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EFFECTS OF ORCHINOL, LOROGLOSSOL, DEHYDROORCHINOL, BATATASIN III, AND 3,4′-DIHYDROXY-5-METHOXYDIHYDROSTILBENE ON ORCHID SEEDLINGS[1]

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Two naturally occurring orchid phytoalexins, orchinol and loroglossol; a synthetic analogue, dehydroorchinol; a possible precursor of orchinol (3,4′-dihydroxy-5-methoxydihydrostilbene); and batatasin III (3,3′-dihydroxy-5-methoxydihydrostilbene) reduced the growth of *Cattleya aurantiaca* seedlings. These compounds had no effect on the length of the first leaves during early stages of development.

Introduction

Relatively little is known about the effects of phytoalexins, especially those from orchids, on higher plants (VANETTEN and PUEPPKE 1976; ARDITTI 1979; STOESSL and ARDITTI 1984). Pisatin (a phytoalexin from *Pisum sativum*) represses the growth of wheat roots (CRUICKSHANK and PERRIN 1961) and is toxic to mammalian cells (OKU et al. 1976) and pea callus cultures (BAILEY 1970), possibly by injuring the plasma membrane (SHIRAISHI et al. 1975). Phaseollin, a phytoalexin from the Fabaceae, inhibits cell suspension cultures of kidney beans (GLAZENER and VANETTEN 1978). The cells were killed within 30 min by 32 μg phaseollin/ml. Phaseollin blocks ATP formation in mitochondria of *Cucumis sativus* hypocotyls (VANETTEN and PUEPPKE 1976). A similar effect was reported for ipomeamarone, a phytoalexin from sweet potato, which prevents oxidative phosphorylation in mung bean mitochondria (URITANI et al. 1954). Seed germination and hypocotyl growth of clover are inhibited by trifolirhizin (CHANG et al. 1969). BAT from dormant bulblets of *Dioscorea batatas* induces dormancy in these organs (HASHIMOTO et al. 1974). Rishitin, a phytoalexin from potatoes, changes the electrical potential of cell membranes of *Nitrella* and *Nitelopsis* (VOROBIEV et al. 1975).

Orchid phytoalexins inhibit a number of fungi and bacteria at concentrations from 10^{-4} to 10^{-2} M. In *Orchis militaris*, ORC may reach a level of 0.5×10^{-2} M in tissues 8 days following infection with *Rhizoctonia repens* (NUESCH 1963). This concentration is well within the toxic range for some bacteria and fungi, but its effects on orchid seedlings—terrestrial or epiphytic, species from temperate or tropical regions—are not known.

Little is known about the effects of phytoalexins on (1) angiosperms in general, (2) the plants that produce them, and (3) species related to the source plants. This is regrettable because phytoalexins or related natural or synthetic compounds could be useful as fungicides, bactericides, or as anticontaminants for tissue or whole-plant cultures in vitro. Therefore, we determined the effects of LOR, BAT, ORC, its analogue DOR, and a possible precursor, DMS, on a model system consisting of germinating orchid seeds and developing seedlings.

Material and methods

ORC, LOR, DOR, DMS, and BAT were synthesized (STOESSL et al. 1974; LEE et al. 1978), dissolved in 70% ethanol, and added to KC in 2 ml aliquots at 0.078, 0.156, 0.313, 0.625, 1.25, and 2.5×10^{-4} M; W and E served as controls. All cultures were replicated three times.

Mature seeds of *Cattleya aurantiaca* were cultured (TAMANAHA et al. 1979). The effects of ORC, DOR, DMS, LOR, BAT, and the controls were evaluated by the growth index (SPOERL 1948) method, which measures seedling development (three determinations per concentration); leaf length measurements (TAMANAHA et al. 1979), using 100 seedlings per culture; and assays of chlorophyll content of entire seedlings (MACKINNEY 1941; ARNON 1949; modified for use with orchids by HARRISON [1973]) in two samples per treatment. The data are averages of these.

Results

Growth indices of seedlings were lower than the E controls on media containing 0.078×10^{-4} M ORC, DOR, and BAT; 0.156×10^{-4} M ORC and DMS; 0.313×10^{-4} M LOR, DMS, and BAT; 0.625×10^{-4} M ORC and DOR; 1.25×10^{-4} M ORC and LOR; and 2.5×10^{-4} M LOR (fig. 1). They exceeded E and W controls on 0.156×10^{-4}

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[1] Abbreviations: BAT = batatasin, 3,3′-dihydroxy-5-methoxydihydrostilbene; DMS = 3,4′-dihydroxy-5-methoxydihydrostilbene; DOR = dehydroorchinol; E = KC plus 2 ml 70% ethanol; KC = Knudson C medium; LOR = loroglossol; ORC = orchinol; W = KC plus 2 ml distilled water.

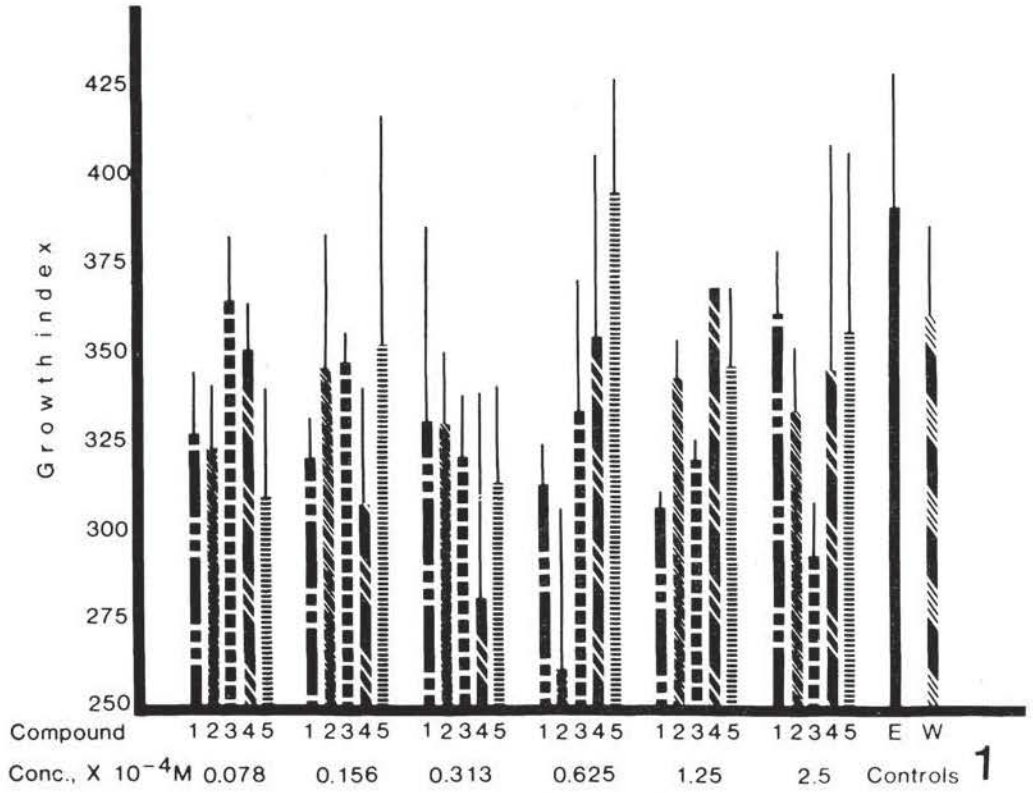


FIG. 1.—Growth indices of *Cattleya aurantiaca* seedlings on KC medium (control) as well as media containing 2 ml 70% ethanol/liter (control), ORC, LOR, DOR, DMS, and BAT. *E* = Knudson C medium plus 2 ml 70% ethanol/liter; *Tot.* = total chlorophyll; *W* = Knudson C medium plus 2 ml distilled water/liter; *1* = ORC, *2* = DOR, *3* = LOR, *4* = DMS, and *5* = BAT. The narrow lines are standard deviations.

M BAT, 0.313×10^{-4} M ORC, 0.625×10^{-4} M DMS and BAT, and 2.5×10^{-4} M DMS and BAT.

First leaves of seedlings at stage 4 of development in E and W were equal (fig. 2). The first leaves of seedlings at stage 4 of DOR (except 0.625×10^{-4} M), LOR, and DMS (except 0.313×10^{-4} M) were longer than or equal to the controls (fig. 2). First leaves of seedlings at stage 4 on ORC were shorter than the controls on all concentrations except 2.5×10^{-4} M (fig. 2). On BAT, first leaves were shorter on all concentrations save 1.25×10^{-4} M (fig. 2). At stage 5 the first leaves were shorter on all phytoalexin-containing media except 0.156×10^{-4} M DOR and LOR, 0.313×10^{-4} M DOR, and 0.625, 1.25, and 2.5 M $\times 10^{-4}$ M LOR (fig. 2).

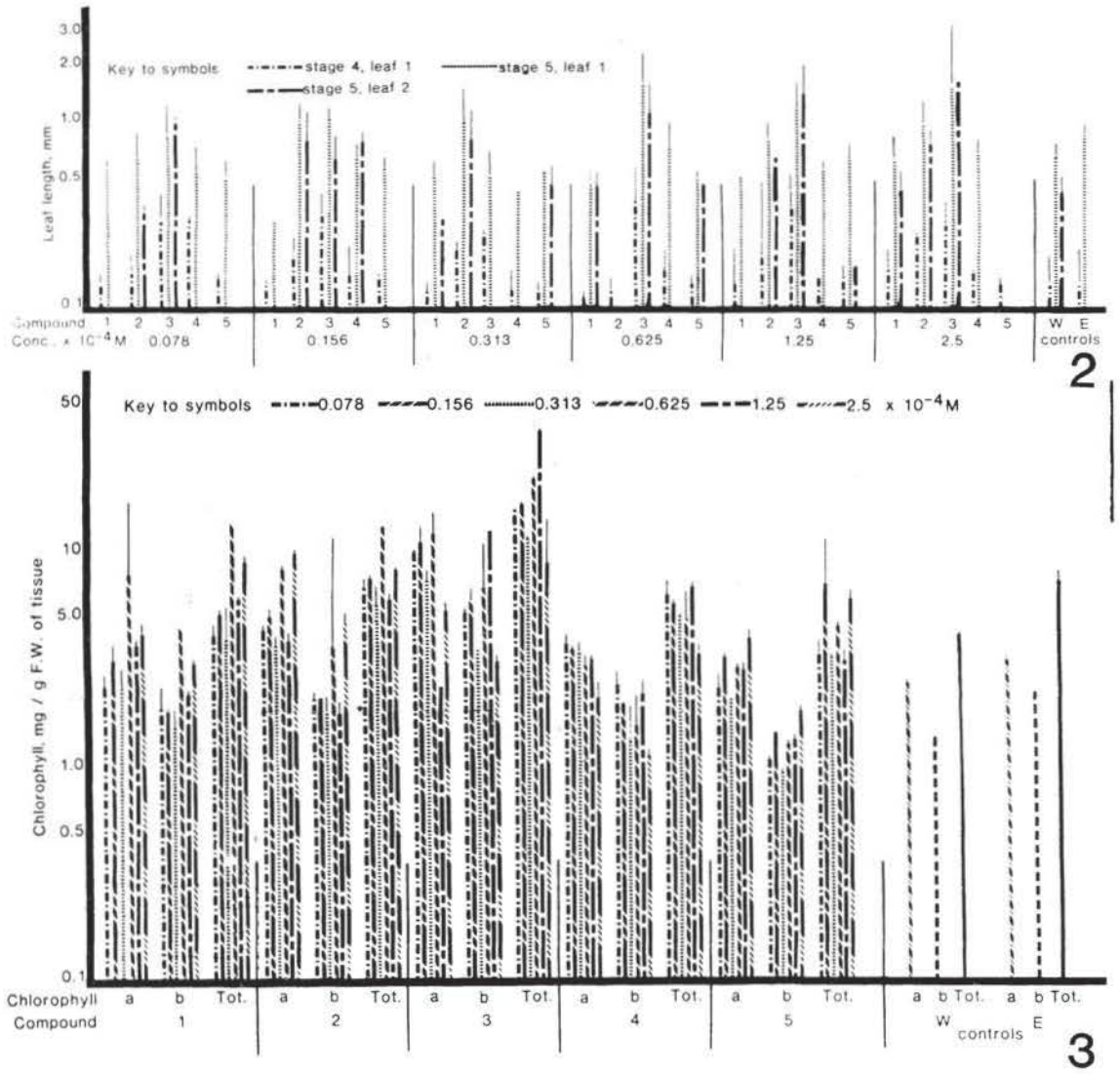
Second leaves at stage 5 were not produced on E; 0.078×10^{-4} M ORC and BAT; 0.156×10^{-4} M ORC and BAT; 0.313×10^{-4} M LOR and DMS; 0.625×10^{-4} M DOR and DMS; 1.25×10^{-4} M ORC and DMS; and 2.5×10^{-4} M DMS and BAT (fig. 2). There were no first leaves at stage 5 on 0.625×10^{-4} M DOR and 2.5×10^{-4} M BAT.

Chlorophyll content (a, b, total) of seedlings on ORC was equal to or higher than on the two controls except that, on 0.078×10^{-4} M and 0.313×10^{-4} M, the total level was lower than on E (fig. 3). On LOR and DOR all chlorophyll levels were equal to or higher than on the controls (fig. 3). On DMS, levels of chlorophyll a varied, as did those of total chlorophyll (fig. 3). Levels of chlorophyll b were similar to or slightly lower than in control seedlings (fig. 3). Chlorophyll a levels on BAT were equal to or somewhat higher than the controls. Chlorophyll b content was lower than in the controls. Total chlorophyll concentration on BAT was equal to that in the controls.

Discussion

Germination of orchids is unlike that of other plants in that the embryo swells (stage 1), bursts through the testa (stage 2), and forms a conical protocorm (stage 3). A shoot tip develops on the protocorm and gives rise to leaves (stage 4) that increase in size (stage 5) and number (stage 6). Roots (stage 6) usually appear after the leaves (TAMANAHA et al. 1979). The growth index (SPOERL 1948) is based on the relative number of each stage in seedling populations and is a good method to measure growth of orchid seedlings because it quantifies development and does not merely record increases in size and mass of protocorms, leaves, and/or roots.

A drawback of the growth index is that it does



FIGS. 2, 3.—Effects of phytoalexins on seedlings of *Cattleya aurantiaca*. Fig. 2, Leaf length of seedlings on KC medium (control) as well as media containing 2 ml 70% ethanol/liter (control), ORC, LOR, DOR, DMS, and BAT. Fig. 3, Chlorophyll content of seedlings on KC medium (control) as well as media containing 2 ml 70% ethanol/liter (control), ORC, LOR, DOR, DMS, and BAT. Explanation of symbols as in fig. 1 and as shown. Narrow lines are standard deviations.

not measure leaf and root growth in terms of size increases or chlorophyll levels. Therefore, the effects of an agent that may not block formation of leaves and/or roots but does inhibit their elongation would remain undetected. To overcome these limitations, we measured the length of the first leaf of seedlings at stages 4 and 5. Since an agent may affect leaf size and number without influencing chlorophyll synthesis, we determined pigment levels. Measurement of leaf length (fig. 2), when combined with the determination of chlorophyll—which is found in protocorms, leaves, and roots of *Cattleya* seedlings (fig. 3)—and growth index (fig. 1), have proved useful in measuring orchid seed germination and seedling development (HARRISON 1973; ARDITTI 1979; TAMANAHA et al. 1979).

ORC is produced by *Orchis militaris*, *Serapias* species, *Loroglossum hircinum*, and *L. longibracteatum; L. hircinum* is also the source of LOR (ARDITTI 1979). These species are European terrestrial orchids that belong to the tribe Ophryoideae, subtribe Platanthereae. *Cattleya aurantiaca*, the assay plant, belongs to the tribe Kerosphaeroideae, Series A: Acranthae, subtribe Laelieae, and is an epiphytic species from Central America (SCHULTES and PEASE 1963). The assay plant and species that produce ORC and LOR are not closely related. Perhaps this distant relationship explains why some concentrations of ORC, LOR, and DOR are somewhat inhibitory to *C. aurantiaca* at concentrations lower than those found in fungus-infected *Orchis* plants. One reason may be that, in infected *Orchis*

plants, the phytoalexins may enter or become bound to the fungus.

A search for phytoalexins in extracts from infected roots and pseudobulbs of *Cymbidium* hybrids failed to detect phenanthrenes (STOESSL et al. 1980). A weak antifungal compound was isolated and identified as linoleic acid-1-monoglyceride (STOESSL et al. 1980). Recently, 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene was isolated from *Coelogyne ovalis* (Kerosphaeroideae, Acranthae, Coelogyneae), a species more closely related to *Cattleya* than to *Orchis* (MAJUMDER and LAHA 1981). This suggests that phenanthrene phytoalexins may be more widespread among the Orchidaceae than believed until now. If so, it is reasonable to expect that these and chemically similar compounds would not be toxic to orchids. This assumption is supported by our findings because (1) reductions in growth indices in their presence, if and when they occurred, were small; (2) leaf length increased on LOR-containing media even at a concentration that inhibited the growth index; and (3) chlorophyll levels were not reduced by ORC and actually increased by LOR.

An obvious question pertains to whether the low solubility of ORC in an agar medium might suppress its biological effects: ORC effects on the seedlings indicated that it was taken up by the orchids because of solubilization by factors that may be present in the plant (GÄUMANN and KERN 1959; STOESSL and ARDITTI 1984).

The effects of DMS on leaf length are generally similar to those of ORC. This suggests either that DMS (1) serves as a precursor to ORC or (2) has the same effects, regardless of whether it is a precursor of ORC.

BAT is reported to induce dormancy in yam bulbils (HASHIMOTO et al. 1974). If its effects on orchid seedlings were to be the same, it is reasonable to expect reduced growth, shorter leaves, and lower chlorophyll levels. This was not so in the concentration range screened by us. Orchid seedlings are not comparable to yam bulbils. Therefore, it is not surprising that a compound that induces dormancy in *Dioscorea batatas* (Dioscoreaceae) affects *C. aurantiaca* (Orchidaceae) differently.

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