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Pladienolides, New Substances from Culture of *Streptomyces platensis* Mer-11107

II. Physico-chemical Properties and Structure Elucidation

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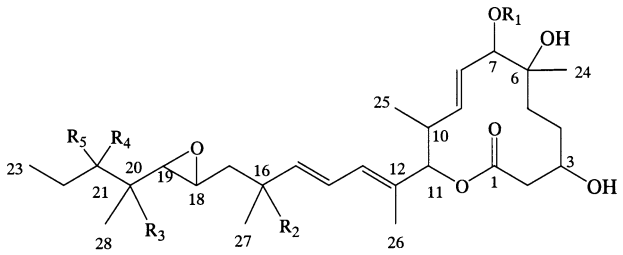
In the course of our screening using fermented broth from soil microorganisms, novel metabolites (pladienolides), possessing inhibitory activity against vascular endothelial growth factor (VEGF) expression and cancer cell proliferation, were isolated from *Streptomyces platensis* Mer-11107. Pladienolides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), and G (**7**) were found to be novel 12-membered macrolides by spectroscopic studies including ^{1}H, ^{13}C NMR, HMQC, HMBC, and NOE experiments. Pladienolides are unusual 12-membered macrolides having a long side chain at the carbon that bears a lactone oxygen.

Natural products are the most consistently successful source of new antitumor drugs, including paclitaxel, podophyllotoxin derivatives, topotecan, vindesine, and adriamycin, which are all now widely used in anticancer therapy[1]. However, a conventional screening method such as testing cytotoxicity to cancer cells is unlikely to lead to the discovery of effective antitumor agents. We therefore aimed to identify a compound that affects a tumor-specific microenvironment, specifically adaptation to hypoxia[2].

During the screening of microbial products for hypoxia signal pathway inhibitors, we found the fermentation broth of *Streptomyces platensis* Mer-11107 to inhibit hypoxia-induced VEGF expression. Subsequent bioassay-guided purification resulted in the isolation of seven new macrolides, which we termed pladienolides. Their taxonomy, fermentation, isolation, and screening are reported in a preceding paper[3].

In this paper, we describe the physico-chemical properties and structural determination of these new antitumor substances (Fig. 1).

Fig. 1. Structure of pladienolides.



	R_1	R_2	R_3	R_4, R_5
pladienolide A (**1**)	H	H	H	H, OH
pladienolide B (**2**)	Ac	H	H	H, OH
pladienolide C (**3**)	Ac	H	H	=O
pladienolide D (**4**)	Ac	OH	H	H, OH
pladienolide E (**5**)	Ac	H	OH	H, OH
pladienolide F (**6**)	H	OH	H	H, OH
pladienolide G (**7**)	H	H	OH	H, OH

Results

Physico-chemical Properties

The physico-chemical properties of **1**～**7** are summarized in Table 1. Pladienolides A～G were soluble in MeOH, acetone, *n*-BuOH, EtOAc, and DMSO, but not in *n*-hexane

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Table 1. Physico-chemical properties of **1**～**7**.

	1	**2**	**3**	**4**	**5**	**6**	**7**
Appearance	Colorless solid	Colorless solid	Colorless solid	Colorless solid	Colorless solid	Colorless solid	Colorless solid
Molecular formula	$C_{28}H_{46}O_7$	$C_{30}H_{48}O_8$	$C_{30}H_{46}O_8$	$C_{30}H_{48}O_9$	$C_{30}H_{48}O_9$	$C_{28}H_{46}O_8$	$C_{28}H_{46}O_8$
Molecular weight	494	536	534	552	552	510	510
HRESI-MS (*m* / *z*)							
Found:	495.3333 $(M+H)^+$	537.3432 $(M+H)^+$	557.3089 $(M+Na)^+$	575.3191 $(M+Na)^+$	575.3176 $(M+Na)^+$	511.3280 $(M+H)^+$	511.3270 $(M+H)^+$
Calcd:	495.3322 (for $C_{28}H_{47}O_7$)	537.3427 (for $C_{30}H_{49}O_8$)	557.3090 (for $C_{30}H_{46}O_8Na$)	575.3196 (for $C_{30}H_{48}O_9Na$)	575.3196 (for $C_{30}H_{48}O_9Na$)	511.3271 (for $C_{28}H_{47}O_8$)	511.3271 (for $C_{28}H_{47}O_8$)
UV (MeOH) λ_{max} nm (ε)	240 (28,800)	240 (31,300)	240 (16,800)	240 (33,100)	240 (26,200)	240 (38,000)	240 (35,800)
IR ν_{max} (KBr) cm^{-1}	3364, 2963, 1732, 1714	3443, 2968, 1732, 1715	3363, 2970, 1732, 1715	3417, 2967, 1732, 1714	3500, 2969, 1732, 1715	3404, 2970, 1731, 1716	3400, 2969, 1730, 1715
$[\alpha]_D^{26}$	-5.2° (*c* 0.3, MeOH)	+5.2° (*c* 1.0, MeOH)	+23.3° (*c* 0.25, MeOH)	-17.7° (*c* 0.2, MeOH)	-2.6° (*c* 0.45, MeOH)	-20.6° (*c* 0.4, MeOH)	-9.8° (*c* 0.16, MeOH)
HPLC (R_t, minutes)*	13.4	15.5	17.3	11.4	12.9	9.0	10.8

* YMC J'sphere ODS M-80 JM-307, 4.6 i.d. x 75 mm; mobile phase: CH_3CN-0.15 % KH_2PO_4 buffer (pH 3.5), 20-80 %, 0-20 minutes; flow rate: 1.0 ml / minutes; detection: UV at 240 nm.

or water. All compounds gave positive color reactions with iodine and H_2SO_4, but were negative to ninhydrin. The UV absorption maxima at 240 nm indicated the existence of a diene system in all compounds. The carbonyl groups were observed in the IR spectra as strong bands (1714～1732 cm^{-1}), while the absorption at 3200～3500 cm^{-1} was attributed to hydroxy groups.

Structure Elucidation

The structures of the seven pladienolides were determined by analysis of ^1H, ^{13}C NMR, MS, IR, and 2D NMR, including HMQC, HMBC, and NOESY spectra. The ^1H and ^{13}C NMR data of pladienolides are summarized in Tables 2 and 3.

Structure of Pladienolide B (**2**)

Pladienolide B (**2**) was isolated as colorless solid. The molecular formula of **2**, $C_{30}H_{48}O_8$ was deduced from the HRESI-MS measurements of its protonated molecular cluster ion (*m*/*z* 537.3432). The IR and UV spectra indicated the presence of hydroxy group (3443 cm^{-1}), carbonyl group (1715, 1732 cm^{-1}), and diene (λ_{max} 240 nm) functionalities in the molecule. The ^1H, ^{13}C NMR, and HMQC spectra (CD_3OD) of **2** revealed the presence of seven methyls, five methylenes, and fourteen methines including five sp^2 and six oxymethines (two of them are derived from an epoxide ring, suggested by their chemical shifts at δ 58.4 and 63.0 respectively), four quaternary carbons including one sp^2, one sp^3 and two carbonyl groups, which account for five of the seven degrees of unsaturation. The remaining two degrees of unsaturation are attributed to two rings. It was also indicated that 45 protons were attached to 26 carbons, while the remaining three protons were those in hydroxy groups. The presence of an acetyl group was inferred from the ^1H signal (observed at δ 2.10), and the ^{13}C signals (observed at δ 172.2 and 21.1). Detailed analyses of the ^1H and ^{13}C NMR spectra with the aid of the ^1H-^1H COSY experiment, coupled with the structural information from the UV and IR spectra, clearly revealed three partial structures (**a**～**c**) consisting of the following fragments: from C-2 to C-5 (**a** unit), from C-7 to C-11 (**b** unit) and from C-13 to C-23 (**c** unit). The connections of these three units and remaining carbonyl (C-1) at δ 171.8 and acetyl carbons at δ 172.2 are suggested by the HMBC correlations (Fig. 2). The methylene (H_2-2) in **a** unit was connected to the ester carbonyl (C-1, δ 171.8), since a long-range correlation between H_2-2 and C-1 was unambiguously observed. The attachment of the remaining methyl group (C-24, δ_C 24.2; δ_H 1.23) to the quaternary carbon (C-6) at δ 74.1 was indicated by the long-range correlations of H_3-24 to C-6 and to C-5 (δ_C 24.2) in **a** unit and to C-7 (δ_C 80.3) in **b** unit and supported by the signal of H_3-24 protons as a sharp

Table 2. ^{1}H NMR data of **1**, **2**, **3**, **4**, **5**, **6**, and **7**.

No.	**1**	**2**	**3**	**4**
2	2.59 (2H, m)	2.56 (2H, m)	2.59 (2H, m)	2.56 (2H, m)
3	3.84 (1H, m)	3.82 (1H, m)	3.82 (1H, m)	3.82 (1H,m)
4	1.42 (1H, m) 1.63 (1H, m)	1.42 (1H, m) 1.62 (1H, m)	1.42 (1H, m) 1.62 (1H, m)	1.42 (1H, m) 1.62 (1H, m)
5	1.42 (1H, m) 1.63 (1H, m)	1.38 (1H, m) 1.65 (1H, m)	1.40 (1H, m) 1.65 (1H, m)	1.38 (1H, m) 1.65 (1H, m)
7	3.77 (1H, d, 9.8)	5.08 (1H, d, 9.8)	5.12 (1H, d, 9.8)	5.10 (1H, d, 9.8)
8	5.78 (1H, dd, 9.8, 15.2)	5.74 (1H, dd, 9.8, 15.2)	5.76 (1H, dd, 9.8, 15.2)	5.74 (1H, dd, 8.3, 15.2)
9	5.45 (1H, dd, 9.8, 15.2)	5.60 (1H, dd, 9.8, 15.2)	5.64 (1H, dd, 9.8, 15.2)	5.60 (1H, dd, 9.8, 15.2)
10	2.64 (1H, m)	2.60 (1H, m)	2.62 (1H, m)	2.62 (1H, m)
11	5.10 (1H, d, 9.8)	5.08 (1H, d, 9.8)	5.12 (1H, d , 9.8)	5.11 (1H, d, 10.8)
13	6.15 (1H, d, 9.8)	6.13 (1H, d, 9.8)	6.18 (1H, d, 9.8)	6.18 (1H, d, 10.8)
14	6.36 (1H, dd, 9.8, 15.2)	6.36 (1H, dd, 9.8, 15.2)	6.40 (1H, dd, 9.8, 15.2)	6.57 (1H, dd, 10.8, 15.2)
15	5.72 (1H, dd, 8.2, 15.2)	5.70 (1H, dd, 8.3, 15.2)	5.72 (1H, dd, 8.3, 15.2)	5.92 (1H, d, 15.2)
16	2.54 (1H, m)	2.52 (1H, m)	2.52 (1H, m)	-
17	1.54 (1H, m) 1.70 (1H, m)	1.48 (1H, m) 1.70 (1H, m)	1.52 (1H, m) 1.72 (1H, m)	1.70 (1H, dd, 6.4, 14.2) 1.90 (1H, dd, 6.4, 14.2)
18	2.80 (1H, dt, 2.4, 5.7)	2.76 (1H, dt, 2.4, 5.7)	2.82 (1H, m)	2.94 (1H, dt, 2.4, 5.7)
19	2.73 (1H, dd, 2.4, 8.3)	2.70 (1H, dd, 2.4, 8.3)	2.80 (1H, m)	2.72 (1H, dd, 2.4, 8.3)
20	1.28 (1H, m)	1.25 (1H, m)	2.38 (1H, m)	1.30 (1H, m)
21	3.58 (1H, dt, 8.3, 4.4)	3.56 (1H, dt, 8.3, 4.4)	-	3.55 (1H, dt, 8.3, 4.4)
22	1.52 (2H, m)	1.52 (2H, m)	2.65 (2H, m)	1.52 (2H, m)
23	1.02 (3H, t, 8.0)	0.98 (3H, t, 8.0)	1.08 (3H, t, 8.0)	0.98 (3H, t, 8.0)
24	1.33 (3H, s)	1.23 (3H, s)	1.25 (3H, s)	1.23 (3H, s)
25	0.97 (3H, d, 7.0)	0.93 (3H, d, 7.0)	0.94 (3H, d, 7.0)	0.93 (3H, d, 7.0)
26	1.82 (3H, s)	1.79 (3H, d, 1.0)	1.81 (3H, s)	1.82 (3H, d, 1.0)
27	1.15 (3H, d, 6.8)	1.12 (3H, d, 6.8)	1.16 (3H, d, 6.8)	1.38 (3H, s)
28	0.98 (3H, d, 6.8)	0.94 (3H, d, 6.8)	1.13 (3H, d, 6.8)	0.95 (3H, d, 6.8)
7-$COCH_3$	-	2.10 (3H, s)	2.12 (3H, s)	2.10 (3H, s)

Table 2. Continued

No.	5	6	7
2	2.59 (2H, m)	2.54 (2H, m)	2.58 (2H, m)
3	3.85 (1H, m)	3.80 (1H, m)	3.82 (1H, m)
4	1.44 (1H, m) 1.66 (1H, m)	1.50-1.70 (2H, m)	1.35 (1H, m) 1.65 (1H, m)
5	1.43 (1H, m) 1.64 (1H, m)	1.50-1.70 (2H, m)	1.35 (1H, m) 1.66 (1H, m)
7	5.12 (1H, d, 9.8)	3.74 (1H, d, 9.8)	3.74 (1H, d, 9.8)
8	5.76 (1H, dd, 9.8, 15.2)	5.78 (1H, dd, 9.8, 15.2)	5.75 (1H, dd, 9.8, 15.2)
9	5.62 (1H, dd, 9.8, 15.2)	5.42 (1H, dd, 9.8, 15.2)	5.60 (1H, dd, 9.8, 15.2)
10	2.63 (1H, m)	2.62 (1H, m)	2.60 (1H, m)
11	5.12 (1H, d, 9.8)	5.10 (1H, d, 9.8)	5.07 (1H, d, 10.2)
13	6.16 (1H, d, 9.8)	6.18 (1H, d, 10.8)	6.12 (1H, d, 10.8)
14	6.38 (1H, dd, 9.8, 15.2)	6.57 (1H, dd, 9.8, 15.2)	6.38 (1H, dd, 10.8, 15.2)
15	5.72 (1H, dd, 8.3, 15.2)	5.91 (1H, d, 15.2)	5.70 (1H, dd, 8.3, 15.2)
16	2.55 (1H, m)	-	2.52 (1H, m)
17	1.60 (1H, m) 1.68 (1H, m)	1.71 (1H, dd, 6.4, 14.2) 1.90 (1H, dd, 6.4, 14.2)	1.55 (1H, m) 1.65 (1H, m)
18	3.02 (1H, dt, 2.4, 5.7)	2.94 (1H, dt, 2.4, 5.7)	3.00 (1H, dt, 2.0, 5.4)
19	2.98 (1H, d, 2.4)	2.70 (1H, dd, 2.4, 8.3)	2.95 (1H, d, 2.0)
20	-	1.32 (1H, m)	-
21	3.36 (1H, m)	3.56 (1H, dt, 8.3, 4.4)	3.35 (1H, m)
22	1.38 (1H, m) 1.76 (1H, m)	1.52 (2H, m)	1.35 (1H, m) 1.76 (1H, m)
23	1.08 (3H, t, 8.0)	0.98 (3H, t, 8.0)	1.06 (3H, t, 7.3)
24	1.25 (3H, s)	1.31 (3H, s)	1.31 (3H, s)
25	0.93 (3H, d, 7.0)	0.94 (3H, d, 7.0)	0.94 (3H, d, 6.4)
26	1.82 (3H, d, 1.0)	1.82 (3H, s)	1.79 (3H, s)
27	1.16 (3H d, 7.0)	1.38 (3H, s)	1.13 (3H, d, 7.0)
28	1.12 (3H, s)	0.95 (3H, d, 6.8)	1.09 (3H, s)
7-$COCH_3$	2.12 (3H, s)	-	-

Data expressed in ppm, with *J* values in Hz.

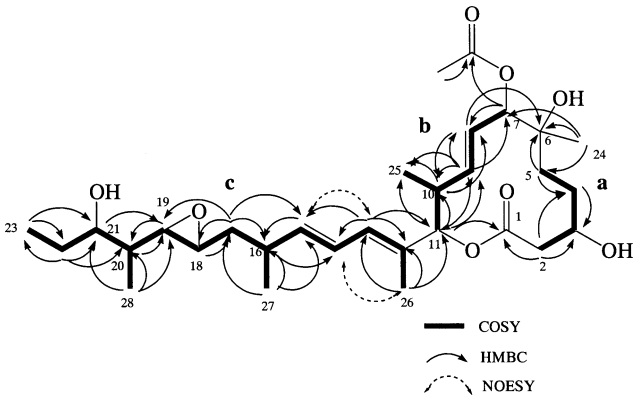
Table 3. ^{13}C NMR Data of **1**, **2**, **3**, **4**, **5**, **6**, and **7**.

No.	1	2	3	4	5	6	7
1	172.0 (s)	171.8 (s)	171.8 (s)	171.8 (s)	171.8 (s)	171.9 (s)	172.0 (s)
2	40.2 (t)	40.1 (t)	40.1 (t)	40.1 (t)	40.1 (t)	40.2 (t)	40.1 (t)
3	70.7 (d)	70.4 (d)	70.4 (d)	70.4 (d)	70.4 (d)	70.7 (d)	70.7 (d)
4	30.5 (t)	30.5 (t)	30.5 (t)	30.5 (t)	30.5 (t)	30.5 (t)	30.5 (t)
5	37.6 (t)	37.5 (t)	37.5 (t)	37.5 (t)	37.5 (t)	37.6 (t)	37.6 (t)
6	74.7 (s)	74.1 (s)	74.1 (s)	74.1 (s)	74.1 (s)	74.7 (s)	74.7 (s)
7	78.2 (d)	80.3 (d)	80.3 (d)	80.3 (d)	80.3 (d)	78.2 (d)	78.2 (d)
8	131.6 (d)	127.1 (d)	127.1 (d)	127.1 (d)	127.1 (d)	131.7 (d)	131.6 (d)
9	137.7 (d)	141.7 (d)	141.7 (d)	141.6 (d)	141.7 (d)	137.7 (d)	137.7 (d)
10	41.8 (d)	41.8 (d)	41.8 (d)	41.8 (d)	41.8 (d)	41.8 (d)	41.8 (d)
11	84.4 (d)	84.3 (d)	84.2 (d)	84.2 (d)	84.3 (d)	84.3 (d)	84.4 (d)
12	132.1 (s)	132.2 (s)	132.1 (s)	131.8 (s)	132.2 (s)	131.7 (s)	132.1 (s)
13	132.6 (d)	132.4 (d)	132.6 (d)	133.8 (d)	132.4 (d)	134.0 (d)	132.6 (d)
14	125.9 (d)	125.8 (d)	126.0 (d)	123.6 (d)	125.9 (d)	123.7 (d)	125.9 (d)
15	142.3 (d)	142.4 (d)	142.2 (d)	143.2 (d)	142.4 (d)	143.2 (d)	142.3 (d)
16	36.7 (d)	36.7 (d)	36.7 (d)	73.1 (s)	36.7 (d)	73.1 (s)	36.7 (d)
17	40.7 (t)	40.7 (t)	40.4 (t)	46.0 (t)	40.4 (t)	46.0 (t)	40.4 (t)
18	58.5 (d)	58.4 (d)	58.4 (d)	56.0 (d)	54.9 (d)	56.0 (d)	54.9 (d)
19	63.0 (d)	63.0 (d)	61.4 (d)	62.5 (d)	63.9 (d)	62.6 (d)	63.9 (d)
20	42.8 (d)	42.8 (d)	50.0 (d)	42.6 (d)	73.0 (s)	42.6 (d)	73.0 (s)
21	75.3 (d)	75.3 (d)	214.7 (s)	75.3 (d)	78.7 (d)	75.3 (d)	78.7 (d)
22	28.6 (t)	28.6 (t)	36.2 (t)	28.6 (t)	24.6 (t)	28.6 (t)	24.6 (t)
23	10.8 (q)*	10.8 (q)*	7.7 (q)	10.8 (q)	11.6 (q)	10.9 (q)	11.5 (q)
24	24.5 (q)	24.2 (q)	24.2 (q)	24.2 (q)	24.2 (q)	24.5 (t)	24.4 (q)
25	17.1 (q)	16.9 (q)	16.9 (q)	16.8 (q)	16.9 (q)	17.0 (q)	17.1 (q)
26	11.9 (q)	11.9 (q)	12.0 (q)	12.0 (q)	11.9 (q)	12.0 (q)	11.9 (q)
27	21.6 (q)	21.6 (q)	21.7 (q)	28.8 (q)	21.6 (q)	28.8 (q)	21.6 (q)
28	10.9 (q)*	10.9 (q)*	13.3 (q)	10.5 (q)	18.7 (q)	10.6 (q)	18.7 (q)
7-$COCH_3$ C=O	-	172.2 (s)	172.2 (s)	172.2 (s)	172.2 (s)	-	-
7-$COCH_3$ CH_3	-	21.1 (q)	21.1 (q)	21.1 (q)	21.1 (q)	-	-

Data expressed in ppm, with J values in Hz, the ^{13}C multiplicity was determined indirectly from HMQC spectra.

* The carbon with similar chemical shifts may be interchanged.

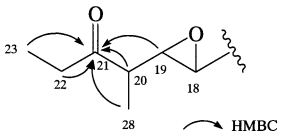
Fig. 2. ^{1}H-^{1}H COSY, HMBC and NOESY data summary for pladienolide B.



singlet. Moreover, C-6 is clearly linked to an oxygen atom based on its chemical shift at δ 74.1; these are indicative of the connection of the **a** and **b** units through the C-6 quaternary oxycarbon. The attachment position of an acetoxy group was inferred from the low-field chemical shift of H-7 (δ 5.08), and its attachment to C-7 was substantiated by the HMBC correlation between H-7 and the acetyl carbonyl. Since the molecule of **2** was inferred, from the degree of unsaturation, to contain one ring other than an epoxide, the C-1 carbonyl had to be linked to the C-11 oxymethine to form a 12-membered lactone ring, which was supported by the low field resonance of C-11 (δ_C 84.3) and H-11 (δ_H 5.08). In the HMBC spectrum, three-bond coupling was observed between H-11 and C-1, which also corroborated the presence of a 12-membered ring. Further, the diene side chain (**c** unit) was connected to the C-11 oxymethine carbon, as evidenced by the long-range correlations of C-11 to olefinic proton (H-13) at δ 6.13 and the methyl proton (H_3-26) at δ 1.79.

The geometry of the olefinic protons (H-8, H-9 and H-14, H-15) was determined to be the (*E*) configuration by the vicinal coupling constants $J_{8,9}$=15.2 Hz and $J_{14,15}$=15.2 Hz, respectively. The high field ^{13}C chemical shift of methyl group (C-26) at 11.9 (q) indicated the (*E*) configuration of the double bond[4] which was supported by NOEs between H-13 and H-15 and between the methyl proton (H_3-26) at δ 1.79 and H-14 (Fig. 2). The epoxide on the side chain was unambiguously assigned as *trans* on the basis of the coupling constant ($J_{18,19}$=2.4 Hz)[5]. Thus, the structure of pladienolide B was elucidated as **2**. The absolute stereostructural elucidation of **2** will be reported elsewhere.

Fig. 3. Key HMBC correlations for **3**.



Structure of Pladienolide C (**3**)

The molecular formula of pladienolide C (**3**) was determined by HRESI-MS to be $C_{30}H_{46}O_8$, giving two fewer protons that of **2**. The ^{1}H and ^{13}C NMR spectral analyses, performed by means of ^{1}H-^{1}H COSY and HMQC experiments, supported the molecular formula. In the ^{13}C NMR spectrum of **3**, the C-21 signal was observed in the low-field region (δ 214.7), unlike that of **2**. Other carbon chemical shifts seen in **3** were in close agreement with those of **2** except for the low-field chemical shifts of C-20 (δ 50.0) and C-22 (δ 36.2) carbons. In the ^{1}H NMR spectrum of **3**, the oxymethine signal corresponding to that of **2** at the C-21 position (δ 3.56) disappeared. These results revealed that the carbon at C-21 of **3** was a keto group. The connectivities through the C-21 carbonyl carbon in **3** were confirmed by the HMBC correlations [C-21/H-19, H-20, H_2-22, H_3-23, H_3-28] as shown in Fig. 3. Thus, the structure of **3** was determined to be a 21-keto analogue of **2**.

Structure of Pladienolide D (**4**)

The molecular formula of pladienolide D (**4**) was determined to be $C_{30}H_{48}O_9$ from the HRESI-MS, which was 16 m.u. (mass unit). larger than that of **2**, suggesting that **4** contained an additional oxygen atom. The ^{13}C NMR and HMQC spectra of **4** revealed that the ^{13}C signals of **4** were identical to those of **2**, except for an additional oxygen bearing quaternary carbon at δ 73.1 and disappearance of a methine carbon (C-16) signal at δ 36.7 in the spectrum of **2**. In the ^{1}H NMR spectrum of **4**, a signal corresponding to a methine proton (H-16) in **2** disappeared. The olefinic proton signal (H-15) at δ 5.92 in the diene moiety was simplified into a doublet; and, further, the signal for H_3-27 protons appeared as a sharp singlet, indicating that C-16 is bearing a hydroxyl group. The expected long-range correlations [C-16/H_2-17, H_3-27 and H-15], in the HMBC spectrum (Fig. 4), were unambiguously observed. From these, the structure of **4** was determined to be a 16-hydroxy analogue of **2**.

Fig. 4. Key HMBC correlations for **4**.

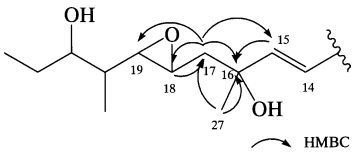
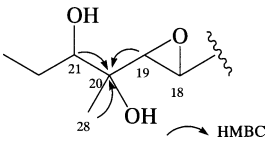


Fig. 5. Key HMBC correlations for **5**.



Structure of Pladienolide E (**5**)

The molecular formula of pladienolide E (**5**) was determined by the HRESI-MS to be $C_{30}H_{48}O_9$, the same as that of **4**, suggesting that **5** also contained an additional oxygen atom to **2**. In the ^{13}C NMR and HMQC spectra of **5**, the C-20 signal (δ 73.1) and C-28 methyl signal (δ 18.7) were shifted to lower field compared with those in **2**, accompanied by a high-field chemical shift of one of the epoxide carbons (C-18). In the ^1H NMR spectrum of **5**, a double doublet signal corresponding to an epoxide proton (H-19) in **2** was simplified into a doublet, and a doublet signal of H_3-28 protons in **2** appeared as a sharp singlet, indicating the C-28 methyl to be attached to a quaternary carbon. These were confirmed by the HMBC correlations [C-20/H-19, H-21 and H_3-28] as shown in Fig. 5. The above observations, with the molecular formula of **5**, allowed its final structure to be determined as a 20-hydroxy analogue of **2**.

Structure of Pladienolides A (**1**), F (**6**) and G (**7**)

HRESI-MS revealed the molecular formula of pladienolide A (**1**) to be $C_{28}H_{46}O_7$, which was 42 m.u. less than that of **2**. The ^1H NMR spectrum of **1** was very similar to that of **2**, but was characterized by the disappearance of the acetyl signal (δ 2.10) in **1** and a high-field chemical shift of the H-7 methine proton (δ 3.77), accompanied by a significant change in the chemical shift of several olefinic protons. The ^{13}C NMR spectrum of **1** was also similar to that of **2** and showed the lack of an acetyl carbon and a high-field shift of the C-7 carbon. Accordingly, the structure of **1** was considered to be a 7-deacetyl analogue of **2**, as shown in Fig. 1. This was finally confirmed by 2D-NMR including ^1H-^1H COSY, HMQC and HMBC analyses.

The molecular formulae of pladienolides F (**6**) and G (**7**) were both found by HRESI-MS to be $C_{28}H_{46}O_8$, which was 42 m.u. less than those of **4** and **5**. The NMR signals of **6** and **7** respectively resembled those of **4** and **5**, except for the significant change in high-field chemical shifts of C-7 (**6**: δ_C 78.2; δ_H 3.74 and **7**: δ_C 78.2; δ_H 3.74). Similar to the case of **1** and **2**, **6** and **7** are 7-deacetyl analogues of **4** and **5**, respectively, as shown in Fig. 1. Finally, the structures of **6** and **7** were confirmed by ^1H-^1H COSY, HMQC and HMBC analyses.

Discussion

Pladienolides were isolated from the fermentation broth of *Streptomyces platensis* Mer-11107 as unique 12-membered macrolides possessing a diene unit and one epoxide moiety. Other nonglycosidic 12-membered macrolides from the Actinomycetes, such as FD-895[6], lactimidomycin[7] and NK30424A, B[8] also contain a long side chain at the carbon that bears a lactone oxygen, and are known to have diverse biological activities. Notably, the planar structure of FD-895, which shows cytocidal activities against several types of cancer cells, is closely related to those of pladienolides. The difference between FD-895 and pladienolide B is that FD-895 has a hydroxy group at the C-17 position and a methoxy group substituted for the hydroxy group at the C-21 position. Although the resemblance of the chemical structures to FD-895, pladienolides are the first 12-membered macrolides reported to inhibit the hypoxia-inducible factor-1 (HIF-1) pathway[9].

Experimental

IR spectra were recorded on a JASCO FT/IR-300E spectrometer. UV spectra were recorded on a Shimadzu UV-2400PC spectrometer. Optical rotations were obtained on a Jasco DIP-1000 Digital Polarimeter. NMR analyses were performed using a Varian Unity INOVA500 spectrometer operating at 30°C. Chemical shifts were referenced to internal standard peaks: δ_H 3.35 for CHD_2OD, δ_C 49.0 for CD_3OD. Mass spectra were

measured on a JMS SX102A spectrometer. High-resolution mass spectra were obtained on a Micromass Q-Tof Ultima Global spectrometer.

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