

Fig. 1. Pismo-infected seed showing appearance of pycnidia on seed coat after germination in moist chamber. $\times 7$. Photograph by R. C. Blackmore

ment would be required for the efficient operation of a thermo-chemical treatment plant.

A full account of this work will be published elsewhere. Thanks are due to Miss E. Milne, Applied Mathematics Laboratory, N.Z. Department of Scientific and Industrial Research, for statistical analysis of results.

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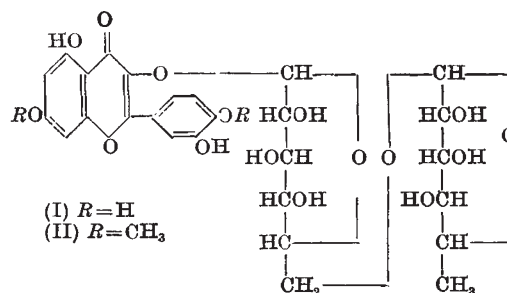
An Antagonist of the Sterility Hormone Rutin in the Green Alga *Chlamydomonas* *eugametos*: Ombuoside=7,4'-Dimethyl- rutin

RUTIN (= quercetin-3-rutinoside) prevents the copulation of male and female gametes in *Chlamydomonas eugametos*¹. Its effect is very specific. Checking more than forty flavonols, it was found that only rutin induces sterility. The sugar at carbon atom 3 (rutinose) seems to be important, as quercitrin (= quercetin-3-rhamnoside) does not influence fertility.

In 1935 a strain was obtained which could not be made to copulate². Filtrates of these *agametos* cells, like rutin, prevented the copulation of *eugametos* gametes. Thus *agametos* cells produce and secrete a sterility hormone. This hormone could be isolated from cells of a certain *agametos* mutant. It was identified as rutin³ (I).

Three enzyme systems dependent on genes are responsible for rutin formation in *agametos* cells: (1) the formation of *l*-rhamnose (gene *rha*⁺), (2) the formation of rutinose from *l*-rhamnose and *d*-glucose (gene *runo*⁺), (3) the formation of rutin from quercetin and rutinose (gene *ru*⁺). *Agametos* has *rha*⁺*runo*⁺*ru*⁺, *eugametos* has *rha*⁰*runo*⁺*ru*⁰⁴. Quercetin could be isolated from cells of a *ru*⁰ *agametos* mutant⁵, suggesting that the biosynthesis of rutin follows the sequence given above.

However, *agametos* cells become able to copulate when the cells are kept in the dark for three days. Then the cells are practically free of rutin. They lose their ability to copulate after a period of illumination of 15–20 min. During the short period of copulation, it is possible to cross *agametos* with *eugametos*. The effect of rutin is not yet clear. It is evident that the formation or the secretion of gamones which induce the last phase of the copulation is disturbed.



We have now been able to examine ombuoside (II), a glycoside that is a 7,4'-dimethyl-rutin, which has been obtained from the leaves of the ombu (*Phytolacca dioica*, L.), where it accompanies rutin⁵. The results of the experiments are: (1) ombuoside (10^{-5} gm./ml.) shows no influence on the copulation of *eugametos* gametes; (2) *agametos* cells obtain the ability to copulate in solutions containing 10^{-5} to 10^{-7} gm./ml. ombuoside; (3) *eugametos* gametes made sterile by rutin (10^{-8} gm./ml.) begin to copulate again after adding ombuoside (10^{-6} gm./ml.); (4) *eugametos* gametes continue to copulate in solutions containing ten times more ombuoside than rutin.

We conclude from these results that ombuoside is able to compensate the rutin effect.

We also tested 7,4'-dimethyl-quercetin or ombuin, the aglycone of ombuoside. It has no influence on *eugametos* gametes. However, we found that ombuin compensates the sterility of *agametos* cells. This effect may indicate that *agametos* cells can form ombuoside enzymatically from ombuin.

The specificity of 7,4'-dimethyl-quercetin was demonstrated by examination of ten other methyl derivatives of quercetin (for which we thank Dr. I. Loew, Max Planck-Institut fuer Medizinische Forschung, Heidelberg), namely: 7-, 5-, 3-, 3', 7,3-, 3,5,3', 3,7,3', 5,7,3', 5,3',4', and 7,3',4'-methyl quercetins were without any effect on *agametos* cells. These results show that 7,4'-dimethyl-quercetin (ombuin) and 7,4'-dimethyl-rutin (ombuoside) have a special importance in this antagonism with rutin.

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