NEW TRIENE-β-LACTONE ANTIBIOTICS, TRIEDIMYCINS A AND B

Sir:

Two new antibioics, triedimycins A and B closely related to oxazolomycin,[1~3] have found in the culture of *Streptomyces* sp. MJ213-62F4 resembling *Streptomyces melanosporofaciens*. The organism was isolated from a soil sample collected at Nanao City, Ishikawa Prefecture, Japan and deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan under the accession No. FERM P-11768. These antibiotics showed potent *in vitro* antitumor activity against murine leukemia cells, but weak antimicrobial activity against limited bacteria.

The strain was inoculated into a medium (110 ml) containing glycerol 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, yeast extract (Dainippon Pharmaceutical) 0.3%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (pH 7.4) in a 500-ml baffled Erlenmeyer flask and cultured for 48 hours at 28°C on a rotatory shaker (180 rpm). The culture (each 2.2 ml) was transferred into 17 flasks containing the same medium and cultivation was carried out for 72 hours under the same condition described above.

The antibiotics in the whole broth (1,700 ml, pH 6.6) were extracted with EtOAc (1,700 ml). The

Table 1. ^{1}H NMR spectra of triedimycins A and B

Proton	Triedimycin A		Triedimycin B		Oxazolomycin	
	δ	J (Hz)	δ	J (Hz)	δ	J (Hz)
2	2.46 q	7.6	2.45 q	7.6	2.47 q	7.6
2-CH_3	1.20 d	7.6	1.20 d	7.6	1.23 d	7.6
3-OH	4.08 s		3.34 s		3.69 s	
4	3.55 t	4.5	3.69 t	5	3.60 t	4.5
4-OCH_3	3.37 s		3.42 s		3.40 s	
5	1.34 m,		1.37 m,		1.39 m,	
	2.01 ddd	4.5, 7, 16	1.99 ddd	4.4, 7, 15	2.04 ddd	4.5, 5.5, 15
6	1.79 m		1.85 m		1.82 m	
6-CH_3	0.99 d	7	0.98 d	7.2	0.99 d	7.4
7	3.99 t	7	3.96 t	8	3.96 br t	7.4
8	5.69 dd	8, 14	5.68 dd	8, 15	5.68 dd	8, 14
9	~6.2		~6.2		~6.2	
10	~6.2		~6.2		~6.2	
11	5.71 dt	6, 14	5.69 dt	6, 14	5.69 dt	6.2, 14
12	3.91 t (2H)	6	3.91 t (2H)	6	3.92 br t (2H)	6.2
NH	6.49 t	5.8	6.44 t	5.6	6.33 br t	5.6
15	4.80 dd	5, 7	4.86 q	6.4	4.39 d	6.4
					4.72 d	6.4
16	4.01 dd,	7, 11,	1.81 d (3H)	6.4		
	4.19 dd	5, 11				
16-OCH_3	3.45 s					
N-CH_3	2.93 s		2.91 s		2.92 s	
2′-CH_3	1.10 s,		1.09 s,		1.10 s,	
	1.34 s		1.34 s		1.36 s	
3′	4.63 br		4.63 br		4.63 d	6.4
3′-OH	4.54 br		4.49 br		4.38 d	6.4
4′-CH_3	1.79 s		1.79 s		1.79 s	
5′	6.42 d	12	6.42 d	12	6.41 d	11
6′	6.22 br t	12	6.22 br t	12	~6.2	
7′	5.94 br t	12	5.94 br t	12	5.95 br t	11
8′	6.63 dd	12, 15	6.63 dd	12, 15	6.63 dd	11, 15
9′	5.76 dt	7.2, 15	5.76 dt	7, 15	5.78 dt	7.4, 15
10′	3.45 d (2H)	7.2	3.44 d (2H)	7	3.52 d (2H)	7.4
12′	6.62 s		6.62 s		6.80 br s	
13′					7.80 s	
13′-CH_3	2.41 s		2.39 s			

δ: ppm from TMS in $CDCl_3$.

EtOAc extract was dehydrated with anhydrous Na_2SO_4 and concentrated to obtain a brownish syrup (806 mg). Triedimycins A and B (36 and 25 μg/ml, respectively) in the syrup were assayed by reverse-phase HPLC using a Resolve C18 column (Waters, Particle size 5 μm, 3.9×150 mm) and a mixture (9:11) of MeCN and H_2O as a mobile phase. When it was operated at a flow rate 1.0 ml/minute, teiedimycins A and B showed Rt 4.8 and 5.1 minutes, respectively, by detection with UV absorbance at 275 nm.

The syrup (805 mg) was chromatographed on a silica gel column (C-200, 40 g, Wako Pure Chemicals) developed with $CHCl_3$-MeOH (50:1 and then 20:1) to give a crude powder (205 mg, A: 134, B: 126 μg/mg). The crude powder was further purified by silica gel column chromatography (C-200, 20 g) developed with EtOAc-Me_2CO (7:1) to yield a pale yellow powder (59 mg) containing 394 μg/mg of triedimycin A and 376 μg/mg of B. Separation of triedimycins A and B in the powder (44 mg) was accomplished by preparative HPLC on a reverse-phase column (Capcell Pak C18, 20× 250 mm, Shiseido) developed with MeCN-H_2O (4:6) and pure triedimycins A and B (13 mg and 10 mg, respectively) were obtained as white powders.

Triedimycin A ($C_{38}H_{55}N_3O_{10}$): MP 70~80°C (gradually dec); $[\alpha]_D^{21}$ +71° (*c* 1.0, MeOH); FAB-MS *m/z* 714 $(M+H)^+$; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 230 (670), 266 (sh, 559), 276 (668), 285 (sh, 514); IR ν_{max} (KBr) cm^{-1} 3370, 2968, 2932, 1826 (β-lactone), 1693, 1641, 1531, 1454, 1387, 1097, 993, 952, 841.

Triedimycin B ($C_{37}H_{53}N_3O_9$): MP 85~95°C (gradually dec); $[\alpha]_D^{21}$ +75° (*c* 0.8, MeOH); FAB-MS *m/z* 684 $(M+H)^+$; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 226 (575), 266 (sh, 482), 275 (575), 284 (sh, 444); IR ν_{max} (KBr) cm^{-1} 3395, 2970, 2940, 1821 (β-lactone), 1691, 1639, 1531, 1452, 1396, 1101, 1000, 952, 833.

Triedimycins A and B are soluble in MeOH, $CHCl_3$, Me_2CO and EtOAc, but almost insoluble in benzene and H_2O.

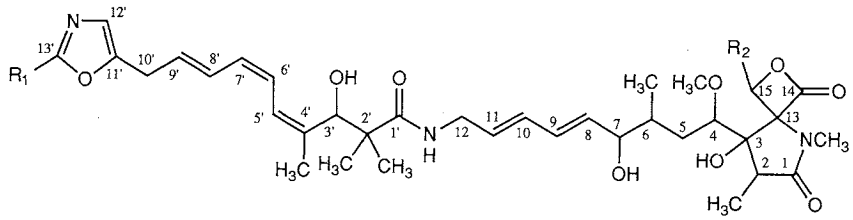
As the UV and IR spectra of triedimycins showed to be very similar to those of resistaphylin[2] or oxazolomycin,[3] the ^1H NMR spectra were directly compared with those of oxazolomycin† as shown in Table 1. The ^{13}C NMR (Table 2), COSY and NOESY data of these antibiotics suggested that triedimycin A substitutes methoxymethyl and methyl groups to the oxazolomycin molecule at the C-15 and C-13′ positions, respectively, and triedimycin B has two methyl groups at the both positions, as shown in the gross structures. Stereochemical assignments of olefins in new antibiotics were determined to be the same as those of oxazolomycin from the coupling constants of ^1H NMR (Table 1) and NOESY data.

Table 2. ^{13}C NMR spectra of triedimycins A and B (δ ppm).

Carbon	A	B	Carbon	A	B
1	175.01	175.07	16-OCH_3	59.60	
2	43.26	43.09	N-CH_3	26.55	26.61
2-CH_3	10.28	10.07	1′	178.01	178.05
3	81.55	81.55	2′	44.64	44.68
4	82.33	82.01	2′-$(CH_3)_2$	21.52,	21.56,
4-OCH_3	56.91	57.28		26.06	26.11
5	32.74	33.14	3′	75.58	75.66
6	37.20	37.09	4′	138.37	138.42
6-CH_3	17.25	17.63	4′-CH_3	19.50	19.54
7	76.87	77.02	5′	124.78	124.83
8	134.11	134.18	6′	123.71	123.74
9	131.10	131.16	7′	127.97	127.99
10	131.56	131.60	8′	128.08	128.13
11	129.60	129.66	9′	129.00	129.06
12	41.19	41.21	10′	29.10	29.16
13	86.22	84.30	11′	150.11	150.14
14	168.91	169.68	12′	122.58	122.64
15	78.29	77.50	13′	160.73	160.76
16	70.54	16.84	13′-CH_3	13.89	13.93

δ: ppm from TMS in $CDCl_3$.

† A crude sample of oxazolomycin was kindly provided by Dr. DAISUKE UEMURA, Shizuoka University and purified by preparative TLC according to his suggestion.



Triedimycin A $R_1=CH_3$ $R_2=CH_3OCH_2$
Triedimycin B $R_1=CH_3$ $R_2=CH_3$
Oxazolomycin $R_1=H$ $R_2=H$

Triedimycins A and B exhibited weak antimicrobial activity against only limited bacteria such as *Micrococcus luteus* FDA16 (MIC on brain heart infusion agar, each 25 μg/ml) and *Pseudomonas aeruginosa* A3 (each 50 μg/ml), and had potent cytotoxicity to murine leukemia P388 cells (IC_{50} 0.06 and 0.19 μg/ml). Solutions of 2.5 μg/ml of triedimycins A and B in 1/15 M phosphate buffer (pH 6.8) showed hazy inhibition zones, 20.0 and 21.5 mm i.d., respectively, by cylinder plate method using *Staphylococcus aureus* FDA 209P as a test organism. Mice (ICR female, 4-week old) tolerated intraperitoneal administration of 6.25 mg/kg of triedimycins A and B, but they were toxic at 12.5 mg/kg.

More details of the structural elucidation and biological properties will be reported in due course.

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