Biosynthesis of a Cyclopentyl Dienyl Isonitrile Acid in Cultures of the Fungus *Trichoderma hamatum* (Bon.) Bain. aggr.

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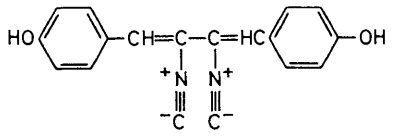
Summary The structure and biosynthetic origin of the isonitrile-containing antibiotic (**2**) was established by experiments with [^{14}C]- and [^{13}C]-tyrosine in cultures of *Trichoderma hamatum* (Bon.) Bain. aggr.

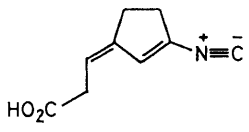
BIOSYNTHETIC studies have linked xanthocillin X (**1**), one of the isonitriles produced by some *Penicillium notatum* strains and some *Aspergillus* species, to tyrosine and the shikimate pathway[1] but the biogenesis of the *Trichoderma* isonitriles remain unknown. These are cyclopentyl isonitriles carrying a C_3 or C_2 substituent in the 3 position and differing in their degrees of unsaturation and oxygenation [*e.g.* the diene-acid (**2**),[2] the epoxy-acid (**3**),[2] and trichoviridin (**4**)[3] which have been found to co-occur in *T. hamatum* strains examined in the course of the biosynthetic experiments under discussion].

Fungal cyclopentanes have been reported to originate from substituted benzenoids by ring contraction processes.

Thus, terrein (**6**) was shown[4] to be derived from acetate *via* 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (**5**) with elimination of C-7. *A priori* a similar biogenesis appeared to be reasonable for the *Trichoderma* isonitriles.



(1)



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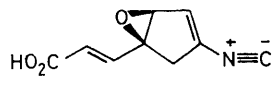

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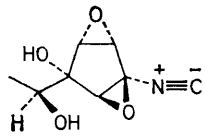

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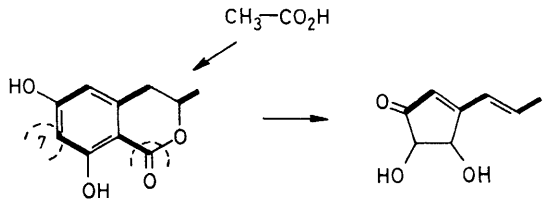


(5) (6)

The *T. hamatum* strain (HLX 1379†) grown as before[2] produced antibiotic (**2**) as the only major metabolite. Cultures of this strain were incubated with a series of potential precursors and the results obtained are listed in the Table. The level of acetate incorporation was an order of magnitude lower than that observed in the biosynthesis of the polyketide terrein (0·59%).

TABLE. Incorporation of ^{14}C, ^{3}H, and ^{13}C labelled precursors into the isonitrile acid (**2**).

Substrate	% Incorporation
$CH_3{}^{14}CO_2Na$	0·05
$^{14}CH_3CO_2Na$	0·035
[*U*-^{14}C]-L-Tyrosine	6·5
[1-^{14}C]-L-Tyrosine	7·6
[1-^{14}C]-[3,5(ring)-$^{3}H_2$]-L-Tyrosine ($^{3}H/^{14}C$ = 6·8)	7·4 (No ^{3}H)
[3-^{13}C]-D,L-Tyrosine	5[a]
[1-^{13}C]-D,L-Tyrosine	5[a]
[1-^{14}C]-L-Phenylalanine	0·14
[1-^{14}C]-3,4-Dihydroxy-L-phenylalanine	0·55

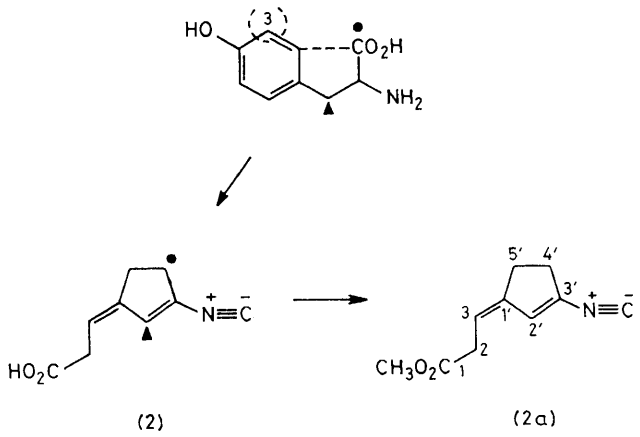
[a] By ^{13}C n.m.r. spectroscopy.

Of the three aromatic amino-acids investigated, tyrosine was incorporated considerably better than the rest (the low incorporation of phenylalanine and 3,4-dihydroxyphenylalanine might suggest the existence of parallel pathways, a phenomenon already observed in biosynthetic experiments with other fungal cultures[5]). The difference in the incorporation between [*U*-^{14}C]- and [1-^{14}C]-L-tyrosine (0·9 and 1·1% respectively, in two runs) agreed well with that expected for the loss of one ring carbon from the [U-^{14}C] labelled tyrosine (0·8%).

The ^{1}H n.m.r. spectrum of (**2a**) has been reported previously[2] and the proton assignments are consistent with the structure (**2a**): δ (^{1}H; $CDCl_3$) 6·51br. (1H, H-2′, s), 5·44 (1H, H-3, $J_{2'3}$ 7·75 Hz, t), 3·69 (3H, OMe, s), 3·13 (2H, H-2, $J_{2,3'}$ 7·75 Hz, d), and 2·67 (4H, H-4′ and H-5′, m). The ^{13}C n.m.r. spectrum was assigned by off-resonance and single-frequency proton-decoupling experiments: δ(^{13}C; $CDCl_3$) 171·7 (C-1, s), 169·4br (–N*C*, s), 144·0 (C-1′, s), 132·6 [C-3′, t, J(^{14}N–C) 12·5 Hz], 128·8 (C-2′, $^{1}J_{CH}$ 174 Hz), 114·8 (C-3, $^{1}J_{CH}$ 160 Hz), 51·9 (OMe, $^{1}J_{CH}$ 147 Hz), 34·6 (C-2, $^{1}J_{CH}$ 129 Hz), 32·7 (C-4′, $^{1}J_{CH}$ 135 Hz), and 28·4 (C-5′, $^{1}J_{CH}$ 133 Hz) p.p.m. C-4′ and C-5′ were distinguished *via* their long-range C–H coupling constants. With the aliphatic protons decoupled, C-4′ exhibits a single long-range splitting ($^{3}J_{CH}$ 7·9 Hz) due to H-2′; C-5′ exhibits long-range coupling to both H-2′ and H-3 (*ca.* 6 Hz to each). C-5′ also shows long-range coupling to H-2.

The alternative cyclopentadiene structures[7] for the diene ester can be eliminated by the presence of long-range C–H coupling between the low-field methylene protons (δ 3·13) and the ester carbonyl carbon (δ 171·7 p.p.m.). The lack of allylic H–H coupling[6] between the low-field methylene protons (δ 3·13) and the low-field olefinic proton (δ 6·51) also militates against the structure (**7**). The *Z*-stereochemistry about the exocyclic double bond in (**2a**) was established by proton–proton nuclear Overhauser effect experiments.[8]

[3-^{13}C]- and [1-^{13}C]-D,L-tyrosine were synthesised by unambiguous routes from 90% enriched $Ba^{13}CO_3$ and $K^{13}CN$ respectively. The purity and label positions in the tyrosine were verified by ^{13}C and ^{1}H n.m.r. and mass spectrometry. 100 flasks were incubated each with 5 mg of [^{13}C]-D,L-tyrosine and culture fluids were extracted with EtOAc, the acid fraction was esterified (CH_2N_2), and the esters were purified by repeated chromatography on Al_2O_3 (neutral) layers.



(2) (2a)

SCHEME

Incubation of *T. hamatum* with [3-^{13}C]tyrosine resulted in an ester (**2a**) with enrichment at C-2′. Similarly incubation with [1-^{13}C]tyrosine gave an ester enriched at C-4′. This demonstrates the unexpected involvement of the tyrosine side chain in the cyclopentane ring (Scheme). Label positions were established by ^{13}C n.m.r. spectroscopy and by the presence of enhanced ^{13}C satellites in the ^{1}H

† Accession number to the culture collection held at The Atlantic Regional Laboratory.

n.m.r. spectrum of the product. In the C-4′ enriched compound, the ^{13}C satellites about the proton signal at δ 2·75 showed H–H coupling to H-2′ but not to H-3, thus verifying the proton and carbon assignments.

The experiments carried out thus far (a) associate the *Trichoderma* isonitrile (**2**) with the metabolism of tyrosine, (b) reveal the unexpected involvement of the tyrosine side chain in the five-membered carbocyclic ring, (c) indicate the probable loss of the aromatic C(3) and the appearance of the aromatic C(4)–C(6) as the side chain of the antibiotic, and (d) unequivocally establish the structure and stereochemistry (**2**) for the antibiotic.

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[8] See for instance H. J. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect', Academic Press, New York, 1971.