Duality of Pathways in the Oxidation of Ergosterol to its Peroxide *In Vivo*

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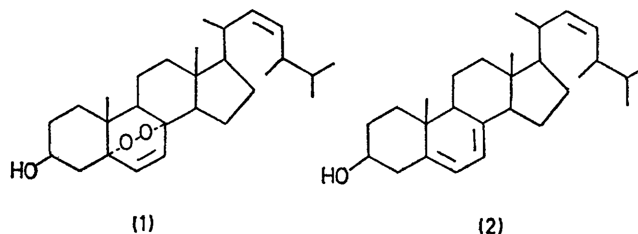
Summary Ergosterol is converted into ergosterol peroxide by simultaneous chemical (photo-oxygenation) and enzymic pathways in *Penicillium rubrum* and *Gibberella fujikuroi*.

ERGOSTEROL PEROXIDE (**1**) occurs widely in fungi and yeasts,[1] and incorporation studies have established that it can be an intermediate in the oxidative metabolism of ergosterol (**2**).[2] In view of the reservation expressed by some[3] that (**1**) could arise by artefactual rather than natural processes, it became essential to ascertain the true origin of this putative metabolite. We report the results of studies with *Penicillium rubrum* and *Gibberella fujikuroi* which demonstrate that ergosterol peroxide is produced by each organism along two distinct pathways, one of which is photochemical and the other not.

[^{3}H]Ergosterol (0·3–1·9 mCi/mmol), prepared as previously described[2b] or by reduction of Δ^4-ergosterone with sodium [$^3H_4$]borohydride, was fed to growing cultures of *G. fujikuroi* and *P. rubrum* under conditions of normal laboratory illumination and in darkness. Ergosterol peroxide, isolated along with other metabolites (accounting for >70% recovery of radioactivity) by extraction of culture media and mycelia, was purified by t.l.c. and crystallized to constant specific activity, and was found to have incorporated up to *ca.* 20% of [^{3}H]ergosterol in the dark-grown



cultures. The extent of photo-oxidative formation of (**1**) was determined by incubation of (**2**) with cultures which had been killed in an autoclave after development of pigmentation (4–5 days). Blank runs, carried out with killed

TABLE. % Incorporation of [^{3}H]ergosterol (2) into ergosterol peroxide (1) under various growth conditions.

Organism	Total incorporation		Photo-oxidative incorporation[a]		Enzymic incorporation	
	Light[b]	Dark	Light[b]	Dark	Light[b]	Dark
G. fujikuroi	46·7	20·9	25·7	0·3	21·0	20·6
P. rubrum	31·5	19·3	12·1	0·6	19·4	18·7

[a] Determined from feeding experiments with killed cultures. [b] Cultures grown under standard laboratory illumination conditions.

cultures under dark conditions, showed negligible incorporation of (2), whereas killed cultures under normal illumination showed appreciable (presumably photosensitized)[4] conversion of (2) into (1). Pigmentation in *P. rubrum* is due principally to the formation of mitorubrin and related compounds[5] and, in fact, mitorubrin has been found to be a relatively efficient (k = 0·2 relative to Methylene Blue) sensitizer for the photochemical oxygenation of (2). A similar role could be ascribed to pigments such as bikaverin and related substances[6] present in *G. fujikuroi*.

Addition of labelled ergosterol to culture extracts during work-up revealed that a substantial proportion of the conversion of (2) into (1) takes place during this phase (up to 24% of total conversion in *G. fujikuroi* and 13% in *P. rubrum*) unless processing is carried out under low-level illumination. The residual incorporation of (2), after subtraction of photo-oxidative formation of (1) during the growth phase and work-up, amounts to *ca.* 21% in *G. fujikuroi* and 19% in *P. rubrum* and, as expected, is approximately equal in light- and dark-grown cultures. Since pigment sensitization is ruled out for this fraction of the conversion, enzymic mediation must presumably be involved. These data are summarised in the Table.

As an *in vitro* model for the enzymic process, the oxidation of ergosterol with 30% H_2O_2 in the presence of horseradish peroxidase was studied. Although the peroxidase oxidation of (2) is much slower (k *ca.* 0·006) than photochemical oxygenation, ergosterol peroxide is a major product, along with other metabolites [cerevisterol,[7] 3β-hydroxy-5α,8α-epidioxyergosta-6,9(11),22-trien-12-one,[8] and ergosta-4,6,8-(14),22-tetraen-3-one[2]] also present in cultures of the two test organisms. The peroxidase reaction with (2) is markedly sensitive to inhibition by *p*-t-butylpyrocatechol and by 2,5-di-t-butylhydroquinone, both known to be free-radical scavengers. In the inhibited reactions, up to 7·5% of the normal metabolism is diverted to products (hydroperoxides) arising from presumed radical intermediates. These results suggest that $^1\Delta_g$ oxygen is not involved in the enzymic conversion of (2) into (1), a conclusion in accord with that reached by Teng and Smith in their study of the oxygenation of cholesterol.[9] The possible intervention of triplet oxygen in this process has a chemical analogy.[10]

Feedings with [^{3}H]-[2b] and [^{14}C]-ergosterol peroxide (1·03 Ci/mmol, from mevalonate-fed *G. fujikuroi* cultures) reveal that this substance is metabolised differently by *P. rubrum* and *G. fujikuroi*. However, in neither organism is the peroxide converted back into ergosterol. This is to be contrasted with the finding of Gaylor and Topham of conversion of (1) into (2) by an enzyme preparation from bakers' yeast.[11] The negligible incorporation of labelled ergosterol peroxide into cerevisterol in *G. fujikuroi* indicates that the latter metabolite is formed from ergosterol by a route which does not involve the peroxide (1).

The demonstration of simultaneous photo-oxidative and enzymic pathways in the transformation of (2) to (1) in two unrelated fungi largely removes the ambiguities[3,12] previously associated with the isolation of ergosterol peroxide and, incidentally, assigns a functional role to pigments in these organisms.

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