Biosynthesis of Cercosporin

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Cercosporin, a deep purple pigment, was first isolated by Kuyama *et al.*[1] in 1957 as a metabolite of *Cercospora kikuchii* [Matsumoto *et* Tomoyasu] Gardner, a pathogenic fungus of purple speck of japanese soybean. The chemical structure was elucidated by Lousberg *et al.*[2] and Yamazaki,[3] one of the present authors, quite independently as shown in **1** of Fig. 1. It was found to be a novel photodynamic pigment which reveals its photosensitizing effect on many organisms, such as mice and microbes, when they were exposed to light in aerobic condition. It was also confirmed to play as a photosensitizer in the photooxygenation reaction of 2,5-dimethylfuran, amino acid residues and other biological substrates.[4]

In the present experiment, we have made clear the course of the biosynthesis of cercosporin, using three kinds of carbon-13 labelled samples which were prepared from mycelia of *Cercospora* incubated in the presence of acetate-1-^{13}C, acetate-2-^{13}C and formate-^{13}C, respectively. The crystals obtained were identified as cercosporin by means of NMR and MS. The isotope peaks in mass spectra proved that the obtained cercosporins prepared from three precursors were equally enriched about 10% with carbon-13, which is in good agreement with the results obtained from carbon-14 experiments previously reported.[3]

TABLE I. ASSIGNMENTS, CHEMICAL SHIFTS AND RELATIVE INTENSITIES OF CARBON-13 IN THE ^{13}C-NMR SPECTRA

No. of Carbon atoms	Chemical shifts[a] (δ ppm)	Relative intensity[b] from Acetate-1-^{13}C	from Acetate-2-^{13}C	from Formate-^{13}C
C-1	23.78 (q)[c]	1.18	9.93*	0.84
C-2	67.59 (d)	9.92*	1.03	0.80
C-3	43.62 (t)	1.27	9.84*	1.00
C-4	137.36 (s)	5.88*	0.65	0.72
C-5	131.72 (s)	0.95	5.70*	1.24
C-6	128.38 (s)	7.28*	1	1.40
C-7	113.09 (s)	1.22	7.78*	1.96
C-8	163.75 (s)	5.59*	0.85	0.94
C-9	109.11 (d)	1.17	7.42*	1.07
C-10	182.26 (s)	6.94*	0.77	0.86
C-11	108.60 (s)	0.89	5.26*	1.43
C-12	167.57 (s)	7.47*	0.83	1.12
C-13	153.32 (s)	0.78	4.53*	0.92
C-14	60.97 (q)	1.00[d]	1.00	8.70*
C-15	93.25 (t)	1.23	0.88	10.60*

* Carbon-13 enriched atoms.

[a] Solvent: pyridine-d_5.

[b] Relative intensities are expressed as ratios in peak height of the corresponding signals of labelled compounds to the original one.

[c] The abbreviations, s, d, t and q indicate singlet, doublet, triplet and quartet, respectively, in the off-resonance spectrum.

[d] The underlined carbons (C-14 and C-3) are settled as 1.00 to calculate the relative intensities of the four spectra.

The spectra of these samples were measured by Fourier Transfer NMR, a JOEL PS-100/PFT-100 Spectrometer. As shown in Table I, only 15 signals are observed in spite of the presence of 29 carbon atoms in the formula. Since cercosporin has a two-fold rotation axis shown by the dotted line in Fig. 1, the diminution in the number of the signals is ascribed to the symmetry of the molecule, *viz.*, the chemical equivalence of the atoms. Therefore, it is sufficient in the following discussions to consider only the carbons numbered from C-1 to C-15 in Fig. 1. The assignments of signals shown in Table I have been established as follows; the signals of carbon attached to protons, C-1, C-2, C-3, C-9, C-14 and C-15 can be easily assigned according to the chemical shifts and splitting patterns of signals in the off-resonance spectrum. Taking the substituent effects on chemical shifts of aromatic compounds into account, the aromatic ring carbons in the molecule are assigned. The signals due to C-8, C-10, C-12, and C-13 are observed downfield as they are attached to oxygen atoms, whereas those due to C-7, C-9 and C-11 which possess the *ortho* position for oxygen-carring carbons are upfield. The enhancement of Nuclear Overhauser Effect gave us a clue to settle the remaining C-4, C-5 and C-6. The assignments were actually done by estimating the distance from each carbon to its nearest neighbouring protons, *i.e.*, the methylene protons attached to C-3. The most enhanced signal is assigned to C-4, while C-6 is seldom expected to be affected by NOE.

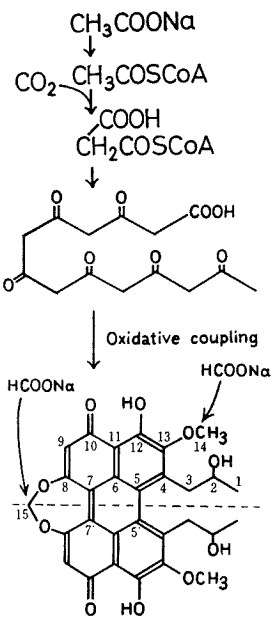

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FIG. 1. Biosynthetic Scheme of Cercosporin.

The ^{13}C-enriched precursors above cited were incorporated efficiently into the molecule, so that the signals of labelled carbon atoms in the spectra can be easily distinguished because of the increase in peak height (marked with asterisks in Table I). Six labelled carbons, C-2, C-4, C-6, C-8, C-10 and C-12 from acetate-1-^{13}C, and seven, C-1, C-3, C-5, C-7, C-9, C-11 and C-13 from acetate-2-^{13}C, are alternately arranged, clearly indicating the formation of the skeleton of the molecule by condensation of acetate units. The relative intensities of the labelled carbons are in the range of 4.5 to 10.6. Whether or not the difference among these values is biosynthetically significant in the incorporation process is now under consideration.

Methoxyl and methylenedioxy groups were not labelled by acetates but formates. The latter composing an unusual seven-membered ring seems to be derived by cyclization in the manner similar to that of *O*-methoxyphenols.[5] Methionine is generally more accepted as the precursor in the transmethylation process. In this case, however, formate was found to be enough to enrich above groups.

Consequently the biosynthetic scheme of cercosporin is shown in Fig. 1. One acetate and six malonates are polymerized *via* the polyketide route to form a polyketomethylene chain followed by decarboxylation, hydroxylation, *O*-methylation including the formation of methylenedioxy bridge, and dimerization through the oxydative coupling[6] of two identical units. The formation of cercosporin from one long polymer was excluded by the fact that all of C-5, C-5′, C-7 and C-7′ combining two naphthalene moieties were originated from methyl carbons of acetates. The isolation of

many mould naphthoquinones[7] originated acetate-polymalonate pathway further supports the sequence of the condensation.

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