Fungal Sex Hormones

By G. W. GOODAY (*Department of Agricultural Science, University of Oxford, Oxford OX1 3PF, U.K.*)

There is much biological evidence for hormonal control of sexual reproduction in a wide range of fungi, but only three hormones have been chemically characterized.

The sexual reproduction of the water mould *Allomyces* involves the formation of motile male and female gametes by the same plant, and the subsequent fusion of actively swimming males with the more sluggish females. Machlis (1958) has shown that the male gametes are chemotactically attracted to the female gametes and has named the attractant 'sirenin'. Sirenin is inactivated by the male gametes (Carlile & Machlis, 1965), and so, to obtain enough for characterization, Machlis *et al.* (1966) have used an interspecific hybrid that is predominantly female, and have used a predominantly male hybrid to assay the hormone concentration by counting the numbers of male gametes attracted to the surface of a dialysis membrane separating them from the test solution. By growing the female hybrid under conditions designed to control the formation and release of the gametes a yield of $1 \mu\text{M}$ -sirenin can be obtained in the culture filtrate, and it has been shown to be a bicyclic sesquiterpenediol, $\text{C}_{15}\text{H}_{24}\text{O}_2$ (Nutting *et al.*, 1968), probably biosynthesized by cyclization of *cis*-farnesyl pyrophosphate. This molecule is active in the bioassay at concentrations down to 0.1 nM . Sirenin has been synthesized by a number of different methods, and the products have been shown to be active (Bhalerao *et al.*, 1970).

Achlya is also a water mould, and Raper (1942) used carefully chosen species to show that hormones control the differentiation of the sexual hyphae that form when the two sexes are grown together. Raper & Haagen-Smit (1942) extracted and purified the hormone that is produced by the female plants and that initiates the sequence of events leading to sexual fusion, but it was not until much later that Arsenault *et al.* (1968) characterized it. It proved to be a sterol, 'antheridiol', related to stigmasterol. The biological activity of chemically synthesized material has confirmed this structure (Edwards *et al.*, 1969). Antheridiol is active on receptive strains at concentrations down to 0.01 nM . Barksdale (1969) has shown that this compound elicits a sequence of distinct responses from the male plants of branching, chemotropic attraction of the branches, delimitation of the antheridia with accompanying meiosis and secretion of hormone B, which is thought to be a closely related sterol and which in turn acts on the female plants causing the formation of oogonia.

The involvement of hormones controlling sexual reproduction in the Mucorales was first demonstrated

by Burgeff (1924). In this group of fungi sexual reproduction occurs between morphologically indistinguishable mating types, (+) and (−), and Burgeff (1924) showed that diffusible substances are responsible for the formation of sexual hyphae when two mating types meet. Burgeff & Plempel (1956) obtained an extract from the culture filtrate of mated *Mucor mucedo* that had hormone activity, and, although this work was carried much further, the hormone was not fully characterized (Plempel, 1963). Plempel (1963) obtained evidence for diffusible factors, 'progamones', released by each mating type and causing the opposite mating type to produce the sex hormones.

A closely related fungus, *Blakeslea trispora*, produces much larger amounts of β -carotene when mated than when either strain is grown separately. A soluble factor present in mated fermentations that causes a large increase in carotenogenesis when added to unmated strains proved to be a mixture of related C_{18} -monocyclic trisporic keto acids, the 'trisporic acids' (Caglioti *et al.*, 1964). That trisporic acids could be sex hormones was suggested when extracts made by van den Ende (1968) from the culture filtrate of mated cultures of *B. trispora*, and by Gooday (1968*a,b*) from mated mycelium of *M. mucedo* grown on nutrient agar, proved to have biological activity in the sex-hormone bioassay with *M. mucedo*. Direct chemical and biological comparisons of chemically pure trisporic acids and the active extract from *M. mucedo* have confirmed their sex-hormone activity (Austin *et al.*, 1969). Down to 20 ng of trisporic acid B or C can be detected in the bioassay. Chemically synthesized trisporic acid B has full biological activity on both (+) and (−) *M. mucedo* (Edwards *et al.*, 1971).

The trisporic acids are only formed when there is free interchange of soluble materials between the two mating types, but the exact mechanism of this collaborative biosynthesis is still in doubt. Mated fermentations in which one of the partners is generally labelled with ^{14}C have shown that (+) and (−) mycelia contribute approximately equally to the final yield of trisporic acid (van den Ende & Stegwee, 1971), and mated fermentations in which one of the partners is inhibited with 5-fluorouracil indicate that RNA-mediated enzyme synthesis in both strains is required for continued trisporic acid biosynthesis (J. D. Bu'Lock, personal communication). Recent experiments have shown that trisporic acid can be detected in the culture filtrate of mycelium of one mating type of *B. trispora* after it has been irrigated with dialysed diffusate from the opposite mating type (H. van den Ende, personal communication). Austin *et al.* (1970), using radioactive mevalonate, β -carotene and retinyl acetate, have obtained good evidence that the trisporic acids are biosynthesized by cleavage and oxidation of β -carotene via retinal

and trisporol C, the primary alcohol corresponding to trisporic acid C.

The three fungal sex hormones that have been characterized are all terpenes, but beyond this show no similarities in structure, biochemistry or biological activity. The activity of each one is confined to species that are closely related to the species producing it. In each case the isolation of the hormones involved has been made possible only by obtaining sexual activity in selected strains of fungi in controlled fermentations, and by reliable bioassay techniques. If these prerequisites can be met by other fungi we can expect to see the identification of their sex hormones.

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Comparative Metabolism of *Claviceps purpurea* *in vivo* and *in vitro*

By R. A. BASSETT, E. B. CHAIN, K. CORBETT, A. G. F. DICKERSON and P. G. MANTLE (Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY, U.K.)

Interest in the metabolism of the fungus *Claviceps purpurea* has concerned the occurrence of pharma-

cologically active alkaloids within the ergot sclerotium when this organism is parasitic on cereals or grasses, and attention has been given in this Department to the possibility of obtaining these metabolites from axenic cultures of the fungus in stirred fermenters. This has proved to be a difficult process, because of the instability of those few strains that have been found to synthesize significant yields of alkaloid *in vitro* and also because, as in the parasitic state, the alkaloid is only produced by sclerotial cells and not by the thin filamentous sphacelial form in which the fungus usually grows in axenic culture.

A better understanding of fermentation conditions has resulted from a comparison of metabolism during both parasitic growth and axenic cultures of alkaloid-producing and non-alkaloid-producing strains. It is recognized that, although fermentation experiments are easier to control, they are more difficult to interpret, owing to the heterogeneity of cell types.

Comparison of dry-weight accumulation with measurements of protein, nucleic acids and total carbohydrate has confirmed that parasitic sclerotia develop by the proximal addition of a standard type of tissue that yields ergots of homogeneous shape and composition.

Experiments in which ergot-infected rye has fixed $^{14}\text{CO}_2$ have shown that the principal sugar supplied by the host to the fungus is sucrose. This sugar is also preferred in fermentations as giving the highest alkaloid yield. A β -fructofuranosidase liberates glucose by transferring the fructose component to form fructo-oligosaccharides. This enzyme is not a typical invertase, but is a cell-bound and inducible fructosyltransferase. Experimental evidence is consistent with the following structures for the four principal oligosaccharides: $\text{Fruf}\beta 2 \rightarrow 6\text{Glc}\alpha$, $\text{Fruf}\beta 2 \rightarrow 6\text{Glc}\alpha 1 \rightarrow 2\text{Fruf}\beta$ (6^O-fructosylsucrose); $\text{Fruf}\beta 2 \rightarrow 1\text{Fruf}\beta 2 \rightarrow 6\text{Glc}\alpha$; $\text{Fruf}\beta 2 \rightarrow 1\text{Fruf}\beta 2 \rightarrow 6\text{Glc}\alpha 1 \rightarrow 2\text{Fruf}\beta$. The same oligosaccharides accumulate during the early stages of fermentations, but only when sucrose is the carbon source. Ultimately, in fermentations exceeding 10 days, the oligosaccharides may be hydrolysed through further mediation by the β -fructofuranosidase, liberating fructose and leaving the disaccharide, $\text{Fruf}\beta 2 \rightarrow 6\text{Glc}\alpha$, on which the enzyme appears to have low activity. Free fructose is utilized for growth, but only in the absence of glucose. Utilization of sucrose by this process is a fundamental metabolic feature of *C. purpurea* irrespective of whether or not alkaloid is being synthesized, but such a mechanism may favour alkaloid synthesis during later stages of fermentation by providing a continuous supply of carbohydrate.

Although inorganic phosphate is totally absorbed from fermentation broths within the first 5 days, the initial concentration is critical if optimum alkaloid yields are to be produced. On the plant the total phosphate concentrations in developing sclerotial