Roquefortine, an Intermediate in the Biosynthesis of Oxaline in Cultures of *Penicillium oxalicum*

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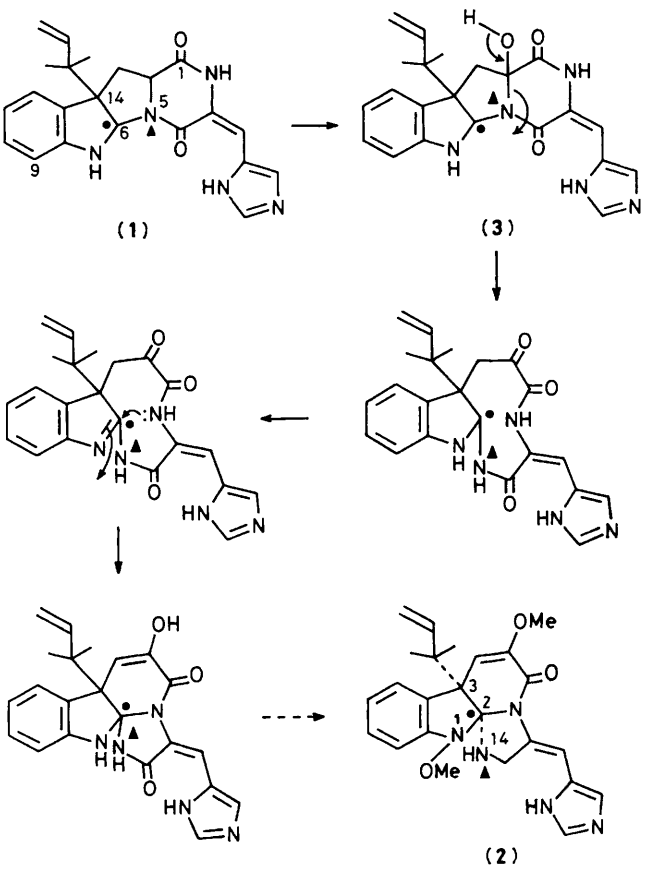
The incorporation of (2*RS*)-[*indole*-2-^{13}C, 2-^{15}N]tryptophan into both roquefortine and oxaline, and the efficient conversion of roquefortine into oxaline, defines a biosynthetic pathway by which N-14 of oxaline is derived from N-5 of roquefortine (and thus from tryptophan) and not from an exogenous nitrogen source.

Previous studies on the biosynthesis of roquefortine (**1**), a neurotoxic metabolite isolated from cultures of *Penicillium roqueforti*[1] and *Penicillium crustosum*[2] and oxaline (**2**), a metabolite of *Penicillium oxalicum*,[3,4] established that the 3-*pro-S* hydrogen atom of (2*S*)-histidine is lost during the *in vivo* formation of the α,β-dehydroamino acid moiety in these two compounds.[5] The mode of incorporation of the 1,1-dimethylallyl group at C-14 in roquefortine (**1**) has been studied with the aid of [1,2-$^{13}C_2$]acetate and (3*RS*)-[2,3-$^{13}C_2$]mevalonic acid lactone.[6]

A unique structural feature of oxaline (**2**) is the presence of a carbon atom, C-2, with three different nitrogen substituents. A plausible mechanism for the formation of this moiety requires that N-14 in oxaline originates from N-5 in roquefortine by an intramolecular rearrangement. The co-occurrence of roquefortine with oxaline in cultures of *P. oxalicum* lends credence to this hypothesis which was investigated using (^{14}C)-, (^{3}H,^{14}C)-, and (^{13}C,^{15}N)-labelled precursors.

Ring closure of *N*-acetyl-(2*RS*)-tryptophan by dicyclohexylcarbodi-imide in pyridine gives the corresponding oxazolone which on addition of tritiated water undergoes rapid exchange at the chiral centre as well as slow hydrolyic opening of the oxazolone ring.[7,8] Basic hydrolysis of the tritiated *N*-acetyltryptophan then afforded (2*RS*)-[2-^{3}H]tryptophan. (2*RS*)-[*indole*-2-^{13}C,2-^{15}N]Tryptophan was prepared from [2-^{13}C]-indole-3-aldehyde and [1-^{15}N]hydantoin[9] (99.0 atom % ^{15}N) using standard methods.[10] The [2-^{13}C]indole-3-aldehyde was in turn prepared from 2-nitrobenzaldehyde and potassium [^{13}C]cyanide (90.0 atom % ^{13}C) using reported procedures.[11]

Incorporation of (2*S*)-[*ring*-2-^{14}C]histidine (4.4%, dilution 15.1†) and (2*S*)-[3-^{14}C]tryptophan (10.8%, dilution 19.8) into roquefortine by cultures of *P. roqueforti* (HPB 061175), on a yeast extract (2%)–sucrose (5%) medium confirmed the biosynthetic origin of the metabolite. Addition of a mixture of



Scheme 1. Proposed biosynthetic pathway for oxaline (● = ^{13}C, ▲ = ^{15}N).

† Dilution values obtained from ^{14}C experiments are defined as in equation (1) where *n* and *m* are the appropriate number of

$$\text{Dilution} = \frac{\text{specific activity (precursor)}}{\text{specific activity (product)}} \times \frac{m(\text{product})}{n(\text{precursor})} \quad (1)$$

labelled sites; *cf.* P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Perkin Trans.* 1, 1981, 1298.

(2*RS*)-[2-^{3}H]- and (2*RS*)-[3-^{14}C]-tryptophan (^{3}H:^{14}C 7.02) to cultures of *P. roqueforti* gave roquefortine with a ^{3}H:^{14}C ratio of 2.01 (incorporation of ^{14}C 5.9%, dilution 12.0). This ratio corresponds to a 71.4% loss of ^{3}H which can be ascribed to a combination of two factors *viz.* the conversion of (2*R*)-[2-^{3}H]tryptophan *via* indole pyruvic acid into (2*S*)-tryptophan with concomitant loss of ^{3}H and exchange of ^{3}H at C-16 in roquefortine with ^{1}H of the medium. Thus in feeding experiments with [2-^{15}N]tryptophan a substantial loss of ^{15}N label can be expected.

The fate of the C-2 nitrogen atom of tryptophan in the biosynthesis of roquefortine was studied by the addition of (2*RS*)-[*indole*-2-^{13}C,2-^{15}N]]tryptophan, admixed with (2*RS*)-[*benzene-ring*-U-^{14}C]tryptophan to provide an independent measure of dilution, to cultures of *P. roqueforti*. Incorporation of the ^{14}C precursor into roquefortine was found to occur with high efficiency (6.1%) and low dilution (8.7). The presence of ^{13}C at C-6 in the enriched roquefortine directly bonded to ^{15}N was evident from the (^{13}C, ^{15}N)-coupling (^{1}J 7.0 Hz) observed for the C-6 resonance at δ 78.3 p.p.m. in the 20.0 MHz proton-noise-decoupled ^{13}C n.m.r. spectrum. The enhanced intensity of the C-6 resonance is ascribed to the presence of (^{13}C, ^{14}N)-labelled roquefortine and indicated that *ca.* 64% of the ^{15}N label of the tryptophan precursor is lost.

The origin of N-14 in oxaline was studied in the first instance by the addition of (2*RS*)-[*indole*-2-^{13}C, 2-^{15}N]tryptophan, admixed with (2*RS*)-[*benzene-ring*-U-^{14}C]tryptophan, to cultures of *P. oxalicum* (MRC 100) on yeast extract (2%)–sucrose (5%) medium. The ^{14}C precursor was incorporated efficiently (3.0%) with low dilution (20.5) into oxaline. The 125.76 MHz broad-band proton-decoupled ^{13}C n.m.r. spectrum of the enriched oxaline pointed to the presence of contiguous ^{13}C and ^{15}N atoms as the resonance at δ 101.60 p.p.m., assigned to C-2, exhibited a one-bond (^{13}C, ^{15}N)-coupling of 10.1 Hz. In addition the enhanced intensity of this resonance, due to the presence of [2-^{13}C, 14-^{14}N]oxaline, showed that *ca.* 37% of the ^{15}N label of the tryptophan precursor is lost. The precursor role of roquefortine in the biogenesis of oxaline by cultures of *P. oxalicum* was confirmed using the [*benzene-ring*-U-^{14}C, 6-^{13}C, 5-^{15}N]roquefortine obtained from cultures of *P. crustosum* (Sol-7) supplemented with (2*RS*)-[*indole*-2-^{13}C, 2-^{15}N]tryptophan (incorporation 4.3%, dilution 10.1). The roquefortine was efficiently incorporated into oxaline (24.3%) but the dilution of 29.8 precluded the observation, even at 125.76 MHz, of the one-bond (^{13}C, ^{15}N)-coupling for the C-2 resonance in the broad-band proton-decoupled ^{13}C n.m.r. spectrum.

A plausible mechanism for the formation of oxaline from roquefortine is presented in Scheme 1. The isolation and characterization (by mass spectrometry and n.m.r. spectroscopy) of a new metabolite, 16-hydroxyroquefortine (**3**), $C_{22}H_{23}N_5O_3$, together with roquefortine from large-scale fermentations of *P. crustosum* (Sol-7) supports the proposed pathway.

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