Tremorgenic Mycotoxins from *Penicillium crustosum*. Biosynthesis of Penitrem A

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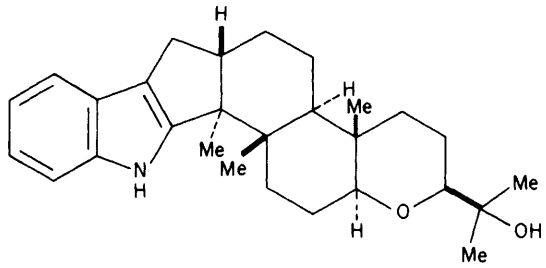
The biosynthesis of penitrem A has been studied with both ^{13}C- and ^{2}H-labelled precursors, *viz.* [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, and [1-^{13}C,2-$^{2}H_3$]-acetate, [2-^{13}C]-, [2,3-$^{13}C_2$]-, [2-$^{2}H_2$]- and [5-$^{2}H_2$]-mevalonate. The results show that penitrem A is derived from tryptophan, which contributes the indole moiety of the metabolite, geranylgeranylpyrophosphate, and two isopentenylpyrophosphate units. A 1,2-bond migration, involving the 2,3-bond of a mevalonate unit, occurs in the course of the biosynthesis and results in the observation of a one-bond (C,C) coupling between two [1-^{13}C]acetate-derived carbon atoms and between two [2-^{13}C]acetate-derived carbon atoms. Analysis of the one-bond (C,C)coupling constants in [2-^{13}C]acetate-derived penitrem A showed that [1,2-$^{13}C_2$]acetate was formed during the fermentation. Although loss of water from an hydroxyisopropyl group to form the isopropenyl function present in penitrem A should proceed with retention of the stereochemical integrity of the two methyl groups, isomerization of the double bond causes equal distribution of ^{13}C label between C-36 and C-38 and precludes any stereochemical deductions.

Penicillium crustosum when grown in surface culture pro



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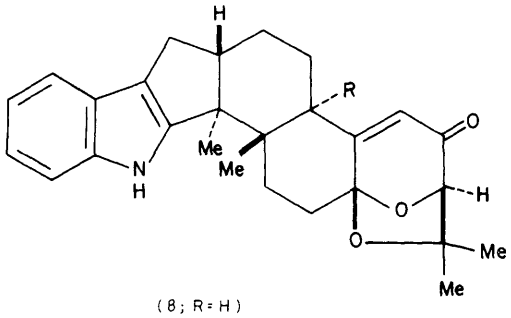
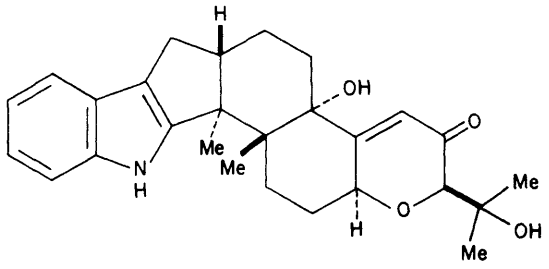


(8; R = H)
(10; R = OH)



(9)

Table 1. 125.76 MHz ^{13}C N.m.r. data for penitrem A

Carbon atom	δ_C [a]	^1J(CC) [b]	^1J(CC) [c]	$\Delta\delta$/p.p.m. [d]
10	35.06 †	(40.3)	40.7	
11	149.48 *	73.9, (40.3)	40.7	−0.180
12	47.01 †	29.9		
13	24.67 *	29.9		−0.148
14	52.71 †	33.6		
15	81.01 *	33.6		−0.058
16	76.09 *	39.1	41.0	−0.149
18	72.44 *	39.7		−0.059
19	58.79 †	39.7		
20	18.56 †	(34.8)		
21	30.59 *	37.8		
22	78.24 †	39.1		
23	66.11 *	(29.9)	29.9	
24	61.92 †	(29.9)	29.8	
25	66.31 *	39.3		−0.058
26	74.67 †	39.6		
28	71.99 †	37.2		
29	28.89 *	37.8		
30	26.91 †	(33.6)	34.0	
31	43.55 *	36.6	34.3	−0.223
32	50.08 *	34.8		−0.210
33	107.10 †	73.9		
34	20.32 †	39.7		
35	31.06 †	(40.3)	41.0	
36	19.70 †	(42.7)	43.0	
37	143.27 *	73.2	73.2, 42.8	
38	111.64 †	73.2	73	
39	18.98 †	36.0		
40	21.35 †	34.8		

[a] Relative to internal Me_4Si; solvent $(CD_3)_2CO$. * = enriched by [1-^{13}C]acetate; † = enriched by [2-^{13}C]acetate. [b] Values obtained from the broad-band proton-decoupled spectrum of penitrem A derived from [1,2-$^{13}C_2$]acetate. Values in parentheses are due to multiple labelling. [c] Values obtained from the broad-band proton-decoupled spectrum of penitrem A derived from [2,3-$^{13}C_2$]mevalonolactone. [d] $^{13}C-^{2}H$ Upfield β-shift in p.p.m.

signals. This result implies that a rearrangement (a 1,2-shift), similar to that observed in the biosynthesis of paspaline,[11] occurs during the course of the biosynthesis of penitrem A.

acetate (C,C) coupling. Analysis of the values for ^1J(CC) indicated the presence of eleven intact acetate units with an

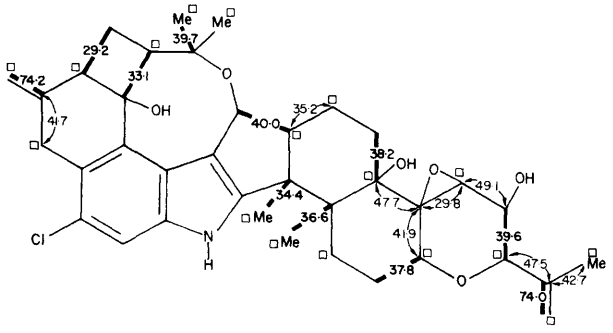


Figure 1. The labelling pattern observed for penitrem A enriched with $^{13}CH_3CO_2H$. The intra-acetate carbon–carbon couplings are indicated by thick lines. The observed inter-acetate and inter-mevalonate couplings are shown by thin arrowed lines. The magnitudes of the coupling constants in Hz are shown on the lines. □ = Carbon atom derived from C-2 of acetate

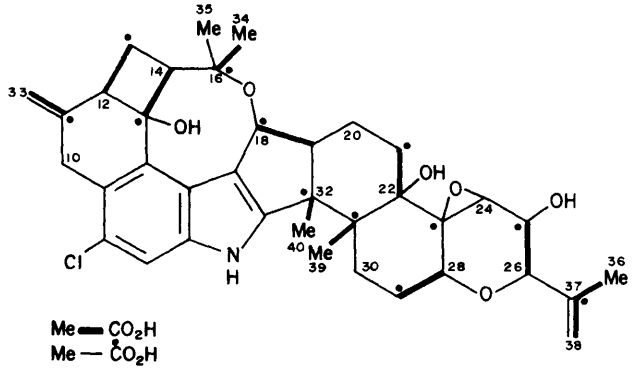


Figure 2. Labelling pattern of penitrem A derived from [1-^{13}C]- and [1,2-$^{13}C_2$]acetate labelling experiments

mevalonate units. These types of one-bond (C,C) couplings (see Figure 1) are particularly evident in the more highly functionalized rings of penitrem A where substantial differences in the various possible ^1J(CC) values allow the resolution of more than one such coupling at a particular site. The observation of some intermevalonate couplings is particularly noteworthy. A similar rearrangement to that mentioned above whereby [2-^{13}C]acetate-derived carbon atoms end up in contiguous positions as a result of a bond migration (1,2-shift) has been observed in the biosynthesis of paspaline.[11]

Some of the carbon atoms of the indole moiety of penitrem A enriched by [2-^{13}C]acetate also exhibited very low intensity satellite signals due to (C,C) coupling, *viz.* C-4 61.8 Hz, C-5 61.1 Hz, C-7 62.0 Hz, C-8 55.9 Hz, and C-9 55.2 Hz; even at this high field strength no (C,C) coupling was observed for C-6. This information indicates coupling of C-4 to C-5, C-8 to C-9, and C-6 presumably to C-7. This observation is best explained by conversion of [2-^{13}C]acetate into glucose which in turn enters the shikimate pathway leading to tryptophan *via* anthranilic acid.[20]

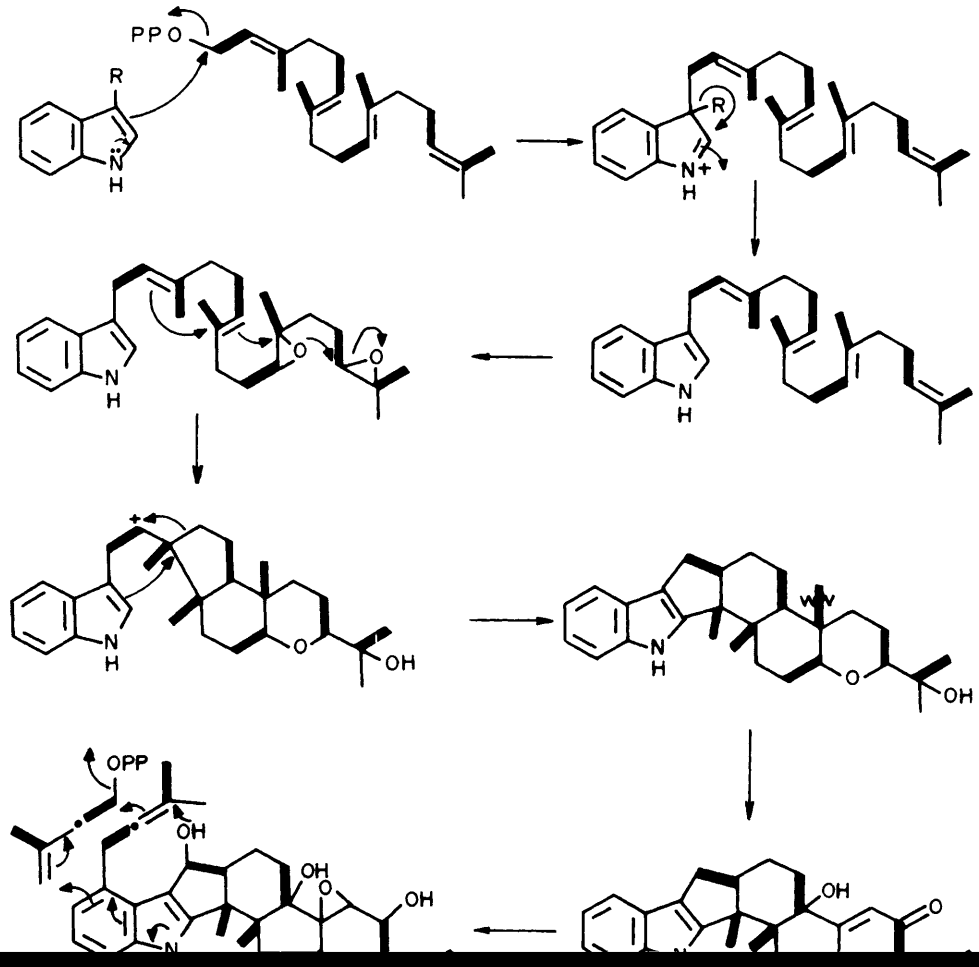
The measured (C,C) coupling constants (Table 1) in the broad-band proton-decoupled ^{13}C n.m.r. spectrum of penitrem A derived from [1,2-$^{13}C_2$]acetate are in accordance with the following intact acetate units (Figure 2): C(11)–C(33), C(12)–C(13), C(14)–C(15), C(16)–C(34), C(18)–C(19), C(21)–C(22), C(25)–C(26), C(28)–C(29), C(31)–C(39), C(32)–C(40), and C(37)–C(38) [or C(36)]. It is of interest to note that the signal due to C-37 exhibited two pairs of satellite peaks of equal intensity due to coupling with C-38 [^1J(CC) 73.2 Hz] and C-36 [^1J(CC) 42.7 Hz]. A similar pair of satellite peaks but of different intensity is observed for the C-11 signal due to coupling with C-10 [^1J(CC) 40.3 Hz] and C-33 [^1J(CC) 73.9 Hz]. One possible explanation for this phenomenon may be found in the following observation. In the broad-band proton-decoupled ^{13}C n.m.r. spectrum of penitrem A derived from (3*RS*)-[2-^{13}C]mevalonolactone seven carbon signals are enhanced, *viz.* C-10, C-20, C-24, C-30, C-35, C-36, and C-38 whereas in the spectrum of penitrem A derived from [1,2-$^{13}C_2$]acetate these signals exhibited lower intensity satellite peaks due to one-bond (C,C) couplings: C-10 (40.3 Hz), C-20 (34.8 Hz), C-24 (29.9 Hz), C-30 (33.6 Hz), C-35 (40.3 Hz), C-36 (42.7 Hz), and C-38 (73.2 Hz) by coupling to C-11, C-10

subsequent experiment in which a culture of *P. crustosum* was supplemented with (3*RS*)-[2,3-$^{13}C_2$]mevalonolactone. The broad-band proton-decoupled ^{13}C n.m.r. spectrum of the enriched penitrem A showed the presence of four intact two-carbon units (see Table 1): C(10)–C(11), C(16)–C(35), C(23)–C(24), and C(30)–C(31). Once again the signals due to C-36 and C-38 displayed equal intensity satellite peaks due to one-bond (C,C) coupling with C-37 (47.2 and 73.2 Hz, respectively). This result also confirms that in the course of the biosynthesis the 2,3-bond of mevalonolactone is cleaved by a 1,2-bond migration as C-20, which is derived from C-2 of mevalonolactone, exhibits no one-bond (C,C) coupling. As a result of this shift a coupling is observed between C-19 and C-20 in the ^{13}C n.m.r. spectrum of [2-^{13}C]acetate-derived penitrem A as well as between C-31 and C-32 in that of [1-^{13}C]acetate-derived penitrem A. A similar result was found in a study on the biosynthesis of paspaline (7).[11]

The incorporation of label from [1,2-$^{13}C_2$]acetate, [2-^{13}C]- and [2,3-$^{13}C_2$]-mevalonolactone to an equal extent at C-36 and C-38 could be due to a non-specific elimination of water from an hydroxyisopropyl group, a process in which the stereochemical integrity of the two prochiral diastereoisotopic methyl groups would be lost. However, in the biosynthesis of paspaline (7) these two methyl groups retain their stereochemical integrity [11] and it would appear that in the course of the isolation and subsequent purification of penitrem A by column chromatography on silica gel, isomerization of the $\Delta^{37,38}$-bond occurs.

On the basis of the results obtained for penitrem A using ^{13}C-labelled precursors a biosynthetic pathway as outlined in the Scheme is postulated. The initial stages of the pathway are similar to those proposed for paspaline [11,21] and paxilline.[12,21] The exact sequence of events is, however, open to speculation. The formation of the isopropenyl function by loss of water from an hydroxyisopropyl group is shown to occur with retention of the stereochemical integrity of the two methyl groups as is indeed the case in paspaline and paxilline biosynthesis. However, in the case of penitrem A we still do not know which two carbon atoms of the isopropenyl group are derived from an intact acetate unit as isomerization of the $\Delta^{37,38}$-bond may occur during the purification of the metabolite (see above). This aspect of the biosynthesis is at present under investigation.

The fate of the hydrogen atoms in the biosynthesis of


PPO
R
N
H
R
N+
H
N
H
O
O
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+
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OPP
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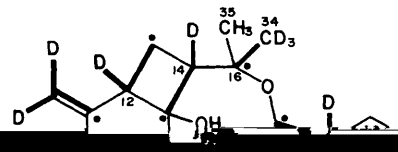

Table 2. Yields of penitrem A (1) from differently labelled precursors

Precursor	Atom % ^{13}C	Amount (mg)	Yield of (1) [a]

16 S. Yoshida, S. Shiraishi, K. Fujita, and N. Takahashi, *Tetrahedron Lett.*, 1978, 1863.
17 C. P. Gorst-Allman, K. G. R. Pachler, P. S. Steyn, P. L. Wessels, and D. B. Scott, *J. Chem. Soc., Perkin Trans. 1*, 1977, 2181.
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