STUDIES ON THE BIOSYNTHESIS OF BASIC 16-MEMBERED MACROLIDE ANTIBIOTICS, PLATENOMYCINS. IV

BIOSYNTHESIS OF PLATENOMYCINS

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To elucidate the biosynthetic pathway of platenomycin (PLM), biosynthetic relationships of platenolides I (PL-I) and II (PL-II), 3-O-propionyl-5-O-mycaminosyl platenolides I (PPL-I-MC) and II (PPL-II-MC), 9-dehydro demycarosyl platenomycin (DDM-PLM), demycarosyl platenomycin (DM-PLM) and 4″-deacyl platenomycin (DA-PLM) were examined with growing cultures or the washed mycelium of blocked mutants of *Streptomyces platensis* subsp. *malvinus* MCRL 0388, a platenomycin-producing organism. As a result, it was revealed that PLM was biosynthesized from PL-I *via* DM-PLM and DA-PLM along the pathways shown in Chart 1. 4″-Isovaleroyl unit of PLM-A_1 and 4″-propionyl unit of PLM-B_1 were respectively derived from L-leucine and L-isoleucine.

In the earlier parts of this series[1,2,3], it was proposed that platenolides I (PL-I) and II (PL-II) produced by blocked mutants of group B and 3-O-propionyl-5-O-mycaminosyl platenolides I (PPL-I-MC*) and II (PPL-II-MC*), 9-dehydro demycarosyl platenomycin (DDM-PLM) and demycarosyl platenomycin (DM-PLM) produced by blocked mutants of group A would be the biosynthetic intermediates of platenomycin (PLM). To clarify the biosynthetic relationships of these compounds, bioconversion of these compounds was examined using growing cultures or washed mycelia of blocked mutants of *Streptomyces platensis* subsp. *malvinus* MCRL 0388 which were classified into 8 groups (A to G and doubtful groups) according to their complementation pattern[1]. The supplemented fermentation of the mutants of groups C, D, E and F indicated that PLM was microbiologically produced from every compound, though the productivity varied with the strain tested. The bioconversion by the washed mycelium of the mutants of groups A, B, C, E and F indicated that among six compounds PL-I was the starting intermediate and DM-PLM was the final one in PLM biosynthesis. Thus, the following three biosythetic routes were established from PL-I to DM-PLM; 1) PL-I→PPL-I-MC→PPL-II-MC→DM-PLM, 2) PL-I→PPL-I-MC→DDM-PLM→DM-PLM, 3) PL-I→PL-II→PPL-II-MC→DM-PLM.

In the above experiments, accumulation of 4″-deacyl platenomycin (DA-PLM) was not recognized, but conversion of DM-PLM to DA-PLM was shown with strain UN-1 of the doubtful group which possessed low bioconversion ability from DA-PLM into PLM. In the bioconversion of DA-PLM to PLM, the precursor for 4″-acyl unit of PLM-A_1 (isovarelic acid) and PLM-B_1 (propionic acid) was found to be L-leucine and L-isoleucine, respectively. Thus, the biosynthetic map from PL-I to PLM was drawn as summarized in Chart 1. Experimental details are dealt with in this paper.

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* PPL-I-MC and PPL-II-MC were communicated before as PL-I-MC and PL-II-MC, respectively[4].

Materials and Methods

Microorganisms

In this study previously described blocked mutants were used which were derived from the strain MCRL 0388 by N-methyl-N′-nitro-N-nitrosoguanidine (NTG) and/or ultra-violet mutation[1]. These mutants were completely blocked at some step(s) on the PLM-biosynthetic pathway and unable to synthesize PLM by themselves alone. They were classified into eight groups (A to G and doubtful groups) based on their blocked step.

Culture media

SC medium was used for preparation of seed culture of which composition was described previously[1]. WM medium for preparation of an intact washed mycelium was composed of 20 g of maltrup (syrup containing 50~60 % maltose, Hayashibara Co., Ltd.), 4 g of sodium chloride and 1,000 ml of soy been meal extract prepared as below, pH being adjusted to 6.5 before autoclaving. Soy bean meal extract was prepared by boiling 40 g of soy bean meal and 3 g of corn gluten (Nihon Shokuhin Co., Ltd.) in 1,000 ml of water at 110°C for 20 minutes in an autoclave, and, after centrifugation (5,000 r.p.m.) for 15 minutes, adding water to the resulting aqueous exract up to 1,000 ml. P medium described previously[2] was used in fermentation experiments with growing culture of blocked mutants.

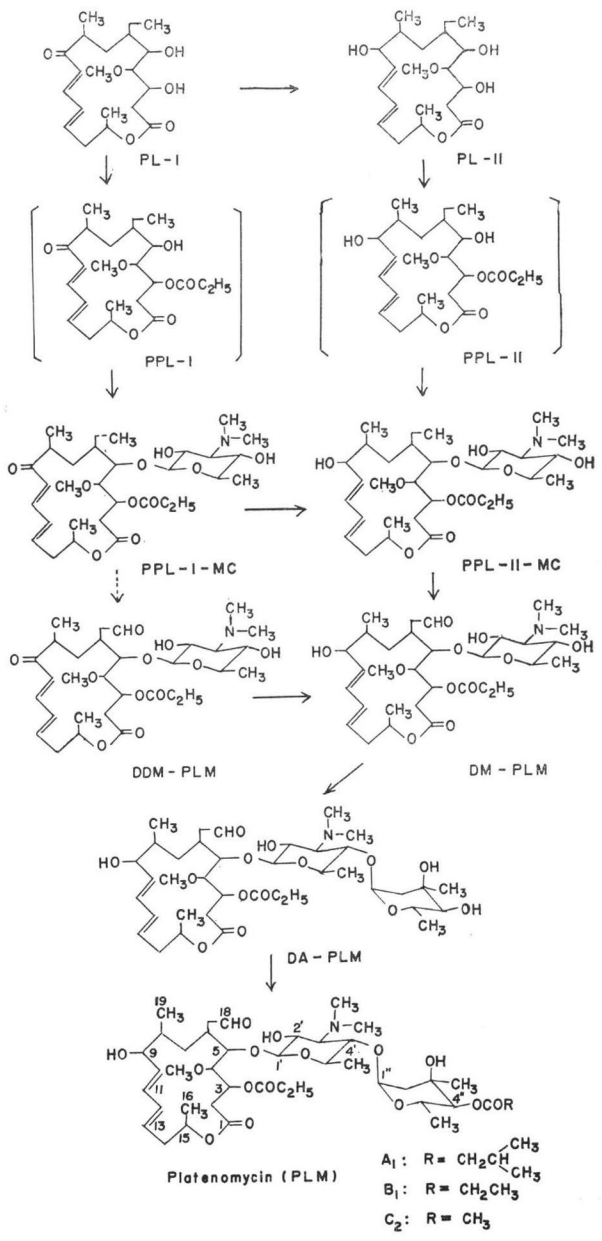
Test compounds

PL-I and PL-II produced by the strain U-92[3], and PPL-I-MC, PPL-II-MC, DDM-PLM and DM-PLM produced by the strain N-90[2] were used in the present experiment. DA-PLM used here was isolated from the cultured broth of the mutant strain MCRL 0769 which was derived from the strain MCRL 0388 by NTG treatment. Formation of DA-PLM by this strain was due to hydrolysis of PLM at the 4″-acyl position by the esterase produced during fermentation[5]. DA-PLM was identical with 4″-depropionyl SF-837 obtained by microbiological hydrolysis of antibiotic SF-837 with *Mucor spinescens*[6].

Preparation of and incubation with washed mycelium

Two ml of vegetative inoculum of

Chart 1. Scheme for PLM biosynthesis.



the test strain harvested in SC medium were transferred into 100 ml of the WM medium prepared in 500-ml SAKAGUCHI flask and incubated for 3 days at 27°C on a reciprocal shaker. Then, the mycelium was collected by centrifugation (4,000 r.p.m., 4°C, 15 minutes) and washed twice with sterile physiological saline solution. The compound as a substrate in the conversion test (200~300 μM) was incubated with the above washed mycelium (cell concentration: 0.4 g wet weight/ml) in sterilized 0.25 % glucose-physiological saline solution at 28°C for 5 hours.

Assay and detection of antibiotic substance(s)

The production of antibiotic substance(s) (PLM) in the supplemented fermentation broth was assayed by an usual cup-plate method on Penassay agar (Kyoei Pharmaceutical Co., Ltd.), using *Sarcina lutea* PCI 1001 as a test organism and crystalline PLM-A_1 (base) as a standard. The antibiotic activity was indicated as PLM-A_1.

The following thin-layer chromatography (TLC) procedure was convinient for demonstrating the derivatives of PLM produced in the broth. Fifty ml of centrifuged broth were extracted twice with 25 ml of ethyl acetate at pH 8.0. The extract was concentrated *in vacuo*. The products in the concentrate were monitered by TLC on an alumina - kieselguhr (6 : 1) plate, using a solvent system of benzene - acetone (7 : 3) mixture. The detection was made by spraying 40 % sulfuric acid followed by heating (Rf-values[7]: PLM-A_1 0.72, PLM-B_1 0.58, PLM-C_2 0.43). Bioautography, if run in pallarel with chemical coloration, would be of use in differentiating antibiotic substance(s) from other inactive products.

Detection of PLM and other bioconverted products

After incubation with washed mycelium, the mycelium was separated by a centrifuge. Then, 3 ml of the supernatant solution were extracted with 4 ml of ethyl acetate at pH 8.0, and 2 ml of extract were concentrated, and then redissolved in 0.1 ml of ethyl acetate, of which 4 μl were spoted with Microcaps (Drummond Scientific Co.) on a thin-layer plate. Before use, a plate was vertically divided into lanes of 1-cm width with a needle so as to avoid any sideways movement of the spot during development. For TLC, alumina - kieselguhr (6 : 1)/benzene - acetone (6 : 4) system and/or silica gel GF/chloroform - methanol - 7 % ammonia water (40 : 12 : 20) system was applied. PL-I and PLM-A_1 were simultaneously spoted on a plate as a standard material. Detection and quantitative determination of the bioconvertants were carried out by surveying a plate with Ultraviolet Spectrophotometer (Hitachi MPF-2A) at 232 and 280 nm. Rf values of the convertants are shown in Table 1. Mass spectrometoric analysis was used for identification of the bioconvertants.

Table 1. Rf values of platenomycins and other biosynthetically related compounds on TLC.

Compound	Rf values	
	I	II
PLM-A_1	0.68	0.86
PLM-B_1	0.65	0.84
PLM-C_2	0.40	0.80
DA-PLM	0.19	0.67
PPL-I-MC	0.27	0.65
PPL-II-MC	0.30	0.50
DDM-PLM	0.20	0.35
DM-PLM	0.12	0.20
PL-I	0.34	0.77
PL-II	0.17	0.59

I: Alumina - Kieselguhr (6 : 1), Benzene - acetone (6 : 4).
II: Silica gel GF, Chloroform - methanol - 7% ammonia water (40 : 12 : 20).

Results and Discussion

Conversion of PL-I, PL-II, PPL-I-MC, PPL-II-MC, DDM-PLM and DM-PLM into PLM by Growing Culture of Blocked Mutants

In the previous paper[1] dealing with the cosynthesis and cofermentation of platenomycins by pairs of blocked mutants, it was suggested that the products (PPL-I-MC, PPL-II-MC, DDM-PLM and DM-PLM) of mutants of group A which were converted into PLM by mutants of groups C, D, E and F and those (PL-I and PL-II) of group B mutants which were transformed

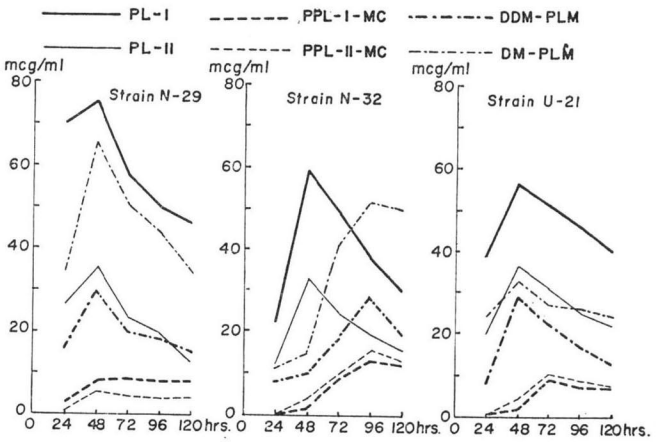
Table 2. Production of PLM in the supplemented fermentation of blocked mutants*.

Test organism		Added substrate						Control
Mutant group	Strain	PL-I	PL-II	PPL-I-MC	PPL-II-MC	DDM-PLM	DM-PLM	
C	N- 1	16.0	5.7	—	—	3.0	7.2	—
	N- 4	18.5	14.0	16.5	13.0	16.0	18.0	—
	N-29	66.5	17.5	15.3	11.5	15.0	36.5	—
	N-32	54.0	20.0	15.0	12.0	25.0	40.0	—
	U-21	48.0	24.0	9.0	8.7	18.0	20.0	—
D	N-9	24.5	10.2	8.0	8.5	21.0	23.8	—
	NU-29	1.0	+	2.0	3.0	11.5	20.0	—
E	N-11	+	2.0	4.0	2.0	6.0	4.0	—
F	N-17	—	—	2.0	+	9.0	8.5	—

* Production (mcg/ml) was determined with PLM-A_1 as standard.
"—" and "+" show no and a little activity, respectively.

into PLM by mutants of groups C, D and E would be intermediates in PLM biosynthesis. As already referred to[2,3], many other minor compounds were simultaneously produced by blocked mutants of group A or B. Therefore, to verify whether or not the above six compounds were real biosynthetic intermediates in the biosynthesis of PLM, supplemented fermentations with these compounds by growing cultures of a mutant of groups C, D, E and F were conducted in P medium. When one of six compounds was added at a level of 100 mcg/ml to 72 hours growing cultures and incubated for an additional 24 hours on a reciprocal shaker, the production of PLM was observed in every case (Table 2). These observations were compatible with the previous cosynthetic results. As blocked mutants of group C, especially strains N-29, N-32 and U-21, showed relatively good production of PLM, conversion of six compounds to PLM with these strains was tested by adding a compounds before inoculation of the P medium with the seed culture. As shown in Fig. 1, the time course of bioconversion of the added compouds to an antibiotic substance (PLM) varied depending upon strains and compounds used. Generally, PL-I and PL-II were rapidly and PPL-I-MC and PPL-II-MC were slowly utilized. TLC and mass analysis showed that the

Fig. 1. Time course of PLM production in the supplemented fermentation of C-group blocked mutants.
Medium: P medium (100 ml/500-ml SAKAGUCHI flask).
Added substrate concentration: 100 mcg/ml.
Incubation condition: 150 c.p.m., 26°C.
Activity was determined as PLM-A_1.



antibiotics produced were mainly PLM-A_1, B_1 and C_2. From the present findings, these six compounds tested are defined as the real intermediates in the biosynthesis of PLM.

Bioconversion by Intact Washed Mycelium of Blocked Mutants

TLC analysis of extracts from the incubation system showed that two platenolides and four platenolide glycosides were generally well utilized, though the yields of bioconverted products were vuite variable depending on the nature and the culture age of the blocked mutant used. The results summarized in Tables 3 and 4 indicate that these precursor compounds are biosynthetically closely related to each other.

(**1**) Bioconversion of PL-I and PL-II

As shown in Table 3, the yield of bioconversion of PL-I and PL-II by washed mycelia ranged from 80 % to 100 %. Mutants of group C gave PLM more abundantly than other groups as in the case of supplemented fermentations. After 5-hour of incubation, mycelia of group C mutants converted PL-I into PL-II (11.2 to 24.1 % of total bioconverted products), PPL-II-MC (8.6 to 19.6 %), DM-PLM (0 to 17.4 %) and PLM (35.2 to 76.2 %), and the starting PL-I had disappeared. They similarly converted PL-II into PPL-II-MC (14.3 to 34.9 %) and PLM (26.8 to 52.8 %), but 13.3 to 58.2 % of starting PL-II was recovered. The previous

Table 3. Bioconversion of PL-I and PL-II by the washed mycelium of blocked mutants.

Mutant		Added sub-strate	Ratio of substrate and bioconverted products (mol. %)									RS	BP
Group	Strain		PL-I	PL-II	PPL-I-MC	PPL-II-MC	DDM-PLM	DM-PLM	PLM-A_1	PLM-B_1	PLM-C_2		
A	N-90	PL-I	14.6	19.8	13.6	41.4		10.6				15.5	106.4
		PL-II		58.9		39.1		2.0				58.5	96.1
	N-33	PL-I	30.8	35.7	5.5	23.5		4.5				35.6	101.5
		PL-II		52.0		45.9		2.1				50.2	96.5
B	N-92*	PL-I	43.1	56.9								37.8	80.3
		PL-II		100.0								97.9	0
C	N-32	PL-I	0	22.6		12.5		17.4	11.0	27.6	8.9	0	81.3
		PL-II		31.7		34.9			9.4	18.3	5.7	28.8	87.0
	U-21	PL-II	0	24.1		19.6		6.9	8.7	19.0	7.5	0	92.3
		PL-II		51.0		14.3			10.1	16.7		58.2	93.4
	N-29	PL-I	0	11.2		8.6			13.3	51.9	15.0	0	87.9
		PL-II		14.9		32.3			8.9	43.9		13.3	87.4
E	N-11	PL-I	77.1	15.0		7.9						67.6	69.9
		PL-II		95.5						4.5		94.1	80.0
F	N-17	PL-I	46.7	53.3								46.0	104.1
		PL-II		100.0								82.8	0

RS: Recovery of substrate (mol. %). BP: Yeild of bioconverted products [mol. %, (Total bioconverted products/(Added substrate - Recovered substrate)) × 100].

Medium: 0.25% glucose-physiological saline solution. Cell concentration: 0.4 g wet weight/ml. Added substration concentration: 200~300 μM. Incubation condition: 28°C, 200 r.p.m., 5 hours.

* The strain U-92 produced PL-I and PL-II even in an absence of a substrate. Therefore, the data shown here was obtained by deducting the amount shown in a blank test.

observation that the strain N-11 of group E is blocked at an earlier step of PLM biosynthesis than the strains of group C and that strains of group C gave far more PLM than that of group E in the above platenolides supplemented fermentation were also supported in the present experiments in which PLM (4.5 %) was produced by group E mutant only when PL-II was used as a substrate, while, when PL-I was supplied, the strain gave no PLM, but gave PL-II (15.0 %). When PL-I or PL-II was incubated with the mycelium of group A mutants, the formation of platenolide glycosides was clearly observed. PL-I was transformed to PL-II (19.8 to 35.7 %), PPL-I-MC (5.5 to 13.6 %), PPL-II-MC (23.5 to 41.4 %) and DM-PLM (4.5 to 10.6 %), while PL-II was converted into PPL-II-MC (39.1 to 45.9 %) and DM-PLM (2.0 to 2.1 %). The washed mycelium of group A mutants did not accumulate any glycoside in the absence of PL-I and PL-II. The strain N-17 of group F could not convert PL-I or PL-II into PLM, but did convert PL-I into PL-II (53.3 %). Thus, it was shown that PL-I and PL-II are real biosynthetic precursors of PLM and that PL-I is a precursor for PL-II. According to UV spectra and mass

Table 4. Bioconversion of PPL-I-MC, PPL-II-MC, DDM-PLM and DM-PLM by the washed mycelium of blocked mutants.

Mutant		Added substrate	Ratio of substrate and bioconverted products (mol. %)									RS	BP
Group	Strain		PL-I	PL-II	PPL-I-MC	PPL-II-MC	DDM-PLM	DM-PLM	PLM-A_1	PLM-B_1	PLM-C_2		
A	N-90	PPL-I-MC			0	100.0						0	105.8
		PPL-II-MC				100.0						104.4	0
		DDM-PLM					31.1	68.9				32.2	105.1
		DM-PLM						100.0				105.0	0
C	N-32	PPL-I-MC			0	88.0		12.0				0	100.0
		PPL-II-MC				72.9		27.1				65.8	71.2
		DDM-PLM					37.0	38.6	5.6	12.5	6.2	37.7	96.5
		DM-PLM						59.2	18.6	20.1	2.1	47.1	61.1
	U-21	PPL-I-MC			0	94.2				5.8		0	86.7
		PPL-II-MC				88.6		5.7				85.6	38.0
		DDM-PLM					25.6	41.4	7.3	19.5	6.2	24.8	95.6
		DM-PLM						86.7	2.9	8.2	2.2	83.9	81.8
	N-29	PPL-I-MC			0	87.6				12.4		0	99.6
		PPL-II-MC				88.8			4.9	6.2		80.6	52.1
		DDM-PLM					24.4	44.9	12.3	15.0	3.4	23.5	95.7
		DM-PLM						72.9	7.1	20.0		68.3	80.0
E	N-11	PPL-I-MC			97.5					2.5		95.9	76.3
		PPL-II-MC				85.6			5.1	7.3	2.0	84.1	89.8
		DDM-PLM					78.6	11.4	4.5	5.5		76.1	80.5
		DM-PLM						81.0	7.1	7.2	4.7	84.1	105.1
F	N-17	PPL-I-MC	23.0	40.6	0	24.6		9.0		2.8		0	105.3
		PPL-II-MC				71.0		29.0				60.9	63.6
		DDM-PLM					87.4	13.6				88.6	111.8
		DM-PLM						77.5	7.2	15.3		75.9	101.7

cf. Footnote of Table 2.

spectrometric analysis, minor products produced by the strain U-92 of group B are suggested to be 3-O-propionyl platenolides I (PPL-I) and II (PPL-II). Therefore, though the bioconversion test leading to these compounds was not conducted with PPL-I and PPL-II (because of the low production of these compounds), propionylation at C-3 hydroxyl of PL-I or PL-II would presumably take place before glycosidation with mycaminose at the C-5 position. Thus, it is proposed that PL-I is converted to PPL-I-MC *via* PPL-I and that PL-II converted from PL-I is transformed to PPL-II-MC *via* PPL-II.

(2) Bioconversion of PPL-I-MC, PPL-II-MC, DDM-PLM and DM-PLM

As shown in Table 4, the strain N-90 of group A rapidly and completely converts PPL-I-MC into PPL-II-MC (100 %). However, no bioconverted product was detected from PPL-II-MC and DM-PLM after 5-hour incubation (PPL-II-MC was converted into DM-PLM (14.7 %) after 24 hours). With the strains of group C, PPL-I-MC was converted into PPL-II-MC (87.6 to 100 %), DM-PLM (0 to 12.0 %) and PLM (0 to 12.4 %). Similarly, PPL-II-MC was converted into DM-PLM (0 to 27 %) and PLM (0 to 11.1 %), DDM-PLM was converted into DM-PLM (38.6 to 44.9 %) and PLM (24.3 to 33.0 %), and DM-PLM was converted only into PLM (13.3 to 40.8 %). In the case of the strain N-11 of group E, PPL-I-MC, PPL-II-MC, DDM-PLM and DM-PLM were all converted into PLM in a low yield. When PPL-I-MC was incubated with the mycelium of the strain N-17 of group F, PL-I and PL-II were detected together with PPL-II-MC, DM-PLM and PLM. Thus, it is suggested that PPL-I-MC is a precursor for PPL-II-MC which is then converted into DM-PLM and the resulting DM-PLM is an intermediate of PLM. However, formation of DDM-PLM was not detected in any case.

Bioconversion of DM-PLM into DA-PLM

From the structural difference between DM-PLM and PLM, two biosynthetic pathways were thought possible. In one, PLM is formed directly by glycosidation of DM-PLM with an acyl

Table 5. Bioconversion of DA-PLM into PLM by the washed mycelium of blocked mutants.

Mutant		Added substrate	Ratio of substrate and bioconverted products (mol. %)					RS	BP
Group	Strain		DA-PLM	PLM-A_1	PLM-B_1	PLM-C_2	UI		
A	N-90	DA-PLM	40.0	15.9	32.5	11.6		30.6	63.8
B	U-92	DA-PLM	0	48.9	28.9	22.2		0	73.3
C	N-29	DA-PLM	0	40.5	51.4	5.6	2.5	0	82.1
	N-32	DA-PLM	0	38.0	47.9	14.1		0	101.7
	U-21	DA-PLM	5.3	32.7	47.4	14.6		5.0	97.3
D	N-9	DA-PLM	17.4	27.9	40.0	22.7	4.3	15.4	80.3
	NU-29	DA-PLM	0	28.7	46.9	24.4		0	98.7
E	N-11	DA-PLM	33.6	15.8	38.2	12.4		30.5	86.7
F	N-17	DA-PLM	10.0	34.3	31.7	17.0	7.0	7.2	83.6
Doubtful	NU-1	DA-PLM	50.0	17.2	24.4	8.4		42.4	73.6

cf. Footnote of Table 2, except below.
UI: Unidentified compounds.
Added substrate concentration: DA-PLM 200 μM.

mycarose donor. Alternately DM-PLM may acquire a mycarose residue to give DA-PLM which is then transformed to PLM by acylation at the C-4″ position. The fact that DA-PLM was not detected in any experiment seemed to argue against the pathway *via* DA-PLM, but this fact might be explained by considering that DA-PLM once formed is converted into PLM so rapidly and completely, that DA-PLM does not accumulate. To clarify this situation, the bioconversion of DA-PLM by blocked mutants was examined. As shown in Table 5, the bioconversion of DA-PLM into PLM was generally very high except the strains N-90 and NU-1. The strain NU-1 of the doubtful group played an important role in defining the final pathway. When DM-PLM was incubated with the washed mycelium of the strain NU-1 in glucose-physiological saline solution, DA-PLM was detected as expected in the solution together with PLM. The yield of DA-PLM was increased when phosphate buffer was used instead of physiological saline solution. Since the deacylation enzyme (esterase) produced by the PLM-producing strain was active at an alkaline pH[5], it was necessary to prove that the strain NU-1 accumulated DA-PLM by glycosidation of DM-PLM and not by enzymatic hydrolysis of 4″-acyl groups of PLM. When DM-PLM and/or PLM-A_1 was incubated with this strain in the buffer solution, PLM-A_1 did not give DA-PLM at all and the starting PLM-A_1 was recovered in a reasonable yield, while DM-PLM, when used alone or in combination with PLM-A_1, gave DA-PLM as shown in Table 6. Thus, the pathway *via* DA-PLM was established.

Table 6. Bioconversion of DM-PLM into DA-PLM by the washed mycelium of the strain NU-1.

Medium	Substrate	Ratio of substrate and bioconverted products (mol %)						RS-D	RS-A	BP
		pH	DM-PLM	DA-PLM	PLM-A_1	PLM-B_1	PLM-C_2			
Saline	DM-PLM	4.5	58.5	6.7	3.2	26.5	5.1	65.8		93.3
PPB, pH 6.0	DM-PLM	5.8	60.6	6.0	3.0	26.0	4.4	61.4		103.2
PPB, pH 7.0	DM-PLM	6.4	55.8	16.3	2.2	23.2	2.5	53.5		98.2
PPB, pH 8.0	DM-PLM	7.1	54.3	19.4	1.7	22.5	2.1	52.4		93.8
Saline	PLM-A_1	4.8			100.0				104.1	0
PPB, pH 6.0	PLM-A_1	5.2			100.0				107.6	0
PPB, pH 7.0	PLM-A_1	6.4			100.0				102.4	0
PPB, pH 8.0	PLM-A_1	7.2			100.0				108.3	0
PPB, pH 6.0	DM-PLM + PLM-A_1	5.2	33.6	3.8	44.3	14.7	3.6	61.5	103.5	100.9
PPB, pH 7.0	DM-PLM + PLM-A_1	6.4	29.0	10.1	44.6	13.9	2.4	53.9	102.1	105.6
PPB, pH 8.0	DM-PLM + PLM-A_1	7.3	28.8	13.1	42.9	13.2	2.0	49.7	105.6	97.4

cf. Footnote of Table 2, except below.
RS-D: Recovery of substrate (DM-PLM, mol. %).
RS-A: Recovery of substrate (PLM-A_1, mol. %).
Medium:
Saline: 0.25 % glucose, 0.25 % L-isoleucine-physiological saline solution.
PPB: 0.25 % glucose, 0.25 % L-isoleucine-0.02 M phosphate buffer solution.
Added substrate concentration: DM-PLM 300 μM, PLM-A_1 250 μM.

Precursors for the 4″-Acyl Unit of PLM-A_1 and B_1

Previously, in an attempt to find a favorable PLM-production medium, it was noticed that PLM-A_1 was produced as the major product when L-leucine was added to the medium, and PLM-B_1 was produced as the major product when L-isoleucine was added. Thus, the effect of adding

Table 7. 4″-Acylation of DA-PLM to PLM by the washed mycelium of the strain U-21.

Added amino acid	Added substrate	Ratio of substrate and bioconverted products (mol. %)				RS	BP
		DA-PLM	PLM-A_1	PLM-B_1	PLM-C_2		
none	DA-PLM	6.7	33.7	47.4	12.2	6.1	91.7
L-Leucine	DA-PLM	7.9	44.8	35.8	11.5	8.1	98.0
L-Isoleucine	DA-PLM	18.2	12.5	56.8	12.5	16.7	90.0

cf. Footnote of Table 2, except below.
Added amino acid concentration: 0.25 %.
Added substrate concentration: DA-PLM 200 μM.

L-leucine or L-isoleucine to the incubation solution of the strain U-21 of group C was examined. As shown in Table 7, the relative production of PLM-A_1 increased in the presence of L-leucine, while that of PLM-B_1 increased in the presence of L-isoleucine. These results suggest that L-leucine is a precursor of isovaleroyl unit in PLM-A_1 and L-isoleucine is a precursor of propionyl unit in PLM-B_1. The former was reported as the precursor of the isovaleroyl unit at 4″-acyl position of carbomycin[8].

The above experimental results lead to the conclusion that PLM is biosynthesized starting from PL-I along the pathways summarized in Chart 1. The pathway from PPL-I-MC to DDM-PLM was expected, but no compounds or no blocked mutant was found which gave DDM-PLM. This step thus must be considered as uncertain.

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