A Role for Paxilline in the Biosynthesis of Indole–Diterpenoid Penitrem Mycotoxins

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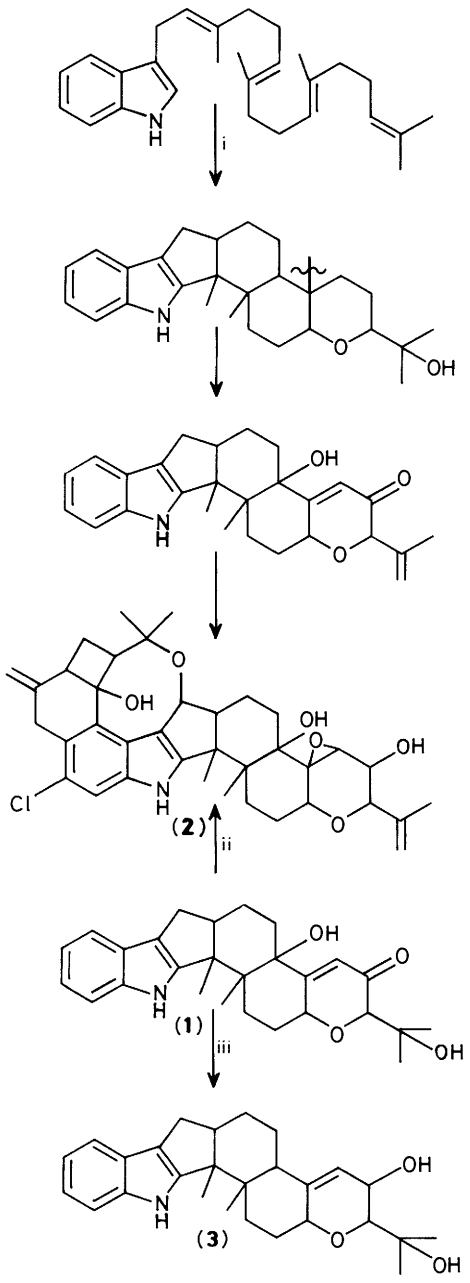
[^{14}C]Paxilline was biosynthetically incorporated into penitrem A (3.1—8.7%), and when calculated to include penitrem E to a greater extent (3.3—17%), in submerged fermentations of *Penicillium janczewskii*; concurrent biotransformation of [^{14}C]paxilline to a hydroxy derivative may indicate another intermediate in penitrem biosynthesis.

A unique group of structurally related natural products, the penitrems[1], janthitrems[2], aflatrem[3], the paspaline/paspalinines[4] and paxilline[5] have an indole–diterpenoid core in common. These compounds, which are generally tremorgenic in animals, are exclusively metabolites of fungi of the genera *Penicillium*, *Aspergillus* and *Claviceps*. The lolitrems[6] which have only been isolated from ryegrass infected with the endophytic fungus *Acremonium loliae* are probably also, at least in part, biosynthetic products of the fungal component.[7] In any one of the fungi involved, biosynthetic interrelationships between their indole–diterpenoid products which have so far been characterised can be deduced but are limited to simple oxidative and reductive steps, chlorination and prenylation. Although theoretical biosynthetic schemes have been proposed for the penitrems[8] and paspalines[9], including similar early steps in the formation of the indole–diterpenoid part, there has been no experimental evidence other than demonstration of their origin from tryptophan and mevalonate precursors.[8–10]

Paxilline (**1**) is one of the simplest indole–diterpenoids and is readily obtained from submerged fermentation of one particular strain of *Penicillium paxilli*.[11] Not only is (**1**) a putative precursor of the elusive lolitrems,[10] requiring only prenylation and epoxidation, but also it is structurally close to two theoretical intermediates in penitrem biosynthesis[8] (Scheme).

Meaningful experiments concerning a putative role for (**1**) as an intermediate in penitrem biosynthesis are conditioned partly by paxilline's hydrophobicity which hinders administration to a fungus in liquid culture *via* the medium. However, the unique facility of penitrem biosynthesis by a strain of *P. janczewskii* in submerged fermentation goes some way towards optimising access of added (**1**) to the idiophase of a finely divided suspension of fungal hyphae and spores.

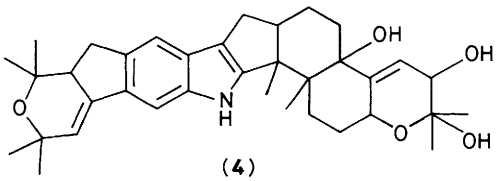
Compound ^{14}C-(**1**), biosynthetically radiolabelled by [2-^{14}C]-mevalonate in the diterpenoid, was administered in varying amounts (Table) to *P. janczewskii* fermentations 2—3 days after inoculation, when biomass had been formed and the idiophase had commenced. Four days later, the principal penitrems extracted from the cells were isolated by preparative t.l.c. on silica gel. The most abundant penitrem A (**2**) was purified by h.p.l.c., particularly to ensure separation from residual ^{14}C-(**1**). Radioactivity in (**2**), determined by scintillation counting and confirmed qualitatively by autoradiography of chromatograms in which (**2**) and (**1**) are well resolved, was 3.1—8.7% of that of the ^{14}C-(**1**) administered which had become cell-associated (Table). Autoradiography showed that other less-abundant penitrems were also radiolabelled and thus enhanced the percentage incorporation value of (**1**) into penitrems (3.3—17%) by including radiolabel in the next most abundant penitrem E (deoxypenitrem A). Compound ^{14}C-(**1**) was partly retained in the broth and was also evident in mycelial extract, some of which could have been that which had become cell-associated but was not taken up into the cells. There is no reason



Scheme. i, Putative pathway;[8] ii, biotransformation demonstrated in *P. janczewskii*; iii, biotransformation demonstrated in *P. paxilli*,[13] *A. loliae*,[13] and *P. janczewskii*

Table. Biosynthetic incorporation of [^{14}C]paxilline into penitrems in five submerged fermentations of *Penicillium janczewskii* differing in penitrem yield and paxilline addition

Fermentation	*P. janczewskii* sub-strain	^{14}C-Paxilline: Specific radioactivity (d.p.m. mg^{-1})	^{14}C-Paxilline: Added to 100 ml culture (mg)	^{14}C-Paxilline: Added to 100 ml culture (d.p.m.)	Total penitrem yield (mg)	[^{14}C]Paxilline incorporated into penitrem A (%)	Cell-associated [^{14}C]paxilline incorporated (%) into: penitrem A	Cell-associated [^{14}C]paxilline incorporated (%) into: penitrems A and E
1	A	2.6×10^5	1	2.6×10^5	1.28	1.19	3.06	5.2
2	A	8.3×10^5	0.36	3.0×10^5	0.60	1.78	5.00	7.2
3	B	8.3×10^5	0.18	1.5×10^5	2.8	2.95	3.28	3.3
4	B	8.3×10^5	0.19	1.6×10^5	1.5	3.80	3.93	6.9
5	C	2.6×10^5	0.15	3.8×10^4	3.2	4.18	8.67	17.0



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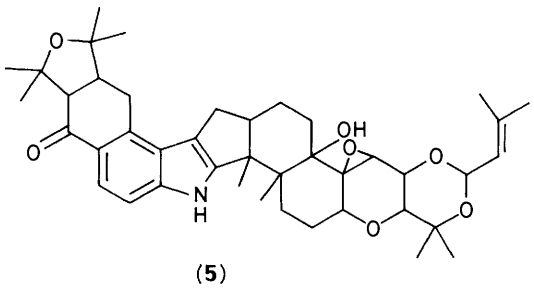

(5)

to expect that *P. janczewskii* has a specific uptake mechanism for (**1**). However, the results are consistent with direct incorporation of the indole-diterpenoid of (**1**) into (**2**) and related compounds. The greatest percentage incorporation value was obtained as expected from the smallest additions of radiolabelled putative precursor in circumstances of highest penitrem yield. Nevertheless, cellular uptake is probably an additionally important limiting factor.

In the *P. janczewskii* fermentation 1, ^{14}C-(**1**) was also transformed significantly to a more polar metabolite which co-chromatographed on t.l.c. with 19-desoxypaxillin-16β-ol (**3**), a novel indole–diterpenoid recently characterised as a minor fermentation product in *P. paxilli* and *A. loliae*.[12] The findings encourage further experimental study of biosynthetic intermediates of penitrems and other substituted indole–diterpenoids, such as janthitrem E (**4**) and lolitrem B (**5**)[13] and the extent to which they form a metabolic grid as has been recognised for a group of tetraketide fungal products.[14]

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References

1 A. E. De Jesus, P. S. Steyn, F. R. Van Heerden, R. Vleggaar, P. L. Wessels, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1847.
2 A. E. De Jesus, P. S. Steyn, F. R. Van Heerden, and R. Vleggaar. *J. Chem. Soc., Perkin Trans. 1*, 1984, 697.
3 R. T. Gallagher, J. Clardy, and B. J. Wilson, *Tetrahedron Lett.*, 1980, 239.
4 R. J. Cole, J. W. Dorner, J. A. Lansden, R. H. Cox, C. Pape, B. Cunfer, S. S. Nicholson, and D. M. Bedell, *J. Agric. Food Chem.*, 1977, **25**, 1197.
5 J. P. Springer, J. Clardy, J. M. Wells, R. J. Cole, and J. W. Kirksey, *Tetrahedron Lett.*, 1975, **30**, 2531.
6 R. T. Gallagher, A. D. Hawkes, P. S. Steyn, and R. Vleggaar, *J. Chem. Soc., Chem. Commun.*, 1984, 614.
7 C. M. Weedon and P. G. Mantle, *Phytochemistry*, 1987, **26**, 969.
8 A. E. De Jesus, C. P. Gorst-Allman, P. S. Steyn, F. R. Van Heerden, R. Vleggaar, P. L. Wessels, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1863.
9 W. Aklin, F. Weibel, and D. Arigoni, *Chimia*, 1977, **31**, 63.
10 I. Laws and P. G. Mantle, *J. Gen. Microbiol.*, in the press.
11 M. Ibba, S. J. C. Taylor, C. M. Weedon, and P. G. Mantle, *J. Gen. Microbiol.*, 1987, **133**, 3109.
12 C. M. Weedon, Ph.D. Thesis, University of London, 1987.
13 P. S. Steyn and R. Vleggaar, *Fortschr. Chem. Org. Naturst.*, 1985, **48**, 1.
14 J. D. Bu'Lock, in 'The Filamentous Fungi,' ed. J. E. Smith and D. R. Berry, Edward Arnold, London, 1975, vol. I, p. 35.

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