UTILIZATION OF $^{13}$C-$^{13}$C COUPLING IN STRUCTURAL AND BIOSYNTHETIC STUDIES. XII

BIOSYNTHESIS OF 2-(BUT-1,3-DIENYL)-3-HYDRO-4-(PENTA-1,3-DIENYL)-TETRAHYDROFURAN, A METABOLITE OF CHAETOMIUM COARCTATUM

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Dedicated to Professor Hamao Umezawa on his 65th birthday

Abstract --- The titled metabolite of Chaetomium coarctatum was proved to derive from seven acetate units. The labeling pattern obtained by the use of $^{13}$C-labeled precursors implies the involvement of epoxide rearrangement to give a tetrahydrofuran ring. Incorporation of $^{2}$H$_{3}$CO$_{2}$Na was also investigated by $^{2}$H-nmr spectrometry.

The fungus Chaetomium coarctatum produces two metabolites, coarctatin$^{2}$ and 2-(but-1,3-dienyl)-3-hydro-4-(penta-1,3-dienyl)-tetrahydrofuran, I$^{3}$. The former compound has been proved to derive from four acetic acid molecules with the introduction of three C$_{4}$ units$^{1}$. In this paper we wish to report the biosynthetic origin of I$^{3}$ revealed by $^{13}$C- and $^{2}$H-spectroscopy.

Although the structure of I was determined by Burrows et al.$^{3}$, severe overlapping of olefinic protons in the 100 MHz $^{1}$H-nmr spectrum (Fig. 1a) prevented to establish the stereochemistry of three double bonds in I. Since the information on the chemical shifts of proton signals was
prerequisite for making the unambiguous assignment of the $^{13}$C-nmr spectrum of I by selective [$^2$H]-$^{13}$C decoupling experiments, we first analyzed the 270 MHz $^1$H-nmr spectrum of I (Fig. 1b). The results show the stereochemistry of the double bonds in question are all trans.

Assignment of the $^{13}$C-nmr spectrum of I (see Table 1 and Fig. 2)

Based on the multiplicity information and chemical shift trend, signals at 17.9 (quartet in the off-resonance decoupled spectrum), 51.2 (doublet), 70.8 (triplet) and 117.8 ppm (triplet) were assigned straightforwardly to C-13, C-3, C-4 and C-8, respectively. Two oxymethines C-1 (84.5 ppm) and C-2 (81.2 ppm), and an olefinic carbon C-9 (127.9 ppm) were distinguishable by selective proton decoupling at H-1 (4.13 ppm), H-2 (3.71 ppm) and H-9 (5.41 ppm). However, due to the close chemical shifts of the remaining olefinic protons in the 100 MHz $^1$H-nmr spectrum, this technique was of limited use for assigning C-5, C-6, C-7, C-10, C-11 and C-12; these could only be separated into the following three groups consisting of C-5 and C-12 (131.2 and 128.9 ppm), C-6 and C-7 (132.9 and 135.8 ppm), and C-10 and C-11 (132.8 and 130.5 ppm) by selective irradiation at 5.70, 6.30 and 6.10 ppm, respectively.

The distinction of these signals within the groups were made as follows. In the $^{13}$C-nmr spectrum of I labeled with $^{13}$CH$_3$,$^{13}$CO$_2$Na (vide infra), five pairs of $^{13}$C-$^{13}$C coupling patterns were observed (Fig. 3) and inter alia the methyl signal (C-13) was coupled to a resonance at 128.9 ppm which, therefore, must be attributed to C-12 ($J_{c-c}=43$ Hz). The magnitude of the $^{13}$C-$^{13}$C coupling constant observed with C-1 ($J_{c-c}=50$ Hz) is consistent with those between sp$^3$ and sp$^2$ carbons, and consequently its counterpart at 131.2 ppm was assigned to C-5. The coupling constant between two sp$^3$
Table 1. $^{13}$C- and $^1$H-chemical shifts and $^{13}$C-$^{13}$C coupling constants of I

<table>
<thead>
<tr>
<th>carbon</th>
<th>$\delta_0$ (ppm)</th>
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<th>$\delta_H$ (ppm)</th>
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<tr>
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<td>36</td>
<td>+</td>
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<td>36</td>
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<td>4</td>
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<td>#</td>
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<td>$\delta_k$</td>
<td>#</td>
</tr>
<tr>
<td>8</td>
<td>117.8 t</td>
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<td>13</td>
<td>17.9 q</td>
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a) multiplicity in the off-resonance decoupled spectrum.
q = quartet, t = triplet and d = doublet.

b) $^{13}$CH$_3$CO$_2$Na, + CH$_3$CO$_2$Na.

carbons at 81.2 and 51.2 ppm ($J_{c-c}$ = 36 Hz) supported the assignments of C-2 and C-3. Apparent AB-type splittings (Fig. 3) assisted to identify two remaining pairs between signals at 132.9 and 135.8 ($J_{c-c}=54$ Hz) and 132.8 and 130.5 ppm ($J_{c-c}=56$ Hz).

Since their coupling constants are characteristic to conjugated diene systems ($=C=)$, these resonances must be ascribed to C-6,7 and C-10,11. Finally, their one to one basis assignments were obtained by the graphical method proposed by Feeney et al. and the results are summarized in Table 1.

Biosynthesis of I

Incorporation of $^{13}$C-labeled precursors

C. coarctatum was surface cultured in 50 ml of Raulin-Thom medium contained in 500 ml Erlenmeyer flasks. $^{13}$C-Labeled samples of I were prepared by separate additions of each ca. 90% $^{13}$C-enriched CH$_3$CO$_2$Na, $^{13}$CH$_3$CO$_2$Na and $^{13}$CH$_3$CO$_2$Na at the level of 15 mg/50 ml medium on 10, 11 and 12 days after inoculation, respectively. The last compound had been diluted to threefold with unlabeled sodium acetate before the addition in order to minimize extraneous couplings due to excess intramolecular labeling. After a further 3 days cultivation, $^{13}$C-labeled metabolites were isolated by solvent extraction as reported previously.

In the $^{13}$C-nmr spectrum of I enriched by CH$_3$CO$_2$Na, the signal intensities of C-2, C-4, C-5, C-7, C-10 and C-12 were increased by ca. fivefold (Fig. 2a), whereas the resonances due to C-1, C-3, C-6, C-8, C-9, C-11 and C-13 were enhanced in the $^{13}$C-nmr spectrum of I labeled with $^{13}$CH$_3$CO$_2$Na (Fig. 2b). It is interesting to note that two adjacent carbons C-3 and C-9 were labeled simultaneously by $^{13}$CH$_3$CO$_2$Na. However, unlike to the cases of polyketides such as aspyrone, sterigmatocystin, and vulgacmycin, no $^{13}$C-$^{13}$C coupling was recognized between the two carbons probably due to the dilution of the labeled precursor by endogenous acetic acid.

In the $^{13}$C-nmr spectrum of I labeled with $^{13}$CH$_3$CO$_2$Na (Fig. 3) were observed five pairs of $^{13}$C-$^{13}$C couplings, C-1,5 ($J_{c-c}=50$ Hz), C-2,3 ($J_{c-c}=36$ Hz), C-6,7 ($J_{c-c}=54$ Hz), C-10,11 ($J_{c-c}=56$ Hz) and C-12,13 ($J_{c-c}=43$ Hz) indicating the intact incorporation of acetic acid molecules into these positions. On the other hand, C-4, C-8 and C-9 did not show $^{13}$C-$^{13}$C couplings except for those caused by the condensation of two $^{13}$CH$_3$CO$_2$H molecules. This labeling pattern can be reasonably inter-
Fig. 2. $^{13}$C-Nmr spectra of I taken in CDCl$_3$ solution at 25.05 MHz. Data points 16 K, spectral width 4 KHz. (a) Labeled by CH$_3^{13}$CO$_2$Na, (b) labeled by CH$_3^{13}$CO$_2$Na and (c) unlabeled control.

Interpretation by assuming that an epoxide intermediate X comprising seven acetate units is transformed to I via epoxide rearrangement which resulted in the cleavage of the C-4 - C-9 bond as shown below. The same mechanism has been suggested for colletotrichins$^{11}$ and aflatoxins$^{12}$.
Fig. 3. The $^{13}$C-nmr spectrum of 1 labeled with $^{13}$CH$_3^{13}$CO$_2$Na. The upper trace is an expansion of the olefinic region. Signals marked with * are couplings caused by the condensation of the labeled acetic acid molecules.

According to this proposal, C-4 and C-9 must be long-range coupled to each other. However, the expected coupling ($J_{c-c}$ = ca. 1 Hz) is too small to be observed due to the overlapping of a strong natural abundance peak. This obstacle has been overcome by $[^{13}\text{C}]^{[^{13}\text{C}}$ homonuclear spin decoupling experiment, in which the irradiation of the C-9 resonance resulted in narrowing of the C-4 signal ($W_{1/2}$ undecoupled: 6.3 Hz, decoupled: 5.5 Hz). Thus, the origin of the two carbons from the same acetic acid molecule has been proved.

Incorporation of $^{2}$H$_3$CO$_2$H

Recently use has been made of $^2$H-nmr spectroscopy for studying stereospecific incorporation or retention of hydrogen atoms in biosynthesis$^{13}$ or biological transformation$^{14}$ of microbial metabolites. In polyketide metabolites labeled by $^{2}$H$_3$CO$_2$H, the isotope was retained on carbons originating from the methyl carbon of acetic acid.

We utilized this method to reveal which one of the two hydrogens on C-8 is enriched by $^{2}$H$_3$CO$_2$H.
A deuterium labeled sample of 1 was prepared by feeding C\textsubscript{2}H\textsubscript{5}CO\textsubscript{2}Na to the fermentation medium in a similar manner as described above. The resulting \textsuperscript{2}H-nmr spectrum (Fig. 4) apparently shows the retention of a deuterium atom at \textit{H-8\textsubscript{trans}} position (5.3 ppm). The other signals observed are as follows: 1.7(H-13), 2.8(H-3), 4.1(H-1), 5.4(H-9), 6.1(H-11) and 6.3 ppm(H-6). Although the separation of \textit{H-8\textsubscript{trans}} and H-9 resonances is not sufficient, the area of deuterium signals seems to increase in the order of H-11, H-9, H-3, H-1, H-6, H-8\textsubscript{trans} and H-13. Except for the terminal methyl, into which three deuterium atoms would be incorporated, this order is consistent with the increasing distance from the starter unit (C-13 and C-12) to the corresponding carbons in a polyketide intermediate such as 5. This result may imply that a deuterium-hydrogen exchange takes place to a certain extent during the elongation of the polyketide chain in \textit{C. coarctatum}; the reaction would occur more frequently at positions closer to the starter unit. This is in sharp contrast to the biosynthesis of griseofulvin\textsuperscript{13}, where almost equal incorporation of deuterium atoms was reported. Further measurement of the \textsuperscript{2}H-nmr spectrum of 1 at a higher magnetic field may be necessary to make a definitive conclusion.

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References


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