

**Dihydroactinidiolide – a potent growth inhibitor from *Eleocharis coloradoensis* (spikerush)**

K. L. Stevens and G. B. Merrill

*Western Regional Research Center, US Department of Agriculture, 800 Buchanan Street, Berkeley (CA 94710, USA), 10 June 1980***Summary.** Dihydroactinidiolide has been isolated from *Eleocharis coloradoensis*, along with p-coumaric acid, ferulic acid and luteolin, and shown to be a potent phytotoxin at low concentration.

Bioassay of components from the aquatic sedge, *Eleocharis coloradoensis* (Britt.) Gilly (spikerush), has revealed a number of substances which are known phytotoxins, viz., p-coumaric acid, ferulic acid and luteolin (fig. 1)<sup>1</sup>. In addition to these mildly toxic substances a potent phytotoxin<sup>2</sup> has been isolated via extraction and gas chromatography and identified by mass spectrometry<sup>3</sup> as dihydroactinidiolide (DAD, fig. 1). To confirm its identity and procure adequate amounts for biological testing, DAD was synthesized by standard procedures<sup>4,5</sup>.

Seed germination tests were performed on *Nasturtium officinale* (watercress) and *Raphanus sativus* (radish, scarlet globe, Germain's Inc.) which gave essentially identical results. The results of the radish seed test is shown in figure 2. Seeds were placed on 9-cm filter paper discs in petri dishes containing varying amounts (0–50 ppm) of test material and incubated at 28 °C (18-h-day) and 18 °C (6-h-night) for 66 h. DAD, at a concentration above 50 ppm, completely prevents germination, while at 10 ppm a 65% reduction is observed with respect to the control. Due to the limited solubility of DAD in water, data above 20–30 ppm are less reliable than the lower concentrations. However,

even at 5 ppm (the concentration of DAD in spikerush) DAD appears to be a potent inhibitor of seed germination. Often the seeds which germinate in the presence of DAD are much slower in development than the controls and frequently exhibit chlorosis. The developing roots are often stunted, discolored and contain fewer root hairs. In general, less vigor is observed in the developing seedlings in the presence of DAD.

DAD has been isolated from *Actinidia polygama*<sup>6</sup>, tea leaves<sup>7</sup>, tobacco<sup>8</sup>, and cassia<sup>9</sup> and shown to be an important aroma constituent and also an effective attractant for Felidae animals in lower order<sup>10</sup>. However, the phytotoxic nature of DAD has not been previously reported and may partially account for the observed allelopathic activity in spikerush<sup>11–13</sup>.

Root length elongation is also retarded by the presence of DAD. Watercress (*N. officinale*) seeds were incubated for 5 days on thin agar (0.33%) containing varying amounts of DAD. At 20 ppm a 30% reduction in root length growth is observed relative to the controls. Solubility of DAD in the agar media prevented testing at higher concentrations. As with the seed germination test, the roots were often discolored, stunted, thickened and devoid of root hairs in contrast to the roots of the control.

The phytotoxic activity of p-coumaric acid, ferulic acid and the flavanoid luteolin show a 10–20% reduction in seed germination at 40 ppm. However, mixtures of these compounds with DAD failed to show any synergistic effects in preventing seed germination. The level of inhibition for the mixture was essentially the sum of the individual components at all levels. Testing at high concentration was again thwarted by solubility. Synergism is thus not a probable mode of action of DAD. Its exact mechanism of action will require further investigation.

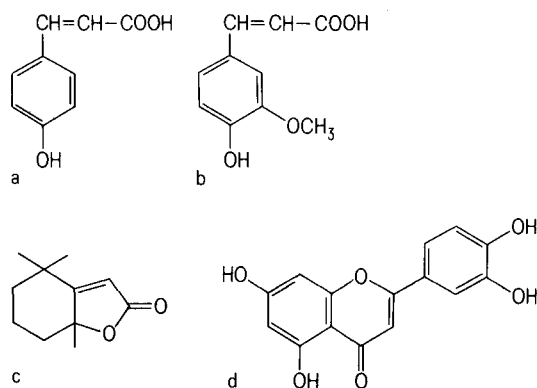


Figure 1. *a* p-coumaric acid, *b* ferulic acid, *c* dihydroactinidiolide (DAD), *d* luteolin.

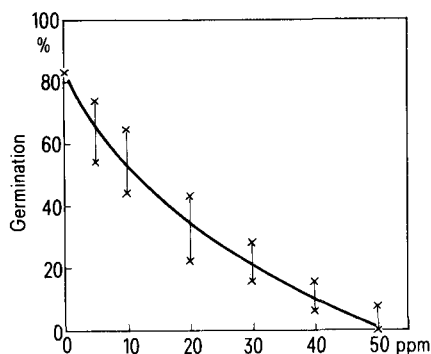


Figure 2. Germination of radish seeds in the presence of DAD.

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