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BIOSYNTHESIS OF CITREOTHIOLACTONE, CITREOPYRONE AND PYRENOCINE B

Yoshikazu Shizuri, Seiji Kosemura, and Shosuke Yamamura*
Department of Chemistry, Faculty of Science and Technology, Keio University
Hiyoshi, Yokohama, Japan
Hideyuki Furukawa, Kazuaki Kawai, and Nobuo Okada
Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya, Japan

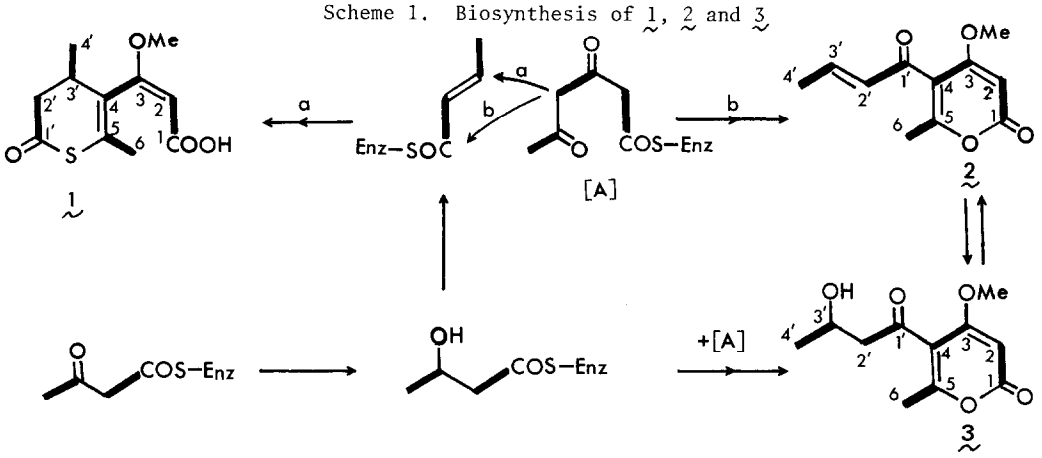
Summary: ^{13}C NMR spectroscopy using INADEQUATE pulse sequence method has been used to deduce the labelling patterns of the three metabolites of Penicillium citreo-viride B. derived from [1,2-^{13}C]acetate, indicating that their carbon skeletons consist of two different units [$CH_3COCH_2COCH_2COS$-Enzyme and CH_3COCH_2COS-Enzyme].

As described in the previous papers, we isolated two novel metabolites, citreothiolactone (1)[1] and citreopyrone (2)[2], from the mycelium of Penicillium citreo-viride B. From a biogenetic point of view, these two metabolites seem to be derived from a common intermediate. Particularly, citreothiolactone (1) is quite interesting because of origin of the sulphur atom constituting a thiolactone moiety. Thus, biosynthetic experiments were carried out under various conditions, as follows.

On addition of cysteine as well as of methionine to the growth medium previously reported, growth rate of P. citreo-viride B. was retarded very much, resulting in the formation of both 1 and 2 in quite low yields. When sodium acetate was added to the growth medium, growth rate of P. citreo-viride B. was slightly retarded and a new result was obtained, in which pyrenocine B (3)[3] previously isolated from the culture filtrate of Pyrenochaeta terrestris was obtained, in addition to the two metabolites (1 and 2). The typical experiment using [1,2-^{13}C]acetate is described.

Polished rice (300 g) in deionized water (800 ml) including [1,2-^{13}C]acetate (1 g) was cooked using an electric rice cooker (99 °C, 17 min), and then transfered into an Erlenmyer flask (3 l), which was pasteurized (120 °C, 20 min at 2 atom), then inoculated with a suspension of mycelium of P. citreo-viride B. (IFO 6200) in a sterilized water and incubated stationarily at 25 °C for 21 days. According to essentially the same procedure as described in the previous paper,[1] the AcOEt extract was separated by a combination of column chromatography (Wakogel C-200; 2% MeOH in $CHCl_3$) and repeated preparative TLC [Kieselgel PF_{254}; hexane - AcOEt (2 : 1 or 4) or $CHCl_3$ - MeOH (12 : 1)] to afford citreothiolactone (1), citreopyrone (2) and pyrenocine B (3) [1 (16 mg); 2 (23 mg); 3 (15 mg)], whose ^{13}C NMR spectra indicated ca.30% enrichment at each carbon atom except for MeO signal. ^{13}C-^{13}C coupled signals due to doubly enriched carbons from [1,2-^{13}C]acetate were observed selectively by means of Freeman's INADEQUATE pulse sequence method.[4] The result is summarized in Table 1, indicating that these three metablites (1, 2 and 3) must be biosynthesized from the common intermediate [A] (see

Scheme 1. Biosynthesis of 1, 2 and 3



Scheme 1), although a possibility is not necessarily ruled out, in which the two pyrones (2 and 3) are derived from the enzyme-bound 3,5,7-trioxooctanethioate and acetyl CoA. The sulphur atom in 1 seems to originate from the enzyme-bound thiocrotonate, although we have not yet obtained any evidence. Further investigation on this point is in progress.

Table 1. ^{13}C NMR data[a] for the incorporation of [1,2-^{13}C]acetate into 1, 2 and 3

Carbon	1 δ (ppm)[b]	J (Hz)	2 δ (ppm)	J (Hz)	3 δ (ppm)	J (Hz)
1	171.4	79.4	162.7	79.4	162.3	79.1
2	94.6	79.4	87.5	79.4	87.8	79.1
3	168.7	67.1	168.3	62.3	168.3	63.0
4	128.6	67.1	113.7	62.3	115.5	63.0
5	156.6	42.7	161.0	52.3	163.8	51.3
6	21.6 (21.7)[c]	42.7	18.2	52.3	18.6	51.3
1'	191.7	42.7	190.0	57.4	201.0	41.0
2'	44.8 (45.4)[c]	42.7	132.7	57.4	52.9	41.0
3'	36.4 (36.5)[c]	35.4	146.4	41.5	64.4	39.6
4'	19.7 (20.0)[c]	35.4	18.4	41.5	22.8	39.6

a) ^{13}C NMR spectra were taken on a JNM-FX 200 NMR spectrometer. b) Relative to TMS in $CDCl_3$. MeO signals in 1, 2 and 3 are observed at δ 56.3, 56.2 and 56.5, respectively. c) These pair of signals are due to two atropisomers.

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