The Biosynthesis of the Sesquiterpenoids, Cyclonerodiol and Cyclonerotriol

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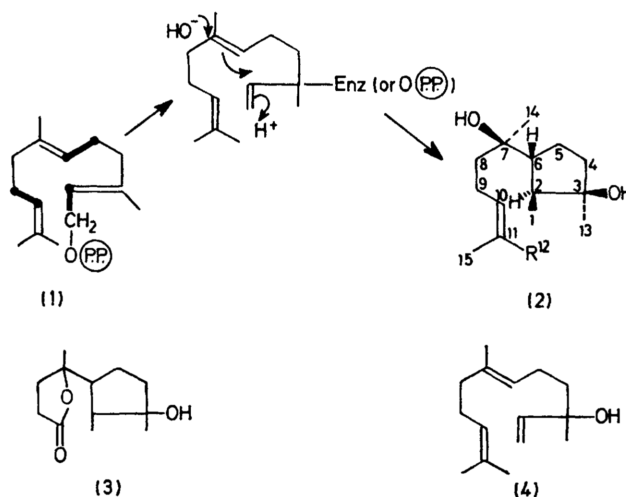
Summary The labelling pattern of cyclonerotriol, biosynthesized from [4,5-$^{13}C_2$]mevalonic acid, has been determined, and the incorporation of [2-$^3H_2$,2-^{14}C]-, [4(*R*)-4-^3H,2-^{14}C]- and [5-$^3H_2$,2-^{14}C]mevalonic acid, farnesyl pyrophosphate and cyclonerodiol are described; nerolidol was not incorporated into these fungal metabolites.

CYCLONEROTRIOL (**2**, R = CH_2OH), a new metabolite of *Fusarium culmorum*,[1] is a member of a group of cyclopentane sesquiterpenoids with a structure similar to the iridoid monoterpenoids. Cyclonerodiol (**2**, R = Me) has also been isolated from *F. culmorum*,[1] *Gibberella fujikuroi*,[2] and *Trichothecium roseum*.[3] We now report on the biosynthesis of these compounds.

[4,5-$^{13}C_2$]Mevalonic acid was synthesized[4] from [1,2-$^{13}C_2$]-acetic acid and fed to *Fusarium culmorum*. In the enriched cyclonerotriol which was formed, the carbon-13 resonances assigned to C(9) and C(10) (23·3 and 127·0 p.p.m., $J_{9,10}$ 44 Hz), C(5) and C(6) (25·2 and 55·5 p.p.m., $J_{5,6}$ 32 Hz) and C(1) and C(2) (15·5 and 45·6 p.p.m., $J_{1,2}$ 37 Hz) were each coupled. The labelling pattern established the manner of folding of farnesyl pyrophosphate (**1**) (Scheme) whilst the retention of three pairs of ^{13}C–^{13}C couplings showed that three isoprene units were incorporated intact. The incorporations of [2-$^3H_2$,2-^{14}C], [4(*R*)-4-^3H,2-^{14}C], and [5-$^3H_2$,2-^{14}C]mevalonic acid into cyclonerodiol and cyclonerotriol by *F. culmorum* and *T. roseum* are shown in the Table.

The atom ratios are those which would be anticipated from this folding of all-*trans*-farnesyl pyrophosphate. However the [2-$^3H_2$,2-^{14}C]mevalonate results, particularly



SCHEME

in the case of the cyclonerodiol obtained from *T. roseum*, revealed the effect of prenyl isomerase in 'washing out' tritium.[5] When the cyclonerotriol (**2**, R = CH_2OH) from the [2-$^3H_2$,2-^{14}C]mevalonate experiment was oxidized to the γ-lactone (**3**) the ^3H : ^{14}C ratio remained at 9·37 : 1 (atom

TABLE. Incorporation of mevalonates into cyclonerodiol and cyclonerotriol

Fungal system	[2-$^3H_2$,2-^{14}C]MVA *T. roseum*	[2-$^3H_2$,2-^{14}C]MVA *F. culmorum*		[4(*R*)-4-^3H,2-^{14}C]MVA *F. culmorum*	[4(*R*)-4-^3H,2-^{14}C]MVA *T. roseum*	[5-$^3H_2$,2-^{14}C]MVA *F. culmorum*
Initial MVA ^3H:^{14}C	14·85:1	10·11:1		6·84:1	5·83:1	12·35:1
Amount ^{14}C fed/μCi	50	54·2		98·5	50	80
Atom ratio	6:3	6:3		3:3	6:3	6:3
Metabolite	diol	diol	triol	triol	diol	triol
^3H:^{14}C ratio	11·75:1	9·39:1	9·44:1	6·82:1	5·94:1	12·37:1
Atom ratio	4·75:3	5·57:3	5·60:3	2·99:3	6·12:3	6·01:3
% Incorporation	3·7	6·4	3·5	6·3	8·1	5·0

ratio 3·70:2) although the specific activity had dropped by a third. Hence the loss of tritium was spread through the molecule and corresponds to the action of prenyl isomerase. Hydroxylation at C(12) could be influenced by an isotope effect leading to a diminished change in the ^3H:^{14}C ratio at this centre. Unfortunately the selective oxidation of C(12) was not successful. All-*trans*-farnesyl pyrophosphate (^3H:^{14}C 14·41:1, 0·83 μCi ^{14}C) (**1**) was prepared enzymatically from [2-$^3H_2$,2-^{14}C]mevalonic acid and fed to *Trichothecium roseum*. The cyclonerodiol (2·7% incorporation) had a ^3H:^{14}C ratio of 14·42:1. Apart from establishing the position of farnesyl pyrophosphate as a precursor, the retention of the ^3H:^{14}C ratio in this case also showed that the loss of tritium in the [2-$^3H_2$,2-^{14}C]mevalonate experiments was due to the action of prenyl isomerase rather than a post-farnesyl pyrophosphate step.

Cyclonerodiol (**2**, R = Me), biosynthetically labelled from [2-^{14}C]mevalonic acid, showed a 15% incorporation into cyclonerotriol in *F. culmorum*. However in three separate experiments (two with *F. culmorum* and one with *T. roseum*), DL-nerolidol (**4**), prepared by the acid-hydrolysis of [^{14}C]-farnesyl pyrophosphate, was not incorporated into cyclonerodiol or cyclonerotriol although these metabolites were produced by the fermentations. Given the relatively high incorporations of the other precursors, this latter result indicates that free nerolidol was not an intermediate in the biosynthesis. This would not exclude nerolidol pyrophosphate or an enzyme bound form of nerolidol acting as the substrate for the cyclization. An alternative possibility involves the direct cyclization of farnesyl pyrophosphate *via* a C(1)–C(3) cyclopropane intermediate.

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