No effect	Maki et al. [91]
	1000 mg/daily	4 weeks	No effect	Maki et al. [92]

1000 mg/day of rebaudioside A for 16 weeks [91] or 4 weeks [92] did not significantly alter blood pressure in subjects with type 2 diabetes mellitus. However, the studies conducted to evaluate the hemodynamic effects of rebaudioside A were performed in normotensive individuals and no evaluation in hypertensive subjects has been performed until now.

The initial clinical studies on the cardiovascular effects of diterpenoids in humans are important and needed since such information is a prerequisite to any rational and safety use of these compounds in the treatment of hypertension.

5. Action of naturally occurring diterpenes in intraocular pressure (IOP)

Glaucoma is a major cause of vision loss throughout the world. The pathogenesis of glaucoma is still poorly understood. However, one common element seems to be the rise of IOP beyond physiologic limits. Treatment for glaucoma consists of reducing IOP to an acceptable target range to prevent further optic nerve damage. A number of pharmacologic agents are available to decrease IOP through distinctly different mechanisms. Since these drugs have their own mechanisms of action, some are used in combination in attempt to reduce the IOP to acceptable levels. Beta-blockers are the treatment of initial choice in most patients with glaucoma. However, in almost 50% of these individuals therapy with beta-blockers alone does not reduce IOP adequately. Therefore, there is a need for new classes of topical IOP-lowering agents that can be used alone or in a combination with beta-blockers [93].

Topical ocular application of forskolin, a diterpene that increases intracellular cAMP by stimulating the enzyme adenylate cyclase, lowered IOP in rabbits, monkeys, and volunteers who were free from eye disease. In man, a topical suspension of 1% forskolin significantly lowered IOP (by 70% on average) in 1 h, the effect reaching a peak at 2 h and remained significant for at least 5 h [18]. Forskolin lowers IOP by reducing aqueous inflow. In rabbits, net aqueous humor inflow decreases, outflow facility remains unchanged, and ciliary blood flow increases [19]. Similarly, in humans forskolin reduces the rate of aqueous humor flow and does not change outflow facility. Thus, the action of forskolin in reducing IOP is the direct result of a reduction in net aqueous flow [21]. A study in normal rabbits using forskolin solutions (from 0.5 to 2%) found significant dose-dependent decreases in IOP within a half hour, peaking in 2–3 h, and lasting up to 10 h [20].

The studies in humans regarding the effect of forskolin on IOP have been limited to healthy volunteers, where forskolin was described to be effective at lowering IOP and decreasing aqueous outflow [22]. In 20 healthy volunteers two instillations of forskolin solution (1%) 5 min apart led to significant decreases in IOP and aqueous flow rate [23]. In eight healthy subjects one drop of forskolin significantly decreased IOP and flow rate was diminished with an average of 34% [21]. While topical use of forskolin in animals and healthy humans appears promising, clinical studies on its use in glaucoma patients are lacking. More research on this important topic is needed.

6. Conclusion

The incidence of arterial hypertension in the world population is very high and a variety of pharmacological preparations are available for therapy including, diuretics, β-adrenergic blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor subtype 1 antagonists, and Ca^{2+} channel blockers. Unfortunately, despite these options, the number of hypertensive patients is steadily increasing, vascular dysfunction persists in many patients, and end-organ injury remains to be a serious complication. As described in this review, the diterpenes are a promising source of new prototypes for the development of novel therapeutic agents for the treatment of hypertension and glaucoma. However, it is important to emphasize that, more biological, toxicological, structure-activity relationships and in vivo studies should be undertaken. In this sense, a multidisciplinary approach involving the discovery of novel diterpenoids from natural products, combined with total or partial synthetic and biosynthetic methodologies as well as detailing the mechanism of action of the most potential diterpenes would provide the best solution to explore the antihypertensive potential of these metabolites.

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