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Seed Dormancy in Red Rice[1]

VII. Structure-Activity Studies of Germination Stimulants

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ABSTRACT

Many chemically dissimilar substances break dormancy of seeds, but the relationship between chemical structure and physiological activity is unknown. In this study, the concentrations of organic acids, esters, aldehydes, alcohols, and inorganic weak acids required to elicit 50% germination of initially dormant, dehulled red rice seeds (*Oryza sativa*) were determined. The activity of most substances was very highly and inversely correlated to lipophilicity as measured by octanol/water partition coefficients; chemicals with the highest partition coefficients required the lowest concentrations to elicit the germination response. Relative efficacy was also dependent upon the functional group; generally, monocarboxylic acids were more effective than aldehydes, esters, hydroxyacids, and alcohols. Relative hydrophobicity plots supported a modulating role of the functional group. Dormancy-breaking activity of methyl formate, formic acid, nitrite, azide, and cyanide was higher than predicted based on lipophilicity and apparently was related to molecular size; compounds with smaller molecular widths were required at lower concentrations to achieve the 50% germination response.

Seed dormancy is broken by many substances (4, 7) including weak acids (9), alcohols (24), aldehydes, nitriles, and ketones (11, 12). The concentrations required to break dormancy vary by as much as five orders of magnitude. What chemical properties of these compounds account for this broad range of activities?

With red rice and several other species, the dormancy-breaking activity of weak acids is pH dependent. GA_3, azide, cyanide, nitrite, salicylhydroxamic acid, or aliphatic, monocarboxylic acids stimulate *ca.* 90% seed germination when applied at incubation medium pH values which favor the neutral form of each substance (5, 7, 9, 10). Therefore, the lack of molecular charge coupled with the weak acid character of dormancy-breaking compounds may be required for physiological activity. However, these properties cannot solely account for the activity of alcohols, aldehydes, esters, and ketones, which do not possess dissociable protons. In this report, we show that relative dormancy-breaking activity of a wide array of substances is primarily related to their lipophilicity but is modulated by the nature of the functional group(s) present or by molecular size.

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MATERIALS AND METHODS

Mature, strawhulled, awnless red rice (*Oryza sativa*) was obtained from the South Farm, Rice Research Station, Crowley, LA in 1985. Harvesting, processing, and storage procedures were those previously described (8). Air-dried seeds were stored at −15°C until use. Seeds were dehulled by hand just prior to treatments.

Germination tests were conducted as described by Cohn and Hughes (10). Lots of 20 dehulled seeds were placed in 50 mL Erlenmeyer flasks containing 2 layers of Whatman No. 1 filter paper and 2 mL of test solution for 24 h. Dilute HCl or NaOH was used to adjust the pH of test solutions buffered with 25 mM citrate-phosphate buffer (pH 3–7) or 2-(*N*-cyclohexylamino)ethanesulfonic acid (pH 9.3). The pH values utilized for weak acid treatments corresponded to their pK except for lactic and succinic acids (pH 3). Esters and aldehydes were applied at pH 7 to minimize conversion to weak acids. Alcohols were incubated at the pK of the parent acid. The pH values of buffer controls corresponded to the pH used to evaluate each chemical. The medium pH of each flask was recorded after chemical incubation to confirm sufficient buffering capacity. All incubations were conducted at 30°C in darkness. After treatment, seeds were rinsed from flasks, washed copiously with running water, briefly blotted with

Table I. *Concentrations (mM) of Monocarboxylic Acids (HA) and Derivatives Required for 90% Germination of Dehulled Red Rice*

Carbon No.	Concentration					
	Acid	Isoacid	Ester	Aldehyde	Alcohol	—OH Acid
1C	10		130		2600	
2C	26		106	42	1000	200
3C	10		30	40	80	315
4C	14	15	NA[a]	NA	NA	NA
5C	10	10				

[a] NA, 90% germination not attainable.

Table II. *Concentrations of Substances Required for 50% Germination of Dehulled Red Rice*

For weak acids, the concentration is that of the free acid (HA). Germination of buffer controls averaged 6%.

Code	Chemical	[mM], 50% germination	Assay pH
A	Salicylic acid	1.2	3.0
B	Caproic acid	2.0	4.8
C	Benzoic acid	2.4	4.2
D	Valeric acid	3.0	4.8
E	Isovaleric acid	7.0	4.8
F	Propionic acid	7.0	4.9
G	Butyric acid	11.0	4.8
H	Isobutyric acid	11.0	4.8
I	Trimethylacetic acid	15.0	5.0
J	Pentanol	22.0	4.8
K	Acetic acid	22.0	4.8
L	Methyl propionate	23.0	7.0
M	Propionaldehyde	29.0	7.0
N	Butyrolactone	40.0	4.8
O	Propanol	41.0	4.9
P	Acetaldehyde	41.0	7.0
Q	Ethyl acetate	65.0	7.0
R	Butanol	80.0	4.8
S	Glycolic acid	135.0	3.8
T	DMO	163.0	6.1
U	Lactic acid	278.0	3.0
V	Succinic acid	460.0	3.0
W	Ethanol	680.0	4.8
X	Isopropanol	690.0	4.9
Y	Methanol	2340.0	3.7

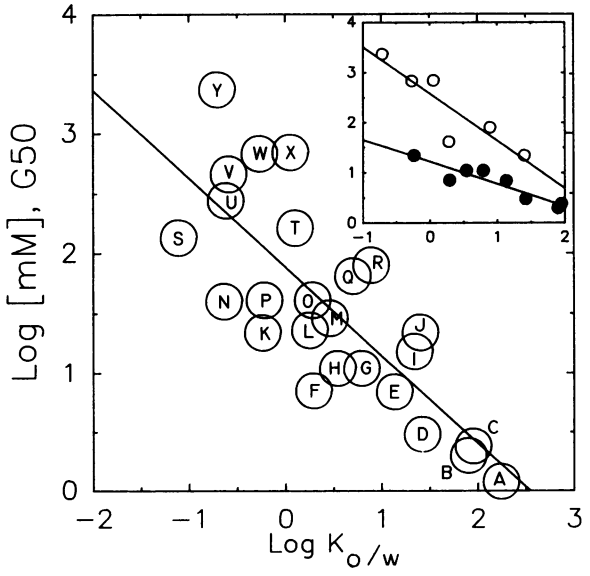

Figure 1. Correlation between the lipophilicity of dormancy-breaking chemicals and concentrations required to elicit 50% germination of initially dormant, dehulled red rice ($y = -0.73x + 1.86$; $r = -0.79$; $P < 0.001$). Inset: (●) monocarboxylic acids ($y = -0.44x + 1.21$; $r = -0.93$; $P < 0.001$), (○) alcohols ($y = -0.94x + 2.57$; $r = -0.90$; $P < 0.05$). Identification code as in Table II.

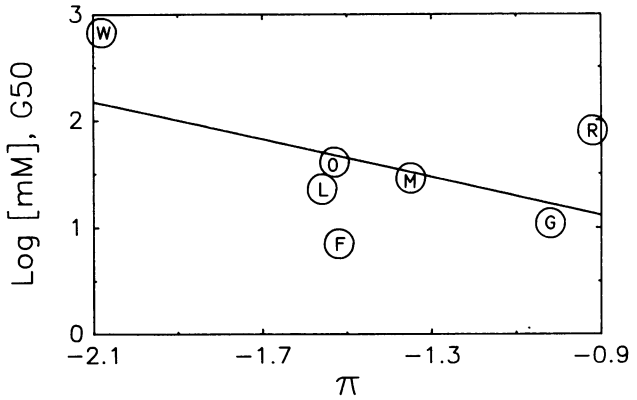

Figure 2. Correlation between relative hydrophobicity (π) of dormancy-breaking chemicals and concentrations required to elicit 50% germination of initially dormant, dehulled red rice ($y = -0.88x + 0.32$; $r = -0.52$). Identification code as in Table II.

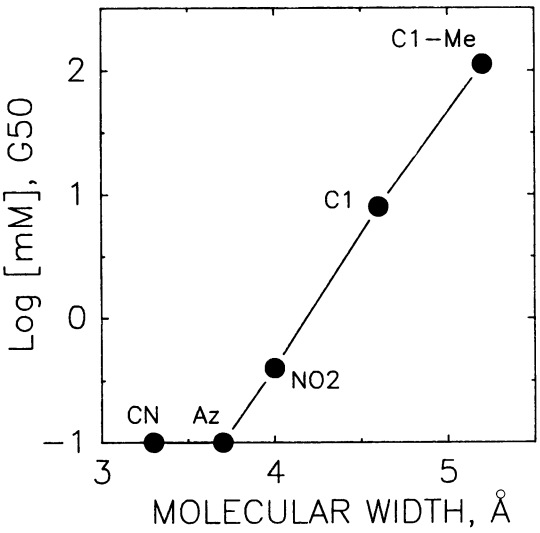

Figure 3. Relationship between molecular width of dormancy-breaking chemicals and concentrations required to elicit 50% germination of initially dormant, dehulled red rice. NO_2, sodium nitrite; Az, sodium azide; CN, potassium cyanide; C1, formic acid; C1-Me, methyl formate.

tissue, and transferred to clean flasks containing filter paper and 2 mL of water for 7 d, 30°C. For each experiment there were five replications of each treatment, and experiments were repeated three times. All chemicals employed were reagent grade. Test solutions were prepared fresh daily.

In experiments which tested the activity of gaseous hydrocarbons, dehulled seeds were incubated in open 6 cm Petri dish halves with 2 mL buffer at pH 7 on 2 layers of Whatman No. 1 filter paper. Dishes were placed in a 9 L desiccator which was continuously flushed with the gas under study for up to 48 h at 25°C. Gases were bubbled through water before passing over the seeds, and the atmosphere in the desiccator was maintained at 100% RH. Seeds were then transferred to Erlenmeyer flasks as described above and incubated for 7 d, 30°C. Butane (2.2% in nitrogen), propane (2.3% in nitrogen), and carbon monoxide (99%) were used as supplied (Airco).

Germination over the time course of each experiment was scored as visible protrusion of the radicle viewed at 10× under a dissecting microscope. Germination percentages presented are relative to the total number of seeds tested. During the optimization of dormancy-breaking concentrations of applied chemicals, viability of ungerminated seeds was evaluated after treatments by growth of isolated embryos.

Concentrations of the undissociated form of weak acids were calculated from the Henderson-Hasselbalch equation. Partition coefficients ($K_{o/w}$[2]) were obtained directly from Hansch and Leo (13) or from regression equations generated from their data. $K_{o/w}$ for DMO was the value determined by Butler (6). Relative hydrophobicities were calculated as the difference between the log $K_{o/w}$ of the chemical under study and the log $K_{o/w}$ of its parent compound (either methane or ethane) (13). Molecular dimensions were estimated from known bond angles, bond lengths, and Van der Waals radii (14, 16). Regression analysis was performed according to Steel and Torrie (22).

RESULTS

Monocarboxylic acids most readily stimulated 90% germination of dehulled red rice (Table I). Isobutyric and isovaleric acids were as active as the linear forms. Nonaromatic hydroxyacids (glycolic and lactic acid) required one order of magnitude higher concentrations for responses similar to their parent acids (acetic and propionic acid). Esters elicited activity at 30 to 130 mM and showed increasing activity with increased carbon number. A similar relationship was observed for the alcohols. Acetaldehyde and propionaldehyde were effective at *ca.* 40 mM. However, several substances of interest broke dormancy but did not elicit 90% germination: butanol, succinic acid, and salicylic acid. Therefore, to facilitate structure-activity comparisons, concentrations required to obtain 50% germination were obtained from dose-response experiments (data not shown) for these chemicals as well as others (Table II).

The log $K_{o/w}$ was plotted versus the log concentration of each substance required for 50% germination (Fig. 1). A significant correlation ($P < 0.001$) between this index of lipophilicity and dormancy-breaking activity was obtained.

The dormancy-breaking activity *within* the monocarboxylic acid group was significantly correlated to log $K_{o/w}$ ($P < 0.001$). A similar relationship was obtained for the alcohols ($P < 0.05$) (Fig. 1, inset). The slopes and intercepts of these two lines were significantly different from each other, as shown by a test of homogeneity of regression coefficients ($P < 0.05$). Correlations between relative hydrophobicity, using ethane (Fig. 2) or methane (data not shown) as the parent compounds, and the log concentration of each substance required for 50% germination were not statistically significant. Forty-eight-hour exposures to propane, butane (each *ca.* 1 mM), or pure carbon monoxide promoted germination no more than 5% above control levels.

The dormancy-breaking activity of substances with smaller size dimensions (cyanide, azide, nitrite, formic acid, and methyl formate) than those reported in Figure 1 was not significantly correlated with lipid solubility and may be a function of molecular width (Fig. 3).

DISCUSSION

Aldehydes, ketones, alcohols, carboxylic acids, esters, and nitriles of various structures are capable of breaking seed dormancy (2, 9, 11, 12, 25). However, no perceivable trends in these data accounted for the broad activity spectrum observed. Now, as a result of this study, we can show a general correlation between dormancy-breaking activity and lipophilicity for representative compounds with the following functional groups: monocarboxylic acids, aldehydes, esters, hydroxyacids, dicarboxylic acids, and alcohols (Fig. 1). Recent data of Taylorson (24) show a correlation between lipophilicity and dormancy-breaking effects of primary alcohols on barnyardgrass, which are consistent with our results. For nondormant seeds, inhibition of germination is also related to the lipophilicity of applied organic chemicals in aqueous solutions (17–19).

While activity generally depended upon lipophilicity (possibly reflecting relative penetration of the caryopsis cuticle [15]), a test for homogeneity of regression coefficients between the alcohol and carboxylic acid series (Fig. 1, inset) indicated that the nature of the functional group modulated the dormancy-breaking response. Consistent with this idea, relative hydrophobicity was poorly correlated with activity (Fig. 2). If derivatives were eliciting a response in the same way, highly significant correlations should have been obtained (3). In addition, butane, propane, and carbon monoxide were inactive at concentrations expected to stimulate 50% germination or greater based upon their lipophilicities. Low mol wt alkanes are also inactive as a dormancy-breaking substances of *Portulaca oleracea* (23) and *Lactuca sativa* (1). Therefore, reasonable lipophilicity alone is insufficient to confer a substance with dormancy-breaking activity. The lack of response to CO also suggests that the dormancy-breaking capabilities of weak acids are not a function of metal chelation.

Inorganic weak acids, formic acid, and methyl formate elicited the standard 50% germination response at lower concentrations than anticipated based solely upon their lipophilicities; relative activity could be expressed as a function of molecular size (Fig. 3). While this relationship may be fortuitous, it is striking that the molecular dimensions of these dormancy-breaking chemicals are smaller than those usually attributed to membrane pores (21).

Is each class of compound breaking dormancy by an independent pathway or via a common mechanism? We have been speculating that proton loading associated with weak acid uptake contributes to dormancy-breaking activity (9), but it is clear from this study that such a generalization has to be tempered until further research is conducted. Dormancy-breaking activity was observed for substances lacking a dissociable proton. Relative activity may be associated with both the ease of penetration and subsequent metabolic conversion to one active functional group. Since the least metabolized substances would be DMO (20), azide, and cyanide, this key functionality may be a weak acid. Alternatively, the intercalation of each substance at a critical concentration

[2] Abbreviations: $K_{o/w}$, octanol/water partition coefficient; DMO, 5,5-dimethyl-2,4-oxazolidinedione.

directly into membrane systems may be the most relevant factor, as has been proposed for alcohols (24).

In summary, the relative activity of many dormancy-breaking chemicals is generally a function of their lipophilicity and further modulated by the nature of the functional groups present. This may account for the large differences in concentration required to elicit equivalent dormancy-breaking activity by a wide range of chemicals.

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