Utilization of ^{13}C–^{13}C Coupling in Structural and Biosynthetic Studies; the Fourier Transform ^{13}C Nuclear Magnetic Resonance Spectrum of Mollisin

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Summary The biosynthetic pathway to mollisin has been clarified by the use of [1,2-^{13}C]acetate and FT ^{13}C-n.m.r.

Two biosynthetic pathways for mollisin, (I), a metabolite of *Mollisia caesia*, have been suggested by Bentley *et al.*[1] based on the labelling pattern obtained by ^{14}C-tracer experiments [Scheme, (a) and (b)]. Pathway (a) was favoured since this route uses an activated methylene for the chlorination reactions. A ^{13}C-labelling study[2] corroborated and amplified the ^{14}C-work.

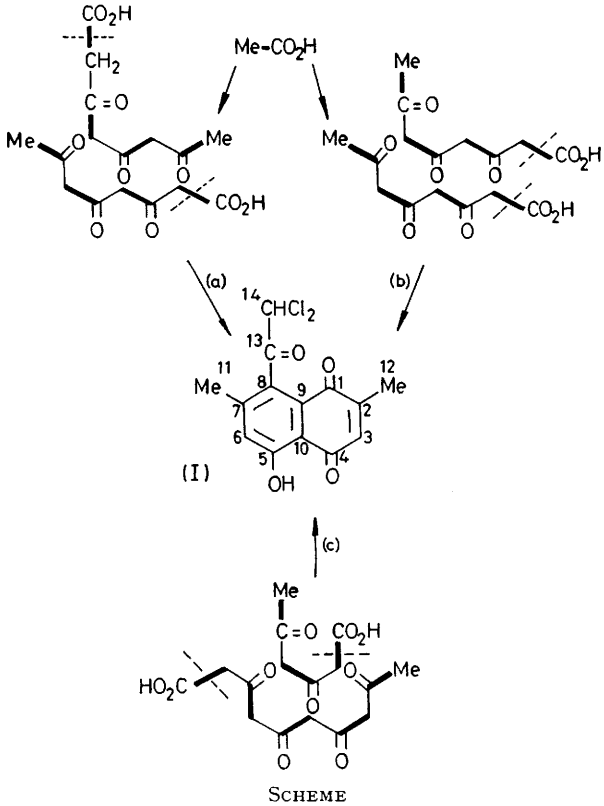
We report the result obtained by using ^{13}C-doubly labelled acetate ($^{13}CH_3{}^{13}CO_2Na$, 90% enriched) which shows another pathway exists for the formation of the metabolite.

If this doubly labelled acetate is incorporated into mollisin without cleavage of the carbon–carbon bond of the acetic acid molecule, ^{13}C–^{13}C coupling should be observed between C-11 and C-7, and C-12 and C-2 but not with C-14 for pathway (a). If pathway (b) takes place coupling between C-11 and C-7, and C-14 and C-13 and no coupling for C-12 should be apparent.

In the FT ^{13}C n.m.r. spectrum of the mollisin obtained from doubly labelled acetate which was prepared and isolated in a manner similar to that reported previously,[2] ^{13}C–^{13}C coupling is observed with C-3, C-6, C-12, and C-14 but not with C-11. The low enrichment level and low mollisin yield resulted in a poor S/N ratio which obscured the ^{13}C–^{13}C coupling of C-2, C-4, C-7, and C-13. ^{13}C–^{13}C coupling constants however could be determined with C-3, C-6, C-12, and C-14 and were as follows: $J_{2,12}$ 45·0, $J_{3,4}$ 52·5, $J_{6,7}$ 61·3, and $J_{13,14}$ 47·5 Hz.

These results show that the pairs of carbons at C-2 and C-12, C-3 and C-4, C-6 and C-7, and C-13 and C-14 (and probably C-5 and C-10, and C-8 and C-9) are derived from the same molecule of acetic acid. Therefore, the operation of either pathway (a) or (b) in mollisin biosynthesis is excluded and another pathway (c) is actually involved in the formation of mollisin.

The following assignment of signals in the ^{13}C n.m.r. spectrum was made with the aid of off-resonance and single frequency decoupling experiments† and from the known



SCHEME

† For the assignment of the ^1H n.m.r. spectrum of mollisin, see ref. 2.

chemical shift of the carbons of naphthoquinone,[3] shanorellin,[4] and rifamycin;[5] δ_C (internal Me_4Si) 189·2, 146·3, 136·1, 186·0, 162·2, 126·0, 148·7, 130·1, 130·7, 112·8, 20·6, 16·3, 191·9, and 70·9, assignable to C-1—C-14, respectively. The assignment of C-1 and C-4, and C-8 and C-9 may be reversed, but these uncertain assignments do not affect the biosynthetic conclusions.

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