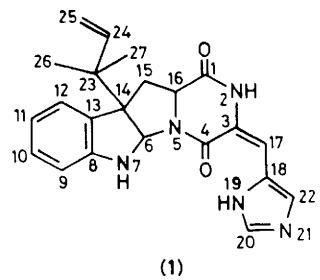
# The Biosynthesis of Roquefortine. An Investigation of Acetate and Mevalonate Incorporation using High Field N.M.R. Spectroscopy

**Charles P. Gorst-Allman,* Pieter S. Steyn, and Robert Vleggaar**

*National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa*

The high field $^{13}C$ n.m.r. spectra of roquefortine derived from [1,2-$^{13}C_2$]acetate and [2,3-$^{13}C_2$]mevalonic acid lactone exhibit previously unobserved couplings, which shed light on the mode of incorporation of the isopentenyl moiety into roquefortine; satellite resonances for numerous carbon atoms in the tryptophan-derived portion of [1,2-$^{13}C_2$]acetate-labelled roquefortine, can be explained *via* the operation of the Krebs' cycle and the shikimate pathway.

The neurotoxic alkaloid roquefortine (**1**), a natural contaminant of blue vein cheeses,[1] is produced by *Penicillium roqueforti*[2] and *Penicillium crustosum*, the latter fungus concurrently producing the penitrems. Roquefortine arises biogenetically from mevalonic acid lactone, tryptophan, and histidine.[3] We present here novel aspects of roquefortine biosynthesis apparent from high field (125.76 MHz) $^{13}C$ n.m.r. examination of roquefortine produced by *P. roqueforti* and *P. crustosum*, grown on liquid media cultures supplemented separately with [1,2-$^{13}C_2$]acetate and [2,3-$^{13}C_2$]mevalonic acid lactone. The $^{13}C$ n.m.r. spectrum[4] of [1,2-$^{13}C_2$]acetate-derived roquefortine showed that both C(26) and C(27) were coupled to C(23) (Table 1). This could be due to multiple-labelling with C(1)–C(2) and C(3)–C(3′) of mevalonic acid lactone arising from contiguous labelled acetate units, but this explanation is untenable since the phenomenon was also observed in the $^{13}C$ n.m.r. spectrum of roquefortine derived from [2,3-$^{13}C_2$]-mevalonic acid lactone.



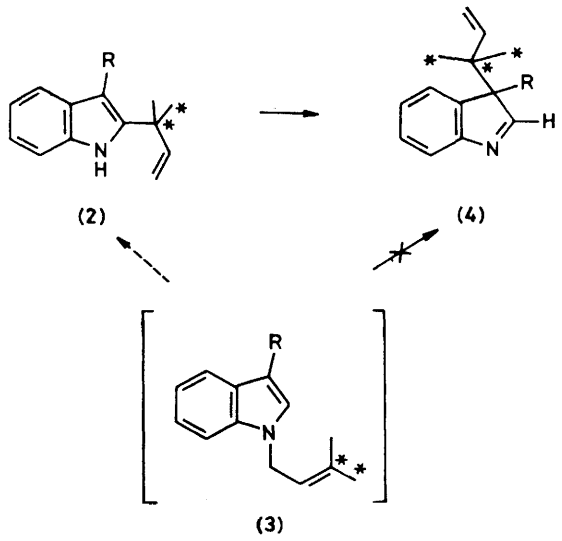

(1)

The mode of incorporation of the isoprenyl group located in the reverse fashion on the 3-position and 2-position of the indole nucleus in roquefortine and echinulin, respectively, has been the subject of much speculation.[5] The current hypothesis is that rearrangement of an intermediate *N*-dimethylallyl grouping occurs, either directly to the 3-position or *via* the 2-position, although direct alkylation at either the 2- or 3-

**Table 1.** $^{13}C$ N.m.r. data for roquefortine (**1**) derived from [1,2-$^{13}C_2$]acetate and [2,3-$^{13}C_2$]mevalonic acid lactone.

| Carbon atom | δ(C) (p.p.m.) [a] | $^1J$(C,C)/Hz (acetate precursor) | $^1J$(C,C)/Hz (mevalonic acid lactone precursor) |
|---|---|---|---|
| 1 | 167.22 | 53.40 | |
| 10 | 129.08 | 58.48 | |
| 11 | 119.10 | 57.07 | |
| 14 | 61.54 | 35.17 | |
| 15 | 36.86 | | |
| 16 | 58.82 | 53.32 | |
| 23 | 40.94 | 36.21 | 36.25 |
| 24 | 143.39 | 70.00 | |
| 25 | 114.72 | 69.98 | |
| 26 | 22.89 [b] | 36.13 | 36.03 |
| 27 | 22.50 [b] | 36.14 | 36.27 |

[a] Relative to internal $Me_4Si$ in $CDCl_3$. [b] These assignments may be interchanged.




**Scheme 1**

position and rearrangement between these positions has not been excluded. The absence of deuterium at the C(6) position in roquefortine, produced by a tryptophan auxotroph of *P. roqueforti* supplemented with multiply deuteriated tryptophan,[3] and the observation that only the (*E*)-methyl groups of the 3,3-dimethylallyl substituents in echinulin are derived from the 2-position of mevalonic acid[6] and do not couple to the adjacent centres, together with our results, suggest that the most likely method of incorporation of the isopentenyl group into roquefortine is that shown in Scheme 1. Here the intermediate (**2**) is formed either by an aza-Claisen-type rearrangement from (**3**) or *via* direct alkylation. Rearrangement to (**4**) then occurs with concomitant loss of the regiospecific integrity of the label. An alternative pathway in which the label is scrambled during the formation of dimethylallylpyrophosphate is precluded as intact isoprene units in the co-metabolite, penitrem A, show no evidence of mixed label.[7] It is noteworthy that with the acetate precursor the satellite resonances for the signal at δ 22.9 are *ca.* twice the intensity of those at δ 22.5, whereas this ratio is reversed for the mevalonic acid lactone precursor. This suggests that the carbon atom resonating at δ 22.9 arises from C(3′) of mevalonic acid lactone and that at δ 22.5 from C(2).

The $^{13}C$ n.m.r. spectrum of roquefortine derived from [1,2-$^{13}C_2$]acetate showed an intact acetate unit at C(1)–C(16) (Table 1). This may be explained in terms of the biosynthesis of tryptophan in which the carboxy-group and the α and β carbon atoms arise from serine. The serine in turn is derived from acetate *via* the Krebs' cycle, oxaloacetate, and 3-phosphoglycerate. The intensity of the satellite signals for C(1) and C(16) is approximately 20% of those observed for C(15). A number of the indole carbon atoms in the $^{13}C$ n.m.r. spectrum of roquefortine derived from [1,2-$^{13}C_2$]acetate exhibit discernible satellite resonances of very low intensity (Table 1). This phenomenon may be explained in terms of the Krebs' cycle, whereby acetate is converted *via* oxaloacetate and phosphoenolpyruvate into glucose which in turn enters the shikimate pathway leading ultimately to tryptophan *via* anthranilic acid. The observation of these couplings is truly remarkable since current theory requires numerous intermediates between acetate and tryptophan.[8] To our knowledge this is the first instance where such couplings have been observed.

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