

Loroglossol: An Orchid Phytoalexin

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ABSTRACT

Loroglossol, a dihydrophenanthrene first isolated from orchids (*Loroglossum hircinum*) after inoculation with fungi, was found to be active against spore germination of *Monilinia fructicola* and *Phytophthora infestans* (ED_{50} approximately 5×10^{-3} M). Previous reports that the compound is inactive presumably were due to its relatively low solubility.

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Loroglossol (Fig. 1, II) and the phytoalexin, hircinol (Fig. 1, III) were isolated by Hardegger et al. (7) and Urech et al. (9) from tubers of the orchid, *Loroglossum hircinum*, after inoculation with *Rhizoctonia versicolor*. The structures of the two compounds have been confirmed recently by Fisch et al (2) and both are very closely related to orchinol (Fig. 1, I), the phytoalexin from the orchid, *Orchis militaris* (4, 5, 6). Both orchinol and hircinol were reported to have appreciable antifungal activity, and to play a role in the defense of orchid tubers against fungal invasion. Loroglossol was described as inactive (2, 7). When a supply of this compound became available from a laboratory synthesis of orchinol (8), the opportunity was taken to re-examine its antifungal activity.

Loroglossol was assayed against spore germination of *Monilinia fructicola* (Wint.) Honey and *Phytophthora infestans* (Mont.) de Bary using the standard slide germination method (1) and other procedures as described in detail elsewhere (10). Loroglossol is sparingly soluble in water and it was made up initially as a 2.5×10^{-2} M solution in ethanol. From this 0.2 ml were rapidly dispersed in 4.0 ml of water forming a fine and apparently stable suspension. After dilution with the spore suspension, this gave a concentration of 1×10^{-3} M. Lower concentrations were obtained by serial dilution of the water suspension.

The results of these assays appeared to confirm previous reports that the compound was inactive. However, it was observed that at concentrations above 0.625×10^{-4} M large numbers of crystals had separated out, indicating that loroglossol rapidly crystallized when transferred from ethanol solution to the water medium, and did not redissolve on subsequent dilution. To avoid this, a dilution series of loroglossol was prepared in ethanol and each dilution was added directly to a spore suspension to give the required concentrations. By this procedure, germination of *P. infestans* zoospores was almost completely inhibited down to 6×10^{-5} M (Table 1). With *M. fructicola*, spore germination was not inhibited below 1.25×10^{-4} M but, at concentrations down to 1.56×10^{-5} M, germ tubes were extremely stunted and greatly distorted, frequently rupturing at their tips. It is unlikely that the majority of such germ tubes were viable.

It appears, therefore, that loroglossol has antifungal activity at least of the same order as orchinol and hircinol [minimum inhibitory dose 10^{-4} - 10^{-5} M, (3, 5)]. These observations justify the warning in the original description of the slide germination assay, that

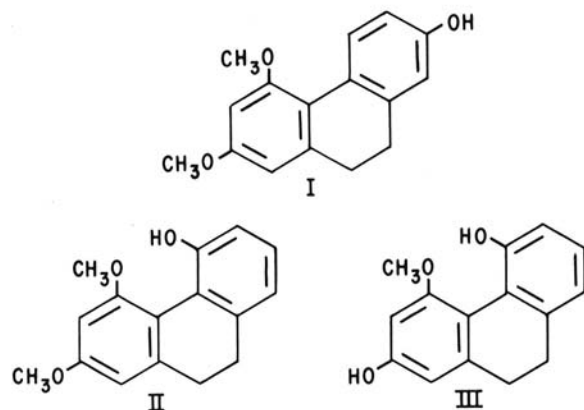


Fig. 1. Structure of orchinol (I), Loroglossol (II) and hircinol (III).

TABLE I. Percentage inhibition of spore germination of *Monilinia fructicola* and *Phytophthora infestans* by loroglossol

Fungus species	Loroglossol ($M \times 10^4$)						
	5.0 ^a	2.5 ^a	1.25 ^a	0.625	0.313	0.156	0.078
<i>Monilinia fructicola</i> ^c	27 ^b	22 ^b	21 ^b	0 ^b	0 ^b	0 ^b	0
<i>Phytophthora infestans</i> ^d	90 ^b	100	93 ^b	92 ^b	20 ^b	0	0

^aCrystals present.

^bDistortion and stunting of germ tubes.

^cGermination was 100% in both water and ethanol controls.

^dGermination was 98% in both water and ethanol controls.

inaccuracies may occur with sparingly soluble compounds. They suggest that the previously reported inactivity of loroglossol may well have been due to its low water-solubility. They also suggest that loroglossol is a

phytoalexin in addition to hircinol in *Loroglossum hircinum*, and its role in the defense reaction of this plant should be reconsidered.

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