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Herbicidal, Plant Growth Inhibitory, and Cytotoxic Activities of Bismuthines Containing Aromatic Heterocycles†

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This work presents the herbicidal and plant growth regulatory activities of tertiary bismuthines containing heterocyclic aromatic rings of the general formula $(2\text{-}C_4H_3X)_3Bi$, where X = S (**3**), O (**1**), or NMe (**2**). Toxicity against *Artemia salina* and herbicidal activity on *Lactuca sativa*, *Trifolium pratense*, and *Lolium multiflorum* were tested. In addition to the effects on mitochondrial respiration obtained from roots of *Phaseolus vulgaris*, these compounds also demonstrated partial radical scavenging properties against 2,2-diphenyl-1-picrylhydrazyl (DPPH). The furyl substituent is the most important structural requirement for the activity measurements observed in this study.

KEYWORDS: Bismuth compounds; plant growth regulation; respiration inhibitor; DPPH

INTRODUCTION

Tertiary bismuthines have not been studied as extensively as their arsine and stilbine counterparts. There are very few reports on the direct attachment of bismuth to an aromatic heterocycle (*1*, *2*). However, bismuth is known for its biological activity and its application in medicinal chemistry. A number of organobismuth compounds have been investigated for various potential uses. For instance, bismuth complexes inhibit *Helicobacter pylori*, producing urease, and the inhibition of urease may play an important role in the antibactericidal activity (*1*). The antimicrobial activity of some organobismuth compounds at low concentrations, their relatively low human cell cytotoxicity, and the new chemistry of bismuth are of interest for the development and/or discovery of new pharmaceutical and/or agrochemical agents. Recently, some well-characterized stilbines and bismuthines containing aromatic heterocycles were reported by Lemus et al. (*3*).

In view of our interest on new sources of plant growth regulation, this work was undertaken. Little is known about the biocoordination chemistry of Bi(III) with proteins, enzymes, and cell membranes, although this could be very important to the biological activity inhibition, as in metabolic systems and cell membrane transport. Studies of plant growth regulation activity show us that the heterocyclic compounds inhibit radicle growth (*4*, *5*) and produce necrotic effects on the foliage (*6*). Also, the bismuthine compounds affect seedling growth and are photosynthetic inhibitors (*7*). In continuation of the search for organometallic compounds with biological activities, we have studied the biological activity of bismuthines. Analogues of these compounds display antitumoral and antibacterial activities (*1*).

This is the first report on the plant growth regulatory (PGR) and herbicidal effects of organometallic compounds from Bi(III). Here we report the biological effects on brine shrimp and plant growth inhibitory activities of three derivatives of Bi(III), namely, tris(2-furyl)bismuth (**1**), tris(1-methyl-2-pyrrolyl)bismuth (**2**), and tris(2-thienyl)bismuth (**3**), on the germination and respiration of seedling growth. The effect on seedling growth and the behavior of these compounds toward metabolic energetic reactions on dicotyledonous and monocotyledonous standard target species are also reported. Our focus was on the development and growth of weedy plants (germination, root and shoot elongation, respiration of seeds, and oxygen uptake of isolated mitochondria), and an attempt was made to correlate these with the antioxidant activity of these compounds.

These parameters are accepted as indirect measures of other physiological processes (*8*) affected by the test compounds. A commercial herbicide, 2,4-D, was included in the bioassay as an internal standard in the plant growth bioassay. In addition, quercetin and gallic acid were used as internal standards in assays concerned with the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and antioxidant measurements, respectively. Our recent data indicate that it is possible to correlate some antioxidant activities (i.e., DPPH) against germination and respiration. These data are important for the physiological interpretation activities of new compounds (*9*).

MATERIALS AND METHODS

Chemicals. The compounds were obtained according to previously reported procedures (*3*), and their molecular weights are (**1**) 410, (**2**) 449, and (**3**) 458, determined by mass spectrometry.

Apparatus. Spectrophotometric measurements were determined with a Spectronic model Genesys 5 spectrophotometer. Oxygen evolution (uptake) was determined with a Clark-type electrode connected to a YSI oxygraph (model 5300). Fluorometric measurements were deter-

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mined with a Turner Barnstead-Thermolyne Quantech S5 fluorometer using 420, 440, 470, 550, and 650 Turner filters.

Seed Germination Bioassays. *Lactuca sativa* L. var. Roman, *Lolium multiflorum* var. Gulf, *Trifolium pratense* var. Kenland, and *Physalis ixocarpa* were purchased from semillas "COBO" S.A. de C. V. Mexico D.F., Mexico. For these experiments 25 seeds of *Lo. multiflorum*, *T. pratense*, and *P. ixocarpa* were placed on a Petri dish; however, 50 seeds of *La. sativa* were required for the assays. The number of seeds used for each experiment was selected so an appreciable change in O_2 uptake could be detected by the oxygraph. Seeds were placed on filter paper (Whatman no.1) in Petri dishes (85-mm diameter). In three replicate experiments, the paper was wet with 8 or 10 mL of deionized water or test solution (MeOH, <1% v/v). The dishes were wrapped with Parafilm (laboratory film) and incubated at 28 °C in the dark at intervals of 48 h. The number of germinated seeds was determined according to the criteria of 1-mm extrusion of the radical. There were three replications for each germination assay. Control seed dishes contained the same amount of seeds and volumes of water and methanol as the test solutions. Seeds were selected for uniformity of size; damaged ones were discarded (*27*).

Growth Bioassays. Coleoptyle or hypocotyle and root lengths for all germinated seeds were measured after 120 h, in three replicates following this design, after which the germinated plants were dried to constant weight at 40 °C (*28*). I_{50} values for plant development of the pure compounds and CH_2Cl_2 extract were obtained by determining the concentration that induced 50% growth inhibition of development of roots and shoots (see **Figure 3** and **Table 3**).

Seeds Respiration. Seed respiration was measured polarographically as oxygen uptake during the germination process. The oxygen uptake, in the presence of different concentrations of tested compounds, was evaluated over 5 and 10 min, in a non-illuminated cell. The requirement for oxygen was plotted as percentage, taking the control as 100% (*17*).

Determination of Mitochondrial Oxygen Consumption. The procedure for the isolation of root bean mitochondria is based on previously described protocols (*29*) with minor modifications, as follows. Approximately 700 g of *Phaseolus vulgaris* seeds were sterilized for 30 min in a 10% (v/v) solution of sodium hypochlorite and then washed with sterile water successively. Seeds were grown in a sterile sphagnum Peat-Moss mixed with vermiculite (1:1) (purchased from Hummert de Mexico, S.A. de C.V., Cuernavaca, Mexico) in complete darkness for 72 h at 28 °C. Roots were excised from the coleoptyle. All subsequent procedures were carried out at 4 °C as rapidly as possible. The chilled tissue was ground in a mortar with silica gel 60 (0.063−0.200 mm) in 2 volumes of grinding buffer (0.4 M sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 30 mM tricine, pH 8.0, with KOH) and filtered through Miracloth into 250-mL centrifuge bottles. The filtrate was then centrifuged at 3000*g* for 5 min in a Sigma-B.Braun model 2-15 rotor, the supernatant was removed, and the mitochondria were pelleted by centrifugation at 10000*g* for 15 min. Wash buffer (1−5 mL; 0.4 M sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 30 mM tricine, pH 7.8, with KOH) was added per tube and the pellet resuspended using a soft paintbrush. The suspension was placed in 50-mL tubes and centrifuged at 3000*g* for 5 min in a Sigma-B.Braun model 2-15 rotor. The supernatant was then carefully transferred to another 50-mL tube and centrifuged at 10000*g* for 15 min to repellet the mitochondria, which were then resuspended in 1−2 mL of resuspension buffer (0.4 M sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 30 mM tricine, pH 7.2, with KOH). To purify the mitochondria from residual contaminating plastids, the crude mitochondrial suspension was loaded onto 26% (v/v) Percoll (Sigma) in resuspension buffer (~40 mL) in a 50-mL polycarbonate tube, and a density gradient was generated by centrifugation at 40000*g* for 90 min in a Sigma-B.Braun model 2-15 rotor. A buff-colored band of mitochondria was visible below a band containing plastids. The upper layer was removed by aspiration and the mitochondrial band recovered (volume ~1−2 mL) and diluted with 20 mL of resuspension buffer. Mitochondria were then recovered by centrifugation at 12000*g* for 15 min in a Sigma-B.Braun model 2-15 rotor. The resulting pellet was very loose, and the supernatant had to be removed by aspiration with care.

This wash procedure was repeated and the final pellet resuspended in 0.4−1 mL of resuspension buffer and stored on ice. Total mitochondrial protein concentration was determined using the Bradford procedure (*30*), adjusted to 0.3−0.5 mg for each experiment, and the freshly prepared mitochondria were used directly in reaction for inhibition. The O_2 uptake of mitochondria was monitored with a YSI model 5300 oxygen monitor connected to a Clark-type electrode.

The integrity of mitochondria was verified using HCN as respiratory chain inhibitor. All compounds were disolved in methanol (=0.5%). Respiration control and ADP:O ratios were calculated for each mitochondrial isolation (*31*). A constant state 3 respiratory rate (*32*), with 10 μM succinate, was obtained before measures with our compounds. The ADP:O ratio for succinate was 1.7.

Reduction of 2,2-Diphenyl-1-picrylhydrazyl [= 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; DPPH] Radical and β-Carotene. For the TLC autographic assay, after developing and drying, TLC plates were sprayed with a 0.2% DPPH solution in MeOH. The plates were examined 30 min after spraying. Active compounds appear as yellow spots against a purple background. In similar form, TLC plates were sprayed with 0.05% β-carotene solution in $CHCl_3$. The plates were examined under UV_{254} light until the background became discolored (bleached). Active compounds appear as pale yellow spots against a white background. For the spectrophotometric assay (*33*, *34*), 50 μL of a solution containing the compound to be tested was added to 5 mL of a 0.004% MeOH solution of DPPH; quercetin was used as internal standard reference. Absorbance at 517 nm was measured after 30 min, and the percent of activity was calculated.

Statistical Analysis. Data shown in the figures and tables are the means of three replicates of seedling growth and DPPH and are presented as means ± standard errors. Data were subjected to analysis of variance (ANOVA) with significant differences between means identified by GLM procedures. The results are given in the text as probability values, with $p < 0.05$ adopted as the criterion of significance. Differences between treatment means were established with a Student−Newman−Keuls (SNK) test. The GI_{50}, RI_{50}, and I_{50} values for each activity were calculated by Probit analysis on the basis of the percentage of inhibition obtained at each concentration of the samples. I_{50} is the concentration producing 50% inhibition. Complete statistical analyses were via the MicroCal Origin 6.0 statistical and graphs PC program.

RESULTS AND DISCUSSION

Bismuthines. The three tertiary bismuthines remain unaffected by water; thus, the Bi−C bonds in the above compounds are not hydrolyzed by water alone. These tris(heteroaryl)bismuth compounds behave like other triarylbismuthines and are thermally stable and melt without decomposition. These compounds show a very slow degree of decomposition at room temperature. The decomposition product could not be characterized because of its insolubility in common solvents. From the IE mass spectrum of the decomposition product of tris(2-furyl)bismuth a polymeric nature may be suggested.

On the basis of the structure of other tertiary bismuthines a pyramidal structure may be assigned to **1**−**3**. In the far-IR spectra and Raman spectra C−Bi vibrations were observed. On the bases of their ^1H and ^{13}C NMR spectra and X-ray diffraction of compound **1**, the final structural analyses was carried out, and the results have published been already (*3*).

Biological Assay: Brine Shrimp (*Artemia salina* L.) Toxicity. The absence of extensive biological data on organobismuth(III) compounds prompted us to undertake a comprehensive study on the structure activity relationship between the most stable organometallic compounds of Bi(III), that is, **1**, **2**, and **3**, obtained for us. The three compounds were tested on a brine shrimp toxicity assay and as plant growth regulator against monocot and dicot seeds and seedlings.

The aim of our study was to investigate the macroscopic effects of these Bi(III) compounds. The compounds were monitored by the survival of brine shrimp and the germination,

Table 1. LC_{50} of Compounds **1**–**3** against *A. salina* Larvae

	1	**2**	**3**
LC_{50} (μM)	99.02	638.3	>1000
LC_{50} (ppm)	40.6	286.6	>458.0

Table 2. Reed–Muench Table for Tris(2-furyl)bismuthin **1**

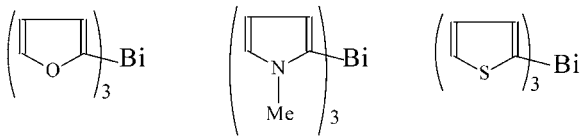
concn	organisms		accumulated		dead/live	%
(μM/ppm)	dead	live	dead	live	rate	mortality
200/82	12	18	40	18	40/58	68.96
100/41	21	9	28	27	28/55	50.90
20/8.2	7	23	7	50	7/57	12.28

$LC_{50} = 99.02\ \mu M$

Table 3. Growth Inhibitor Activity of Compounds **1**–**3** on Germination of *La. sativa*, *T. pratense*, and *Lo. multiflorum*

	I_{50}[a] (μM)		
compd	*L. sativa*	*T. pratense*	*L. multiflorum*
1	16.1	4.6	20.0
2	34.0	7.5	33.8
3	49.7	12.4	36.0

[a] I_{50} = concentration that inhibits 50% germination.



1 **2** **3**

Figure 1. Structures of tris(2-furyl)bismuth (**1**), tris(1-methyl-2-pyrrolyl)-bismuth (**2**), and tris(2-thienyl)bismuth (**3**).

growth, and development of seedling bioassay tests. The brine shrimp lethality assay detects a broad range of bioactivity of natural and synthetic compounds. It is a simple and convenient prescreen for antitumor activity (*10*, *11*). Both tests are extensively used for the determination of active compounds. Two of the three compounds were positive to the brine shrimp test at different concentrations.

The more water soluble and stable compound, **1** (furyl), showed the highest toxicity to the brine shrimp with an $LC_{50} < 99\ \mu M$ and 100% lethality at 500 and 1000 μM (**Table 1**).

Tris(2-thienyl) bismuthine, **3**, did not show toxicity, even at the highest concentration tested ($LC_{50} > 1000\ \mu M$).

On the other hand, tris(1-methyl-2-pyrrolyl)bismuthine, **2**, exhibited an intermediate toxic activity relative to the other two compounds (data not shown). However, the Reed−Muench table shows that at a low concentration (20 μM) of **1**, the death/live rate is very low, compared with the other two compounds (data not show), indicating the potency of compound **1** (**Table 2**).

Herbicidal Activity against Seeds of *Lactuca sativa*, *Trifolium pratense*, and *Lolium multiflorum*. *Growth Inhibition/Seed Germination.* **Table 3** summarizes the seed germination activity of compounds **1**−**3**. Compound **1** showed the highest inhibition activity with GI_{50} values at 4.6, 16.1, and 20.0 μM for *T. pratense*, *La. sativa*, and *Lo. multiflorum*, respectively (**Figure 2**; **Table 3**).

The assays above 200 μM show a high herbicidal activity. For this reason the assays were carried out at <100 μM for compound **1**.

Figure 2 shows seed germination inhibition effects of compounds **1**−**3**. The greatest inhibition was observed with furyl bismuthine, **1**, followed by bismuthine **2** and bismuthine **3**. In general, bismuthine **1** was more active toward dicot seeds; that is, *T. pratense* was more sensitive to this compound given ~100% inhibition above 18.3 μM; for *La. sativa* it shows inhibitory effects above 36.6 μM and for *Lo. multiflorum* above 48.8 μM. The low germination GI_{50} values indicate that these compounds are very good inhibitors for seed germination of *P. ixocarpa* and *T. pratense*. According to Hatfield and Karlen (*12*) and Mohr and Schopfer (*13*), pre-emergence selective inhibitors are applied after crops are planted but before emergence of weeds or crops, as was done in this work.

These results suggest that the furyl moiety of these compounds interferes with some physiological systems. Compounds **1** and **2** may be playing an important role in the inhibition activities, which may be due either to their lipophilicity or to the fact that the hydrophilicity of **1** and **2** at physiological pH makes it easy for these compounds to reach the target.

As observed in **Table 3** and **Figure 2**, concentrations of bismuthine **1** >48.8 μM are lethal. These results permit us to carry out bioassays at lower concentrations, that is, 0, 5.0, 25.0, 50.0, and 75.0 μM and 0, 0.5, 1, 3, 5, 7.5, and 10 μM, and the results are shown in **Tables 4** and **5**, respectively.

On the other hand, we measured the length of shoot and root of seedling growth under these new conditions, and the results are shown in **Tables 6** and **7**. In these tables there are some values out of the normal tendency (bold); they represent a regulatory activity of these compounds at those concentrations. The average of the shoot/root length at control was 45/50, which shows a total length of 95 mm; by comparison of that value with the total average of the seedlings, at the higher concentration, a very significant inhibitory effect on the growth was observed, compound **1** being the most potent. These results are in agreement with the results obtained in the brine shrimp bioassay.

Growth was reduced by all of the compounds, but bismuthine **1** seems to be the most effective.

Monocot and Dicot Growth. Root development is more sensitive to a larger extent based on IC_{50} values, as compared with coleoptyle or hypocotyle development. Growth of monocots apparently was less sensitive to inhibition by compounds **1**−**3** as based on I_{50} values (**Tables 4**−**7**; **Figures 3** and **4**).

The *N*-methylpyrrolyl derivative promotes root and hypocotyle development for the monocot (data not shown). Hypocotyle growth was slightly stimulated at lower concentrations up to 10 μM for *L. sativa*, and to 15 μM for *T. pratense*, and thereafter, partially inhibited by this compound.

Compounds **2** and **3** have the lowest inhibitory effects on growth (30% inhibition or lower) at 5 μM for monocots (data not show), similar to the effects shown on seed germination, whereas for compound **1** 100% inhibition was achieved above 20.0 ppm or 49 μM, respectively, on dicots (**Figures 3C** and **4**).

The results shown here regarding the differences in behavior of furyl, thienyl, and *N*-methylpyrrolyl derivatives indicate that the mechanisms of action of these compounds may be different for growth and germination (*14*). 2,4-D showed a pronounced effect on root and shoot length, as well as on the germination of seeds. One hundred percent inhibition was obtained at 70 μM with I_{50} values of 0.4 and 0.5 μM for root and shoot, respectively [Anaya (*15*) has also reported similar results]; therefore, these bismuthines are more potent than 2,4-D.

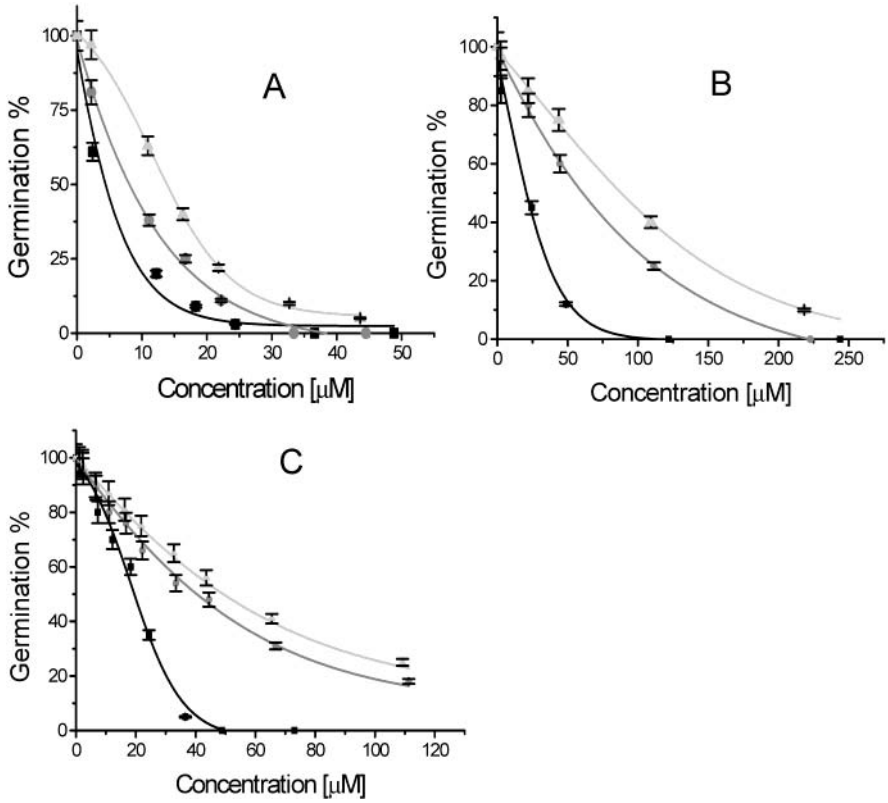


Figure 2. Effects of bismuthines **1** (■), **2** (●), and **3** (▲) on germination of *T. pratense* (A), *La. sativa* (B), and *Lo. multiflorum* (C) seeds, expressed as percent of control germination.

Table 4. Average Length of Seedling Growth of *La. sativa* at Different Doses of Bismuthins[a]

compd	control	5.0 μM	10 μM	25 μM	50 μM	75 μM
1	27/19	24/32	11/7	0	0	0
2	31/18	29/30	9/11	0	0	0
3	31/18	33/36	9/5	7/3	5/2	0

[a] Rate shoot/root (mm).

Table 5. Percentage of Germination at 48 h of Incubation at Different Concentrations of Bismuthines

compd	0 μM	0.5 μM	1.0 μM	3.0 μM	5.0 μM	7.5 μM	10 μM
1	100	95	95	80	70	35	35
2	100	85	85	80	76	65	60
3	100	90	90	85	80	70	60

Table 6. Average Length of Seedlings of *Lo. multiflorum* at Different Doses at 48 h[a]

compd	control	0.5 μM	1.0 μM	3.0 μM	5.0 μM	7.5 μM	10 μM
1	45/50	37/39	34/35	36/43	25/33	24/22	11/15
2	45/50	42/56	38/39	37/38	30/23	29/30	13/17
3	45/50	38/35	37/44	32/26	34/38	21/25	17/15

[a] Shoot/root.

Seed Respiration during Seed Germination. The respiratory rates of all seeds decrease with exposure to the three compounds in a concentration-dependent manner (**Figure 5**), as well as with increasing imbibition time. Thus, these compounds may act as metabolic inhibitors at lower concentrations, inhibiting either energy transduction or respiration redox enzymes but at higher concentrations. RI_{50} values (the concentration that induces 50% seed respiration inhibition) for **1**, **2**, and **3** against seed respiration of *T. pratense* are 6.3, 8.4, and 13.5 μM, respectively. These values show that bismuthines **1** and **2** are good energetic inhibitors of this important physiological pathway. According to their RI_{50} values (data not shown), dicot seeds (*P. ixocarpa* and *T. pratense*) are more sensitive to inhibition against bismuthines. On the other hand, *Lo. multiflorum* seeds showed the highest resistance to respiration inhibition.

Table 7. Percentage of Average Length Reached by Seedlings of *Lo. multiflorum* in the Presence of Different Concentrations of Bismuthines

compd	control	0.5 μM	1.0 μM	3.0 μM	5.0 μM	7.5 μM	10 μM
1	100/100	82/78	75/70	79/86	55/66	53/44	24/30
2	100/100	93/112	84/78	82/76	66/46	64/60	28/34
3	100/100	84/70	82/88	71/52	75/76	46/50	37/30

Mitochondrial Oxygen Uptake Inhibition. The effect of bismuthines on mitochondrial respiration is shown in **Figure 6**. I_{50} values for **1**, **2**, and **3** were 10.0, 17.1, and 35.0 μM, respectively. These data corroborate the results obtained with seed respiration for *La. sativa*, *T. pratense*, and *Lo. multiflorum*. An increase in oxygen uptake for concentrations as high as 7.5 and 10 μM of bismuthines **2** and **3**, respectively, was observed. As in the seed respiration, bismuthine **1** ($I_{50} = 1.1$ μM) is more potent even at the lower concentration. Washing mitochondria with buffer restored full respiratory activity, annulling the effect of the compounds. These results show that bismuthines **1** and **2** are powerful inhibitors of mitochondria respiration, at concentrations similar to those reported for phenolics by Moreland and Novitzky (*16*). The mechanism of action is under study.

Radical Scavenging Properties. Radical scavenging properties of compounds **1**−**3** were evaluated against the DPPH radical reduction as a TLC spray reagent. Compound **1** (5.0−100 μM) appeared as yellow spots against a purple background, whereas the same amounts of **2** and **3** did not react with the radical.

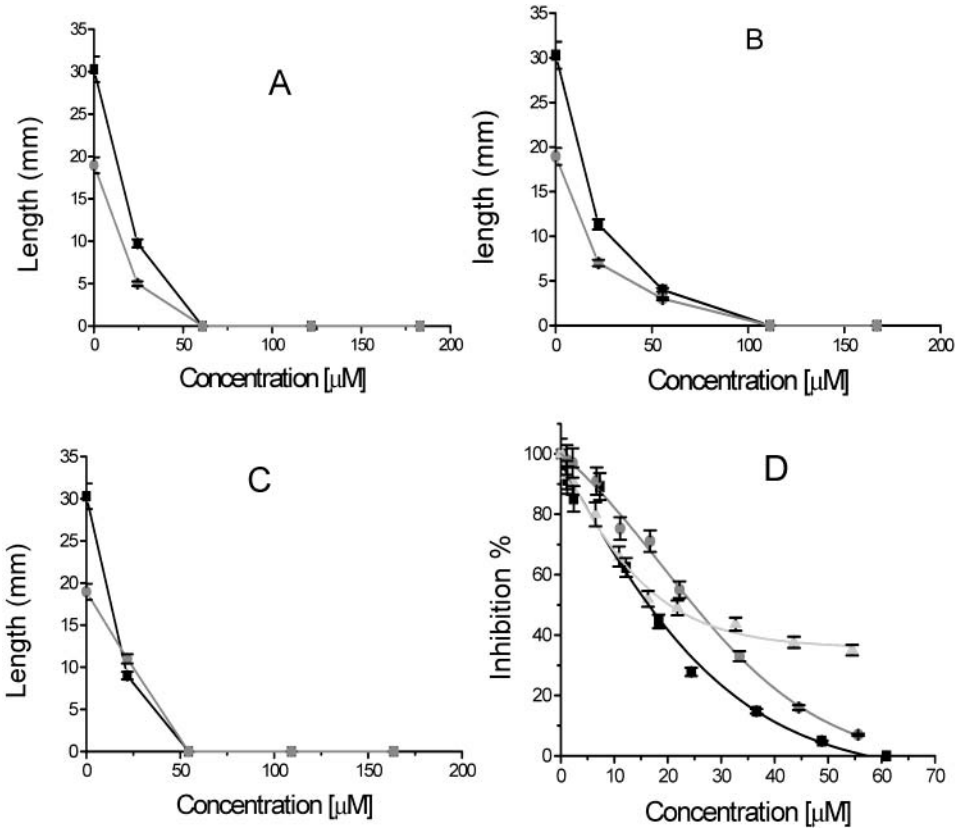


Figure 3. Effects on seedling growth inhibitory activity by bismuthins **1** (C), **2** (A), and **3** (B) on development of root (■) and shoot (●) of *La. sativa*. Compared with the effect on shoot elongation of *Lo. multiflorum* of **1** (■), **2** (●), and **3** (▲), all graphs are the results of three measurements.

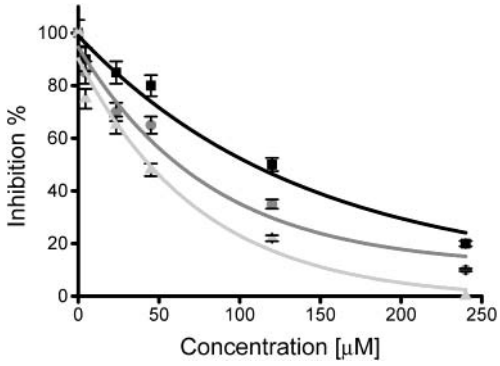


Figure 4. Inhibition by solutions of bismuthines **1** (▲), **2** (●), and **3** (■) on dry weight of seedling growth of *La. sativa*. Each value represents mean ± SE ($N = 5$) in error bars. The inhibition efficacy was expressed as a percentage of bismuthin activity inhibited compared with the control value (100%).

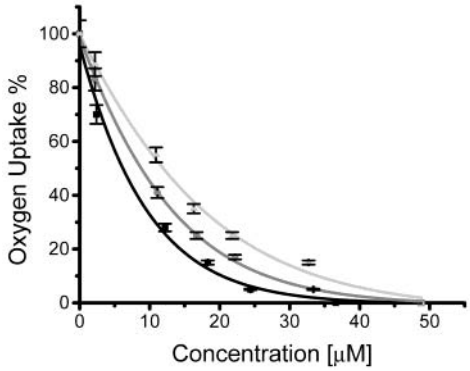


Figure 5. Effects of bismuthines **1** (■), **2** (●), and **3** (▲) on seed respiration of *T. pratense* (dicot) (means of three experiments). Values (at 72 h) correspond to the concentration that inhibits seed respiration during germination.

Compounds **1**−**3** were also tested against DPPH in a spectrophotometric assay as previously reported (*17*). This method confirmed that compound **1** exhibited the strongest radical scavenging activity. Compounds **2** and **3** were less reactive than **1**. It is worth mentioning that quercetin, a flavonol with five hydroxyl groups, was used as a reference compound that possesses strong antioxidant properties (*18*, *19*) and that a solution with DMSO (100%) was used as blank (**Figure 7**).

Concluding Remarks. The three compounds examined in this study have similar molecular structures, the differences resting in the substitution of the furyl (**1**), thienyl (**3**), and *N*-methylpyrrolyl (**2**) substituents (**Figure 1**). Of the three compounds tested for growth inhibition of three plant species, **1** showed the greatest effect on the dicot weed (*P. ixocarpa* and *T. pratense*). **2** had similar inhibitory effects on the root growth of *Lo. multiflorum* (**Figure 3**), whereas **3** had a greater effect on shoots than **2** (**Figure 3**). At amounts >20 μg/g (ppm) of all compounds, the shoots were severely malformed and the thickness was significantly lesser than ($p < 0.05$ ANOVA) than controls.

The effects of these compounds have certain similarities with some natural products such as artemisinin, ovatifolin, costunolide, and parthenolide and their derivatives (*4*, *17*, *20*, *21*).

In addition, it was possible to correlate the antioxidant activity with the seedling growth inhibitory activity. Interestingly, sulfhydryl and pyrrolyl derivatives did not show a significant radical scavenger activity against DPPH. In contrast, the furyl form is the most active compound as radical scavenger. More importantly, these compounds showed an interesting mode of action in seedling development as the inhibition of mitochondrial

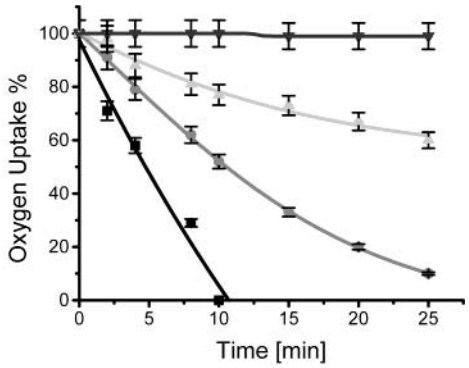


Figure 6. Oxygen uptake of mitochondrial respiration in the presence of **1** (●), **2** (▲), and **3** (▼) at 50 μM; control (■) (conditions given under Materials and Methods).

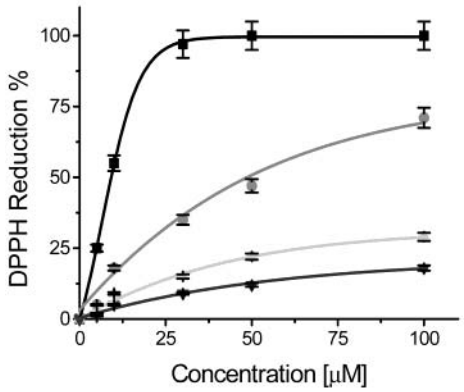


Figure 7. Scavenging activity of quercetin (■), **1** (●), **2** (▲), and **3** (▼) on radical reduction of DPPH. Measurements were performed at 517 nm after 30 min.

respiration, and in the same way bismuthin derivatives could have some interaction in cell proliferation or energetic pathways.

In conclusion, our data indicate that bismuthines **1**−**3** are more selective and potent respiratory inhibitors toward dicots than to monocots. Respiration processes are involved in the interference action, as these processes were inhibited in a parallel manner by 2,4-D and the compounds assayed. At the same doses, a higher inhibition was observed on seed germination than on seed respiration, and it was concluded that these compounds have more than one target site.

The treatment concentrations for the compounds that reduced seedling growth were low (10−40 μM) compared to allelopathic chemicals that have been previously studied under laboratory conditions (*22*, *23*). Well-known secondary metabolites have potency in the range from 100 to 1000 μM for growth reduction by many phenolic acids, or around 10 μM for sorgoleone, or at the micromolar level for juglone, which suppresses the growth of several herbaceous species (*24*−*26*).

Whatever the mechanism(s) of action of the bismuthines, the furyl form has proved to be a good inhibitor of plant growth. They show pre-emergent phytotoxic properties by inhibiting germination and growth. They also show some degree of selectivity by inhibiting dicotyledonous species more drastically.

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