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

PRODUCTION, ISOLATION AND STRUCTURES OF PLATENOLIDES I AND II

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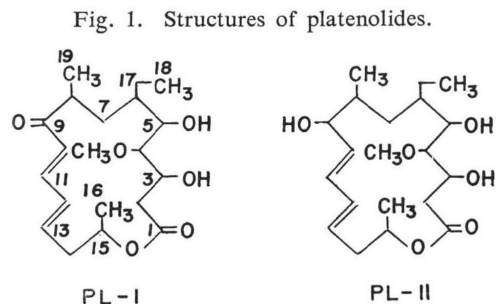
Two neutral macrocyclic lactones designated platenolides I and II have been isolated as the major products from the fermentation broth of the blocked mutants of *Streptomyces platensis* subsp. *malvinus*. These two compounds were isolated by solvent extraction and purified by column chromatography. Both platenolides [PL-I: $C_{20}H_{32}O_6$, PL-II: $C_{20}H_{34}O_6$] are closely related to the platenomycin aglycone.

In the previous paper of this series[1] four glycosidic metabolites produced by the blocked mutants of *Streptomyces platensis* subsp. *malvinus*, group A, derived from the platenomycin-producing strain MCRL 0388 were described. The present report concerns the production, isolation and structure elucidation of two novel macrocyclic lactones produced by blocked mutants of *S. platensis* subsp. *malvinus*, group B. These compounds are named platenolides I (PL-I) and II (PL-II) and their structures are reported as shown in Fig. 1.

Fig. 1. Structures of platenolides.



The biogenetic roles of PL-I and PL-II together with those of the metabolites produced by mutants of group A will be discussed in a succeeding paper.

Materials and Methods

Organisms and media

The mutant strains (N-22 and U-92) used in this study were derived from *Streptomyces platensis* subsp. *malvinus* MCRL 0388 as described previously.[2] These mutants are completely blocked mutants belonging to group B and produce substances PL-I and PL-II dealt with in this paper together with some minor compounds including 3-O-propionyl platenolides I (PPL-I) and II (PPL-II).

Media for maintenance of the strains, for seed culture (SC medium) and for production of metabolites (P medium) used in this study were the same as those described in the preceding papers.[1,2]

Production of platenolides

For the preparation of a vegetative inoculum, mature spores from a maintenance agar medium were inoculated into 500 ml, of Erlenmeyer flasks containing 100 ml of SC medium and cultivated at 28°C for 2 days on a rotary shaker. Then, 150 ml of vegetative inoculum were trans-

ferred to 13 liters of P medium prepared in a 30-liter jar fermentor and submergedly cultured.

Assay of platenolides by thin-layer chromatography (TLC)

Five ml of the fermentation broth were extracted with 5 ml of ethyl acetate. The extract was concentrated *in vacuo* and the dried residue was dissolved in 0.1 ml of ethyl acetate for TLC analysis. Four μl of this solution were spoted on the TLC plate (adsorbent: silica gel - kieserguhr (6 : 1) or silica gel sheets (Woelm)) and developed (solvent system: benzene - acetone, 7 : 3). The amounts of PL-I and PL-II on a TLC plate were determined with a ultraviolet Spectrophotometer (Hitachi MPF-2A) at 232 nm or 280 nm, comparing absorbancy with that of the respective standard sample.

Results and Discussion

Production of Platenolides

In shaken culture the strain U-92 was superior to N-22 in productivity (productivity (mcg/ml): PL-I=62, PL-II=38 by N-22 and PL-I=85, PL-II=65 by U-92), so that the strain U-92 was fermented in a 30-liter jar fermentor and in a 200-liter fermentor. The time course of PL-I and PL-II production are shown in Fig. 2. In a jar fermentor, with the temperature at 26~28°C, PL-I appeared in the culture fluid at about 24 hours and reached a concentration maximum at about 48 hours and then decreased. PL-II appeared at about 40 hours and slowly continued to increase in concentration. In a 200-liter fermentor at 23~25°C, the production of both PL-I and PL-II gradually increased to a maximum. The temperature may affect the production.

Isolation of Platenolides

Platenolides were extracted from the filtered culture broth with an organic solvent and purified chromatographically through a silica gel column followed by a Sephadex LH-20 column as shown in Fig. 3. Thus, from 220 liters of the filtered broth, 12 g of PL-I and 4.6 g of PL-II were obtained which respectively gave a single spot on TLC.

Fig. 2. A typical time course of platenolides production by the blocked mutant (strain U-92).

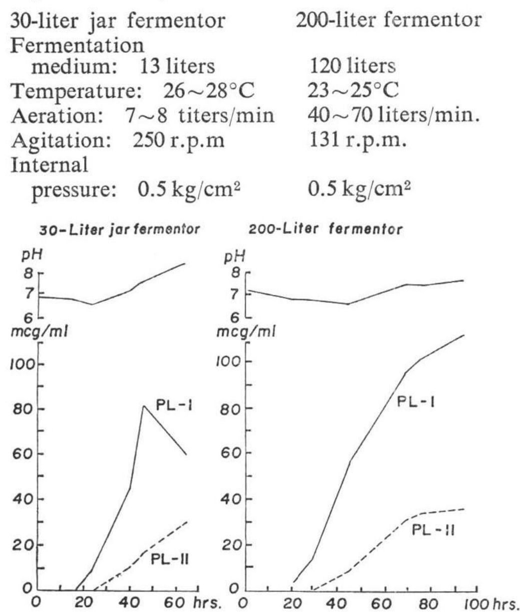


Fig. 3. Isolation and purification of platenolides.

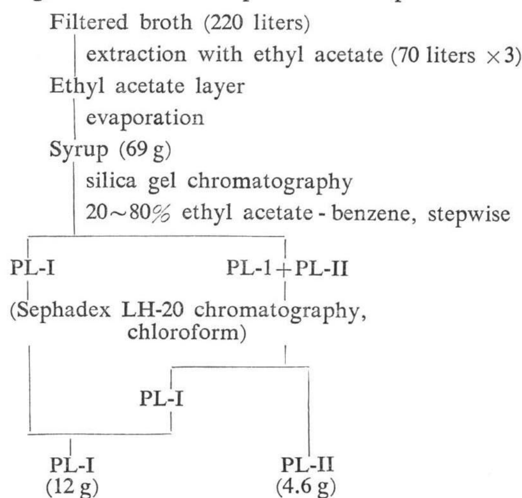
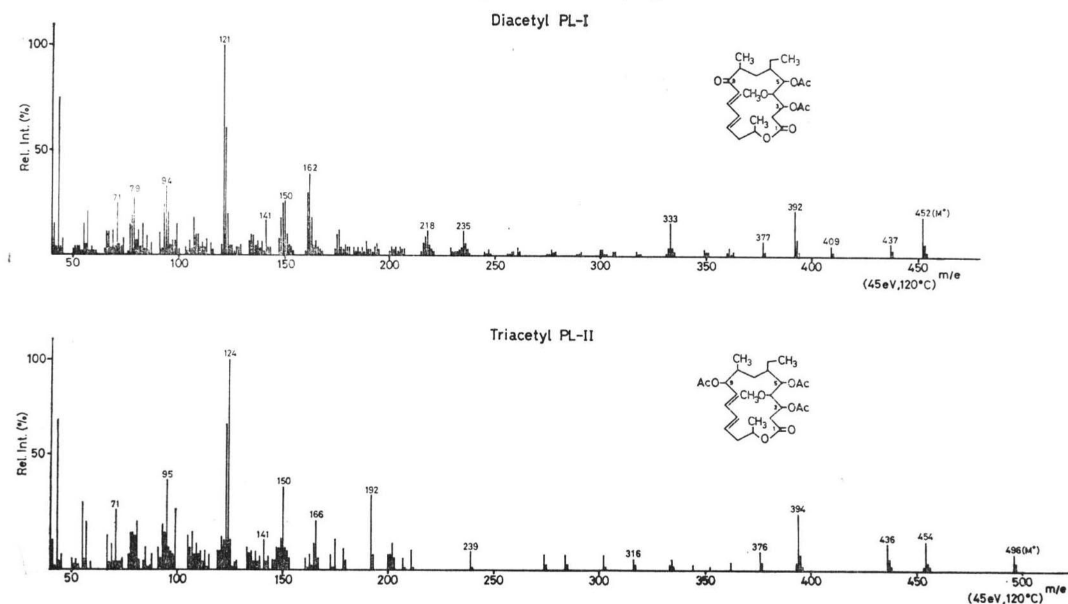


Table 1. Physicochemical properties of platenolides I and II.

Component		PL-I	PL-II
Appearance		colorless needles	colorless prisms
m.p.		140~142°C	65~67°C
Formula		$C_{20}H_{32}O_6$	$C_{20}H_{34}O_6$
Mol. Wt. (MS)		368 (M^+)	370 (M^+)
Elem. Anal. (%)		Obsd. Calcd.	Obsd. Calcd.
		C 65.38 65.21	C 65.10 64.86
		H 8.78 8.69	H 9.27 9.19
UV λ_{max}^{EtOH} (nm) (log ε)		279.5 (4.38)	232 (4.48)
$[\alpha]_D^{25}$ (c 0.4, $CHCl_3$)		+46.2°	+24.8°
IR (nujol) (cm^{-1})		3470, 1725, 1680, 1630, 1590, 1295, 1280, 1250, 1220, 1200, 1180, 1145, 1125, 1115, 1095, 1070, 1040, 1015, 1005, 970, 955, 940, 920, 905, 895, 880, 845.	3450, 1740, 1710, 1660, 1620, 1285, 1210, 1175, 1125, 1100, 1070, 1050, 1010, 990, 960, 900, 875, 865, 835.
Rf values*			
Silica gel GF sheet (Woelm)	II	0.33	0.26
	III	0.54	0.52
Alumina-Kieserguhr (6 : 1)	I	0.58	0.40
	II	0.32	0.20
	III	0.56	0.34
	IV	0.48	0.32

* Solvent system: I: Benzene - acetone (1 : 1), II: Benzene - acetone (7 : 3), III: Ethyl acetate - acetone (8 : 2), IV: Benzene - acetone - water (70 : 29 : 1).

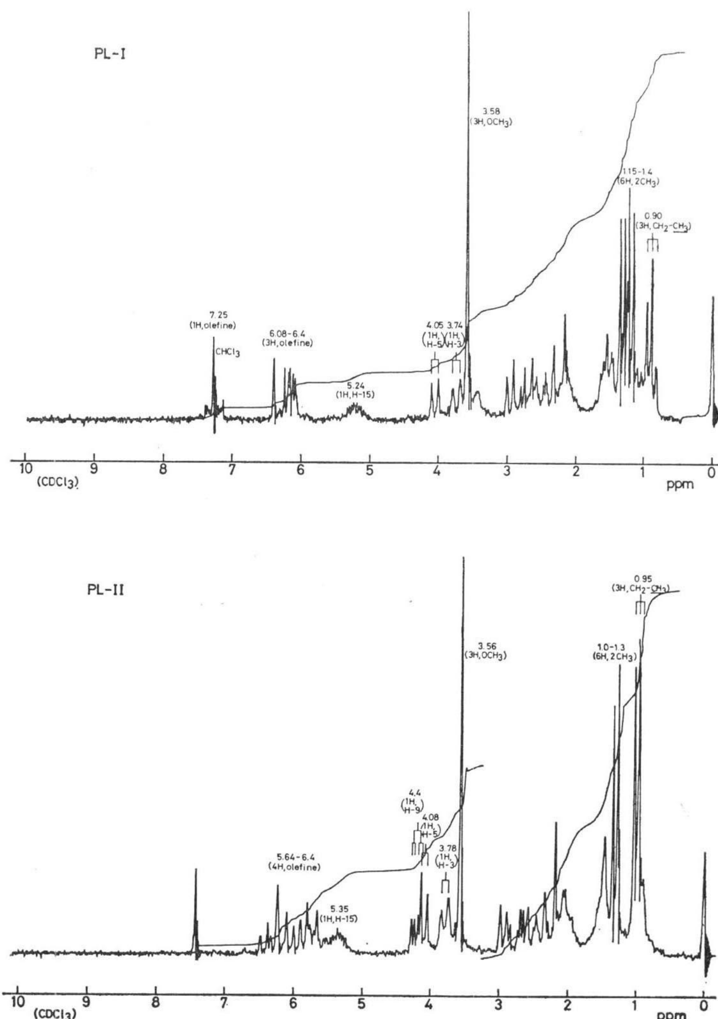
Fig. 4. Mass spectra of acetyl platenolides.



Physicochemical Properties and Structures of Platenolides

PL-I and PL-II obtained as above were recrystallized from benzene - cyclohexane as colorless crystals. The platenolides were visualized on a TLC plate as yellow (PL-I) or brown (PL-II) spots after spraying with 40% sulfuric acid followed by heating. Rf values and some physicochemical properties are shown as Table 1. PL-I and PL-II were neutral in nature and soluble in lower alcohols, lower alkyl acetates, chloroform, acetone and benzene, and almost insoluble in water, petroleum ether, *n*-hexane and cyclohexane. PL-I showed strong IR absorption bands at 3470 (hydroxyl), 1725 (lactone), 1680 (ketone), 1630 and 1590 cm^{-1} (double bond), while PL-II showed IR bands at 3450 (hydroxyl), 1740~1710 (lactone), 1660 and 1620 cm^{-1} (double bond). PL-I gave a diacetate and PL-II gave a triacetate derivatives with an usual acetylation procedure. Neither IR spectrum did showed the presence of a tertiary hydroxyl group. Mass spectra of the acetates are shown as Fig. 4. Abundant peaks were observed at *m/e* 452 (M^+), 392 (M^+-CH_3COOH) and 333 ($M^+-CH_3COOH-CH_3COO\cdot$) with PL-I diacetate and at *m/e* 496 (M^+), 436 (M^+-CH_3COOH), 376 ($M^+-2\times CH_3COOH$) and 316 ($M^+-3\times CH_3COOH$) with PL-II triacetate. The ^{1}H-NMR spectra of PL-I and PL-II measured in $CDCl_3$ are shown as Fig. 5. PL-I showed a triplet methyl signal (δ 0.90) at C-18, two secondary methyl signals (δ 1.15~1.40) at C-16, C-19, an O-methyl (δ 3.58) signal, a doublet due to H-3 (δ 3.74, J=10.5 Hz), a doublet due to H-5 (δ 4.05, J=9.3 Hz), a multiplet signal due to H-15 at δ 5.24, as well as three olefinic due to H-10, H-12, H-13 (δ 6.40~6.80) and an olefinic due to H-11 (δ 7.25) protons. A proton due to H-11 in PL-I diacetate shifted to δ 7.32 (multiplet), separating from the chloroform signal. Similarly, PL-II showed the following reso-

Fig. 5. ^{1}H-NMR spectra of platenolides in $CDCl_3$.



nances; a triplet at δ 0.95 ($-CH_2CH_3$); a multiplet at δ 1.00~1.30 ($2\times C\text{-}CH_3$); a singlet at δ 3.56 ($-OCH_3$); a doublet at δ 3.78 (H-3, J=10.5 Hz); a doublet at δ 4.08 (H-5, J=9.0 Hz); a double doublet at δ 4.40 (H-9, J=9.6, 4.5 Hz); a multiplet at δ 5.35 (1 H, H-15) and a multiplet at δ 5.64~6.50 (4H, olefinic).

The UV maximum at 279.5 nm in PL-I indicated the presence of an $\alpha,\beta,\gamma,\delta$-dienone chromophore as present in DDM-PLM[1] and PPL-I-MC[1] and a maximum at 232 nm in PL-II indicated the presence of an $\alpha,\beta,\gamma,\delta$-diene alcohol chromophore as with DM-PLM[1] and PPL-II-MC.[1] Further, the molecular formula suggested that PL-I is a 9-dehydro derivative of PL-II. This was supported by the IR spectrum of PL-I which exhibited a ketonic band (1680 cm^{-1}) and also by the fact that PL-II was converted into PL-I by manganese dioxide oxidation.

The structure of PL-I was fully supported by a comparison of its NMR and mass spectra with those of DDM-PLM and PPL-I-MC. Instead of the aldehyde signal (δ 9.55) observed in DDM-PLM, PL-I showed a triplet methyl signal at δ 0.90 as in the case of PPL-I-MC (δ 0.74). Thus, similarly to PPL-I-MC, PL-I was concluded to have a methyl residue in place of an aldehyde function on the aglycone skelton of DDM-PLM. Moreover, PL-I did not show the presence of an N-dimethyl signal due to mycaminose or a triplet methyl signal ascribed to a propionyloxy function. In the mass spectrum, diacetyl PL-I gave no fragment ions corresponding to diacetyl mycaminose as shown in diacetyl PPL-I-MC. In addition, the fragment ion at *m/e* 333 which was due to the loss of two acetyloxy functions in diacetyl PL-I was corresponding to the ion (*m/e* 333 ($AGL^+-C_2H_5COOH$)) of the aglycone in diacetyl PPL-I-MC. Based on the above findings, PL-I was defined as a compound which lacks both the mycaminose and propionyl functions of PPL-I-MC. Thus, the structures of PL-I and PL-II were elucidated as depicted in Fig. 1.

Experimental

Isolation of platenolides

After 96 hours of cultivation of the strain U-92, the culture broth was adjusted to pH 6.0 with 1 N HCl and filtered with Celite-545 (1.2 kg). The filtered broth (220 liters) was extracted with ethyl acetate (70 liters ×3). The extract was concentrated *in vacuo* and the resulting syrup (69 g) triturated with benzene (200 ml), the insoluble material removed by filtration, and the extract roughly fractionated into components by column chromatography on silica gel (1 kg) prepared in benzene. Fractions eluted with benzene containing 20~80% ethyl acetate were monitered by TLC. Crude PL-I and PL-II thus obtained were further purified using Sephadex LH-20 columns prepared in chloroform. Eluted fractions giving a single spot of PL-I or PL-II on TLC were combined and evaporated. The resultant PL-I (12.0 g) or PL-II (4.6 g) was recrystallized from benzene - cyclohexane. Thus, pure PL-I (10.9 g) and PL-II (4.3 g) were obtained respectively as colorless crystals.

From the fermentation broth (8 liters) obtained by cultivating another mutant strain (N-22) for 5 days under shaking —the procedures were the same as those applied to the strain U-92— 250 mg of PL-I and 175 mg of PL-II were obtained respectively as colorless crystals. IR, UV and mass spectra of these products showed a good agreement with those produced by the strain U-92.

Oxidation of platenolide II with manganese dioxide

PL-II (50 mg) was oxidized in 3 ml of acetone with 500 mg active manganese dioxide by 3 hours stirring at room temperature. After removing the oxidant, the solvent was evaporated, and the residue was chromatographed over Sephadex LH-20 with chloroform. The fractions

containing the desired product were combined and concentrated to dryness. Recrystallization from benzene - cyclohexane gave colorless needles (31.4 mg), m.p. 140~142°C. This product was identified with PL-I by mixed m.p., UV, MS and TLC.

Diacetyl platenolide I

PL-I (200 mg) was dissolved in 2 ml of dry pyridine, and 2 ml of acetic anhydride was added. The reaction mixture was kept at room temperature overnight and then poured onto cracked ice. The solution was extracted with ethyl acetate (30 ml ×2). The extract was washed with water (30 ml) and dried over anhydrous sodium sulfate, and then evaporated. Recrystallization of the residue from benzene - *n*-hexane gave colorless needles of diacetyl PL-I (120 mg). m.p.: 180~181°C. Anal. Calcd. for $C_{24}H_{36}O_8$: C 63.70, H 8.02. Found: C 63.78, H 8.01. MW: 452 (M^+, *m/e*). UV (EtOH): 279.5 nm (log ε 4.40). IR (nujol): 1755, 1685, 1635, 1600, 1315, 1240 and 1190 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.05 (3 H, s, $C\underline{H}_3CO-$), δ 2.08 (3 H, s, $C\underline{H}_3CO-$).

Triacetyl platenolide II

PL-II (250 mg) was dissolved in 3 ml of dry pyridine, and 3 ml of acetic anhydride was added. Work-up as above gave a yellow oil (290 mg) and addition of *n*-hexane afforded crystalline material. Recrystallization from benzene - *n*-hexane gave colorless needless of triacetyl PL-II (156 mg). m.p.: 157~158°C. Anal. Calcd. for $C_{26}H_{40}O_9$: C 62.90, H 8.06. Found: C 62.97, H 8.05. MW: 496 (M^+, *m/e*). UV (EtOH): 232 nm (log ε 4.48). IR (nujol): 1745, 1730, 1660, 1620, 1305, 1245, 1230 and 1180 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.05 (3 H, s, $C\underline{H}_3CO-$), δ 2.12 (3 H, s, $C\underline{H}_3CO-$), δ 2.15 (3 H, s, CH_3CO-).

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