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*

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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: $\text{Fru}\beta 2 \rightarrow 6\text{Glc}\alpha$, $\text{Fru}\beta 2 \rightarrow 6\text{Glc}\alpha 1 \rightarrow 2\text{Fru}\beta$ (6^O-fructosylsucrose); $\text{Fru}\beta 2 \rightarrow 1\text{Fru}\beta 2 \rightarrow 6\text{Glc}\alpha$; $\text{Fru}\beta 2 \rightarrow 1\text{Fru}\beta 2 \rightarrow 6\text{Glc}\alpha 1 \rightarrow 2\text{Fru}\beta$. 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, $\text{Fru}\beta 2 \rightarrow 6\text{Glc}\alpha$, 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

tissues (approx. $23\text{ }\mu\text{mol}/100\text{mg}$ dry wt.) are similar whether or not alkaloid is being produced.

The appearance of the first traces of purple pigment in superficial layers of the parasitic mycelium coincides with a sharp increase in the total lipid content of sphaelial cells from approx. 10% of the dry weight to approx. 30%. This increase represents the accumulation within new sclerotial cells of a complex triglyceride oil in which the principal fatty acid is ricinoleate. This substance is absent from the oil of sphaelial cells. Thus the appearance of ricinoleate is the first metabolic indicator of the morphogenesis of sclerotial cells. When alkaloid is synthesized it can be detected within 5 days of the differentiation of parasitic sclerotial cells under field conditions, and in the present studies reached 0.4% of the dry weight. Mycelium in fermentation may synthesize up to ten times this amount, but most of the product is found in the broth.

The differentiation of sphaelial hyphae into sclerotial-like cells during fermentation is an essential prerequisite for alkaloid biosynthesis, although, as in the parasitic state, this tissue does not always synthesize alkaloids. This morphogenesis is similarly accompanied by an accumulation (up to 40% of the dry weight) of triglyceride oil rich in ricinoleate, but, in contrast with parasitic tissue, the purple pigment is absent.

C. purpurea, both in the parasitic and in the saprophytic state, readily utilizes amino and ammonium nitrogen, and shows a protein content of sphaelial tissue (approx. 20% by weight) that falls to approx. 12% as developing sclerotial cells become filled with lipid reserves. Thus the proportion of amino acid metabolism that may also be involved in alkaloid biosynthesis is comparatively small. Changes in the concentrations of individual free amino acids throughout parasitic development on rye have been followed in two successive years. Fluctuations of both total amino acids and the relative proportions of individual components were observed, and these were compared with similar measurements during fermentations. Although specific incorporation of radioactive amino acids (alanine, phenylalanine, proline and tryptophan) into ergotamine has been demonstrated, no valid conclusions could be drawn about a consistent relationship between observed changes in amino acid concentrations and the accumulation of ergot alkaloids. However, this study has centred attention on certain amino acids that would merit investigation with respect to specific metabolic turnover. Aspects of the control of alkaloid biosynthesis, particularly involving the concentrations of tryptophan and its derivatives, may best be investigated in axenic culture, but parasitic growth is better suited to studies on the metabolic control of the change from sphaelial growth to sclerotial growth.

The most notable and consistent feature of amino acid analyses during parasitic development is the occurrence of an unidentified substance, the structure of which is being investigated. This occurs only as a minor component before alkaloid is detectable, but accumulates progressively, coincident with alkaloid synthesis, to become the principal free amino acid. This is only a feature of those strains of *C. purpurea* that produce ergoline alkaloids in their sclerotia. Accumulation of the unidentified amino acid in tissues is not observed in fermentations, but this may be due to the large volume of fluid in which the mycelium is suspended.

Viruses of *Penicillium* and *Aspergillus* Species

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The discovery of viruses in several species of *Penicillium* was due, not to any effect on host morphology or growth rate, but to the antiviral activity of extracts of the infected moulds. Lampson *et al.* (1967) postulated that double-stranded RNA, an interferon inducer isolated from *Penicillium funiculosum*, may have arisen from a virus infection. Ellis & Kleinschmidt (1967) found that the antiviral activity of statolon, a fermentation product of *Penicillium stoloniferum*, was associated with polyhedral virus-like particles. Banks *et al.* (1968) isolated virus particles from *P. stoloniferum*, showed that the viral double-stranded RNA induced the formation of interferon in mice and also isolated morphologically similar particles from *P. funiculosum*. Later virus particles were also isolated from *Penicillium cyaneo-fulvum* (Banks *et al.*, 1969b), *Penicillium chrysogenum* (Banks *et al.*, 1969a; Buck *et al.*, 1971) and *Penicillium brevi-compactum* (Wood *et al.*, 1971). Borré *et al.* (1971) have found virus particles associated with lytic plaques in colonies of *Penicillium citrinum* and *Penicillium variable* grown in media of high sugar content.

Following the observation that a high content of cell-wall galactosamine seemed to be associated with the presence of virus particles in *P. stoloniferum* (Buck *et al.*, 1969), a number of fungi reported to contain cell-wall galactosamine were screened with the result that virus particles were found in *Aspergillus niger* and in a closely related species, *Aspergillus foetidus* (Banks *et al.*, 1970). A procedure for the isolation of viruses from *Penicillium* and *Aspergillus* species on a pilot-plant scale has been described (Banks *et al.*, 1971).

Examination of negatively stained preparations of all the known viruses of *Penicillium* and *Aspergillus* species by electron microscopy has shown poly-