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Studies of *ent*-kaurane diterpenes from *Oyedaea verbesinoides* for their inhibitory activity on vascular smooth muscle contraction☆

Stefan Müller[a], Carlos R. Tirapelli[b], Ana M. de Oliveira[c], Renato Murillo[d], Victor Castro[d], Irmgard Merfort[a,*]

[a]*Institute of Pharmaceutical Biology, University Freiburg, Stefan-Meier-Str. 19, 79104 Freiburg, Germany*
[b]*Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, Brazil*
[c]*Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, USP, Ribeirão Preto, SP, Brazil*
[d]*Escuela de Quimica and CIPRONA, Universidad de Costa Rica, San José, Costa Rica*

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Abstract

From the aerial parts of *Oyedaea verbesinoides* nine *ent*-kauranes and a sesquiterpene were isolated. *ent*-9α-Hydroxy-kaur-16-en-19-oic acid, *ent*-15β-tigloyloxy-9α-hydroxy-kaur-16-en-19-oic acid, *ent*-15β-angeloyloxy-9α-hydroxy-kaur-16-en-19-oic acid, *ent*-16α-hydroxykaurane and 1α-angeloyloxy-carotol are new for the genus or the species and *ent*-15β-angeloyloxy-7α,9α-dihydroxy-kaur-16-en-19-oic acid is reported for the first time. Structure elucidation was based on one and two dimensional NMR as well as ESI and CI-MS analysis. Some diterpenes were proven to exhibit inhibitory effects on smooth muscle contraction on rat aorta.

Keywords: *Oyedaea verbesinoides*; *ent*-Kauranes; Smooth muscle; Rat aorta

1. Introduction

The genus *Oyedaea* (Asteraceae, tribe Heliantheae, subtribe Verbesiinae (Karis and Riding, 1994) is known for the occurrence of *ent*-kaurane-type diterpenes (Bohlmann and Zdero, 1979; Seaman et al., 1990). Here, we report the results of a phytochemical reinvestigation of *O. verbesinoides* DC., a tree growing in Central America, and its diterpene pattern (Stokes et al., 1992).

Recently, it was shown that *ent*-kaur-16-en-19-oic acid inhibited the in vitro contractility of rat carotid artery induced by the selective α_1-adrenergic agonist phenylephrine (Phe) (Tirapelli et al., 2002) and that this kaurenoic acid as well as three closely related derivatives also exhibited an inhibitory effect on the contractile activity of rat uterus induced by acetylcholine, oxytocin and serotonin (Campos-Bedolla et al., 1997). To gain more information about structure–activity relationships three of the *ent*-kauranes from *O. verbesinoides* were investigated on the influence of the rat aorta contraction.

2. Results and discussion

2.1. Identification of diterpenes 1–9 and sesquiterpene 10

The lipophilic extract of the aerial parts of *O. verbesinoides* afforded the *ent*-kaurane derivatives *ent*-kaur-16-en-19-oic acid (**1**), *ent*-kaur-16-en-19-al (**2**), *ent*-kaur-16-en-19-ol (**3**), *ent*-9α-hydroxy-kaur-16-en-19-oic acid (**4**), *ent*-15β-tigloyloxy-9α-hydroxy-kaur-16-en-19-oic acid (**5**), *ent*-15β-angeloyloxy-9α-hydroxy-kaur-16-en-19-oic acid (**6**), *ent*-15β-angeloyloxy-7α,9α-dihydroxy-kaur-16-en-19-oic acid (**7**), *ent*-kaur-16β-ol (**8**), *ent*-kaur-9(11),16-dien-19-oic acid (**9**) as well as the carotane sesquiterpene 1α-angeloyloxy-carotol (**10**) (for structures see Fig. 1). Nomenclature is according to the recommendations of the IUPAC (Moss, 1989). All

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* Corresponding author. Tel.: +49-761-203-8373; fax.: +49-761-203-8383.
E-mail address: irmgard.merfort@pharmazie.uni-freiburg.de (I. Merfort).

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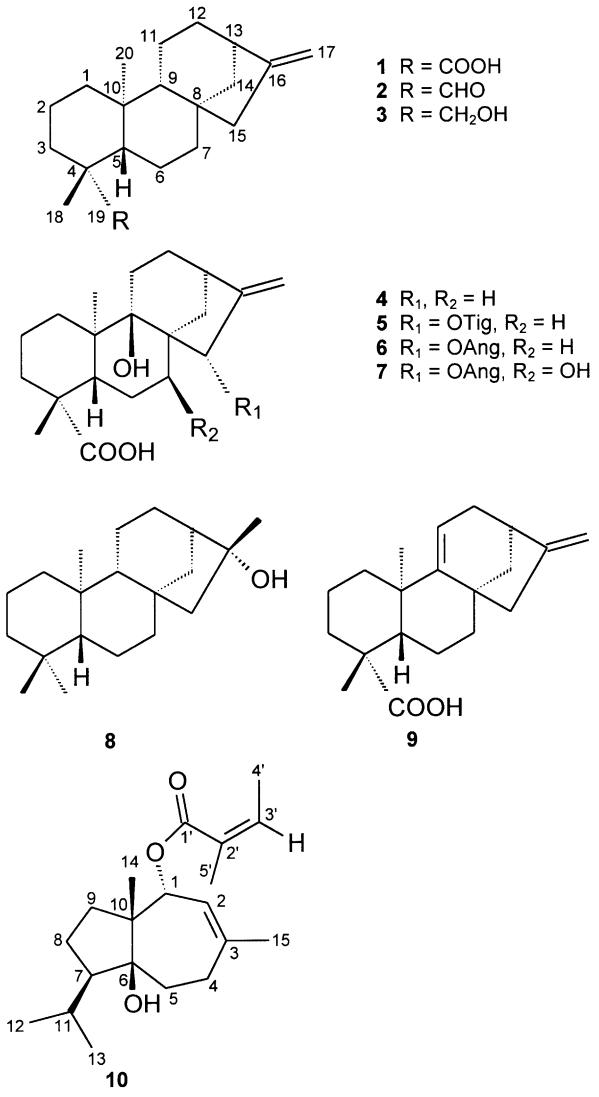

Fig. 1. Structures of the isolated diterpenes **1**–**9** and the sesquiterpene **10**.

known compounds were identified by comparison of their spectral properties (EI-MS, ^{1}H NMR and, except for compound **4**, ^{13}C NMR) with those reported in literature (Bohlmann and Zdero, 1979; Enriquez et al., 1997; Gonzales et al., 1981; Hanson et al., 1976; Hayman and Weavers 1990; Herz et al., 1983; Lu et al., 1993; Piozzi et al., 1971). ^{13}C NMR data for compound **6** are reported for the first time, those from **5** were corrected (Alvarez et al., 1985; Da Costa et al., 1996) (see Table 1).

The structure of compound **7** followed from its NMR (^{1}H NMR, ^{1}H–^{1}H COSY, ^{13}C NMR, GHSQCR, GHMQCR) (Tables 1 and 2) and mass spectral data. High resolution EIMS data indicated the molecular formula $C_{25}H_{36}O_6$ and a molecular mass of 432.2516 which was confirmed by the mass spectra in the ESI and EI mode. The ESI mass spectrum showed a weak pseudomolecular ion peak at *m*/*z* 433, the respective sodium adduct at *m*/*z* 455 and a dimer adduct at *m*/*z* 888 $[2\times M+Na+H]^+$. The EI mass spectrum exhibited

Table 1
^{13}C NMR data of diterpenes **5**–**7** (75 MHz, $^{\$}CDCl_3$, $^{\#}CD_3OD$, δ in ppm relative to TMS)

C	**5**$^{\#}$ δc	**6**$^{\#}$ δc	**7**$^{\$}$ δc
1	33.40	33.41	31.86
2	20.27	20.88	18.88
3	38.91	38.93	37.36
4	44.83	44.84	43.52
5	50.62	50.60	42.14
6	22.19	22.20	28.36[b]
7	38.91	38.93	72.45
8	54.37	54.15	55.25
9	77.74	77.77	78.29[a]
10	45.69	45.69	44.59
11	29.56[a]	29.62[a]	28.25[b]
12	31.21	31.27	33.32
13	42.68	42.68	40.88
14	34.99	35.00	35.93
15	80.87	80.61	78.42[a]
16	157.45	157.66	154.31
17	110.45	110.49	111.15
18	29.62[a]	29.57[a]	28.89
19	182.09	182.07	184.21
20	18.16	18.16	17.68
1′	169.43	169.46	168.61
2′	130.24	129.76	127.93
3′	138.19	137.73	138.55
4′	14.35	15.92	15.82
5′	12.26	20.27	20.59

Where there are letters ([a,b]) assignments may be interchanged.

characteristic peaks at *m*/*z* 432, 414, 332 and 314 resulting from $[M]^+$, $[M–H_2O]^+$, $[M–C_5H_8O_2]^+$ and $[M–C_5H_8O_2–H_2O]^+$, respectively. This fragmentation indicated the presence of a C_5-unsaturated acyl acid residue which was identified as angelate according to the signals in the ^{1}H and ^{13}C NMR spectra (see Tables 1 and 2). The remaining ^{1}H and ^{13}C NMR data were very similar to those reported for *ent*-7α,9α,15β-trihydroxykaur-16-en-19-oic acid (Jakupovic et al., 1989) except for those of C-15 and 16 as well H-15. In the ^{13}C NMR spectrum the signal for C-15 was slightly shifted upfield, that for C-16 downfield. A pronounced downfield shift of $\delta = 1.2$ ppm was observed for H-15 in the ^{1}H NMR spectrum. These shifts are in agreement with an esterification of the C-15 hydroxyl group which was additionally confirmed by the correlation shown in the GHMQCR spectrum between C-1′ and H-15. The stereochemistry at C-15 followed from the chemical shift of C-15 (δ 78.42), which should be shifted upfield to about δ 75 in the case of a β-axial orientated OH-function (Buchanan et al., 1996). The location of the 7-OH group was confirmed by the correlations in the GHMQCR spectrum between H-7 and C-15 as well as H-7 and C-5, its axial configuration by the broad singulet for the carbinol methine proton H-7 in the ^{1}H NMR spectrum. The shape of the singulet resulted from

Table 2
^{1}H NMR data of diterpene **7** (300 MHz, $CDCl_3$)

H	7
1	1.52[a], 1.89[a]
2	1.88[a], 1.52[a]
3	1.09[a], 2.17[a]
5	2.06[a]
6	1.98[a], 2.08[a]
7	3.93 *s(br)*
11	1.98[a], 2.08[a]
12	1.61[a], 1.66[a]
13	2.83 *s(br)*
14	1.66[a], 1.93[a]
15	6.20 *s*
17	5.10 *s*, 5.27 *s*
18	1.25 *s* [b]
20	1.08 *s* [b]
3′	6.06 *qq* (1.5, 7.2)
4′	1.98 *dq* [b] (1.5, 7.2)
5′	1.88 *s(br)* [b]

J (Hz) in parentheses; *δ*-values (in ppm relative to TMS) determined from centre of cross peaks in the GHSQCR spectrum.
[a] Multiplicity not determined, overlapping signals.
[b] Three protons.

an unresolved doublet with a very small coupling constant due to an equatorially orientated proton at C-7 (Buchanan et al., 1996; Lobitz et al., 1998).

Whereas the *ent*-kaurane derivatives **1**–**3** and **9** are already known from this species (Stokes et al., 1992), compounds **4** and **10** are new for the genus *Oyedaea* and **5**, **6** and **8** for *O. verbesinoides*. To the best of our knowledge, diterpene **7** is new. Altogether, the diterpene pattern of *O. verbesinoides* is characteristic for the genus (Bohlmann and Zdero, 1979; Seaman et al., 1990). The sesquiterpene lactones from the melampolide type, which were previously described from *O. verbesinoides*, (Stokes et al., 1992) could not be detected. It has to be clarified whether the recent studied plant material was correctly identified, because sesquiterpene lactones are not characteristic compounds of the subtribe *Verbesiinae* to which the genus *Oeydaea* belongs according to Karis and Riding (1994).

2.2. Inhibitory activity on vascular smooth muscle contraction

The *ent*-kauranes **2**, **6** and **8** assayed in this study inhibited the rat vascular smooth muscle contraction induced by the selective α_1-adrenergic agonist phenylephrine (Phe) (see Table 3). Pre-treatment with compound **8** (10^{-5} and 10^{-4} mol/l) inhibited Phe-induced contraction in a time- and concentration-dependent manner. A concentration of 10^{-5} mol/l of **8** reduced aortic contraction after an incubation time of 30, 45, 60 and 90 min, but not of 5 or 15 min. The maximal inhibition was observed after 60 and 90 min of incubation. The higher concentration of 10^{-4} mol/l achieved maximal inhibition after 60 min of incubation.

Phe-induced contractions were inhibited by the diterpene **2** at 10^{-5} mol/l in a time-dependent manner with the maximal inhibition after 45 min of incubation. In contrast, the inhibitory activity observed at 10^{-4} mol/l was not time-dependent, since the maximum effect was already achieved after an incubation time of 5 min.

As shown in Table 3, exposure of aortic rings to compound **6** (10^{-5} and 10^{-4} mol/l) inhibited the Phe-induced contraction with a maximum after incubation for 5 min. The lower concentration (10^{-5} mol/l) induced no time-dependent inhibitory activity. This was the case with the higher concentration of 10^{-4} mol/l.

A comparison among the maximal effect elicited by these three kauranes at 10^{-4} mol/l showed that they inhibited the vascular contraction of aortic rings to the

Table 3
Percentage inhibition induced by *ent*-kauranes **2**, **6** and **8** upon phenylephrine (Phe)-induced contractility in isolated rat aorta using different incubation times

Min	**2**		**6**		**8**		Nifedipine
	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-6}
5	12.8±2.9[a] (5)	48.0±10.0[ac] (6)	28.8±7.9[a] (7)	52.2±11.5[ac] (5)	4.0±5.1 (4)	41.1±13.8[ac] (5)	–
15	18.0±5.1[a] (5)	35.9±11.3[ac] (8)	21.9±5.7[a] (5)	35.0±13.9[a] (6)	1.6±2.9 (4)	34.8±12.0[ac] (4)	–
30	23.8±9.1[a] (5)	34.1±7.8[ac] (5)	27.4±9.5[a] (5)	36.8±16.9[a] (6)	16.3±9.4[ad] (5)	39.3±16.1[ac] (5)	57.8±9.9[a] (8)
45	35.1±5.6[ab] (5)		25.7±7.3[a] (6)	–	16.9±14.9[ad] (5)	43.3±9.0[ac] (4)	–
60	33.2±13.5[ab] (6)		–	–	33.1±7.4[ade] (5)	67.8±13.6[acf] (5)	–
90			–	–	25.6±9.7[ade] (6)	–	

The rings were initially stimulated with Phe 10^{-7} mol/l (control group, 100% contraction) and a second stimulation was performed after incubation with the respective *ent*-kaurane. The numbers of trials are given in parenthesis, concentration in mol/l.
[a] Significant difference from respective control (0% inhibition).
[b] Significant difference from pre-incubation for 5, 15 and 30 min at 10^{-5} mol/l.
[c] Significant difference from respective period of incubation at 10^{-5} mol/l.
[d] Significant difference from pre-incubation for 5 and 15 min at 10^{-5} mol/l.
[e] Significant difference from pre-incubation for 30 and 45 min at 10^{-5} mol/l.
[f] Significant difference from pre-incubation for 5, 15, 30 and 45 min at 10^{-4} mol/l.

same extent. However, the period of incubation, necessary for **8** to induce this effect, was 60 min while **2** and **6** displayed their maximal effect already after a pre-incubation of 5 min.

Smooth muscle cells in the walls of blood vessels contain calcium channels and the activation of α_1-adrenergic receptors promotes calcium influx by these channels. The main calcium channel present in the smooth muscle cells is the L subtype (Orlov et al., 1996). Since extracellular calcium influx by L-type calcium channels is important in the contraction induced by α_1-adrenergic receptors, we compared the effects of the *ent*-kauranes with the effect of nifedipine, which is an L-type calcium entry blocker. It was noted that the three *ent*-kauranes and nifedipine inhibited the vascular contraction of aortic rings to the same extent. However, nifedipine was added to the medium bath at a concentration of 10^{-6} mol/l while the *ent*-kauranes were added at 10^{-4} mol/l indicating that nifedipine is more potent than the diterpenes **2**, **6** and **8**. From our data it cannot be concluded whether the diterpenes exert their inhibitory effects in the same way as nifedipine. To clarify the mode of action further studies are necessary.

However, some structure–activity relationships can be drawn from our results. Recently, it was reported that the occurrence of a free carboxyl group at C-19 is a prerequisite for an inhibitory activity on the contractions of rat uterus induced by serotonin (Campos-Bedolla et al., 1997). Here we show that diterpenes, such as compound **8**, which miss this structural element, also reduce the contraction of rat aorta, but the potency is less pronounced and a longer incubation time is necessary, 60 min compared to 5 min with compounds **2** and **6**. Together with previous results (Campos-Bedolla et al., 1997; Tirapelli et al., 2002) our studies show that diterpenes of the *ent*-kaurane type display antispasmodic activity on vascular and non-vascular smooth muscle. Therefore, it can be discussed whether these compounds may have also some effects on arterial blood pressure in vivo.

3. Experimental

3.1. General

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in CD_3OD and $CDCl_3$, using an Unity 300 (Fa. Varian) instrument. Chemical shift references were obtained by addition of TMS. Analytical and semi-preparative HPLC were carried out using a Waters 510 pump and a Waters 996 photodiode array detector. A S1990 Pump (Latek) was used for MPLC, together with a glass column (Latek) filled with Eurosil Bioselect 100-30, C-18 (100 Å, 20–45 μm); gradient: 50–100%, H_2O:MeOH, at 2.5 ml/min. A Waters Symmetry C_8 (4.6×250 mm; 5 μm particle size) column with a 15 min linear gradient of 50–100%, H_2O:MeOH, at 1 ml/min was used for analytical HPLC. The semi-preparative HPLC was carried out by a Waters Symmetry Prep C_8 (7.8×150 mm) with a flow rate of 3 ml/min, MeOH-H_2O (57:43), isocratic mode. MS data were obtained at following instruments: EIMS: MAT (Finnigan), 70 eV; ESI-MS: TSQ 7000 (Finnigan), high resolution MS: MAT 95 SL (Finnigan). UV spectra were recorded on an UVIKON 933 UV/vis spectrophotometer (Kontron Instr.) in EtOH.

3.2. Plant material

Aerial parts of *Oyedaea verbesinoides* were collected near Rio Corinto, Guápiles, Costa Rica, and identified by L. Poveda, Professor of Botany, Universidad Nacional, Costa Rica. Voucher specimens (No. JVR8102) are deposited at the Herbarium Journal Valerio, Universidad Nacional, Costa Rica.

3.3. Extraction and isolation

Dried arial parts of *O. verbesinoides* (1536 g) were extracted with Et_2O–MeOH (9:1). The crude extract (20.3 g) was treated with MeOH at −20 °C. The soluble part (18.9 g) was dried under reduced pressure, resolved in MeOH and fractionated by column chromatography on Sephadex LH 20 with methanol yielding 9 frs. CC of fr. 5 (14 g) on Sephadex LH 20 with cyclohexane–CH_2Cl_2–MeOH (7:4:1) followed by MPLC (RP 18, 20–45 μm, using MeOH-H_2O mixtures) gave a crude fraction from which compound **1** (1400 mg) was crystallized from MeOH. In a subfr. compounds **2** (25 mg), **3** (47 mg) and **8** (22 mg) were isolated by CC on silica gel with *n*-hexane and increasing amounts of Et_2O. In a further subfr. compounds **5** (18 mg) and **6** (26 mg) were separated by HPLC (RP_8, MeOH–H_2O (57:43), isocratic mode). Fr. 5.3 was fractionated by CC on Sephadex LH 20 with cyclohexane–CH_2Cl_2–MeOH (7:4:1), followed by MPLC (RP 8, 20–45 μm, with an aeq. MeOH mixture and increasing amounts of MeOH) leading to compounds **4** (3,7 mg), **9** (2,3 mg) and **10** (1,9 mg). CC of fr 5.6 on silica gel (n-hexane with increasing amounts of Et_2O) and finally crystallization from MeOH yielded compound **7** (57 mg).

3.4. ent-15β-Angeloyloxy-7α,9α-dihydroxy-kaur-16-en-19-oic acid (7)

Mp 189 °C (uncorr.) ; $[\alpha]_D$ −32.75° (MeOH; *c* 1.83); λ_{max} nm (log ε): 207 (3,8); ESIMS *m*/*z* (rel. int.): 433 $[M+H]^+$ (16), 455 $[M+Na]^+$ (58), 888 $[2\times M+Na+H]^+$ (100); EIMS *m*/*z* (rel. int): 432 $[M]^+$ (5), 414 $[M–H_2O]^+$ (5), 332 $[M–C_5H_8O_2]^+$ (26), 314 $[M–H_2O–C_5H_8O_2]^+$ (98), 300 (42), 285 (32), 269 (33),

260 (47), 213 (31), 193 (27), 161 (100); HR-EIMS *m/z*: 432.2516 (calc. for $C_{25}H_{36}O_6$); for ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.5. Activity on vascular smooth muscle contraction

3.5.1. Preparation of vessel rings

Male Wistar rats (200–250 g; 50–60 days old) were sacrificed in accordance with the Ethical Animal Committee from the Campus of Ribeirão Preto (University of São Paulo). Their thoracic aorta was quickly removed, cleaned of adherent connective tissues and cut into rings (4 mm). Two stainless-steel stirrups were passed through the lumen of each ring. One stirrup was connected to an isometric force transducer (Letica Scientific Instruments) to measure tension in the vessels. The rings were placed in a 10 ml organ chamber containing Krebs solution, maintained at 37 °C. The composition of Krebs solution was as follows (mmol/l): NaCI (118.0); KCI (4.7); KH_2PO_4 (1.2); $MgSO_4$ (1.2); $NaHCO_3$ (15.0); glucose (5.5); $CaCI_2$ (2.5).

The rings were initially stretched until a basal tension of 1.0 g and then allowed to equilibrate for 60 min with the bath fluid being changed every 15–20 min. The solution was bubbled with a gas mixture of 95% O_2–5% CO_2 in the organ baths to give a pH of 7.4. Endothelial integrity was qualitatively assessed by the degree of relaxation caused by acetylcholine (ACh, 10^{-6} mol/l, distilled H_2O; Sigma, St. Louis, MO, USA) in the presence of contractile tone induced by phenylephrine hydrochloride (Phe, 10^{-7} mol/l; distilled H_2O; Sigma, St Louis, MO, USA). If relaxation with ACh was not 80% or greater, the ring was discarded.

3.5.2. Experimental protocol

After the ACh test, the rings were washed four times for 30 min in Krebs solution gassed with 95% O_2–5% CO_2. After returning to the resting tension, the rings were contracted with Phe 10^{-7} mol/l (control). The rings were washed out and pre-incubated with compound **2** (10^{-5} mol/l) for 5, 15, 30, 45 or 60 min, **8** (10^{-5} mol/l) for 5, 15, 30, 45, 60 or 90 min and **6** (10^{-5} mol/l) for 5, 15, 30 or 45 min. After these periods of incubation a new stimulation was performed with Phe. In a second set of experiments, the same experimental protocol was used to investigate the effects of pre-incubation with **2** (10^{-4} mol/l) for 5, 15 or 30 min, **8** (10^{-4} mol/l) for 5, 15, 30, 45 or 60 min and **6** (10^{-4} mol/l) for 5, 15 or 30 min. The effect of the calcium channel blocker nifedipine (10^{-6} mol/l, 30 min) was also analyzed.

The two stimulations with Phe were determined on the same ring, so that each ring served as its own control. The first stimulation determined with the diterpenoids before the pre-incubation was considered as control. Vessel rings from the same animal that were not exposed to the diterpenoids served as time controls. The three diterpenoids were dissolved in ethanol and added to the medium bath. Assays carried out using only ethanol showed that this solvent did not affect the vascular contractility induced by Phe.

3.5.3. Statistical analysis

Data were calculated as percentage of inhibition and shown as mean ± S.E.M. Comparison with the control group was performed by the paired Student's *t*-test and comparison of mean values between two independent treated groups was performed by the unpaired Student's *t*-test. Statistical analysis among more than three groups was evaluated using one way analysis of variance (ANOVA) followed by Newman–Keuls post-hoc test. $P < 0.05$ was considered as significant.

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References

Alvarez, L., Mata, R., Delgado, G., Romo de Vivar, A.R., 1985. Sesquiterpene lactones from *Viguiera hypargyrea*. Phytochemistry 24, 2973–2976.

Bohlmann, F., Zdero, C., 1979. Ein neues Kaurensäure- und ein Euparin-Derivat aus *Oyedaea*-Arten. Phytochemistry 18, 492–493.

Buchanan, M.S., Connolly, J.D., Kadir, A.A., Rycroft, D.S., 1996. Sesquiterpenoids and diterpenoids from the liverwort *Jungermannia truncata*. Phytochemistry 42, 1641–1646.

Campos-Bedolla, P., Campos, M.G., Valencia-Sanchez, A., Ponce-Monter, H., Uribe, C., Osuna, L., Calderon, J., 1997. Effect of kauranes from *Montanoa* spp. on rat uterus. Phytotherapy Research 11, 11–16.

Da Costa, F.B., Vichnewski, W., Herz, W., 1996. Constituents of *Viguiera aspilloides* and *V. robusta*. Biochemical Systematics and Ecology 24, 585–587.

Enriquez, R.G., Barajas, J., Ortiz, B., Lough, A.J., Reynolds, W.F., Yu, M., Leon, I., Gnecco, D., 1997. Comparison of crystal and solution structure and ^1H and ^{13}C chemical shifts for grandifloric acid, kaurenoic acid, and monoginoic acid. Canadian Journal of Chemistry 75, 342–347.

Gonzales, A.G., Fraga, B.M., Hernandez, M.G., Hanson, J.R., 1981. The ^{13}C NMR spectra of some *ent*-18-hydroxykaur-16-enes. Phytochemistry 20, 846–847.

Hanson, J.R., Siverns, M., Piozzi, F., Savona, G., 1976. The ^{13}C NMR magnetic resonance spectra of kauranoid diterpenes. Journal of the Chemical Society Perkin Transactions 1, 114–117.

Hayman, A.R., Weavers, R.T., 1990. Terpenes of foliage oils from *Halocarpus bidwilli*. Phytochemistry 29, 3157–3162.

Herz, W., Kulanthaivel, P., Watanabe, K., 1983. *ent*-Kauranes and other constituents of three *Helianthus* species. Phytochemistry 22, 2021–2025.

Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M., Bohlmann, F., 1989. Twenty-one acylphloroglucinol derivatives and further constituents from South African *Helicrysum* species. Phytochemistry 28, 1119–1131.

Karis, P.O., Riding, O., 1994. Tribe Heliantheae. In: Bremer, K. (Ed.), Asteraceae—Cladistics and Classifications. Timber Press, Portland, OR, pp. 559–624.

Lobitz, G.O., Tamayo-Castillo, G., Poveda, L., Merfort, I., 1998. Kaurene diterpenes from *Mikania vitifolia*. Phytochemistry 49, 805–809.

Lu, I., Parodi, F.J., Vargas, D., Quijano, L., Mertooetomo, E.R., Hjortso, M.A., Fischer, N.H., 1993. Sesquiterpenes and thiarubrines from *Ambrosia trifida* and its transformed roots. Phytochemistry 33, 113–116.

Moss, G.P., 1989. The nomenclature of steroids. European Journal of Biochemistry 186, 429–458.

Orlov, S.N., Tremblay, J., Hamet, P., 1996. cAMP signaling inhibits dihydropiridine-sensitive Ca^{2+} influx in vascular smooth muscle cells. Hypertension 27, 774–780.

Piozzi, F., Passannanti, S., Paternostro, M.P., 1971. Kauranoid diterpenes in *Espeletia grandiflora*. Phytochemistry 10, 1164–1166.

Rüngeler, P., Brecht, V., Tamayo-Castillo, G., Merfort, I., 2001. Germacranolides from *Mikania guaco*. Phytochemistry 56, 475–489.

Seaman, F., Bohlmann, F., Zdero, C, Mabry, T.J., 1990. Diterpenes of Flowering Plants, Compositae. Springer-Verlag, Heidelberg, New York.

Stokes, S., Castro, V., Poveda, L., Papastergiou, F., Jakupovic, J., 1992. Melampolides from *Oyedaea verbesinoides*. Phytochemistry 31, 2894–2896.

Tirapelli, C.R., Ambrosio, S.R., Da Costa, F.B., De Oliveira, A.M., 2002. Inhibitory action of kaurenoic acid from *Viguiera robusta* (Asteraceae). Fitoterapia 73, 56–62.