High-field ^{13}C N.M.R. Evidence for the Formation of [1,2-^{13}C]Acetate from [2-^{13}C]Acetate during the Biosynthesis of Penitrem A by *Penicillium crustosum*

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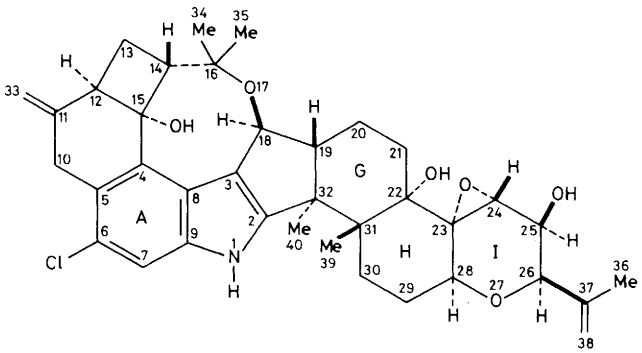
Analysis of one-bond carbon–carbon coupling constants in [2-^{13}C]acetate-derived penitrem A showed that [1,2-^{13}C]acetate was formed during the fermentation.

We have recently reported the structure of penitrem A (**1**), a novel tremorgenic mycotoxin from *Penicillium crustosum* (Sol-7).[1] The proposed relative stereochemistry and conformation of (**1**) are based on nuclear Overhauser effect (n.O.e.) connectivities[2] and as a result the previously reported chirality[1] of the 23,24-epoxide should be amended to that shown for (**1**). The indicated absolute configuration of (**1**) was determined by the partial resolution method of Horeau.[3] In the structural studies extensive use was made of ^{1}H and ^{13}C n.m.r. spectroscopy; labelling studies using [1-^{13}C]- and [1,2-^{13}C]-acetate showed that six isoprenoid units made up the non-indole part of penitrem A. We now report the formation of [1,2-^{13}C]-acetate from [2-^{13}C]acetate by *P. crustosum* during the biosynthesis of penitrem A.



(1)

P. crustosum was grown for 10 days at 25 °C in a stationary culture in ten Erlenmeyer flasks (500 ml), each containing 100 ml of Czapek medium enriched with 2% yeast extract. Sodium [2-^{13}C]acetate (1.15 g, 90.5 atom % ^{13}C) in sterile water (150 ml) was added continuously to the cultures from day 3 to day 9 by means of a peristaltic pump.

The 125.76 MHz proton noise decoupled (p.n.d.) ^{13}C n.m.r. spectrum (Bruker WM-500 n.m.r. spectrometer; solvent [$^{2}H_{6}$]acetone) of the [2-^{13}C]acetate-derived penitrem A showed carbon–carbon couplings for several carbon signals and intensity enhancement of 17 carbon signals *viz.* C(10), C(12), C(14), C(19), C(20), C(22), C(24), C(26), C(28), C(30), C(33), C(34), C(35), C(36), C(38), C(39), and C(40). The intensities of the satellite peaks due to carbon–carbon coupling vary from one site to the next, reflecting the different probabilities of the biosynthetic processes. The observed carbon–

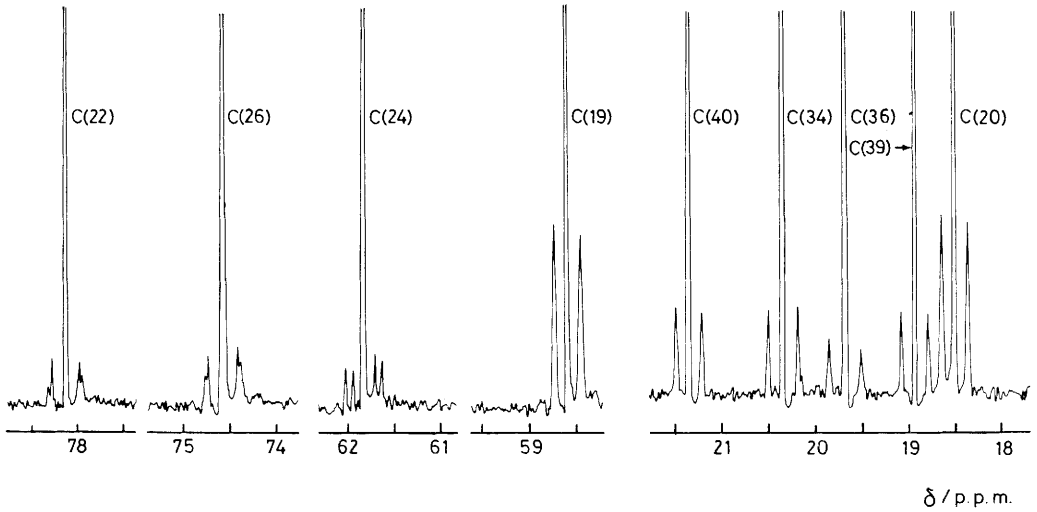


Figure 1. A portion of the 125.76 MHz p.n.d. ^{13}C n.m.r. spectrum of [2-^{13}C]acetate-derived penitrem A.

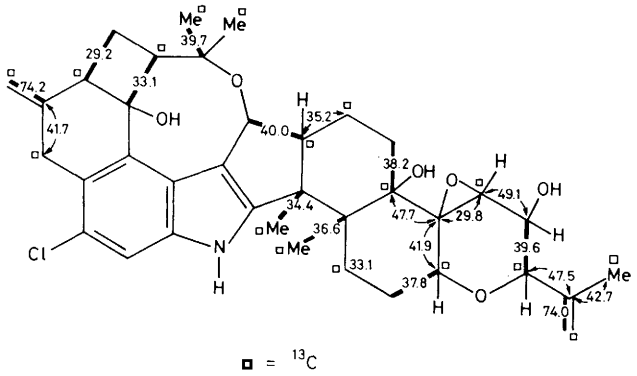


Figure 2. The labelling pattern observed for penitrem A enriched with $^{13}CH_3CO_2H$. The intra-acetate carbon–carbon couplings are indicated by thick lines. The observed interacetate and intermevalonate couplings are shown by thin arrowed lines. The magnitudes of the coupling constants in Hz are shown on the lines.

carbon couplings are shown in Figure 1. The highest intensities were observed for the satellites of the signals representing C(19) and C(20) [^1J(CC) 35.2 Hz]. Analysis of the one-bond (C,C)-coupling constants (see Figure 2) indicated the presence of eleven intact acetate units with an arrangement identical to that observed in [1,2-^{13}C]acetate-derived penitrem A. In addition the spectrum revealed one-bond interacetate and intermevalonate ^{13}C-labelling. Substantial differences in the various possible ^1J(CC) values for individual carbon atoms in the highly functionalized rings (G—I) of penitrem A allow resolution of more than one coupling at such sites. The signals which represent C(22) and C(24) are shown in Figure 1: C(22) displays intermevalonate, C(22)–C(23) and intra-acetate couplings, C(21)–C(22) whereas C(24) displays intermevalonate, C(24)–C(25) and interacetate couplings, C(23)–C(24). Figure 1 also depicts the signal of C(26) which displays intra-acetate, C(25)–C(26) and interacetate couplings, C(26)–C(37). Intra-acetate coupling refers to spin–spin coupling within an intact acetate unit.

Another interesting feature of the spectrum is that couplings are observed for some indole carbon atoms: C(4) 61.8, C(5) 61.1, C(7) 62.0, C(8) 55.9, and C(9) 55.2 Hz. Similar low intensity satellite peaks were observed in the p.n.d. spectrum of [1,2-^{13}C]acetate-derived penitrem A. This may be attributed to the multistep conversion of acetate into glucose which in turn enters the shikimate pathway, leading to tryptophan *via* anthranilic acid.[4]

It is evident that some [1,2-^{13}C]acetate, albeit little, is formed during the fermentation by the frequent recycling of [2-^{13}C]-acetate in the Krebs citric acid cycle.[4] Yoshida *et al.*[5] proposed that similar effects were observed in the 25.2 MHz ^{13}C n.m.r. spectrum of [2-^{13}C]acetate-derived piericidin A, isolated from *Streptomyces mobaerensis*; however, the absence of some of the expected ^1J(CC) couplings in their reconstructed spectrum of piericidin A is disconcerting.

The formation of [1,2-^{13}C]acetate from [2-^{13}C]acetate is probably a general biosynthetic process which has not been recognized in studies at low magnetic field strength. A case in point is [2-^{13}C]acetate-derived averufin which did not display (C,C)-coupling at 25.2 MHz.[6] However, we observe extensive (C,C)-coupling at 125.76 MHz owing to the much improved sensitivity.

The information to be gained from the various ^1J(CC) couplings could prove invaluable in biosynthetic and structural studies of fungal metabolites.

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