

## Preparation and Phytotoxicity of Novel Kaurane Diterpene Amides with Potential Use as Herbicides

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Novel kaurane diterpene monoamides were synthesized in good yields directly from kaurenoic (**1**) and grandiflorenic (**2**) acids and unprotected symmetrical diamines, using a modified protocol for monoacylation. Amides from **1** and **2** and monoamines were also obtained and tested against seed germination and growth of radicle and shoot of *Lactuca sativa* (lettuce), at  $10^{-3}$ ,  $10^{-5}$ , and  $10^{-7}$  M. Amides from symmetrical diamines showed significant inhibitory activity at higher concentrations.

**KEYWORDS:** Kaurane diterpene amides; phytotoxicity; *Lactuca sativa*

### INTRODUCTION

Bioactive natural products often offer new and diverse chemical templates, which may be elaborated by limited synthesis, to produce environmentally compatible agrochemicals, such as potential herbicides and plant growth regulators, among others (*1*).

Kaurane diterpenoids, containing a rigid tetracyclic skeleton, are a very important class of natural products and are widespread in the plant kingdom. They are intermediates in the biosynthesis of a number of plant and fungal metabolites, including gibberellins, well-known fungal plant growth hormones used in agriculture. They possess a considerable number of important biological activities, such as antimicrobial, antiparasitic, insect antifeedant, cytotoxic, anti-HIV, antifertility, hypotensive, antiinflammatory (*2*), and plant growth regulation (*3–9*). Kaurenoic acid (**1**) can be easily isolated in good yields from plants of the Annonaceae (*10*) and Asteraceae (*11*) families and from seedless sunflower heads, discarded as waste in the edible sunflower industry (*12*). The ready availability of **1** from natural sources makes it a suitable starting material for synthesis of novel derivatives for biological screening.

We have already prepared and tested kaurenoic (**1**) and grandiflorenic acids (**2**), isolated from *Wedelia paludosa* (Asteraceae), some derivatives obtained from these acids (alcohols, esters, amides, lactones, oximes), and other naturally occurring kaurane diterpenes for their action on the growth of radicle and shoot of lettuce (*Lactuca sativa* L.). Gibberellic acid, GA<sub>3</sub> (**3**), a commercially available gibberellin, was also tested. Some of the compounds showed remarkable activity either in inhibiting or stimulating *L. sativa* growth, and this activity was even higher than that of GA<sub>3</sub> in some cases (*13*).

Herein, we report the preparation of novel monoamides **4–19** (Figure 1), obtained from the reaction of kaurenoic (**1**) and grandiflorenic (**2**) acids with monoamines and symmetrical diamines, and the effects of these compounds on germination and growth of lettuce (*Lactuca sativa* L.), from  $10^{-3}$  to  $10^{-7}$  M.

### MATERIALS AND METHODS

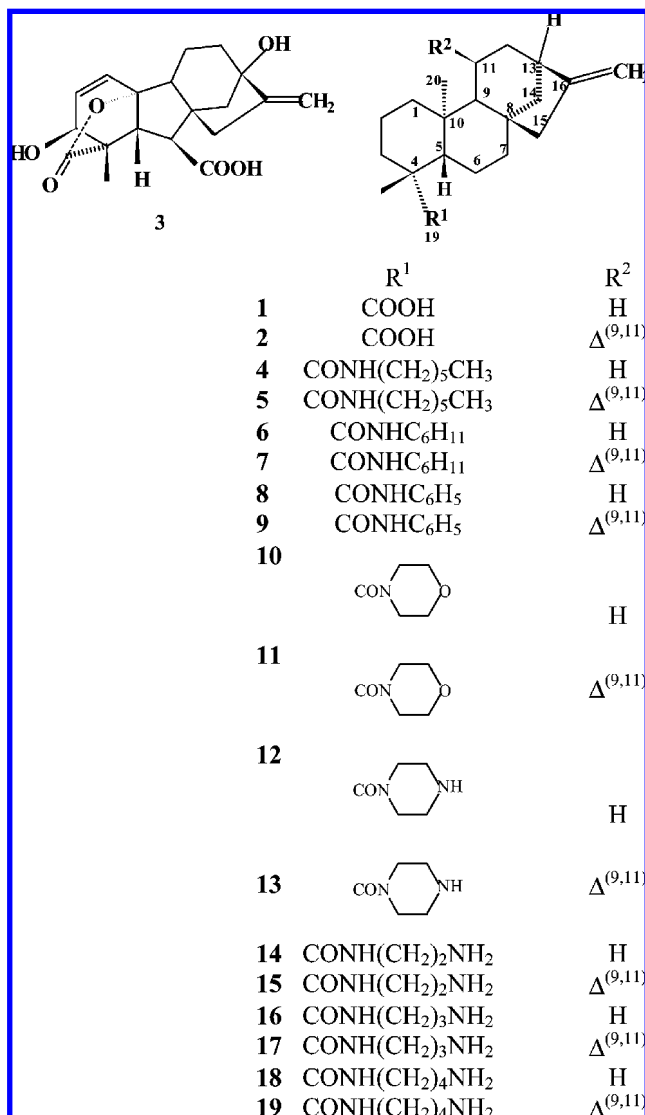
**Instrumentation.** Melting points were determined with a Kofler hot plate apparatus and are uncorrected. Infrared (IR) spectra were recorded on a FT-IR, model MB102 from ABB Bomem (Quebec, Canada). Nuclear magnetic resonance (NMR) spectra (1D and 2D) were recorded in CD<sub>3</sub>OD or CDCl<sub>3</sub>, at room temperature, on a Bruker Avance DRX 200 MHz spectrometer (<sup>1</sup>H NMR, 200 MHz; <sup>13</sup>C NMR, 50 MHz) from Bruker Analytic, Ettlingen, Germany. Electronic impact mass spectra (EIMS) of amides **4–13** were obtained from a HP 6890 gas chromatograph coupled with an HP 5989A mass spectrometer (Palo Alto, CA) at 70 eV. Electrospray ionization mass spectrometry (ESIMS) of amides **14–19** was performed using a Waters MICROMAS Q-TOF (Milford, MA). The optical rotations were measured on a Perkin-Elmer 341 polarimeter (Waltham, MA) and the osmotic pressures on a micro-mometer of Precision Systems Inc. (Natick, MA). The controlled temperature growth chamber was purchased from Quimis (S. Paulo, Brazil).

**Chromatographic Materials.** Silica gel Merck (Darmstadt, Germany) 100–200 and 200–425 mesh were used for column chromatography, and silica gel Merck 60G was used for thin-layer chromatography. Sephadex LH-20 was from Sigma Chemicals Co (St. Louis, MO). All solvents used were of PA and HPLC grade and purchased from Vetec (Brazil) and Sigma, respectively.

**Chemicals.** Gibberellic acid, piperazine, ethylenediamine, butylenediamine and 2-(*N*-morpholino)ethanesulfonic acid (MES) were purchased from Sigma, and propylenediamine was purchased from Fluka (USA).

**Plant Specimen. Souce of **1** and **2**.** *ent*-Kaur-16-en-19-oic acid (**1**) and *ent*-kaur-9(11),16-dien-19-oic acid (**2**) were isolated (9.6 and 2.4%,

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**Figure 1.** Structures of GA<sub>3</sub> (1), kaurane diterpene acids 2 and 3, and amides 4–19.

respectively, of ethanol extract) from aerial parts of *Wedelia paludosa*, Asteraceae (13). The plants were collected in Usiminas garden, Belo Horizonte, Minas Gerais, Brazil.

**General Synthetic Procedure for Amides 4–19.** A mixture of triphenylphosphine (30.0 mmol) and carbon tetrachloride (50.0 mL) was refluxed for 5 h under nitrogen. The solution was then cooled to room temperature, and kaurenoic acid (1) or grandiflorenic acid (2) (3.0 mmol), in carbon tetrachloride, was added. The reaction mixture was heated under reflux for 0.5 h (14). Then, the amine (60.0 mmol) (a) when liquid, was directly added to the residue containing the unstable acid chloride or (b) when solid, was dissolved in carbon tetrachloride, before addition; the mixture was kept, under agitation (a) for 24 h, at room temperature, in the case of monoamines or (b) for 0.5 h at 0 °C, for diamines. After removal of excess of amine and solvent, the residue was submitted to chromatography on a silica gel column with ascending polarities of hexane/ethyl acetate/methanol (for amides from monoamines) and to flash chromatography on silica gel column with ethyl acetate/methanol 7:3 (for amides from diamines). Column chromatography on Sephadex LH-20 (chloroform/methanol 6:4) was used in final purifications of the latter.

**Bioassay.** *Lactuca sativa* (cv Grand Rapids) seeds were purchased from Isla Pak, RS, Brazil. All undersized and damaged seeds were discarded. Germination and growth were conducted in 100 mm Petri dishes containing a 9.0 cm sheet of Whatman no. 1 filter paper as suport. Then, 25 lettuce seeds were placed per dish with 10 mL of a test (10<sup>-3</sup>, 10<sup>-5</sup>, and 10<sup>-7</sup> M) or a control solution. All solutions were prepared

with deionized 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. Concentrations lower than 10<sup>-3</sup> M were obtained by dilution series. All tests were triplicated. Dishes were covered with Parafilm to reduce evaporation and incubated in the dark at 25 °C, in a controlled-environment growth chamber, for 5 days. After this time, numbers of germination seeds were counted (a seed was considered to be germinated when the radicle was at least 0.2 mm long) and the lengths of radicle and shoots were measured (using a pachymeter). During the measurement process, the dishes were kept at 4 °C to avoid subsequent growth. The osmotic pressure values were measured on a microsmometer and ranged between 30 and 38 mOsmolar (13).

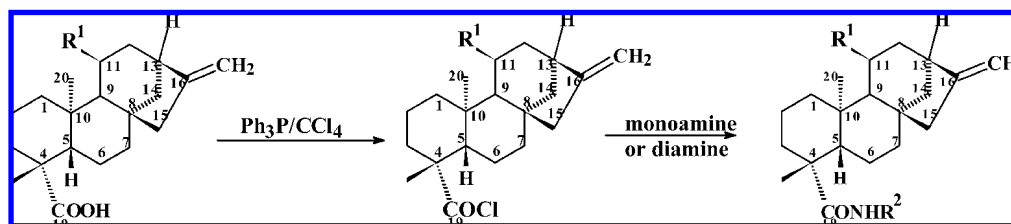
**Data Analysis.** The effects on germination and growth are given as percent differences from control, and they consist of the differences (in cm) between mean values of seeds with tested compounds and mean values for control (seeds grown without addition of tested compounds)/mean values for control × 100. Thus, zero represents the control, positive values represent stimulation of the studied parameter, and negative values represent inhibition.

The data were evaluated using Student's *t*-tests, and the differences between the experiment and control were significant at a value of *P* ≤ 0.05.

## RESULTS AND DISCUSSION

Monoamides 4–11 (Figure 1), from kaurenoic acid (1), grandiflorenic acid (2), and monoamines (hexylamine, cyclohexylamine, aniline, and morpholine), were obtained according methodology based on ref 14. The acid chloride intermediate was prepared by reaction of triphenylphosphine with carbon tetrachloride (Scheme 1), in neutral medium in order to avoid hydrochloric acid addition on exocyclic double bond of kaurane skeleton. Good yields of monoamides 12–19 (Figure 1) with little contamination of diamides could be obtained by careful control of reaction conditions when the symmetrical diamines piperazine, 1,2-ethylenediamine, 1,3-propylenediamine, and 1,4-butylenediamine reacted with 1 and 2. This is a unique achievement, since synthesis of monoamides from symmetrical diamines and carboxylic acid derivatives has been very problematic, due to the competition with bis-amidation. Therefore, a number of alternative strategies (15–19) involving, for example, a protection–acylation–deprotection sequence (18) were developed, aimed to obtain only the desired monoamide. No trace of monoamide 13, from kaurenoic acid (1) and piperazine, using methodologies described in refs 15–17 was found. Amides 4–19 are described for the first time, to the best of our knowledge. <sup>13</sup>C NMR data for all amides are listed in Tables 1 and 2.

The effects of amides 4–19 on germination and radicle and shoot growth of *L. sativa* were evaluated. Lettuce seeds (together with other crops such as cress, onion, and tomato), due to its ready availability and fast, complete, and uniform germination, are being traditionally and widely used in phytotoxic bioassays that specifically focus on detection of allelochemicals (20, 21). The choice of concentrations and duration of the experiments were based on our former results (13). Kaurenoic (1) and grandiflorenic (2) acids and gibberellic acid (3) had been already tested: 3 induced shoot growth at the three concentrations, but stimulated radicle growth only at 10<sup>-7</sup> M. Kaurenoic (1) and grandiflorenic (2) acids strongly stimulated radicle growth, but the latter acted also on shoot growth at all concentrations. Interestingly, gibberellic acid (3) inhibited radicle growth at higher concentrations, contrary to kaurenoic acid (1), which acted in an opposite way (13). Evaluation of putrescine, known to stimulate plant germination and growth (22, 23), as well as the other symmetrical diamines were also done. They showed good stimulatory effect on radicle and shoot growth at 10<sup>-5</sup>

Scheme 1. Synthesis of Amides 4–19<sup>a</sup><sup>a</sup> R<sup>1</sup> = H or Δ<sup>(9,11)</sup>.**Table 1.** <sup>13</sup>C NMR (CDCl<sub>3</sub>: 4–12, CD<sub>3</sub>OD: 14–18, 50 MHz, δ) Chemical Shift Values for Diterpenes Amides from Kaurenoic Acid (1)

C	4	6	8	10	12	14	16	18
1	41.2	41.3	41.0	41.8	41.9	42.3	42.3	42.4
2	19.4	19.6	19.2	20.2	20.2	20.6	20.6	20.7
3	38.4	38.5	38.3	39.7	39.7	38.8	38.4	38.9
4	43.7	43.7	44.1	46.3	46.2	45.1	45.1	45.0
5	57.4	57.5	57.3	61.4	61.6	59.0	59.1	59.0
6	22.5	22.6	22.5	23.1	23.1	23.5	23.6	23.5
7	41.5	41.7	41.4	42.3	42.3	42.8	42.8	42.8
8	44.3	44.4	44.6	44.5	44.6	45.5	45.5	45.5
9	55.1	55.2	55.0	56.4	56.4	56.6	56.6	56.5
10	39.6	39.8	39.5	40.3	40.2	40.9	40.9	40.5
11	18.4	18.6	18.4	18.6	18.6	19.6	19.6	19.5
12	33.0	33.0	32.9	33.3	33.3	34.3	34.3	34.3
13	43.8	43.9	43.6	44.1	44.0	45.3	45.3	45.2
14	39.6	39.7	39.6	40.2	40.4	38.8	38.9	39.7
15	48.9	49.1	48.7	49.1	49.1	50.1	50.2	50.1
16	155.8	155.9	155.5	156.2	156.3	156.8	156.8	156.7
17	102.9	103.7	103.1	103.0	102.9	103.8	103.9	103.9
18	30.1	30.3	29.6	28.1	28.1	30.3	30.6	30.5
19	176.7	175.9	175.1	177.0	176.9	181.4	180.8	179.6
20	15.7	16.0	15.7	18.2	18.3	16.7	16.9	16.8
1'	39.5	47.9	137.9	46.9	47.1	38.7	40.8	40.5
2'	31.4	33.2	120.1	67.1	46.3	40.8	28.7	27.3
3'	29.3	25.1	128.9	#	#	#	38.4	25.9
4'	26.8	25.8	124.0	#	#	#	#	39.7
5'	22.5	#	#	#	#	#	#	#
6'	13.9	#	#	#	#	#	#	#

**Table 2.** <sup>13</sup>C NMR (CDCl<sub>3</sub>: 5–13, CD<sub>3</sub>OD: 15–19, 50 MHz, δ) Chemical Shift Values for Diterpenes Amides from Grandiflorenic Acid (2)

C	5	7	9	11	13	15	17	19
1	39.6	41.4	41.3	41.6	41.4	39.5	40.1	40.9
2	20.6	20.6	20.6	20.9	20.9	21.6	20.1	21.6
3	38.8	38.9	39.0	39.1	39.0	39.1	39.6	39.6
4	44.2	44.1	45.1	46.8	46.7	45.5	45.6	45.5
5	47.5	47.9	47.8	50.4	50.3	48.7	48.2	48.7
6	19.0	19.0	18.6	19.6	19.8	20.1	21.6	20.1
7	29.4	30.2	30.2	30.0	29.9	31.2	31.2	31.3
8	42.3	42.3	42.2	42.6	42.5	43.4	43.5	43.5
9	158.7	158.9	158.6	158.9	158.7	159.5	159.5	159.6
10	38.9	39.0	38.5	39.6	39.5	40.0	39.0	40.1
11	114.9	115.0	115.6	113.7	113.8	116.2	116.2	116.2
12	38.0	38.1	38.0	38.1	37.9	38.6	38.0	39.0
13	41.3	41.4	41.4	41.7	41.5	42.6	42.6	42.7
14	45.1	45.2	45.2	44.8	44.9	46.3	46.3	46.4
15	50.8	50.9	50.9	50.6	50.4	51.6	51.6	51.6
16	156.2	156.3	156.3	157.8	157.5	157.5	157.5	157.5
17	105.5	105.5	106.9	105.2	105.2	106.2	106.2	106.2
18	29.6	29.7	29.4	28.1	27.0	29.6	29.9	29.8
19	177.1	176.1	175.6	175.1	175.0	181.3	181.0	180.0
20	24.0	24.1	24.1	27.1	27.7	22.7	25.0	25.0
1'	41.4	47.6	138.4	46.6	44.7	38.4	38.4	40.5
2'	31.6	33.2	121.0	67.2	44.7	41.1	28.8	27.4
3'	30.1	25.8	129.3	#	#	#	36.9	26.1
4'	26.8	25.0	124.6	#	#	#	#	40.5
5'	22.7	#	#	#	#	#	#	#
6'	14.1	#	#	#	#	#	#	#

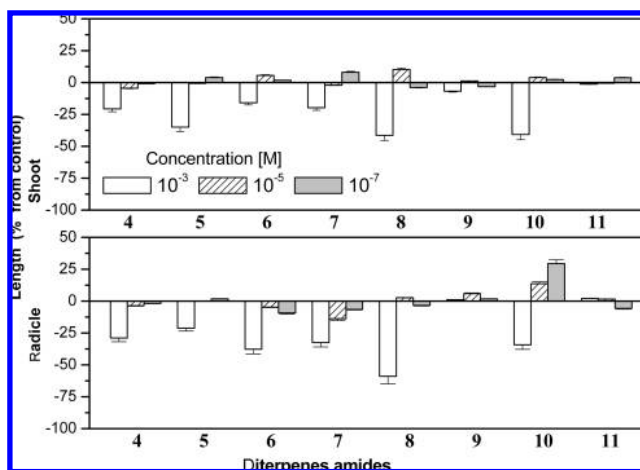
and 10<sup>-7</sup> M, besides high inhibitory effect at 10<sup>-3</sup> M, exception for putrescine, which acted in an opposite way on shoot growth.

In general terms, the effects of amides 4–19 on lettuce were more evident on radicles than on shoots. Amides derived from monoamines 4–11 (Figure 2) significantly inhibited radicle and shoot growth at 10<sup>-3</sup> M as well monoamides from symmetrical diamines 12–19 (Figure 3) at 10<sup>-5</sup> M. Amides from 1,3-propylenediamine and putrescine showed the greater inhibition percentages on radicle growth. Only amides from piperazine (12 and 13) stimulated radicle and shoot growth at 10<sup>-5</sup> and 10<sup>-7</sup> M. Total similarity of effects on radicle and shoot was observed for amides 12, 14, 16, and 18, from kaurenoic acid (1).

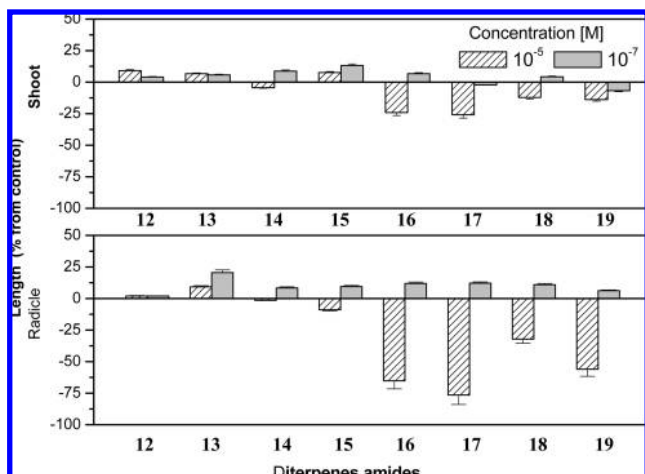
Practically, all tested amides exhibited germination inhibition at three concentrations, except for 18 and 19, which were derived from putrescine and slightly stimulated germination at 10<sup>-7</sup> M (Figure 4). All amides 12–19 gave total germination inhibition at 10<sup>-3</sup> M. Kaurenoic (1) and grandiflorenic (2) acids slightly inhibited lettuce seeds germination, and gibberellic acid (3) promoted slight stimulation at 10<sup>-3</sup> M (13). Putrescine showed the same effect on germination as amides 18 and 19, and the other diamines stimulated germination at all concentrations, differently from diamines 12–19 which totally inhibited *L. sativa* seeds germination.

During the past 30 years, great effort has been dedicated to the discovery of new natural plant toxins with potential application in weed management. The biocides developed from

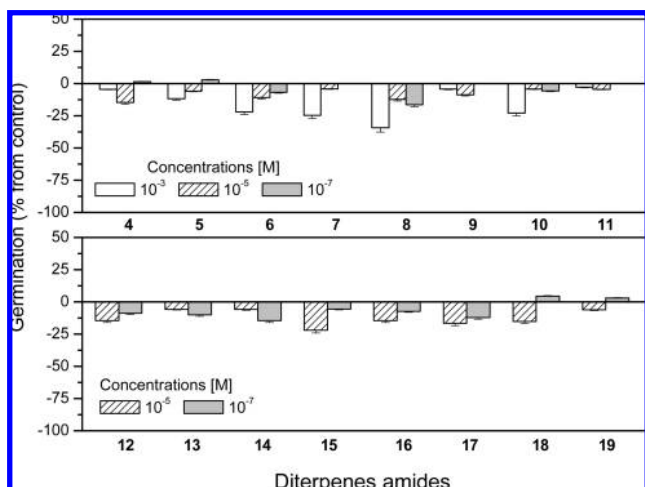
them have important advantages with respect to traditional herbicides: they have new modes of action, high biodegradability, and also low environmental impact (24). Furthermore, the high levels of inhibitory activity shown by monoamides 4–19



**Figure 2.** Effect of amides (from monoamines) 4–11 on (A) radicle and (B) shoot length of *L. sativa*. Values are presented as percentage differences from the control: zero representing an observed value identical to the control, a positive value representing stimulation, and a negative value representing inhibition.



**Figure 3.** Effect of amides (from diamines) 12–19 on (A) radicle and (B) shoot length of *L. sativa*. Values are presented as percentage differences from the control: zero representing an observed value identical to the control, a positive value representing stimulation, and a negative value representing inhibition.



**Figure 4.** Effect of amides 4–19 on germination of *L. sativa*. Values are presented as percentage differences from the control: zero representing an observed value identical to the control, a positive value representing stimulation, and a negative value representing inhibition.

on *L. sativa*, especially monoamides from diamines, suggest that they might be lead compounds for new herbicides.

**Supporting Information Available:** Physical data and  $^1\text{H}$  and  $^{13}\text{C}$  NMR, EIMS, and ESIMS spectral data for compounds 4–19. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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