Effects of Indole Amides on Lettuce and Onion Germination and Growth

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Auxins, such as indole-3-acetic acid (IAA), are important in plant germination and growth, while physiological polyamines, such as putrescine, are involved in cell proliferation and differentiation, and their concentrations increase during germination. In this work, novel indole amides were synthesized in good yields by monoacylation of morpholine and unprotected symmetrical diamines with indole-3-carboxylic acid, a putative metabolite of IAA, possessing no auxin-like activity. These amides were tested for their effects on seed germination and growth of the radicles and shoots of *Lactuca sativa* (lettuce) and *Allium cepa* (onion) seedlings, at 100.0, 1.0, and 0.01 μM concentrations. Germination was generally stimulated, with the exception of amide **3**, derived from morpholine, at 100 μM. On radicle and shoot growth, the effect of these compounds was predominantly inhibitory. Compound **3** was the best inhibitor of growth of lettuce and onion, at the highest concentration. Amides, such as propanil, among others, are described as having herbicidal activity.

Key words: Phytotoxic Activity, Indole-3-carboxylic Acid, *Lactuca sativa*

Introduction

Auxins are the plant hormones discovered first and have been extensively examined for many decades. In bioassays, it has been shown that auxins play a critical role in plant growth and development, being involved in processes such as cell elongation, induction of root formation, flower and fruit development, and fruit ripening. Auxins belong to chemically diverse groups, most of which have an aromatic system such as an indole, phenyl or naphthalene ring with a side chain containing a carboxy group attached. Indole-3-acetic acid (IAA, **1**) is the natural auxin commonly occurring in all vascular and lower plants. Most of a plant's endogenous IAA is found not in the free and biologically active form, but conjugated at the carboxy group to single amino acids, peptides or protein *via* an amide bond or to monosaccharides or polysaccharides *via* an ester bond. Hydrolysis of endogenous conjugates of IAA is likely to be an important source of free IAA (Bajguz and Piotrowska, 2009).

Indole-3-carboxylic acid (**2**), structurally an auxin-related compound, has no auxin-like activity and is considered a putative decarboxylated metabolite of IAA, although it has been rarely identified as an endogenous constituent in plants (Honda *et al.*, 2005; Ljung *et al.*, 2002). According to Sarr *et al.* (2003), indole-2-carboxylic acid, a related compound, has a rate constant in photodegradation, opposed to IAA, and, consequently, a long half-life. The compound therefore tends to accumulate in the plant in higher concentration, thereby becoming toxic.

Spermidine and spermine, together with their biosynthetic precursor putrescine (1,4-diaminobutane), are intimately involved in cell proliferation and differentiation and are named physiological polyamines (Eliassen *et al.*, 2002). Plants are able to produce polyamines and to metabolize them differentially, depending on the phase of their life. A marked increase in polyamine content is usually observed during seed germination (Sinska, 1988; Sinska and Lewandowska, 1991; Glória *et al.*, 2005).

The bioactivity of these compounds has encouraged research on their derivatives as potential agents affecting germination and plant growth.

In the present work, we report the preparation, in good yields, of indole monoamides, obtained from the reaction of indole-3-carboxylic acid (**2**) with morpholine and symmetrical diamines (putrescine, propylenediamine, ethylenediamine, and piperazine), and the evaluation of their effects on germination and growth of the radicles and shoots of lettuce (*Lactuca sativa*) and onion (*Allium cepa*) at 100, 1.0, and 0.01 μM concentrations.

Materials and Methods

General experimental procedures

Nuclear magnetic resonance (NMR) spectra (1D and 2D) were recorded in CD_3OD, at room temperature, on a Bruker Avance DRX 200 MHz spectrometer (^{1}H NMR, 200 MHz; ^{13}C NMR, 50 MHz) (Bruker Analytic, Ettlingen, Germany). Electronic electrospray ionization mass spectrometry (ESIMS) of amides **3**–**7** was performed using a Waters MICROMAS Q-TOF instrument (Milford, MA, USA). The osmotic pressures were determined with a micro-osmometer of Precision Systems Inc. (Natick, MA, USA). The controlled temperature growth chamber was purchased from Quimis (São Paulo, Brazil).

Chromatographic materials

Silica gel (Merck, Darmstadt, Germany), 100–200 mesh and 60G, were used for column chromatography and thin-layer chromatography, respectively. Sephadex LH-20 was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All solvents used were purchased from Vetec (Rio de Janeiro, Brazil).

Chemicals

Indole-3-carboxylic acid, morpholine, piperazine, ethylenediamine, butylenediamine, propylenediamine and 2-(*N*-morpholino)ethanesulfonic acid (MES) were purchased from Sigma.

*General synthetic procedure for amides **3**–**7***

A mixture of indole-3-carboxylic acid (**2**) (1.2 mmol) and thionyl chloride (30.2 mmol) in tetrahydrofuran (THF) (4.4 mL) was kept at 0 °C for 1.5 h; thereafter, the excess of $SOCl_2$ was evaporated under reduced pressure. Then, the amines were directly added to the residue containing the unstable acid chloride, and the resulting mixture was kept under agitation a) for 10 min at –30 °C, by means of a dry ice/EtOAc bath, for liquid diamines (33.0 mmol); b) for 10 min at 0 °C, for solid piperazine (8.9 mmol), in THF; c) for 0.5 h at 0 °C, for liquid morpholine (33.0 mmol). After this time, water was added to stop the reaction. After removal of excess of amine and solvent, the residue was subjected to chromatography on a silica gel column eluted with *n*-hexane/ethyl acetate/methanol mixtures of ascending polarity. Column chromatography on Sephadex LH-20 (chloroform/methanol, 6:4) was used in final purifications of monoamides **3**–**7**.

N-Morpholine-1H-indole-3-carboxamide (**3**): Yield: 73%. – M. p. 222 °C. – IR (KBr): ν_{max} = 3.142 (NH str. assym.), 1.588 (CO str., amide I), 1.567 cm^{-1} (NH bend, amide II). – ^{1}H NMR (CD_3OD): δ = 3.68–3.76 (bs, 4H, H-1'), 3.68–3.76 (bs, 4H, H-2'), 7.11–7.23 (m, 1H, H-6), 7.11–7.23 (m, 1H, H-5), 7.60–7.70 (m, 1H, H-4), 7.43 (m, 1H, H-7), 7.60–7.70 (m, 1H, H-2). – ^{13}C NMR: see Table I. – ESI-MS: *m/z* = 231.08 (100) $[M+H]^+$, 144.00 (17) $[M–C_4H_9NO]^+$.

N-Piperazine-1H-indole-3-carboxamide (**4**): Yield: 46%. – M. p. 226 °C. – IR (KBr): ν_{max} = 3.123 (NH str. assym.), 3.033 (NH str. sym.), 1.588 (CO str., amide I), 1.566 cm^{-1} (NH bend, amide II). – ^{1}H NMR (CD_3OD): δ = 3.29 (bs, 4H, H-1'), 4.02 (bs, 4H, H-2'), 7.08–7.22 (m, 1H, H-5), 7.08–7.22 (m, 1H, H-6), 7.42 (m, 1H, H-7), 7.62–7.72 (bs, 1H, H-2), 7.62–7.72 (bs, 1H, H-4). – ^{13}C NMR: see Table I. – ESI-MS: *m/z* = 229.98 (42.0) $[M+H]^+$, 143.98 (100.0) $[M–C_4H_{10}N_2]^+$, 87.05 (92.0) $[M–C_9H_5ON]$.

N-(Ethyl-2'-amino)-1H-indole-3-carboxamide (**5**): Yield: 45%. – M. p. 236 °C. – IR (KBr): ν_{max} = 3.181 (NH str.), 1.587 (CO str., amide I), 1.574 (NH bend, amide II), 1.548 cm^{-1} (NH bend, amine). – ^{1}H NMR (CD_3OD): δ = 3.10 (t, *J* = 5.8 Hz, 2H, H-2'), 3.60 (t, *J* = 5.8 Hz, 2H, H-1'), 7.07–7.15 (m, 1H, H-6), 7.07–7.15 (m, 1H, H-5), 7.38 (m, 1H, H-7), 7.91 (s, 1H, H-2), 8.04 (m, 1H, H-4). – ^{13}C NMR: see Table I. – ESI-MS: *m/z* = 203.98 (100.0) $[M+H]^+$, 187.02 (45.0), $[M–NH_3+1]^+$, 144.00 (52.0) $[M–C_2H_8N_2]^+$.

N-(Propyl-3'-amino)-1H-indole-3-carboxamide (**6**): Yield: 44%. – M. p. 242 °C. – IR (KBr): ν_{max} = 3.336 (NH str. assym.), 3.181 (NH str. sym.), 1.622 (CO str., amide I), 1.614 (NH bend, amide II), 1.540 cm^{-1} (NH bend, amine). – ^{1}H NMR (CD_3OD): δ = 1.84 (q, *J* = 6.0 Hz, 2H, H-2'), 2.89 (t, *J* = 6.0 Hz, 2H, H-3'), 3.38 (t, *J* = 6.0 Hz, 2H, H-1'), 7.01–7.09 (m, 1H, H-5), 7.01–7.09 (m, 1H, H-6), 7.30 (m, 1H, H-7), 7.82 (s, 1H, H-2), 7.99 (m, 1H, H-4). – ^{13}C NMR: see Table I. – ESI-MS: *m/z* = 218.04 (100.0) $[M+H]^+$, 201.31 (45.0), $[M–NH_3+1]^+$, 144.16 (52.0) $[M–C_3H_{10}N_2]^+$.

N-(Butyl-4'-amino)-1H-indole-3-carboxamide (**7**): Yield: 40%. – M. p. 244 °C. – IR (KBr): ν_{max} = 3.299 (NH str.), 1.599 (overlap CO str. and NH bend), 1.541 cm^{-1} (NH bend, amine). – ^{1}H NMR (CD_3OD): δ = 1.32–1.58 (bs, 2H, H-2'), 1.32–1.58 (bs, 2H, H-3'), 2.68 (bs, 2H, H-4'), 3.15 (bs, 2H, H-1'), 6.89–6.92 (m, 1H, H-5), 6.89–6.92 (m, 1H, H-6), 7.19 (m, 1H, H-7), 7.73 (bs, 1H, H-2), 7.85 (m, 1H, H-4). – ^{13}C NMR: see Table I. – ESI-MS: *m/z* = 232.17 (100.0) $[M+H]^+$, 214.17 (15.0), $[M-NH_3+1]^+$, 144.00 (8.0) $[M-C_4H_{12}N_2]^+$.

Bioassay

Lactuca sativa (cv. Grand Rapids) and *Allium cepa* seeds were purchased from Isla Pak (Porto Alegre, RS, Brazil). All undersized and damaged seeds were discarded. Germination and growth were conducted in 10-cm Petri dishes containing a 9.0-cm sheet of Whatman no. 1 filter paper as support. Then, 25 lettuce or onion seeds, respectively, were placed per dish with 10 mL of a test (100, 1.0, and 0.01 μM) or a control solution, respectively. 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 100 μM were obtained by dilution. All tests were done in triplicate. Dishes were covered with Parafilm to reduce evaporation and incubated in the dark at 25 °C, in a controlled-environment growth chamber, for 5 d in the case of lettuce and 7 d in the case of onion. After this time, numbers of germinated seeds were counted (a seed was considered to be germinated when the radicle was at least 0.2 mm long), and the lengths of radicles 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 was measured with a micro-osmometer and ranged between 30 and 38 mosmolar (Boaventura *et al.*, 2008).

Data analysis

The effects on germination and growth are given as percentage differences from the control, and consist of the differences (in cm) between the mean length of seeds grown in the presence of a test compound and the mean length of the control (seeds grown without addition of tested compounds) relative to mean values of the control · 100:

$$\text{length (\% control)} = \frac{A - C}{C} \cdot 100,$$

where *A* is the value for germination or growth of a treated sample, and *C* is the value of the respective control sample. 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 differences between the experiment and the control were significant at a value of $P \leq 0.05$.

Results and Discussion

Synthesis of indole amides

Indole amides **3**–**7** (Fig. 1) were synthesized from indole-3-carboxylic acid (**2**) *via* its acid chloride (Sears *et al.*, 2005), followed by the reaction of the latter with morpholine and the symmetrical diamines piperazine, ethylenediamine, propyl-

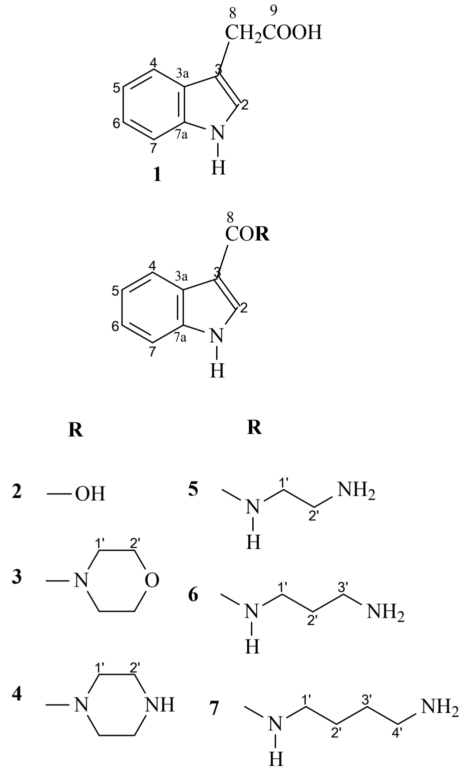

Fig. 1. Chemical structures of indole-3-acetic acid (**1**), indole-3-carboxylic acid (**2**), and amides **3**–**7**.

Table I. ^{13}C NMR (CD_3OD, 50 MHz, δ) chemical shifts for indole-3-carboxylic acid (literature and **2**) and indole amides **3**–**7**.

C	Indole-3-carboxylic acid[a]	**2**	**3**	**4**	**5**	**6**	**7**
2	133.4	131.7	128.2	128.8	128.3	128.3	128.0
3	108.7	107.0	109.7	108.9	110.7	110.0	110.5
3a	127.6	125.8	125.9	125.9	126.8	125.9	125.9
4	122.0	120.7	120.9	121.1	120.7	121.0	120.8
5	122.4	121.9	122.6	122.8	122.6	122.4	122.3
6	123.6	120.3	119.9	119.9	121.2	120.5	120.5
7	112.9	111.2	112.0	112.1	111.9	111.8	111.8
7a	138.2	136.5	136.6	136.6	136.6	136.8	136.8
8	-[b]	167.6	168.4	168.4	168.5	167.8	167.8
1’	-	-	46.0	43.6	40.5	37.2	39.3
2’	-	-	67.1	42.3	37.4	27.9	26.6
3’	-	-	-	-	-	35.6	24.8
4’	-	-	-	-	-	-	38.3

[a] Hagemeier *et al.* (2001). [b] Signal not detected.

enediamine, and butylenediamine (putrescine), respectively. A large excess of the liquid symmetrical diamines was added to the unstable acid chloride with careful control of temperature and a minimal reaction time, to prevent formation of symmetrical diamides. Isolation of the latter was achieved previously using the above diamines and kaurane diterpene acids (Boaventura *et al.*, 2008), however, careful control of the reaction conditions prevented diamide formation, and monoamides were obtained in good yields. Those results are an important achievement, since synthesis of monoamides from symmetrical diamines and carboxylic acid derivatives described in the literature has been very problematic due to the competition with bis-amidation (Boaventura *et al.*, 2008).

For amides **5**–**7**, from liquid diamines, the reaction conditions were –30 °C and 10 min reaction time, and the yields of monoamides were found to be in the range of 40–50%. No symmetrical diamides were isolated, but part of the carboxylic acid **2** was recovered. For piperazine, a solid material, the above conditions could not be maintained due to the poor solubility of this compound in the solvent THF at –30 °C. The reaction conditions for amide **3**, from morpholine, a monoamine, were less rigid than for the others, and the amide yield reached 70%. IR, ^{1}H NMR, and ESI-MS data are listed in Materials and Methods. ^{13}C NMR data for acid **2** and amides **3**–**7** and are listed in Table I. Compound **3** has already been cited (Kutschy *et al.*, 1999), without NMR and MS data, but amides **4**, **5**, **6**, and **7** are described here for the first time, to the best of our knowledge, as well as the effects of all compounds on plant growth.

Biactivity-phytotoxicity bioassay

The effects of the indole amides **3**–**7** on germination and radicle and shoot growth of *L. sativa* (lettuce) and *A. cepa* (onion) were evaluated (Fig. 2). According to Macías *et al.* (2000), lettuce and onion seeds (together with other crops) are traditionally and widely used in the search for allelochemicals, due to their ready availability and fast, complete, and uniform germination. The choice of bioassay conditions was based on our former experiments (Vieira *et al.*, 2005; Boaventura *et al.*, 2008). Acid **2** was also tested (Fig. 2), as well as the diamines (Boaventura *et al.*, 2008). The latter showed a medium stimulatory effect on radicle and shoot growth at lower concentrations, besides a high inhibitory effect at higher concentrations, except putrescine that acted in an opposite way on shoot growth.

In general, amides **3**–**7** had stronger effects on germination, radicle and shoot growth of *L. sativa* and *A. cepa* than the free acid **2** (Fig. 2). Amide **3**, at 100 μM, inhibited germination of *L. sativa* by 73%. The amides inhibited shoot and radicle growth of lettuce more strongly than of onion: Amide **5** inhibited shoot growth by 38% at 0.01 μM, and amide **3** inhibited 73% of radicle growth of *L. sativa* at 100 μM, compared to the control. Also, for *A. cepa*, the effect of this amide on shoot and radicle growth was inhibitory in all

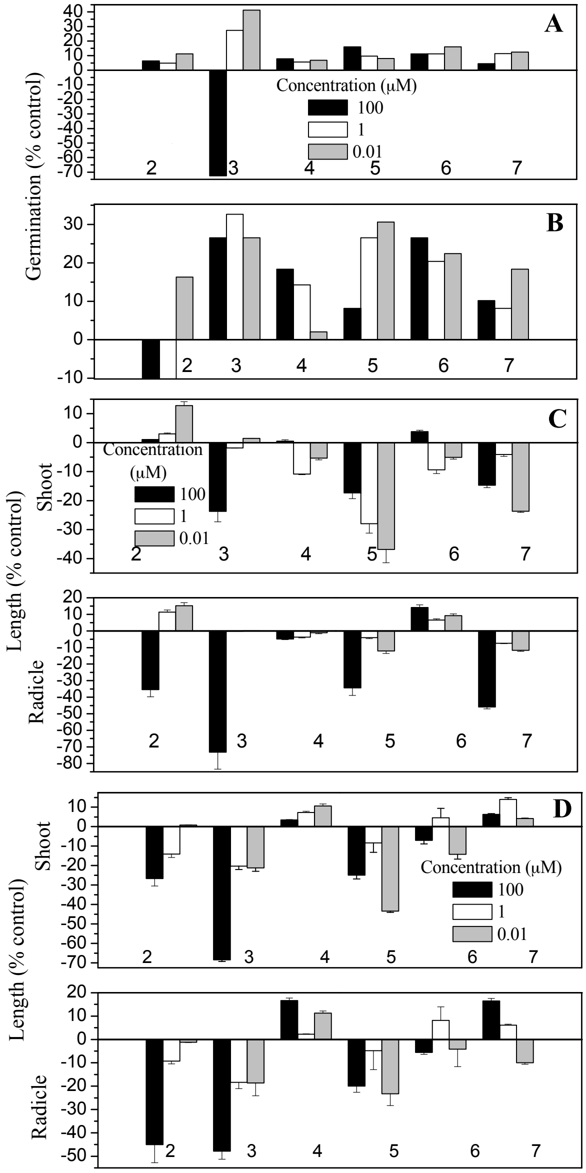

Fig. 2. Effects of indole-3-carboxylic acid (**2**) and indole amides **3**–**7** on (A) germination of *L. sativa*, (B) germination of *A. cepa*, (C) radicle and shoot length of *L. sativa*, (D) radicle and shoot length of *A. cepa*. Bars indicate S. D. (n = 3). Values are presented as percentage differences from the control, zero representing an observed value identical to the control, a positive value representing stimulation, and a negative value representing inhibition.

three tested concentrations (at 100 μM 68% and 48% inhibition, respectively).

Amide **3** produced the best stimulatory effect on germination of *L. sativa*, at the two lower concentrations (44% at 0.01 μM). It was also observed that amides **3**, **5**, and **6** showed an analogous activity pattern on radicle and shoot growth of *A. cepa*, which was not observed for *L. sativa*.

In conclusion, amide **3**, from morpholine, strongly inhibited shoot and radicle growth of both *A. cepa* and *L. sativa* and germination of the latter at the highest concentration. At the lower concentrations, the effect was stimulatory. When a nitrogen atom replaced an oxygen atom in **3**, resulting in amide **4**, both the stimulatory and inhibitory activity, respectively, were drastically reduced. Also, it was observed that the presence of the amide function enhanced the inhibitory activity on shoot and radicle growth of both species compared to the free acid **2** and polyamines, respectively, in all concentrations, principally for *L. sativa*.

Amides have been described as possessing herbicidal activity, such as propanil (Garrido *et al.*, 2003). A series of sarmentine (a phytotoxic pyrrolidine amide isolated from *Piper longum* fruit) analogues were synthesized to study the structure-activity relationship, and it was observed that the amide bond with a secondary amine seems to be necessary for the activity. Sarmentine and its analogues appeared to directly disrupt the cell membrane and then initiate peroxidation reactions (Huang *et al.*, 2010).

The growth inhibitory effects of amides **3**–**7** on germination and growth of *L. sativa* are stronger than those observed for the starting material **2** alone and illustrate the phytotoxicity of these derivatives, suggesting that they might be lead compounds for new herbicides. Further research will be needed to establish their mechanism of action and to assess their complete potential usefulness in weed management. The development of pesticides from natural products is believed to provide new modes of action and a more specific interaction with the pest and be more environmentally friendly than most synthetic ones (Macías *et al.*, 2010).

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