Biosynthesis of Terrein, a Metabolite of *Aspergillus terreus* Thom

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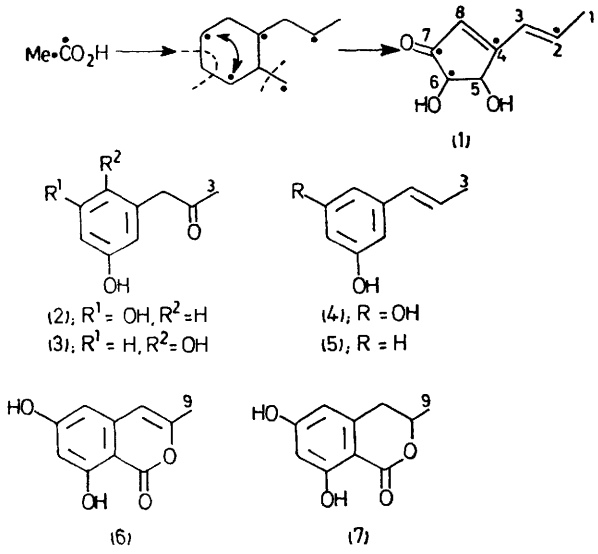
Summary Terrein, a metabolite of *Aspergillus terreus* Thom, is biosynthesised from 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin by contraction of the aromatic ring.

IT has been suggested[1,2] that the biosynthesis of terrein (**1**), a metabolite of *Aspergillus terreus* Thom (IMI 44339), involves contraction of the six-membered ring of a polyketide intermediate (Scheme 1). We have now confirmed this hypothesis by carrying out incorporation experiments with the potential aromatic precursors listed in Table 1. One of them, 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (**7**), was incorporated with high efficiency and specificity. This compound has also been isolated as a co-

metabolite of terrein[3] and so its status as a true intermediate is well supported. Two other compounds, (**2**) and (**3**), were incorporated with comparable efficiency but in



SCHEME 1

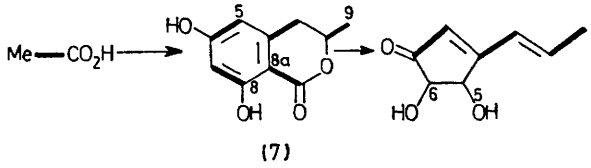
each case the resultant terrein carried only 25% of its total activity at the expected position. This result is consistent with degradation of these potential precursors to [2-^{14}C]acetate prior to incorporation. We have also examined the incorporation of [1-^{13}C]acetate and [1,2-$^{13}C_2$]-acetate; the power of this approach for probing skeletal change in polyketide biosynthesis has previously been amply demonstrated.[4] A full assignment of the ^{13}C n.m.r. spectrum of terrein is given in Table 2; the assignments are consistent with reported chemical shift data[5] and have been confirmed by off-resonance experiments.[6]

TABLE 1. Incorporation of intermediates into terrein

	Incorporation/%	% label at C-1[a]
$Me^{14}CO_2H$	0·59	24·1
[3-^{14}C]-1-(3′,5′-dihydroxyphenyl)-propan-2-one (**2**)	0·19	24·4
[3-^{14}C]-1-(2′,5′-dihydroxyphenyl)-propan-2-one (**3**)	0·75	24·5
[3-^{14}C]-1-(3′,5′-dihydroxyphenyl)prop-2-ene (**4**)	<0·01	—
[3-^{14}C]-1-(3′-hydroxyphenyl)prop-2-ene (**5**)	<0·01	—
[9-^{14}C]-6,8-dihydroxy-3-methylisocoumarin (**6**)	0·04	24·3
[9-^{14}C]-3,4-dihydro-6,8-dihydroxy-3-methyl isocoumarin (**7**)	2·27	92·3[b]

[a] By Kuhn-Roth degradation. Acetic acid isolated as *p*-bromophenacyl acetate. [b] Schmidt degradation of the acetic acid showed that all the activity resided in the methyl group.

The ^{13}C n.m.r. spectrum of terrein labelled with [1-^{13}C]-acetate shows enrichment at C(2), C(4), C(6), and C(7). In addition the signals due to C(6) and C(7) shows a ^{13}C–^{13}C coupling of 42 Hz confirming that these two adjacent carbons are derived from C(1) of acetate. This acetate labelling pattern is in agreement with that found by Birch.[1] As a final check terrein labelled from [1-^{14}C]acetate was degraded using periodate;[7] the formic acid produced from C(6) was found to contain 25% of the total activity.



SCHEME 2

In the spectrum of terrein labelled by [1,2-$^{13}C_2$]acetate six of the eight signals showed (in addition to the usual singlet arising from ^{13}C present at natural abundance) a doublet which can be attributed to ^{13}C–^{13}C coupling in doubly labelled molecules. Thus C(1)–C(2), C(3)–C(4), and C(7)–C(8) show intense couplings of 42, 52, and 56 Hz respectively. In contrast, there was no intense ^{13}C–^{13}C coupling associated with the signals from C(5) and C(6), which proves that these two carbons are derived from separate acetate units.

TABLE 2. Carbon chemical shifts and ^{13}C–^{13}C coupling

Carbon	Carbon chemical shifts /p.p.m. (rel. to Me_4Si)	^{13}C-^{13}C coupling in terrein labelled with [1,2-$^{13}C_2$]acetate/Hz
1	18·7	42
2	139·5	42
3	125·1	52
4	168·3	52
5	77·2	[a]
6	81·7	[a]
7	202·6	56
8	125·7	56

[a] Singlet.

We propose the labelling pattern shown in Scheme 2 (where intact acetate units are indicated by a heavy line). From this we conclude that (a) C(5) and C(6) of terrein are derived from C(8a) and C(8) of the intermediate (**7**), (b) that symmetrical intermediates can be ruled out and (c) that it is unlikely that C(5) of (**7**) is hydroxylated in a subsequent step of the pathway.

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