SYNOPSIS

Chapter I. Introduction:

The recent developments in the biochemistry of penicillins have been briefly reviewed. The discovery of 6-aminopenicillanic acid has led to the preparation and testing of several semi-synthetic penicillins, particularly on resistant bacterial strains which seem to pose a serious problem among the staphylococci. Another notable advance in the field pertains to the characterisation of new penicillins and penicillin-like compounds e.g., cephalosporin N (penicillin N), penicillin M (isopenicillin M) and cephalosporin C. Our knowledge on the biosynthesis and mode of action of penicillin has been greatly extended and the several mechanisms that have been suggested on the formation of penicillin by Penicillium chrysogenum are referred to.

The property of producing penicillins is shared by members of several genera of fungi including a strain of Streptomyces species. Its formation by the thermophilic fungus Malbranchea pulchella seems rather unique in that it demonstrates the possibility of penicillin formation, albeit in small quantities, at elevated temperatures.

The object of the present investigation has been
stated. During screening of thermophilic microorganisms of interest to fermentations in tropical countries, a strain apparently similar to *M. pulchella* ATCC 9989, was encountered in local composites, which forms penicillin in stationary cultures. The earlier work of the author disclosed a striking departure of *M. pulchella* from *P. chrysogenum* in producing no marked elevation in penicillin titres in presence of added phenylacetic acid. The iodometric assays, nevertheless, gave high penicillin titres as against bioassays and the addition of phenylacetic acid did not materially narrow down the discrepancy between the two values. These studies have therefore been undertaken to characterise the substance or substances that interfere in the iodometric assays of penicillin in these cultures.

**Chapter II. Materials and Methods.**

The microbiological, chemical and analytical procedures including radiotracer technique used presently have been described. Fractionation and characterisation of the $^{35}$S-labelled constituents of *M. pulchella* broths and mycelia harvested at 120 and 168 hours, corresponding to end of growth and penicillin forming phases in synthetic media with and without added phenylacetic acid, has been essentially carried out by the procedures described by Ballio et al.
Chapter III. Characterisation of sulphur containing compounds elaborated by M. pulchella:

The main features of sulphur metabolism of M. pulchella in presence and absence of added phenylacetic acid have been elucidated using $^{35}$S-sodium sulphate as the sole source of sulphur in modified Soltero and Johnson's medium containing glucose. The organism is grown in stationary cultures at 45°C. When compared with P. chrysogenum in shake cultures, the uptake of inorganic sulphate by this organism is much less. There is also less accumulation of inorganic sulphate in the mycelium, whatever is taken up being mostly converted into organic forms. The contribution of penicillin–sulphur is however very small which is not materially influenced by the presence of phenylacetic acid. It resembles somewhat the high-yielding strains of P. chrysogenum in respect of considerable uptake of inorganic sulphate but differs from them in not converting the organic sulphur largely to penicillin.

The uptake of inorganic sulphate, particularly in the presence of phenylacetic acid sharply distinguishes M. pulchella from the low-yielding strains of P. chrysogenum.

Examination of the labelled constituents of broths and mycelia after 120 and 168 hours fermentation with and without incorporation of phenylacetic acid disclosed the presence of all the compounds, except 6-aminopenicillanic acid, recognised
by Ballio et al. in cultures of *P. chrysogenum* WIs.51-20e.

By fractionation on Dowex-1x8 and Dowex-50x8 resins, these have been separated and characterised as cystine, methionine, sulphanylocholine, glutathione, 2-hydroxy-4-methylmercapto-butyric acid and 2,2-dimethyl-5-oxo-hexahydroimidazo(5,1-b)-thiosolo-3,7-dicarboxylic acid. Since in no case an increase in penicillin titre has been observed following treatment of broths and mycelial extracts with phenylacetylchloride under conditions described by Arastein, the absence of 6-aminopenicillanic in these cultures has been established.

In addition to the above mentioned labelled substances, a tripeptide, previously described by Arastein in *P. chrysogenum* cultures, as well as a substance presently referred to as Compound B have been found. The evidence on hand suggests that the tripeptide may be identical with 6-(α-aminoadipyl)-cysteinylvaline. Compound B gives rise to a sharp peak (Rf 0.3) in radiochromatograms, approximately overlapping peaks 3 and 4 in radiochromatograms of Tardrew and Johnson and peaks 2 and 3 in the elution diagrams of Ballio et al.

It is readily extracted into ether from acidified broths and on alkali treatment is converted to benzylpenicilloic acid. Although an analytically pure sample has not been obtained, the purest preparation on hand is a colourless waxy solid melting around 40°C. Its behaviour and properties are not inconsistent with the structure N-phenylacetyl-2,2-dimethyl-
5-oxo-hexahydropyrimidino(5,1-b)thiosolo-3,7-di-carboxylic acid.
The organism resembles Penicillium chrysogenum Wis.20-73 and Wis.51-20c
in producing 2,2-dimethyl-5-oxo-hexahydropyrimidino(5,1-b)-
thiosolo-3,7-di-carboxylic acid (Tardrew and Johnson's
Compound VI), referred to as Compound G.

The main effect of incorporation of phenylacetic acid
in broths of these cultures seems not so much to produce
more penicillin as to give rise to a homogenous Compound B.
The effect on incorporation of phenylacetic acid on the
formation of other constituents has also been discussed.

Compound B resembles penicillin in iodometric assays.
Apparently this is one of the major constituents, if not
the constituent, that interferes with the iodometric assay
of penicillin. On the other hand Compound G readily reacts
with iodine even before treatment with alkali and hence does
not contribute to iodometric assay values of penicillin.

A substantial amount of the label is associated with
the "decomposition products of penicillin". If these have
arisen as a result of high incubation temperature, they should
reflect penicillin that is formed in situ. Since the formation
of compounds B and G bear little relationship to these
substances, it would appear that the formation of compounds
B and G need not necessarily involve penicillin as an inter-
mediate. On the other hand, should 6-aminopenicillanic acid
be an intermediate in penicillin biosynthesis in \textit{M. pulchella}
(despite the failure to detect its presence) Compound 6 would
follow enzymatic or non-enzymatic fixation of carbon dioxide
and Compound B on acylation (assuming that the structure
assigned for Compound B is correct). Nevertheless, a clear
picture as to the precise steps involved in their formation
as well as penicillin in this mould needs further experimen-
tation.

\textbf{Chapter IV. Malbranchins A and B:}

Apart from penicillin, \textit{M. pulchella} forms small amounts
of two other antibiotics, malbranchins A and B, which however
do not contain sulphur. Malbranchin A has been obtained in
larger quantities and hence examined in some detail. Its
isolation, purification and properties along with some factors
affecting its formation have been presently reported.
Malbranchin is found largely concentrated in the mycelium,
only a small fraction being excreted in the broths. There is
also some evidence for stimulation of its formation by, and
incorporation of, phenylacetic or phenoxyacetic acids. Further,
some evidence has been adduced to indicate its (or a closely
allied substance) presence in broths of \textit{P. notatum} 832 but not
in \textit{P. chrysogenum} Q176 or Wis 20-F3.

Malbranchin A as obtained, is a pale brown amorphous
powder m.p. 205\textdegree C (decomp.). It is weakly acidic, dissolving
in sodium carbonate but not sodium hydrogen carbonate solutions. It shows a characteristic absorption at 262 mp and a strong band at 5.75 μ in the infra-red spectrum. While it gives negative reactions with ferric chloride, Tollens’, Molsch’s and ninhydrin, it reacts with periodic acid. It resembles penicillin in chromatographic mobilities in a number of solvent systems but can be distinguished from penicillin, apart from solubility characteristics, by its stability to penicillinase action. Acid hydrolysis of the chromatographically homogeneous material gives a mixture of amino acids vis., leucine, valine, alanine and glycine. In view of the negative ninhydrin reaction of malbranolin A, it is likely that these are present in the form of a cyclic peptide, but whether they form an integral part of the antibacterially active molecule or/derived from an impurity is not yet clear. Malbranolin A is active against Gram-positive bacteria including some strains of mycobacteria. Its acute intraperitoneal toxicity in mice appears to be low, no mortality occurring at 500 mg/kg levels, the highest dose tested.

Chapter V. Malbranohosterol, a sterol from R. pulchella:

Ether extracts obtained during the extraction of malbranolin from the mycelium of R. pulchella contain two sterol-like components as revealed on paper chromatograms. One of these which forms the major component, designated
malbranchoasterol, crystallises in long colourless needles from aqueous acetone and appears to be the first sterol from thermophilic organisms to be reported.

Malbranchoasterol, resembles ergosterol, the characteristic sterol of fungi, in its ultra-violet absorption characteristics, chromogenic reactions and mobilities on paper and thin-layer chromatograms. It however differs from ergosterol in melting point, optical rotation and molecular formula in have an extra C₇H₁₀O moiety. While ergosterol is levorotatory, malbranchoasterol is dextrorotatory. Both their acetates are levorotatory. On irradiation of malbranchoasterol with ultra-violet light the absorption in the 270-290 μ region increases, in contrast to the decrease observed with ergosterol and therefore a transformation similar to ergosterol → calciferol seems unlikely.

The ultra-violet absorption spectrum, the Lieberman-Burchard and Tortelli-Jaffe colour reactions indicate the presence of a homocyclic Δ₅,₇ diene system and the formation of a precipitate with digitonin, that of a free hydroxyl group. The sterol forms only a monoacetate and there is found no clear hydroxyl vibration band in the infra-red spectrum of the acetate. While the nature of the second oxygen function is not clear, the infra-red spectrum of malbranchoasterol suggests the possibility of an oxide linkage. The equatorial conformation of the hydroxyl group at C-3 is indicated by the 9.4-μ band in the infra-red
spectrum of the sterol and the simple 8.0 μ band in the infra-red spectrum of the acetate. By reference to members of the ergosterol series it is believed that the 0-3 hydroxyl and 0-10 methyl groups may have the α-configuration.

Malhranchasterol presents several unusual features and studies are presently in progress to elucidate its structure and its role in the metabolism of the fungus.