Biosynthesis of Cochlioquinones

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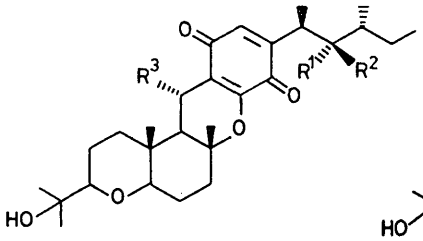
Summary The two oxygen atoms of the 2-(2-hydroxypropyl)tetrahydropyran system of cochlioquinones A (**1**) and B (**2**) are derived from two different oxygen molecules at separate steps on the biosynthetic pathway.

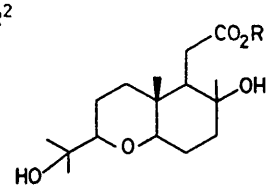
PREVIOUS studies have elucidated the biosynthetic origin of the carbon skeleton of cochlioquinones A (**1**) and B (**2**), minor metabolites of *Cochliobolus miyabeanus*,[1] but details of the steps involved in their biosynthesis have not yet been elucidated. Cochlioquinones A and B both contain the uncommon 2-(2-hydroxypropyl)tetrahydropyran unit; some metabolites of vegetable origin contain the 2-(2-hydroxypropyl)benzodihydrofuran system which appears to be biosynthesised *via* attack of a preformed phenolic hydroxy-group on an epoxide.[2] In this mechanism, the oxygen atoms derive from two different molecules of oxygen. Other mechanisms, involving for example a 1,2-dioxyethane intermediate formed through the dioxygenase-catalysed addition of an O_2 molecule to a double bond, could intervene in the formation of the 2-(2-hydroxypropyl)tetrahydropyran system.[3]

To investigate the type of mechanism involved in the biosynthesis of the cochlioquinones, it was necessary to

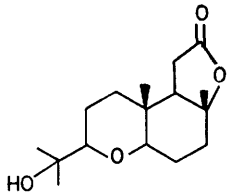
determine the origin of both oxygen atoms in this system. Accordingly, we carried out the fermentation in an atmosphere consisting of nitrogen (79%), $^{16}O_2$ (10·5%), and $^{18}O_2$ (10·5%) (v:v). The composition of the atmosphere in the fermentation flask was kept constant by feeding under constant pressure the isotopically enriched oxygen mixture and absorbing on potassium hydroxide the carbon dioxide produced.



(1) R^1 = OAc; R^2 = H; R^3 = OH
(2) R^1, R^2 = O; R^3 = H
(3) R^1 = OAc; R^2 = H; R^3 = H



(4) R = H
(5) R = Me



(6)

The mass spectra of the resulting cochlioquinones do not enable the origin of these oxygen atoms to be identified; however, this can be determined from the mass spectra of the methyl ester (**5**) obtained by treatment of the degradation product (**4**) with CH_2N_2. Compound (**4**) can be obtained from cochlioquinone B (**2**) by degradation with alkaline hydrogen peroxide or from cochlioquinone A (**1**) by hydrogenolysis to the deoxy-derivative (**3**) followed by similar degradation. The mass spectra of the methyl ester obtained from the cochlioquinones derived from fermentations carried out under normal conditions show an abundant peak at *m/e* 314 (4·11%; M^+); in the mass spectra of the methyl ester obtained from the cochlioquinones formed in an isotopically enriched atmosphere, this peak is accompanied by a peak of almost double intensity at *m/e* 316 (8·15%; $M+2$) and a peak of almost equal intensity at *m/e* 318 (4·15%; $M+4$). This result can only be explained by assuming the presence of two atoms of $^{18}O_2$ in the molecule of (**5**).

These atoms were probably the oxygen atoms of the 2-(2-hydroxypropyl)tetrahydropyran system; the oxygen atoms of the methoxycarbonyl group are derived from oxidative degradation occurring in the aqueous medium, while the remaining tertiary hydroxy-group is likely to be derived from the carbonyl oxygen of an acetogenin. Mass spectral studies show that only one of the two hydroxy-groups of (**5**) is labelled with ^{18}O since two peaks of equal intensity are observed at *m/e* 278 (8·37%) and 280 (7·93%) in the mass spectra of (**5**) derived from the cochlioquinones biosynthesized in a $^{18}O_2$ atmosphere while only the peak at *m/e* 278 is present in the mass spectra of the same compound derived from unlabelled cochlioquinones; therefore, the oxygen atom of the tetrahydropyran ring must be labelled. Analysis of the fragmentation of (**5**) indicates that the labelled hydroxy-group should be ascribed to the hydroxypropyl system since the peak at *m/e* 59, characteristic of tertiary alcohols of the $Me_2C(OH)R$ type, is present in the mass spectra of unlabelled (**5**) while a peak at *m/e* 61 of similar intensity is obtained, together with the peak at *m/e* 59, in the mass spectra of labelled (**5**).

This interpretation is confirmed by the mass spectra of the lactone (**6**). The relationships between the isotopic peaks in the decomposition products (**5**) and (**6**) therefore indicate that the two oxygen atoms of the 2-(2-hydroxypropyl) tetrahydropyran system are derived from elemental oxygen, and more specifically from two different oxygen molecules in the course of two separate oxidative stages on the biosynthetic pathway.

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