Mycinamicin Biosynthesis: Intact Incorporation of an Intermediate by a Chain-elongation Process in *Micromonospora griseorubida*

Hideaki Suzuki,[*,a] **Satoshi Takenaka,**[b] **Kenji Kinoshita,**[a] **Yuji Kogami,**[a] **Tatsuro Fujiwara**[a] **and Toshiro Morohoshi**[a]
[a] *Research Laboratories, Toyo Jozo Co., Ltd., Ohito-cho, Shizuoka 410-23, Japan*
[b] *Development Division of Fermentation Technology, Toyo Jozo Co., Ltd., Ohito-cho, Shizuoka 410-23, Japan*

The *N*-acetylcysteamine thioester† of a hypothetical intermediate in the biosynthesis of mycinamicin was incorporated intact into mycinamicin aglycone in cooperation with dotriacolides in *Micromonospora griseorubida*.

Mycinamicin II is a 16-membered macrolide antibiotic composed of a branched lactone and two sugars, desosamine and mycinose. It is produced by *Micromonospora griseorubida*, and has strong antibacterial activity against Gram-positive bacteria.[1] Macrolide compounds, related to mycinamicin II were isolated from the culture filtrate of *M. griseorubida* and characterized by physicochemical methods.[2-5] Bioconversion analysis using a blocked mutant identified these compounds as intermediates in the biosynthesis of mycinamicin, and thus the biosynthetic steps leading from protomycinolide IV to mycinamicin II were delineated.[6] Protomycinolide IV,[4] the first macrolide intermediate in the biosynthesis of mycinamicin II, is assembled from three acetates and five propionates, as identified by incorporation analysis with ^{13}C-labelled precursors.[7]

It is currently believed that the biosynthetic pathway of macrolactone formation resembles that of fatty acids. Cane and Yang[8] and Yeu *et al.*[9] independently demonstrated that ^{13}C-labelled *N*-acetylcysteamine thioesters† (Scheme 1), the activated forms of hypothetical intermediates in the biosynthesis of macrolide antibiotics, were incorporated into the macrolactone rings in their intact forms when they were fed to producing cultures of the relevant microorganism. These results support the hypothesis that, in macrolactone formation, the thioester of a β-keto fatty acid undergoes a series of reduction–dehydration–reduction steps before the next chain elongation reaction. Mycinoic acids, considered to be biosynthetic intermediates in the formation of the macrolactone, have been isolated from *M. griseorubida*,[10,11] and similar compounds have also been obtained from *Streptomyces fradiae*.[12]

Recently, we discovered that *M. griseorubida* produces large amounts of dotrialcolides[13] (Fig. 1), accompanied by mycinamicin production (data not shown). In this communication, we report the intact incorporation of the *N*-acetylcysteamine thioester† of (2*R*,3*R*)-2-[^{13}C]methyl-3-hydroxypentanoate **1**, an elaborated branched-chain fatty acid, into mycinolide IV **3**[4] (Scheme 2), a mycinamicin aglycone, in combination with dotriacolides. Although compound **1** has not been isolated from *M. griseorubida*, the data reported here strongly suggest that the macrolide carbon skeleton is also assembled by a chain-elongation process in *Micromonospora* spp.

The 100 MHz ^{13}C NMR spectroscopic data for the bioconversion experiments are summarized in Table 1. Compound **1** did not ^{13}C-enrich the C-21 of compound **3** to a greater extent than four methyl groups (C-17, C-18, C-19 and C-20) without the addition of dotriacolides, indicating that the precursor had most likely been degraded to [3-^{13}C]propionate followed by

† *S*-(2-Aminoethyl) thioester.

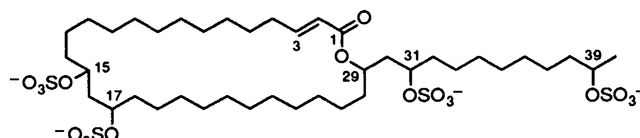
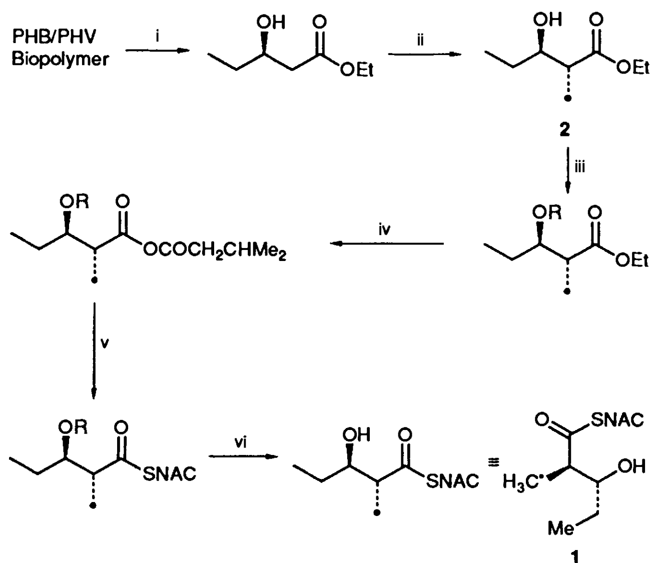
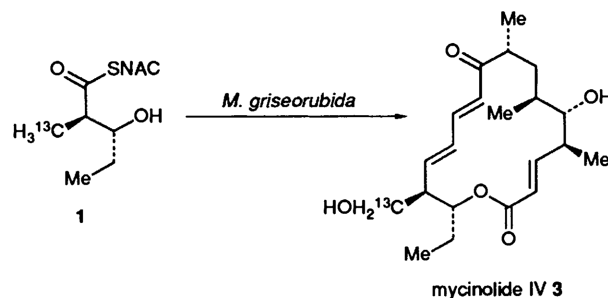


Fig. 1 Structure of dotriacolide



Scheme 1 Synthesis of a *N*-acetylcysteamine thioester of intermediate; • = ^{13}C, R = tetrahydropyranyl, NAC = *N*-acetylcysteamine. *Reagents*: i, H_2SO_4, EtOH; ii, lithium diisopropylamide, tetrahydrofuran, $^{13}CH_3I$; iii, dihydropyran, pyridinium toluene-*p*-sulfonate, CH_2Cl_2; iv, $ClCO_2CH_2CHMe_2$; v, $HSCH_2CH_2NHAc$; vi, CF_3CO_2H, MeOH.



Scheme 2 Intact incorporation of a chain-elongation intermediate into mycinamicin aglycone in *M. griseorubida*; NAC = *N*-acetylcysteamine

Table 1 Effect of dotriacolides on incorporation of **1** into **3** estimated by 100 MHz ^{13}C NMR spectroscopy (in $CDCl_3$)

	Relative peak heights[b]				
Compound added	C-17	C-18	C-19	C-20	C-21
1	3.65	2.99	2.95	3.15	3.18
1 + dotriacolides	2.19	2.10	2.45	2.18	3.11

[a] The values were the mean obtained from three independent experiments. [b] The relative peak heights with ^{13}C-enrichment were calculated as the proportions to natural abundance.

its general incorporation into **3**. However, **1**, after feeding to strain B-120-9 with the addition of dotriacolides, resulted in compound **3** specifically ^{13}C-enriched at C-21, based on the decrease in the height of the signals for C-17, C-18, C-19 and C-20. *M. griseorubida* produces a large amount of dotriacolides under antibiotic fermentation conditions (data not shown). These results suggest that the macrolide carbon skeleton in *M. griseorubida* is assembled in a stepwise manner by a chain-elongation process as shown in *S. fradiae*[9] and *S. erythreus*,[8] and that dotriacolides would play an important role in mycinamicin biosynthesis in *M. griseorubida*. There has not been reported a compound such as the dotriacolides in *Streptomyces*. This suggests that the nature of macrolactone biosynthesis and/or the structure of cell surface between *Micromonospora* spp. and *Streptomyces* spp. might have some differences. Further investigation to grasp the biosynthesis of mycinamicin and to elucidate the role of dotriacolides in *M. griseorubida* are in progress.

Experimental

Synthesis of **1**.—Compound **1** was synthesized as shown in Scheme 1. The microbial polyester containing 70–80% of 3-hydroxybutanoate and 20–30% of 3-hydroxyvalerate (PHB/PHV) was hydrolysed in ethanol with a catalytic amount of sulfuric acid to give monomeric esters.[14] Lithium diisopropylamide (3.0 cm^3 of a 2.0 mol dm^{-3} solution in hexane, 6.0 mmol) was added to tetrahydrofuran (THF) (15 cm^3) and stirred at -75 °C for 10 min. A solution of ethyl (3*R*)-3-hydroxypentanoate (monomeric ester of PHV) (0.73 g, 5.0 mmol) in THF (0.5 cm^3) added dropwisely at -75 °C. After warming the mixture to -20 °C and stirring for 0.5 h, it was cooled again to -75 °C and [^{13}C]-methyl iodide (0.31 cm^3, 5.0 mmol) was added dropwisely. The mixture was stirred at -75 °C for 2 h then at -5 °C for 16 h. The reaction mixture was concentrated *in vacuo* to remove most of THF. The residue was dissolved in ethyl acetate (50 cm^3) and washed with water and brine. The organic layer was dried ($MgSO_4$) and the filtrate was concentrated *in vacuo* after filtration. The residue was purified by silica gel chromatography, eluting with hexane–ethyl acetate (30:1), to give 0.62 g (77%) of ethyl (2*R*,3*R*)-2-[^{13}C]-methyl-3-hydroxypentanoate **2**. The ^{13}C-enrichment level of **2** was more than 95%. Compound **2** was converted into **1** using the mixed anhydride method.[9] Spectral data of **1** including $[\alpha]_D$ were coincident with that of compound obtained during bioconversion experiments in the biosynthesis of tylactone.[9]

Culture for Microbial Conversion of **1**.—*M. griseorubida* B-120-9, a blocked mutant of a high mycinamicin II producing industrial strain, produces **3** singly under antibiotic fermentation conditions. Strain B-120-9 was inoculated into a 150 cm^3 Erlenmeyer flask containing seed medium (soluble starch, 2.0%; yeast extract, 0.5%; NZ Amine type A, 0.5%: tryptose, 0.5%; $CaCO_3$, 0.1%; $FeSO_4 \cdot 7H_2O$, 0.002%; pH 7.2) (20 cm^3), and cultivated on rotary shaker at 28 °C for 48 h. This seed culture was then inoculated (10% by volume) into a 150 cm^3 Erlenmeyer flask containing production medium (glucose, 2.0%; Casitone, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.6%; $CaCo_3$, 0.3%; K_2HPO_4, 0.2%; $FeSO_4 \cdot 7H_2O$, 0.001%; $CoCl_2 \cdot 6H_2O$, 0.0002%; $ZnSO_4 \cdot 7H_2O$, 0.0001%; pH 7.0) (20 cm^3). After 48 h at the same culture conditions, **1** (0.002 g) or **1** (0.002 g) with dotriacolides (0.001 g) were added to the flask and further cultivation was continued for 120 h. ^{13}C-Labelled **3** was extracted from the culture fitrate with ethyl acetate at pH 9.0 and the organic extract was concentrated to afford a crude powder. By repeated crystallization from acetone–hexane, ^{13}C-labelled **3** (0.002 g) was obtained as colourless crystals. Its purity was more than 90% (HPLC).

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