The Biosynthetic Interrelationships of Fungal Phenalenones

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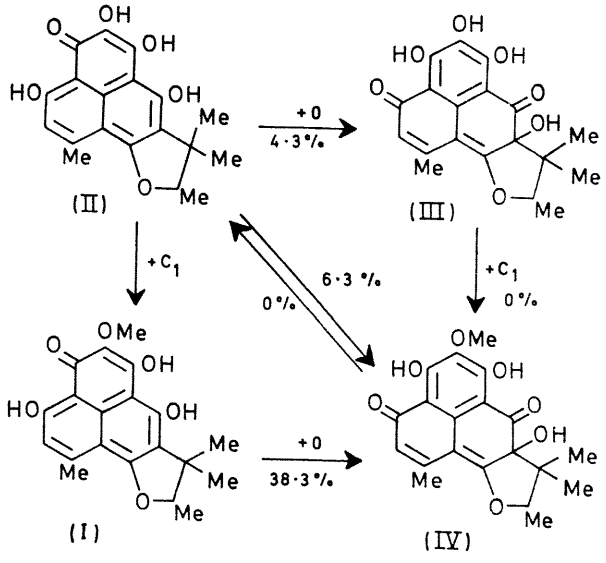
Summary Labelling studies with *Penicillium herquei* have shown that atrovenetin is incorporated into herqueinone *via* deoxyherqueinone, requiring sequential methylation and oxidation: norherqueinone is derived oxidatively from atrovenetin, but is not methylated to form herqueinone.

THE recent characterisation of deoxyherqueinone (I) as a metabolite of *Penicillium herquei*,[1] was of particular interest in relation to the biosynthesis of the previously known fungal phenalenones[2] (perinaphthenones) atrovenetin (II), norherqueinone (III), and herqueinone (IV). Structural considerations had appeared to indicate a direct pathway [(II)→(III)→(IV)] involving sequential oxidation and methylation, whereas the subsequent isolation of deoxyherqueinone favoured a possible alternative sequence [(II)→(I)→(IV)]. Evidence strongly supporting the preferential biosynthesis of herqueinone (IV) by this latter pathway has now been obtained through studies of the metabolic interconversions of all four phenalenones.

Since norherqueinone is known to be derived from acetate and mevalonate units,[3] a simple biosynthetic procedure was used to obtain samples of ^{14}C-labelled (II), (III), and (IV) from sodium [1-^{14}C]acetate. As the fermentation yield of deoxyherqueinone (I) was low, this labelled compound was prepared from [^{14}C]herqueinone (IV) by reduction with zinc and acetic acid.[2]

Atrovenetin was accumulated by *P. herquei* when grown on Raulin–Thom medium at 24° in submerged culture, whereas satisfactory yields of norherqueinone and herqueinone could only be obtained in surface culture. Under the latter conditions, incorporation of the labelled phenalenones was considerably improved by dissolving in minimum volumes of either acetone or pyridine together with the "Tween 80" and distilled water (5:1:15 v/v), prior to adding to the growing cultures.

The products (II), (III), and (IV) were subsequently isolated and purified to constant specific activities by



SCHEME

Specific activities (d.p.m./mg) of the ^{14}C-labelled substrates: deoxyherqueinone (I), $2{\cdot}8 \times 10^4$; atrovenetin (II), 14×10^4; norherqueinone (III), $3{\cdot}3 \times 10^4$; herqueinone (IV), $4{\cdot}5 \times 10^4$ (5—10 mg aliquot portions were added to each 100 ml culture at an appropriate stage of growth).

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standard procedures based on previous studies,[2,3] and the interconversions observed under these conditions are shown in the Scheme. The conversion of labelled deoxyherqueinone to norherqueinone was also examined and found to be negligible. Indirect incorporation of activity following prior metabolism of the [^{14}C]phenalenones to [^{14}C]acetate was apparently insignificant, since physcion anthrone, which was characterised as an acetate-derived metabolite of *P. herquei*,[4] was recovered unlabelled in all of the above experiments.

These results demonstrate the incorporation of atrovenetin (II) into norherqueinone (III) and herqueinone (IV). While the low yield of deoxyherqueinone (I) precluded more than a qualitative confirmation of its formation from atrovenetin, when tested as a substrate (I) was found to serve as an efficient precursor of herqueinone (38·3% incorporation) in contrast to the non-utilisation of norherqueinone. If the zero incorporation values are not a consequence of selective impermeability of the fungal cell wall, it follows that under these experimental conditions, atrovenetin is converted into herqueinone *via* deoxyherqueinone [(II)→(I)→(IV)] rather than norherqueinone.

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