Biosynthesis of the Neurotoxin Alkaloid Roquefortine

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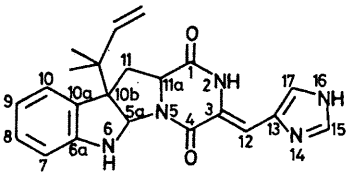
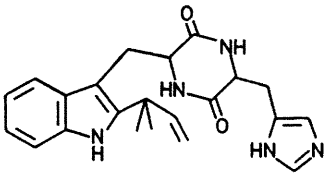
Summary [^{14}C]-Labelled mevalonic acid, tryptophan, and histidine have been incorporated into roquefortine and quantitative formation of roquefortine from multiply deuteriated tryptophan by an auxotropic mutant of *Penicillium roqueforti* has shown that the hydrogen at the 2-position of the indole ring is lost in this conversion.

THE neurotoxic alkaloid roquefortine (I) has been isolated from cultures of *Penicillium roqueforti*[1] and has been detected in blue vein cheese of diverse origins.[2] We have isolated roquefortine (*ca.* 3 mg l^{-1}) from cultures of *P. roqueforti* derived from Stilton cheese. Feeding [2-^{14}C]-mevalonic acid lactone, [methylene-^{14}C]-tryptophan, and

[2-^{14}C]-histidine gave incorporation of 0·08, 0·15, and 1·12%, respectively, into roquefortine, confirming the biosynthetic origins expected for such a structure.



(I)



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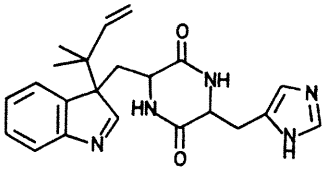
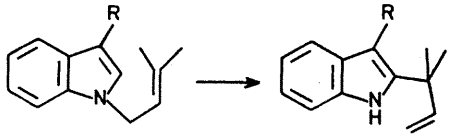


(III)

Diketopiperazines of tryptophan and another amino acid, modified by isoprenylation and other reactions, have been found in many *Penicillium* and *Asperillus* spp. and include echinulin and related compounds,[3] the austamides,[4] the brevianamides,[5] lanosulin,[6] and oxaline.[7] A common occurrence in this class of compounds is the presence of a 'reverse' substituted dimethylallyl grouping at the 2-position of the indole ring. Current speculation is that this substituent could be formed by rearrangement of an intermediate *N*-dimethylallyl grouping (Scheme) which can be achieved *in vitro*.[8]



SCHEME

We have investigated the involvement of the 2-position of the indole ring in the biosynthesis of roquefortine by using a mutant strain and deuterium labelling. A tryptophan auxotroph, that is a mutant unable to grow in the absence of tryptophan or its immediate precursors, was isolated from our strain of *P. roqueforti* by mutagenesis with u.v. light.[9] This mutant appears to be defective in anthranilate synthetase, and its inability to grow on minimal medium is overcome by supplementation with tryptophan, indole, or anthranilic acid. Roquefortine is still produced by this mutant, but in reduced yield. Clearly with this mutant all cellular constituents that contain tryptophan, both proteins and secondary metabolites, must be formed entirely from the added tryptophan. Growth of this mutant on multiply deuteriated tryptophan[10],† yielded deuteriated roquefortine. The ^{1}H n.m.r. spectrum showed the aromatic protons H-7—10 to be reduced to the expected *ca.* 25% of the undeuteriated sample, but the signal at δ 5·70 for H-5a, which is the original 2-position of the indole ring, was of undiminished intensity and contained no (<5%) deuterium. The tryptophan fed contained >95% deuterium at the 2-position and so at some stage in the biosynthetic sequence leading to roquefortine the proton at this position has been lost. One possible explanation is that an intermediate such as (II) containing the reverse dimethylallyl grouping at the 2-position is involved in the biosynthetic sequence. Rearrangement would lead to the 3-substituted derivative (III) which could cyclise to roquefortine (I). Our results also are in accordance with the earlier hypothesis of Bycroft[11] which involved sulphonium intermediates at the 2-position of the indole ring. However, the cell free system obtained from *Aspergillus amstelodami* that carries out the incorporation of a reversed isoprene unit at C-2 in echinulin biosynthesis from dimethylallyl pyrophosphate and *cyclo*-alanyltryptophan[12] would not seem to involve such intermediates. The involvement of *N*-substituted intermediates referred to above[8] and in the Scheme is based on chemical evidence and the loss of the 2-hydrogen and 3-substitution could occur by direct attack of dimethylallyl pyrophosphate at the 2- or 3-positions and rearrangement between these positions.

Such a use of auxotropic mutants has rarely been applied to the study of secondary metabolites. The advantages of these mutants are the quantitative derivation of the product from the added precursor which allows easy, unambiguous identification of the sites of labelled positions by spectroscopic means without resorting to lengthy degradative methods.

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† Mass spectral analysis showed that the tryptophan contained 2% $^2H_0$, 1·6% $^2H_1$, 3·6% $^2H_2$, 17·8% $^2H_3$, 38·6% $^2H_4$, and 36·4% $^2H_5$. The ^{1}H n.m.r. spectrum showed that there was >95% ^{2}H at the 2-position.

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