Note

Biosynthesis of 2-(But-1,3-dienyl)-3-hydro-4-(penta-1,3-dienyl)-tetrahydrofuran, a Metabolite of *Chaetomium coarctatum*

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The fungus *Chaetomium coarctatum* produces two metabolites. One is coarctatin¹⁾ (Fig. 1) which has been proved to be derived from four acetic acid molecules with the introduction of three C₁ units.²⁾ The other is 2-(but-1,3-dienyl)-3-hydro-4-(penta-1,3-dienyl)-tetrahydrofuran (I). The structure of (I) determined by Burrows *et al.*³⁾ suggests its biosynthetic origin to be derived either from the condensation of two polyketide chains or a single chain involving intramolecular rearrangements.⁴⁾

In a preliminary communication, we reported the biosynthetic studies of (I) investigated by means of ¹³C- and ²H-NMR spectroscopy and assumed, based on the experimental results, that a straight chain epoxide intermediate comprising seven acetate units would be transformed to (I)

via an epoxide rearrangement⁴⁾ (Fig. 1). In this paper, we wish to report in detail the biosynthetic mechanism of (I) corroborated by an incorporation of ¹⁸O₂ gas.

(a) *Reinvestigation of the incorporation pattern of C²H₃CO₂H by 60 MHz ²H-NMR spectroscopy.* Previously we discussed the incorporation pattern of C²H₃CO₂H into (I) based on its 15 MHz ²H-NMR spectral analysis.⁴⁾ However some problems still remained unclear because of insufficient separation of the important signals; for instance, although the isotope seemed to be retained much more at the C-8 *trans* position than the *cis* position, the exact area of these signals could not be obtained.

In order to solve these problems, we re-analyzed the 60 MHz ²H-NMR spectrum of (I) labeled with C²H₃CO₂H which enabled us to make an accurate measurement of the area of all the signals as shown in Fig. 2.

The degree of deuterium atom retention is summarized in Table I. The signal intensities were normalized to H-13, assuming the incorporation of three deuterium atoms into this position. The lower incorporation of deuterium into H-1 to H-11 (expected value = 1.0) as compared with H-13 may be reasonably explained by the exchange of deuterium by water at the level of malonyl CoA. It is noted that the isotope was incorporated into H-6, H-8 *cis*, H-8 *trans* and H-9 almost to the same degree. The exact area of the H-8 *cis* and *trans* signals could not be determined due to insufficient separation of these resonances. The lower incorporation into H-3 may be due in part to the loss of the isotope during the epoxide rearrangement (Fig. 1), since this position is activated in the hypothetical aldehyde intermediate (*vide infra*). However, we can not at present find any reasonable explanation for the loss of deuterium

FIG. 1. Biosynthetic Pathway of (I) and Coarctatin.

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FIG. 2. The 60 MHz ^2H -NMR Spectrum of (I) Labeled with $\text{C}^2\text{H}_3\text{CO}_2\text{Na}$ Taken in CHCl_3 Solution. CDCl_3 due to the natural abundance peak was used as the internal standard. Spectral width 3 K Hz, and data points 8 K.

TABLE I. THE ^2H -CHEMICAL SHIFTS AND SIGNAL INTENSITIES OF (I) LABELED WITH $\text{C}^2\text{H}_3\text{COOH}$

	Chemical shift ^a	Signal intensity ^b
1	4.14	0.60
3	2.86	0.55
6	6.38	0.80
8 <i>cis</i>	5.16	0.80
8 <i>trans</i>	5.28	0.85
9	5.50	0.80
11	6.08	0.20
13	1.73	3.00

^a In ppm relative to the solvent peak of chloroform (7.30 ppm).

^b Signal intensities were normalized to ^2H -13 assuming the incorporation of three deuterium atoms into this position.

TABLE II. MASS SPECTRA OF (I) UNLABELED AND LABELED WITH $^{18}\text{O}_2$ GAS

	<i>m/z</i>	Relative signal intensity	
		Labeled	Control
M^+	206	100	100
$\text{M}^+ + 1$	207	60	17
$\text{M}^+ + 2$	208	144	4
$\text{M}^+ + 4$	210	71	
$\text{M}^+ - \text{CH}_3$	191	60	26
$(\text{M}^+ + 2) - \text{CH}_3$	193	47	
$(\text{M}^+ + 4) - \text{CH}_3$	195	29	
$(\text{M}^+ + 2) - \text{H}_2\text{O}$ or $(\text{M}^+ + 4) - \text{H}_2^{18}\text{O}$	190	68	
$(\text{M}^+ + 2) - \text{H}_2^{18}\text{O}$ or $\text{M}^+ - \text{H}_2\text{O}$	188	126	43

(b) *Incorporation of $^{18}\text{O}_2$ gas.* Since there was no direct experimental proof with regard to the involvement of epoxide intermediates such as (II) and (III) in Fig. 1, the incorporation of $^{18}\text{O}_2$ gas into (I) was investigated in order to shed light on the formation mechanism of (I). In the mass spectrum of (I) labeled with $^{18}\text{O}_2$ gas (Table II), the strong labeled peaks of $(\text{M} + 2)^+$ and $(\text{M} + 4)^+$ due to incorporation of $^{18}\text{O}_2$ were observed together with the corresponding fragment peaks caused by demethylation or dehydration. Since the two oxygen atoms in (I) proved to be labeled by $^{18}\text{O}_2$, it can be reasonably assumed that an epoxide intermediate such as (II) produced by air oxidation of a polyketide intermediate is transformed into (I) via an epoxide rearrangement accompanying the cleavage between C-4 and C-9. Thus, the biosynthetic pathway of (I) is summarized as shown in Fig. 1.

from H-1.

The area of the H-11 signal is extraordinarily small. This finding may imply that acetoacetyl-CoA, which would form C-10 to C-13 of the skeleton of (I), is considerably longer in its life span than any other intermediates involved in the elongation of the polyketide chain of (I) and, as a result, subjected to extensive loss of deuterium.

These results are in sharp contrast to the biosynthesis of griseofulvin⁵⁾ where the incorporation of deuterium was described to be almost equal.

Although we reported that the deuterium atom was retained more at the H-8 *trans* position than the H-8 *cis* position, it has been proved by the 60 MHz ^2H -NMR spectrum of (I) that the deuterium atom was retained almost equally at these two positions.

EXPERIMENTAL

C. coarctatum was surface-cultured at 27°C in 500 ml Erlenmeyer flasks each containing 50 ml of Raulin-Thom medium supplemented with 1% yeast extract. The ²H-labeled sample of (I) was prepared by additions of C²H₃CO₂Na at the level of 15 mg/50 ml medium at 10, 11 and 12 days after inoculation.

The ¹⁸O-labeled sample of (I) was prepared in two 30 ml flasks with a side chamber containing 10 ml of the medium under a closed aeration condition, where metabolically produced CO₂ was removed by 10-N NaOH placed in the side chamber and 10 ml of ¹⁸O₂ gas were supplemented at 5 and 9 days after inoculation.

After 15 days cultivation, the ²H or ¹⁸O-labeled metabolites were isolated by solvent extraction as previously reported.²⁾

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