[Chem. Pharm. Bull. 33(10)4296—4298(1985)]

Biosynthesis of Silvaticamide, a Toxin from *Aspergillus silvaticus*

YUKIO MAEBAYASHI and MIKIO YAMAZAKI*

Research Institute for Chemobiodynamics, Chiba University, 8–1, Inohana-1, Chiba 280, Japan

(Received February 27, 1985)

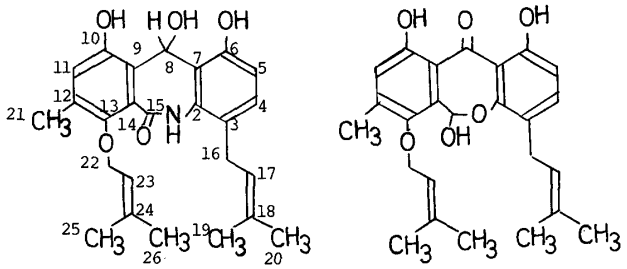
Biosynthetic incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]acetates into silvaticamide (**1**) was investigated in *Aspergillus silvaticus*. The incorporation experiments unambiguously demonstrate that silvaticamide is biosynthesized through the acetate-malonate pathway.

Keywords——biosynthesis; silvaticamide; [1-^{13}C]acetate; [1,2-$^{13}C_2$]acetate; acetate-malonate pathway; *Aspergillus silvaticus*

Introduction

Silvaticamide (**1**) is a toxic metabolite of *Aspergillus silvaticus* IFO 8173 and its chemical structure has been elucidated in our laboratory.[1] The structure of **1** is closely related to those of arugosins A—C from *A. rugulosus*,[2] emericellin from *A. nidulans*,[3] and shamixanthones from *A. variecolor*.[4] In particular, the structure of **1** was very close to that of arugosin B (**2**), except that it contains one nitrogen atom in the molecule (Fig. 1). These fungal metabolites, biogenetically regarded as "seco-anthraquinone," have been reported to be biosynthesized through the acetate-malonate pathway.[5,6] It was therefore thought that **1** may be biosynthesized through the acetate-malonate pathway in spite of the presence of the amide bond.

In this paper we report the results of biosynthetic studies on **1** using [1-^{13}C]- and [1,2-$^{13}C_2$]acetates.



1 2

Fig. 1

Results and Discussion

In the enrichment experiments, [1-^{13}C]acetate (1 g, 93.2% ^{13}C) and [1,2-$^{13}C_2$]acetate (1 g, 90.0% ^{13}C] were added to 7 day-old cultures of *A. silvaticus*. After growth for a further 19 d, the cultures were harvested and the dried mycelia were extracted with acetone. The ^{13}C-labelled **1** was isolated by column chromatography on silica gel and purified by preparative thin layer chromatography (TLC). The carbon-13 nuclear magnetic resonance (^{13}C-NMR)

TABLE I. ^{13}C-NMR Data for Silvaticamide Derived from [1-^{13}C]- and [1,2-$^{13}C_2$]Acetates

Carbon No.	δ_C[a]	^1J (CC)/Hz[b]	Carbon No.	δ_C	^1J (CC)/Hz
2	155.15 s (+)[d]	s	15	172.88 s	s
3	134.54 s	67.9	16	28.86 t (+)	44.7
4	108.78 d (+)	62.3	17	123.95 d	43.6
5	129.76 d	60.2	18	133.33 s (+)	45.6
6	126.49 s (+)	60.2	19	25.98 q	—
7	112.12 s	47.8	20	17.82 q	43.6
8	51.72 d (+)	47.8	21	16.21 q	44.6
9	148.73 s	72.2	22	72.49 t (+)	48.8
10	121.25 s (+)	70.6	23	122.13 d	47.8
11	121.84 d	—[c]	24	138.71 s (+)	41.4
12	133.12 s (+)	41.6	25	25.91 q	—
13	148.93 s	72.2	26	18.12 q	41.6
14	156.24 s (+)	71.6			

a) Relative to internal Me_4Si. *b*) Value [*J* (CC)/Hz] obtained from the spectrum of **1** labelled with [1,2-^{13}C]acetate. *c*) Could not be evaluated because of overlapping. *d*) Enrichment by [1-^{13}C]acetate.

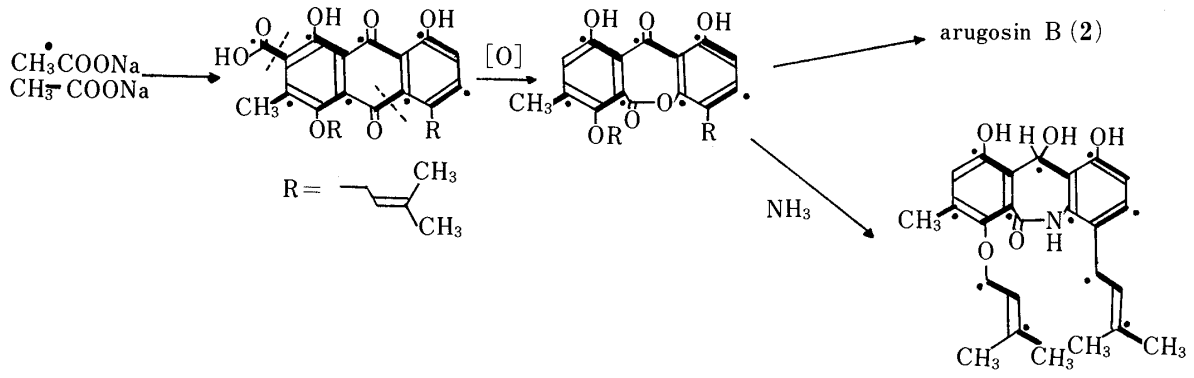


Chart 1. The Biosynthesis of Silvaticamide (**1**)

spectra of samples of **1** derived by incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]acetates showed enrichment of individual atoms and ^{13}C–^{13}C couplings as summarized in Table I. The assignments of the resonances in the ^{13}C-NMR spectra were made on the basis of standard shift data, off-resonance multiplets, and the magnitudes of ^{13}C–^{13}C couplings in **1** labelled with [1,2-$^{13}C_2$]acetate.

The proton-noise-decoupled ^{13}C-NMR spectrum of **1** derived from [1-^{13}C]acetate showed eleven enhanced signals (average enrichment factor 1.32) attributed to C(2), C(4), C(8), C(10), C(12), C(14), C(16), C(18), C(22), and C(24). This result indicated the involvement of eleven acetate units in the formation of **1**. The proton-noise-decoupled ^{13}C-NMR spectrum of **1** obtained by addition of [1,2-$^{13}C_2$]acetate to the culture exhibited the expected spin–spin couplings between carbon atoms derived from intact acetate units. The measured ^1J(CC) values of these couplings are given in Table I and prove the presence of ten intact acetate units arranged as shown in Chart 1: C(3)–C(4), C(5)–C(6), C(7)–C(8), C(9)–C(10), C(21)–C(12), C(13)–C(14), C(26)–C(24), C(23)–C(22), C(20)–C(18), and C(17)–C(16). On the other hand, the resonances corresponding to C(15) and C(2) were clearly singlets. These results indicate that oxidative cleavage of the ring between C(2) and C(15) had occurred.

The above results indicate that silvaticamide (**1**) may be formed through the acetate-malonate pathway *via* the formation of seco-anthraquinone as indicated in Chart 1.

Experimental

The proton-noise-decoupled ^{13}C-NMR spectra were measured with a JEOL GX-270 spectrometer. ^{13}C-Labelled acetates were purchased from Merck Sharp and Dohme Canada Ltd.

Aspergillus silvaticus IFO 8173 was grown in stationary culture at 25 °C in liquid medium of the following composition: glucose 50 g, malt extract (Difco) 20 g, yeast extract (Difco) 20 g, corn steep liquor 20 ml, H_2O 1000 ml, pH 4.7.

Incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]Acetates——The fungus was cultured in Roux's flasks containing 125 ml of the medium. [1-^{13}C]Acetate (1 g, 93.2% ^{13}C) and [1,2-$^{13}C_2$]acetate (1 g, 90% ^{13}C) dissolved in sterilized water were separately added to 1000 ml aliquots of medium containing 7 d culture of the fungus and culture was continued for a further 19 d. The mycelia were separated from the culture liquid by filtration, dried and extracted with acetone. After removal of acetone *in vacuo*, the extracts were chromatographed on silica gel using hexane–acetone as the eluant. Crude **1** was obtained from the fraction eluted with hexane–acetone (1 : 1) by preparative TLC on silica gel and recrystallized from benzene–acetone to give silvaticamide (22.5 mg) labelled with [1-^{13}C]acetate and silvaticamide (29 mg) labelled with [1,2-$^{13}C_2$]acetate.

Acknowledgement The authors are indebted to Dr. Y. Horie of this institute for providing *Aspergillus silvaticus* IFO 8173.

References

1) M. Yamazaki, H. Fujimoto, and Y. Ohta, *Heterocycles*, **15**, 889 (1981).
2) K. K. Chexal, J. S. E. Holker, T. J. Simpson, and K. Young, *J. Chem. Soc., Perkin Trans. 1*, **1975**, 543; J. A. Ballantine, V. Ferrito, C. H. Hassall, and M. L. Jenkins, *J. Chem. Soc., Perkin Trans. 1*, **1973**, 1825.
3) M. Ishida, T. Hamasaki, Y. Hatsuda, K. Fukuyama, T. Tsukihara, and Y. Katsube, *Agric. Biol. Chem.*, **39**, 291 (1975).
4) K. K. Chexal, C. Fouweather, J. S. E. Holker, T. J. Simpson, and K. Young, *J. Chem. Soc., Perkin Trans. 1*, **1974**, 1584.
5) J. S. E. Holker, R. D. Lapper, and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, **1974**, 2135.
6) Bardshiri and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, **1981**, 195.