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A Theoretical Analysis of the Relationship between the Electronic Structure of Indole Derivatives and Their Phytotoxicity against *Lactuca sativa* seeds.

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ABSTRACT

Allelopathic effects of a chemical compound strongly depends on the target species, dose or concentration employed, structure of the molecule and physical properties, such as water solubility, lipophilicity and others. In this work, we present the results of a preliminary quantum-chemical analysis of the possible relationships between the electronic structure of a series of indole derivatives and the percentage of germination inhibition (I) of *Lactuca Sativa* seeds. The results allow predicting phytotoxicity activity of these compounds, the structural features of an ideal indole derivative, as well as some structural characteristics of the active site.

Keywords: QSAR, *Lactuca sativa*, allelochemicals, allelopathy, gramine, indole derivatives.

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INTRODUCTION

Allelopathic interactions between plants are usually caused by plant produced allelochemicals. Research on allelopathic interactions have focused in agricultural crops as an option for the development of integrated weed management strategies, reducing environmental effects and cost of crop production [1-6]. Allelopathy in field conditions may arise from natural metabolites and from decomposition of vegetal residues, when it is released to the soil and degraded by biotic and abiotic processes. Besides, the allelopathy effect of a given allelochemical strongly depends, among others, on the target species, dose or concentration, structure of the molecule and physical properties, such as water solubility, lipophilicity and others [7-10]. The effectiveness of a given allelochemical is a highly complex property, and their mode of action is not easy to predict or understand. Seed germination is determined by a combination of the degree of dormancy and environmental factors such as light, oxygen, and temperature. Of these environmental aspects, temperature is a primary feature that influences seed germination [11-17]. Alkaloids containing the indolic skeleton, such as gramine (Fig. 1), triptamine and other derivatives, are present in several species of gramineae, leguminous and other plant families [18-20]. They are responsible not only of various deleterious effects on mammals, insects, fungi and bacteria [21-24], but also of allelopathic effects against competitive weeds and cereals [25, 26]. The phytotoxic effects of these molecules may arise from indole derivatives, formed by degradation of the indolic fragment contained in naturally occurring alkaloids.

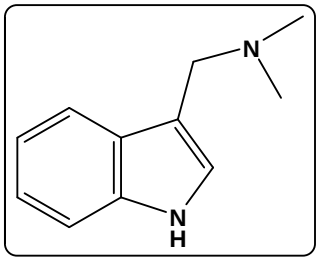

Figure 1: Structure of gramine

In this work, and as an effort to reveal some of the principles that rule the potential phytotoxic activity of a series of indole derivatives substituted in the aromatic ring, we have used molecular orbital theory to obtain an equation predicting the phytotoxicity of a series of molecules shown in Fig. 2. The study was carried out to predict experimental data from germination inhibition percentage (I) assays performed in *lactuca sativa* seeds.

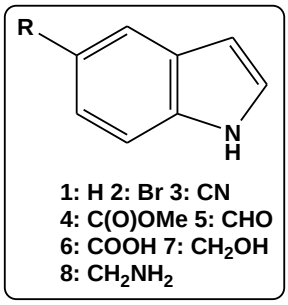

Figure 2: Molecules used in this study.

EXPERIMENTAL

Chemicals

All indole derivatives were purchased from Aldrich Chemical Co. at the highest purity available and were used as received, without further purification.

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Germination Assay

Phytotoxicity was measured using the germination inhibition methodology performed in lettuce seeds. 45 lettuce seeds (*Lactuca Sativa* L.) were uniformly distributed in 5 Petri dishes and covered with a cotton film. In order to provide the same concentration of indolic derivative for each compound to be tested, each plate was watered with 10 ml of an aqueous solution containing 50 mg/ml of the respective compound. The plates were sealed, incubated at 25±2°C and subjected to 6 days of continuous 8:16 hours of light:dark cycles. Light periods were provided by a cold white fluorescent light with intensity of 300 ft-c. Seeds were obtained from commercial sources. Controls were incubated only with distilled water. Each assay was replicated 3 times and the effectiveness was expressed as a percentage of the control experiment. Each value is the average of three assays. Errors were smaller than 5% in all cases. The data was analyzed by ANOVA.

CALCULATIONS

The formal model to relate electronic structure with biological activity has been fully presented and explained in detail in this and several other Journals [27-31]. It is based on the following master equation:

$$\log(\mathrm{I}) = a + bM_{D_i} + c\log\left[\sigma_{D_i} / (ABC)^{1/2}\right] + \sum_j \left[e_j Q_j + f_j S_j^E + s_j S_j^N\right] +$$
$$+ \sum_j \sum_m \left[h_j(m) F_j(m) + x_j(m) S_j^E(m)\right] + \sum_j \sum_{m'} \left[r_j(m') F_j(m') + t_j(m') S_j^N(m')\right] +$$
$$+ \sum_j \left[g_j \mu_j + k_j \eta_j + o_j \omega_j + z_j \varsigma_j + w_j Q_j^{\max}\right] \qquad (1)$$

Where I is the percentage of germination inhibition, M is the molecular mass, σ its rotational symmetry number, ABC the product of the molecular moments of inertia about the three principal axes of rotation, Q_j is the net charge on atom j, S_j^E and S_j^N are the total atomic electrophilic and nucleophilic superdelocalizabilities of atom j, respectively, $F_{j,m}$ ($F_{j,m'}$) is the Fukui index (electron population) of the occupied (vacant) MO m (m') localized on atom j. $S_j^E(m)$ is the atomic electrophilic superdelocalizability of MO m localized on atom j, $S_j^N(m)$ is the atomic nucleophilic superdelocalizability of MO m localized on atom j. The total atomic electrophilic superdelocalizability of atom j corresponds to the sum over occupied MOs of the $S_j^E(m)$'s and the total atomic nucleophilic superdelocalizability of atom j is the sum over vacant MOs of the $S_j^N(m)$'s. μ_j is the local atomic electronic chemical potential of atom j, η_j is the local atomic hardness of atom j, ω_j is the local atomic electrophilicity of atom j, ς_j is the local atomic softness of atom j, and $Q_j^{\max}$ is the maximal amount of electronic charge that atom j may accept from another site. $HOMO_p$* refers to the highest occupied molecular orbital localized on atom p and $LUMO_q$* refers to the lowest empty MO localized on atom q. This method was originally developed for the estimation of equilibrium constants for receptor binding affinities. During year 2013, and based on theoretical considerations, it was extended to any kind of biological activity provided that the group of studied molecules have the same mechanism of action [32]. The practical application of this model to very different biological activities and series of molecules have provided excellent results (see [33-36] and references therein). Here we work within the common skeleton hypothesis, involving the existence of a series of molecules in which there is a group of atoms common to all of them, which are responsible for most part of the variation in the activity throughout the series. The common skeleton is shown in Fig. 3.

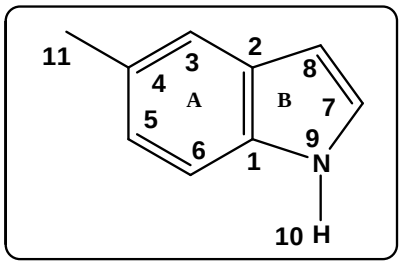

Figure 3: Common skeleton numbering.

All geometries were fully optimized at the B3LYP/6-31G(d,p) level with the Gaussian03 package of programs [37]. The D-Cent-QSAR software [38] was employed to obtain the local atomic reactivity indices from the electronic structure calculations, including corrections to the Milliken population analysis [39]. Given that the number of experimental data is inferior to the number of local atomic reactivity indices, we employed the usual statistical tools for this situation. A linear multiple regression analysis (LMRA) was performed with the biological activity as the dependent variable and the set of local atomic reactivity indices of the common skeleton atoms as the independent variables. The Statistica software [40] was used for this purpose.

RESULTS AND DISCUSSION

Table 1 shows the logarithm of germination inhibition percentages, from the experiments described in the experimental section. All compounds display moderate activity. A closer inspection to this table reveals that the phytotoxicity depends on the structural modifications of the aromatic ring.

Table 1: Molecules and log of germination inhibition percentage (I)

Mol.	R	log(I) 50 µg/ml
1	-H	1.39
2	-Br	2.00
3	-CN	1.98
4	-C(O)OMe	1.15
5	-CHO	0.26
6	-COOH	0.23
7	$-CH_2OH$	0.95
8	$-CH_2NH_2$	0.70

Linear Multiple Regression Analysis (LMRA)

The best equation obtained by the LMRA is:

$$\log(I)=2.17-6.72F_6(LUMO+2)^*-3.91S_{10}^E(HOMO)^*-2.85Q_4 \quad (2)$$

with n=8, R=0.99, R^2=0.99, adj-R^2=0.98, F(3,4)=101.23 (p<0.0003) and SD=0.10. No outliers were detected and no residuals fall outside the ±2σ limits. Here, $F_6(LUMO+2)^*$ is the Fukui index (electron population) of the third lowest vacant MO localized on atom 6, $S_{10}^E(HOMO)^*$ is the electrophilic superdelocalizability of the highest occupied MO localized on atom 10 and Q_4 is the net charge of atom 4 (Fig. 1). There is no significant correlation between any pair of independent variables (not higher than 10%). The beta values and the result of the *t*-test for the significance of the coefficients in Eq. 2 (not shown) indicate that the importance of the variables is $F_6(LUMO+2)^* >> S_{10}^E(HOMO)^* > Q_4$. The associated statistical parameters of Eq. 2 show that this equation is statistically significant and that the variation of a set of three local atomic reactivity indices belonging to the common skeleton (Fig. 1) explains about 98% of the variation of log(I). Fig. 2 shows the plot of predicted *vs*. observed log(I) values.

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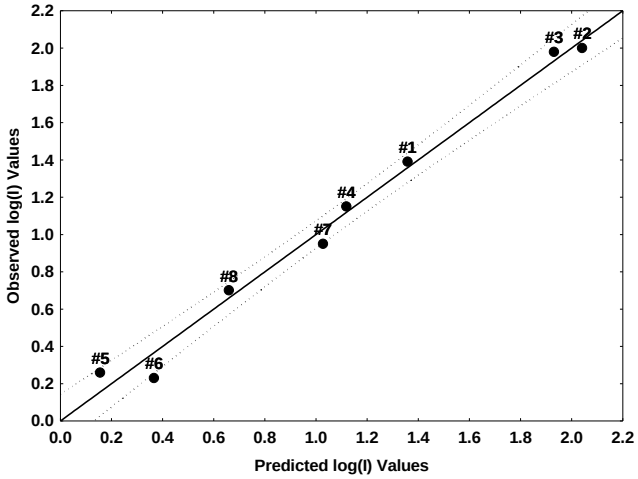

Figure 2: Plot of observed log(I) *vs*. predicted values according to equation 2.

In Fig. 2, an adequate correlation between experimental and predicted activity is observed. The molecular mechanism of action of these compounds is not known. Parameters such as electrophilicity, nucleophilicity and H-bond interactions with macromolecules at the active site, can be involved in the action mechanism. Table 2 displays the local molecular orbital contributions to the structure of orbitals (HOMO-2), (HOMO-1), (HOMO) - (LUMO), (LUMO+1), (LUMO+2), at atoms 6, 7, 9 and 10.

Table 2: Local molecular orbital structure of atoms 6, 7, 9 and 10

Mol	Atom 6 (C)	Atom 7 (C)	Atom 9 (N)	Atom 10 (H)
1 (31)	29π30π31π-32π 33π34π	29π30π31π-32π33π34π	29π30π31π-32π33π34π	21σ22σ27σ-35σ36σ37σ
2 (48)	46σ47π48π-49π50π51π	44π47π48π-49π51π52π	45π47π48π-49π51π52π	37σ39σ43σ-53σ55σ57σ
3 (37)	35π36π37π-38π39π40π	35π36π37π-38π39π41π	35π36π37π-39π41π43π	24σ27σ31σ-42σ43σ45σ
4 (46)	44σ45π46π-47π48π49π	43π45π46π-47π48π49π	43 45π46π-47π48π49π	30σ31σ32σ-50σ52σ54σ
5 (38)	35π37π38π-39π40π41π	35π37π38π-39π40π41π	35π37π38π-39π40π41π	26σ28σ33σ-42σ45σ46σ
6 (42)	40σ41π42π-43π44π45π	39π41π42π-43π44π45π	39π41π42π-43π44π45π	29σ32σ36σ-46σ50σ52σ
7 (39)	36π38π39π-40π41π42π	36π38π39π-40π41π42π	37π38π39π-40π41π42π	26σ27σ28σ-43σ44σ46σ
8 (39)	37π38π39π-40π41π42π	37π38π39π-40π41π42π	37π38π39π-40π41π42π	27σ28σ34σ-43σ44σ46σ

A variable-by-variable analysis of Eq. 2 shows that a high inhibition of germination is associated with a low value for $F_6(LUMO+2)^*$, a negative net charge on atom 4 and a high negative value for $S_{10}^{E}(HOMO)^*$. Atom 6 is a carbon in ring A (Fig. 3) and Table 2 shows that the three lowest vacant local MOs are of π nature. A low value for this local atomic reactivity index suggest that $(LUMO+2)_6^*$ is engaged in a repulsive interaction with vacant MOs of the partner. As the appearance of this MO indicates that $(LUMO+1)_6^*$ and $(LUMO)_6^*$ are also interacting with the site, we suggest that this atom is interacting with an electron rich center through its first two lowest vacant local MOs. Atom 10 is the hydrogen bonded to N9 (Fig. 1). Table 2 shows that the three highest occupied MOs are of σ nature. Let us remember that, if this atom becomes engaged in a hydrogen bond interaction of the N-H…X kind with the partner, the σ MOs transform into π MOs. Therefore it is suggested that atom 10 forms an H bond with a N or O atom of the active site. Another possibility is that H10 participates in a σ-σ, a cation-π or a cation-anion interaction. The substitution of H10 by a methyl group could help to clarify this fact. Atom 4 is a carbon in ring A (Fig. 1). A negative charge indicates

that this atom is participating in an electrostatic interaction with a positively charged residue of the partner. All these suggestions are encompassed in the two dimensional (2D) partial pharmacophore shown in Fig. 3.

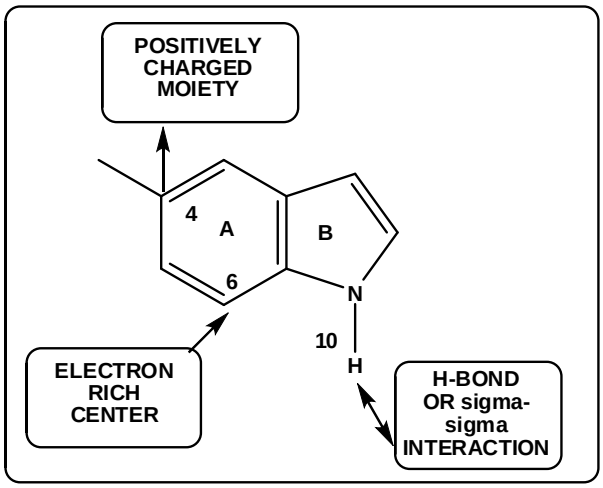

Figure 3: 2D partial pharmacophore for log(I).

CONCLUSIONS

The use of MLRA to reveal the interactions that rule the germination inhibition of *lactuca sativa* by indole derivatives appears to be an appropriate methodology for this purpose. According to our results, electro-attractive substituents on position 4, favors the deficiency of electron density in that position, and decreases the strength of the H-bond with N9, favoring the possible H-bond formation with suitable groups at the active site, increasing the phytotoxic activity. In agreement with that, the Br and CN derivatives are the two substituents with the highest electron-acceptor properties and the most effective compounds. These results allow predicting the phytotoxic activity of these molecules, the structural features of an ideal indole derivative, as well as structural characteristics of the active site. Part of the allelopathic potential of natural indole alkaloids could arise from interactions similar to that described in this work.

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