APPENDIX

Antifungal Tests

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Gladiolic acid was found by Brian, Curtis & Hemming (1948) to inhibit, at low concentrations, spore germination in a number of species of fungi. Most of their assays, however, were carried out with *Botrytis allii* Munn. as test organism, using a method described earlier by Brian & Hemming (1945). In view of the close chemical relationship of gladiolic acid to dihydrogladiolic, cyclopolic and cyclopaldic acids, it was decided to compare the inhibitory effects of all four substances on the germination of conidia of *B. allii*. A culture of *B. allii* was kindly

supplied by Dr P. W. Brian, of Imperial Chemical Industries Ltd., Butterwick Research Laboratories, Welwyn.

such a low solubility that the highest concentration which could be obtained was 40 $\mu\text{g./ml.}$, and the 160 $\mu\text{g./ml.}$ aqueous solution used to make the first dilution had to be kept warm in order to avoid crystallization. The results obtained are given in Table 1, the degrees of inhibition being those adopted by Brian & Hemming. 0 = No germination; (1) = less than 1% germination; 1 = 1–5% germination; 2 = 5–50% germination; 3 = 50–90% germination; 4 = 90–100%, i.e. complete germination; — = not tested.

Table 1. *Fungistatic activities of gladiolic, dihydrogladiolic, cyclopolic and cyclopaldic acids at pH 3.5*

Acid	Concn. of acid ($\mu\text{g./ml.}$)										
	320	160	80	40	20	10	5	2.5	1.25	0.625	0.312
Gladiolic	0	0	0	0	0	(1)	2	3–4	—	—	—
Dihydrogladiolic	4	4	4	4	4	—	—	—	—	—	—
Cyclopolic	3–4	4	4	4	4	—	—	—	—	—	—
Cyclopaldic	—	—	—	0	0	0	0	(1)	2	3	4

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In a preliminary experiment, dilutions were made in the 'germination medium' used by Brian & Hemming (1945). Gladiolic acid showed only very slight activity at 20 $\mu\text{g./ml.}$, the highest concentration used. Cyclopolic acid and dihydrogladiolic acid were completely inactive at 80 $\mu\text{g./ml.}$, but cyclopaldic acid gave almost complete inhibition at 10 $\mu\text{g./ml.}$, indicating that it is appreciably more active than gladiolic acid. Brian *et al.* (1948) found that at pH 3.5 gladiolic acid gives over 95% inhibition at a concentration of 7.8 $\mu\text{g./ml.}$, but that activity falls off as the pH is increased. The pH of the 'germination medium' used was 5.6, which would explain the low activity of gladiolic acid in this experiment.

In a second series of tests Czapek-Dox solution, adjusted to pH 3.5, was used for making dilutions. The highest concentrations which could be tested were limited by the comparatively low solubilities of the substances. Cyclopaldic acid, in particular, has

The concentration of gladiolic acid permitting less than 1% germination is 10 $\mu\text{g./ml.}$, which agrees well with the figure, 7.8 $\mu\text{g./ml.}$, given by Brian *et al.* (1948). Cyclopolic acid, at the highest concentration tested, showed a slight effect, in that a few conidia had not germinated and, in the case of those which had, the germ tubes were much shorter than in the controls. Dihydrogladiolic acid was completely inactive, the amount of growth, at the highest concentration, being equal to that in the controls. Cyclopaldic acid, as was indicated in the first experiment, is about four times as active as gladiolic acid.

SUMMARY

1. Cyclopaldic acid, at a concentration of 2.5 $\mu\text{g./ml.}$, permits less than 1% germination of the spores of *Botrytis allii* Munn. in Czapek-Dox medium at pH 3.5.

2. Cyclopolic acid, under the same conditions and at a concentration of 320 $\mu\text{g./ml.}$, shows little fungistatic activity.

REFERENCES

- Brian, P. W., Curtis, P. J. & Hemming, H. G. (1948). *J. gen. Microbiol.* **2**, 341.
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