Effects of Kaurane Diterpene Derivatives on Germination and Growth of *Lactuca sativa* Seedlings

Henriete S. Vieira, Jacqueline A. Takahashi, Lúcia P. S. Pimenta, and Maria Amélia D. Boaventura*

Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte – MG, Brazil. Fax: 55 31 34 99 57 00. E-mail: dianadb@dedalus.lcc.ufmg.br

* Author for correspondence and reprint requests

Z. Naturforsch. **60 c,** 72–78 (2005); received May 5/August 23, 2004

Kaurenoic and grandiflorenic acid, isolated from *Wedelia paludosa* (Asteraceae), some derivatives from these acids (alcohols, esters, amides, lactones, oximes) and other naturally occurring kaurane diterpenes were tested for their action on the growth of radical and shoot of *Lactuca sativa*. Gibberellic acid, GA_3, a commercially available phytohormone, belonging to the same class of diterpenes, was also tested. Some of the tested substances showed a remarkable activity either in the inhibition or in stimulation of *L. sativa* growth. The activity, in some cases, was even higher than that of GA_3.

Key words: Gibberellic Acid, Kaurenoic Acid, Allelopathic Activity

Introduction

A number of natural products with allelopathic activity has been reported and used in agriculture, as for example the gibberellins, a group of diterpene lactones. Kaurane diterpenes containing a rigid tetracyclic skeleton are intermediates in the biosynthesis of a number of plant and fungal metabolites, including gibberellins, and are widespread in the plant kingdom. Bioassay-guided fractionation in plant study made this class of diterpenes to be "rediscovered", due to their many biological activities (Ghisalberti, 1997), including plant growth regulation (Torrenegra and Tellez, 1996; Villalobos *et al.*, 1994; Hanson *et al*., 1980; Becker and Kempf, 1976; Hüneck and Scheiber, 1972; Cross *et al*., 1970; Katsumi *et al*., 1964).

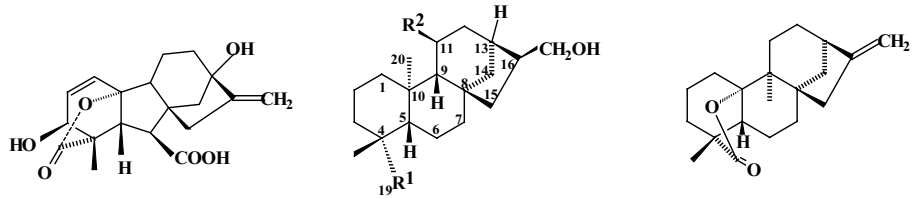
In the phytochemical study of *Wedelia paludosa* (Asteraceae) we have isolated a high amount (ca. 10% of crude extract, in some cases) of *ent*-kaur-16-en-19-oic acid [kaurenoic acid, (**2**)], together with a minor proportion (ca. 2–3% of crude extract) of *ent*-kaur-$\Delta^{9(11)}$,16-dien-19-oic acid [grandiflorenic acid (**3**)], among other kaurane diterpenes **11**–**13** (Fig. 1); *ent*-kauran-16βH-17-ol (**5**) was isolated from *Xylopia frutescens* (Anonnaceae). Some bioassay systems were applied on these compounds (and derivatives obtained by chemical transformations of **2** and **3**, Fig. 1), as for example, trypanocidal (Vieira *et al*., 2002) and allelopathic evaluations. We describe here the results of the lettuce (*Lactuca sativa*) hypocotyl assay.

Results and Discussion

Compounds **2**–**20** (Fig. 1) were obtained by isolation from plant extracts and/or chemical transformations (Vieira *et al*., 2001, 2002; Takahashi *et al*., 1995, 2001). Lactones **21** and **22** are described here for the first time to our best knowledge. The structure of lactone **23** was previously elucidated by X-ray (Doriguetto *et al*., 2002). Their syntheses are showed in Scheme 1. Intermediary ketones **24** and **25** were obtained from **2** and **3**, respectively, initially by exocyclic double bond oxidation (Castellaro *et al*., 1990), followed by Baeyer-Villiger rearrangement (Anastasia *et al*., 1985). Lactone **22** was obtained from triol **8** by oxidation with $KMnO_4/CuSO_4$ (Jefford and Wang, 1988).

The choice of concentrations to be used in allelopathic experiments was based on the work of Macías *et al.* (1994, 2000). According to them, substances with inhibitory activity against species used in standard allelopathic bioassays show a strong inhibitory effect (alternative herbicides) only at concentrations between 10^{-2} and 10^{-3} M; at lower concentrations (10^{-5}–10^{-9} M) this effect disappears or becomes stimulatory. Plants were measured after 5 d standing in the dark, according procedures described by Hoad *et al.* (1981) and Macías *et al*.

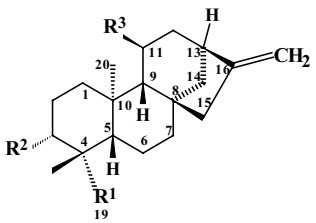
0939–5075/2005/0100–0072 $ 06.00

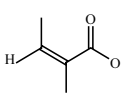
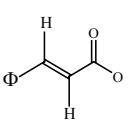
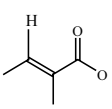


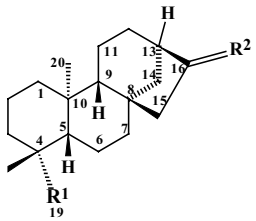
1

	R^1	R^2
5	CH_3	H
7	CH_2OH	H
8	CH_2OH	OH

20



	R^1	R^2	R^3
2	COOH	H	H
3	COOH	H	Δ(9,11)
4	COOH	OH	H
6	CH_2OH	H	H
9	$COOCH_3$	H	H
10	$COOCH_3$	OH	H
11	COOH		H
12	COOH		H
13	COOH		H

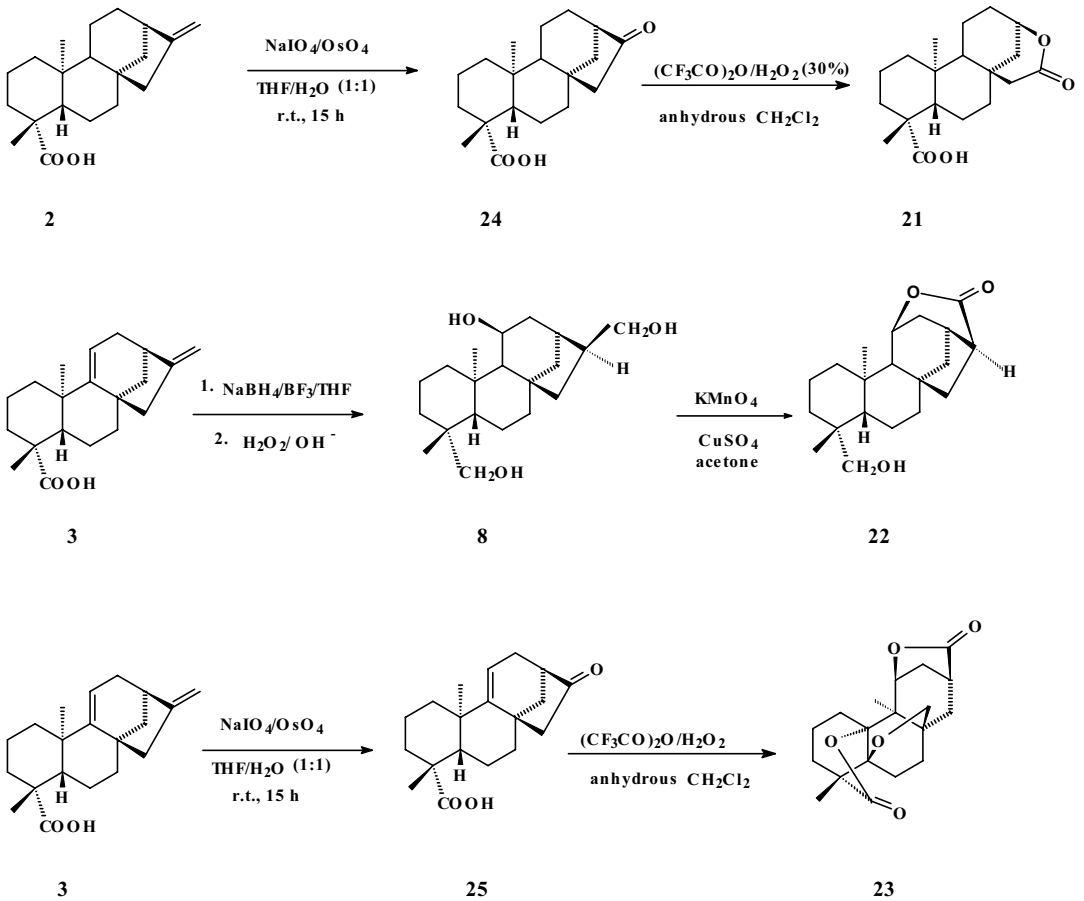


	R^1	R^2
14	$COOCH_3$	NOH (Z)
15	$COOCH_3$	NOH (E)
16	CH_2OH	NOH(Z)
17		CH_2
18		CH_2
19	$CON(CH_2CH_3)_2$	CH_2

Fig. 1. Structures of gibberellic acid (**1**) and kaurane diterpenoids **2**–**20**.

(2000). The experimental results of germination, radical and shoot length in the lettuce hypocotyl assay for the kaurane diterpenoids **2**–**23** and gibberellic acid, GA_3 (**1**), are shown in Fig. 2–5. The numbers are expressed as percent units from the control: zero represents an observed value identical to the control, a positive value represents stimulation and a negative value represents inhibition.

Gibberellins are physiologically defined by their ability to induce shoot elongation in certain dwarf plants which was demonstrated in various plant extracts using dwarf maize plants and dwarf peas. With *Lactuca sativa*, in the dark, Frankland and Wareing (1960) demonstrated that hypocotyl growth was very little affected by gibberellic acid treatment at 10^{-4} M.



Scheme 1.

The acids **1**–**4** presented stronger action on radical than on shoot growth (Fig. 2). The best activity for **1** was at 10^{-7} M and for **3** at 10^{-3} M, although the latter was active in all three tested concentrations. Gibberellic acid (**1**) inhibited radical growth at higher concentrations, contrary to kaurenoic acid (**2**), that acted in an opposite way. Brian *et al*. (1967) did not find activity for **2** in the lettuce hypocotyl assay at 10 ppm (3.3×10^{-5} M) concentration.

Acids **1**–**3** were active on shoot growth (Fig. 2); compound **4** (*ent*-3β-hydroxy-kaur-16-en-19-oic acid) showed activity only at 10^{-7} M, the presence of the hydroxyl group at C-3 being the differentiating structural factor. The stereochemistry of hydroxyl groups in gibberellins is important: 3β-hydroxy gibberellins, in the absence of a 2β-hydroxy group, are more active than the correspondent 3α-hydroxy derivatives (Hoad *et al*., 1981). Therefore, the small activity of compound **4**, compared to the

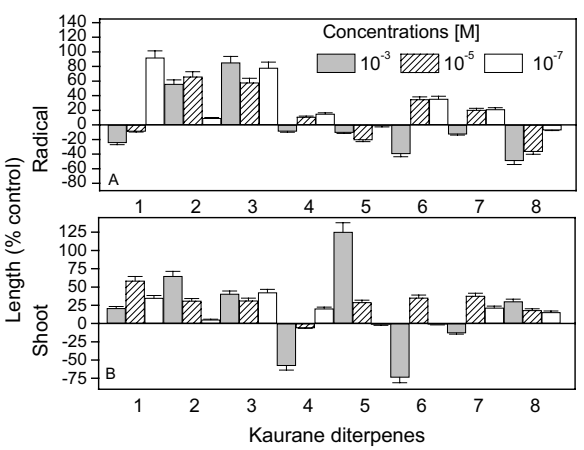


Fig. 2. Effect of gibberellic acid (**1**), kaurane diterpene acids **2**–**4** and alcohols **5**–**8** on: (A) radical and (B) shoot length of *L. sativa.*

other acids, could be also associated with the α stereochemistry of the hydroxyl group at C-3.

Oppositely to the acids, the alcohols **5**–**8** showed better stimulating activity on shoot growth (Fig. 2), mainly *ent*-kauran-16βH-17-ol (**5**) with the higher stimulatory effect at 10^{-3} M; in contrast, Brian *et al.* (1967) cited the slight activity presented by **5** in the lettuce hypocotyl assay at 10 ppm (3.5×10^{-5} M) concentration. *Ent*-kauran-16-en-19-ol (**6**), the higher inhibitor among alcohols, and diol **7** showed analogous activity on radical elongation; triol **8** acted in the opposite way on promoting shoot growth and inhibiting radical growth. Only gibberellic acid (**1**) stimulated the germination of *L. sativa.* (Fig. 3A). Among the esters (**9**–**13**), *ent*-3β-tygloyloxykaur-16-en-19-oic acid (**13**) was the most active on radical growth, at 10^{-7} M, the lower dosis (Fig. 4). Methyl *ent*-kaur-16-en-19-oate (**9**) was the best inhibitor on shoot growth at 10^{-3} M (Fig. 4). According to Villalobos *et al.* (1994), the presence of an angeloyloxy group at C-18, associated with a methyl ester at C-19, improves the activity. Both *E* oximes **15** and **16** showed analogous results on radical and on shoot growth. Here, the *E* stereochemistry of the oxime group seems to be the determinant factor for growth activity. The kaurenoic ester **9** showed the best germination results, at 10^{-5} M, among all tested compounds, followed by tygloyloxy ester **13** and ester *Z* oxime **14** (Fig. 3B). Amide **18** (*ent*-kaur-16-en-19-pyrrolidinamide) was active in both

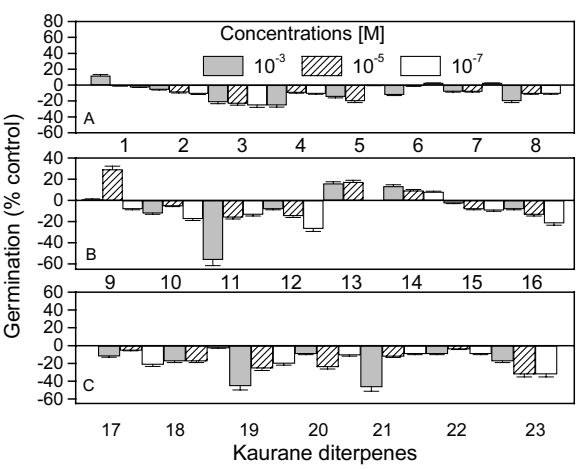


Fig. 3. Effect of (A) gibberellic acid (**1**), kaurane diterpene acids **2**–**4** and alcohols **5**–**8**; (B) kaurane diterpene derivatives esters **9**–**13**, ester oximes **14**–**15**, alcohol oxime **16**; (C) kaurane diterpene amides **17**–**19** and lactones **20**–**23** on the germination of *L. sativa*.

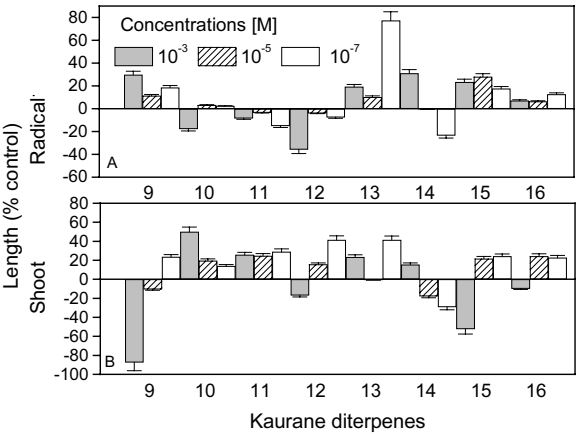


Fig. 4. Effect of kaurane diterpene derivatives esters **9**–**13**, ester oximes **14**–**15** and alcohol oxime **16** on: (A) radical and (B) shoot length of *L. sativa.*

shoot and radical elongation and in all three concentrations (Fig. 5). In general, all three amides **17**, **18** and **19** showed a total coherence in their results for radical and shoot growth. Tetrachirin (**20**) and lactone **23** showed similar activities in both radical and shoot growth (Fig. 5). Lactone **21**, from kaurenoic acid, presented the best stimulatory (10^{-5} M) and the best inhibitory (10^{-3} M) effect on shoot elongation of *L. sativa* (Fig. 5).

Finally, only grandiflorenic acid (**3**) and *ent*-kaur-16-en-19-pyrrolidinamide (**18**) showed action on both shoot and radical length in all concentrations; kaurenoic acid (**2**) had analogous action in radical and shoot growth; gibberellic acid (**1**) acted

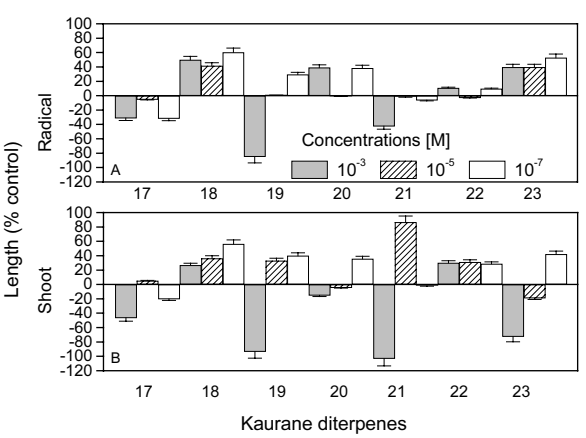


Fig. 5. Effect of kaurane diterpene derivatives amides **17**–**19** and lactones **20**–**23** on: (A) radical length of *L. sativa* and (B) shoot length of *L. sativa.*

in an opposite way compared to lactone **23**: its profile activity in radical corresponds to the shoot in **23** and vice-versa. Results found for the inhibitory effect for most of the tested substances corroborate with the proposition found in the literature (Macías *et al*., 2000) that inhibition occurs mainly at concentrations around at 10^{-3} M. These substances are consequently potential alternative herbicides.

Experimental

General procedure

Melting points were determined with a Kofler hot plate apparatus and are uncorrected. The optical rotations were measured on a Perkin Elmer 341 polarimeter. IR spectra were recorded on a Shimadzu IR-408 spectrophotometer. IR absorption bands are expressed in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker Advance DPX 200 operating at 200 and 50 MHz, respectively. The chemical shifts are reported in δ values (ppm) relative to the solvent $CDCl_3$ (δ = 7.26 for ^1H NMR and 77.01 ppm for ^{13}C NMR). Mass spectra (GC-EI-MS) were obtained from a GCQ Finnigan-ION TRAP instrument and they were performed with an ionizing energy of 70 eV. Silica gel used for flash chromatography was obtained from Merck (WC4790-005, 230–400 mesh) and celite from Labsynth Ltda., São Paulo, SP, Brazil.

Gibberellic acid (**1**) was purchased from Sigma (USA). *Ent*-kaur-16-en-19-oic acid (kaurenoic acid, **2**) was isolated from green fruits of both *Xylopia frutescens* (Takahashi *et al*., 1995) and *Xylopia sericea* (Takahashi *et al*., 2001) and aerial parts of *Wedelia paludosa* (9.6% of ethanolic crude extract). Grandiflorenic acid (**3**) was isolated from ethanolic extracts of aerial parts of *Wedelia paludosa* (2.4% of crude extract). *Ent*-3β-hydroxy-kaur-16-en-19-oic acid (**4**) was obtained by alkaline hydrolysis from *ent*-3β-cinamoyloxykaur-16-en-19-oic acid (**12**, yield: 84%). *Ent*-kauran-16βH-17-ol (**5**) was isolated from the hexanic extract of green fruits of *Xylopia frutescens* (2.2% of crude extract). *Ent*-kauran-16-en-19-ol (**6**) was obtained by reduction of **9** with $LiAlH_4$ (yield: 86%). *Ent*-kauran-16βH-17,19-diol (**7**) and *ent*-kauran-16βH-11α,17,19-triol (**8**) were obtained from **2** and **3**, respectively, by treatment with diborane/H_2O_2 (Vieira *et al*., 2002). Methyl *ent*-kaur-16-en-19-oate (**9**) and methyl *ent*-kaur-3β-hydroxy-16-en-19-oate (**10**) were obtained by treatment of **2** and **4**, respectively, with diazomethane (quantitative yield). *Ent*-3β-angeloyloxykaur-16-en-19-oic acid (**11**, 2.4% of crude extract), *ent*-3β-cinamoyloxykaur-16-en-19-oic acid (**12**, 1.9% of crude extract) and *ent*-3β-tygloyloxykaur-16-en-19-oic acid (**13**, 0.065% of crude extract) were isolated from ethanolic extracts of aerial parts of *Wedelia paludosa* (Vieira *et al*., 2001). Methyl *ent*-16*Z*-oxime-17-norkauran-19-oate (**14**), methyl *ent*-16*E*-oxime-17-norkauran-19-oate (**15**), *ent*-16*E*-oxime-17-norkauran-19-ol (**16**), *ent*-kaur-16-en-19-piperidinamide (**17**) *ent*-kaur-16-en-19-pyrrolydinamide (**18**) and *ent*-kaur-16-en-19-*N*,*N*-diethylamide (**19**) were obtained according to the route described by Vieira *et al.* (2002). Tetrachirin (**20**, 0.068% of crude extract) was isolated from ethanolic extracts of aerial parts of *Wedelia paludosa* (Vieira *et al*., 2001).

Ent-kauran-16-oxo-17-nor-19 oic acid (**24**) *and ent-kaur-16-oxo-17-nor-11(9)-en-oic acid* (**25**) (Castellaro *et al*., 1990). To a suspension containing a mixture (500 mg) of kaurenoic acid (**2**) and grandiflorenic acid (**3**) and 1.6 g (8.0 mmol) of $NaIO_4$ in 50 ml of THF/H_2O 1:1 v/v a crystal of OsO_4 was added. After overnight stirring at room temperature, work-up (treatment by $NaHSO_3$) and flash chromatography, 173 mg of **24** and 198 mg of **25** were obtained.

Ent-13α-hydroxy-17-nor-13,16-seco-kauran-16,19-dioic acid 16→13-lactone (**21**) and *ent-5α,15α-epoxy-9,10-friedo-10β,11β-dihydroxy-16,11α:19,10β-diseco-17-norkauran-16,19-dioic acid 16→11:19→10-dilactone* (**23**) (Anastasia *et al.*, 1985). Trifluoroacetic acid was generated *in situ* by adding 148 mmol of trifluoroacetic anhydride to 31 mmol of 30% hydrogen peroxide at 0 °C in anhydrous CH_2Cl_2. Ketone (0.99 mmol), dissolved in anhydrous CH_2Cl_2, was added and the solution stirred for 1 h at room temperature. Work-up (2% K_2CO_3 solution) and flash chromatography (*n*-hexane/ethyl acetate) furnished pure lactones (yields: **21**: 80% and **23**: 19%).

Ent-13α-hydroxy-17-nor-13,16-seco-kauran-16, 19-dioic acid 16→13-lactone (**21**): White powder, $C_{20}H_{30}0_4$ (334). M.p. 218–219 °C. – $[\alpha]_{25}^{D}$ –110° (c = 0.002, $CHCl_3$). – IR (KBr) ν_{max} = 3400, 1725, 1690, 1250–1111 cm^{-1}. – ^1H NMR ($CDCl_3$, 200 MHz): δ = 0.97 (3H, s, 20-CH), 1.26 (3H, s, 18-CH), 4.68 (1H, br s, 13-CH). – ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 40.9, 18.9, 37.3, 43.5, 56.6, 19.8, 42.9, 33.8, 53.0, 39.5, 16.3, 28.7, 75.8, 33.0,

47.9, 172.4, 28.9, 184.0, 16.3. – EI-MS: *m/z* (rel. int.) = 320 (20) [$M^{\cdot+}$], 302 (80) [$M^+ - H_2O$], 260 (40) [$M^+ - CH_3CO_2H$], 215 (100) [$260 - CO_2H$].

Ent-11α,19-dihydroxy-16β-kauran-17-oic acid 17→11-δ-lactone (**22**) (Jefford and Wang, 1988). To a solution containing 48.3 mmol of triol **8** in acetone, $KMnO_4$ (1.0 mmol) and $CuSO_4 \cdot 5H_2O$ (0.06 mmol) were added and the mixture was stirred for 2 d at room temperature. After removing of acetone, the residue was chromatographed (celite followed by silica gel) and the lactone **22** was eluted with CH_2Cl_2 (47% yield) as a white powder, $C_{20}H_{30}O_3$ (318). – M.p. 239–240 °C. $[\alpha]_{25}^{D}$ –27° (c = 0.01, $CHCl_3$). – IR (KBr) ν_{max} = 3300, 1725 cm^{-1}. – ^1H NMR ($CDCl_3$, 200 MHz): δ = 0.98 (3H, s, H-20), 3.46 (1H, d, J = 11.0 Hz, 19a-CH_2), 3.75 (1H, d, J = 11.0 Hz, 19b-CH_2), 0.99 (3H, s, 18-CH), 2.97 (1H, dd, J = 8.0 and 10.0 Hz, 16a-CH_2), 2.69 (1H, br s, 13-CH), 4.58 (1H, br s, 11-CH). – ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 40.1, 18.1, 36.1, 38.6, 56.5, 20.4, 38.8, 44.2, 59.4, 37.4, 75.7, 35.2, 34.3, 41.9, 48.3, 42.5, 177.3, 27.2, 65.2, 17.9. – EI-MS: *m/z* (rel. int.) = 318 (18) [$M^{\cdot+}$], 300 (10) [$M^+ - H_2O$], 287 (100) [$M^+ - CH_2OH$], 269 (40) [$300 - H_2OH$], 241 (35) [$287 - HCO_2H$], 225 (20) [$269 - CO_2$].

Bioassay

Seeds of *Lactuca sativa* (cv. Grand Rapids) were purchased from Isla Pak, RS, Brasil. All undersized and damaged seeds were discarded. In 100 mm Petri dishes containing a 90 mm sheet of Whatman no. 1 filter paper and 10 ml of a test (10^{-3}, 10^{-5} and 10^{-7} M) or control solution 25 lettuce seeds were added. Test and control solutions were prepared with dionized water and their pH values [buffered with 10 mM 2-(*N*-morpholino)-ethanesulfonic acid, MES] were adjusted to 6.0–6.5 with NaOH solution. Lower concentrations than 10^{-3} M were obtained by dilution of the previous solution. There were 3 replicates for each concentration and for control. The dishes were sealed and incubated in the dark at 25 °C for 5 d. After this time the dishes were frozen during the measurement process to avoid subsequent growth (Macías *et al*., 2000). The osmotic pressure values were measured on a microosmometer (Precision Systems Inc., Natick, Mass. USA**)** and ranged between 30 and 38 mosmolar (Macías *et al*., 1994).

Statistic treatment

Statistic treatment was done according to Macías *et al*. (1994) and the results, presented in Fig. 2–5, consist of the differences (in cm) between mean values of seeds grown with tested compounds **1**–**23** and mean values for control (seeds grown without addition of tested compounds). Mean values of the controls (in mm) were: Fig. 2 = 2.1645×10^3; Fig. 4 = 2.6871×10^3; Fig. 5 = 2.3217×10^3. The germination, radical and shoot length values were tested by the Student's t-test and the differences between the experiment and control were significant at a value of P = 0.05.

Aknowledgement

To CNPq for Henriete S. Vieira's grant.

Anastasia M., Allevi P., Ciuffreda P., Fiecchi A., and Scala A. (1985), Synthesis of (2*R*,3*S*,22*R*,23*R*)- and (2*R*,3*S*,22*S*,23*S*)-2,3,22,23-tetrahydroxy-B-homo-7a-oxa-5α-ergostan-7-one, two new brassinolide analogues. J. Org. Chem. **50**, 321–325.

Becker H. and Kempf T. Z. (1976), Untersuchung der Grandiflorensäure [kaura Δ9(11),16-dien-18-carbonsäure] auf Gibberellinaktivität. Z. Pflanzenphysiol. **80**, 87–91.

Brian P. W., Grove J. F., and Mulholland T. P. C. (1967), Relationships between structure and growth-promoting activity of the gibberellins and some allied compounds, in four test systems. Phytochemistry **6**,1475–1499.

Castellaro S. J., Dolan S. C., MacMillan J., and Willis C. L. (1990), Deuterium labeling of *ent*-kaur-16-en-19-oic acid at carbon-6 and -7. Phytochemistry **29**, 1823–1831.

Cross B. E., Stewart J. C., and Stoddart J. L. (1970), 6β,7β-Dihydroxykaurenoic acid: its biological activity and possible role in the biosynthesis of gibberellic acid. Phytochemistry **9**, 1065–1071.

Doriguetto A. C., Vieira H. S., Ellena J. A., Takahashi J. A., Boaventura M. A. D., and Mascarenhas Y. P. (2002), A novel diterpene lactone. Acta Cryst. E**58**, 1392–1394.

Frankland B. and Wareing P. F. (1960), Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature **185**, 255–256.

Ghisalberti E. I. (1997), The biological activity of naturally occurring kaurane diterpenes. Fitoterapia **LXVIII**, 303–325.

Hanson J. R., Willis C. L., and Parry K. P. (1980), The inhibition of gibberellic acid biosynthesis by *ent*-kauran-16β,17-epoxyde. Phytochemistry **19**, 2323–2325.

Hoad G. V., Phinney B. O., Sponsel V. M., and MacMillan J. (1981), The biological activity of sixteen gibberellin A_4 and gibberellin A_9 derivatives using seven bioassays. Phytochemistry **20**, 703–713.

Hüneck S. and Schreiber K. (1972), Wachstumsregulatorische Eigenschaften von Flechten- und Moosinhaltsstoffen. Phytochemistry **11**, 2429–2434.

Jefford C. W. and Wang Y. (1988), Selective heterogeneous oxidation of alcohols and diols with potassium permanganate. J. Chem. Soc. Chem. Comm., 634–635.

Katsumi M., Phinney B. O., Jefferies P. R., and Henrick C. A. (1964), Growth response of the d-5 and an-1 mutants of maize to some kaurene derivatives. Science **44**, 849–850.

Macías F. A., Simonet A. M., and Esteban M. D. (1994), Potential allelopathic lupane triterpenes from bioactive fractions of *Melilotus messanensis*. Phytochemistry **36**, 1369–1379.

Macías F. A., Castellano D., and Molinillo J. M. G. (2000), Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. J. Agric. Food Chem. **48**, 2512–2521.

Takahashi J. A., Boaventura M. A. D., Bayma J. C., and Oliveira A. B. (1995), Frutoic acid, a dimeric kaurane diterpene from *Xylopia frutescens*. Phytochemistry **40**, 607–609.

Takahashi J. A., Vieira H. S., and Boaventura M. A. D. (2001), Mono and diterpenes from seeds of *Xylopia sericea*. Quim. Nova **24**, 616–618.

Torrenegra R. D. and Tellez A. A. N. (1996), Phytochemistry of *Espeletia killipii Cuatr*., and gibberellic activity of some isolated compounds. Rev. Latinoam. Quim. **24**, 2–6.

Vieira H. S., Takahashi J. A., and Boaventura M. A. D. (2001), Constituents from aerial parts of *Wedelia paludosa*. Fitoterapia **72**, 854–856.

Vieira H. S., Takahashi J. A., Oliveira A. B., Chiari E., and Boaventura M. A. D. (2002), Novel derivatives of kaurenoic acid: preparation and evaluation of their trypanocidal activity. J. Braz. Chem. Soc. **13**, 151–157.

Villalobos N., Martín L., Macías M. J., Mancheño B., and Grande M. (1994), Gibberellin-like activity of some tetracyclic diterpenoids from *Elaeoselinum* species and their derivatives. Phytochemistry **37**, 635–639.