Communications to the editor

CHIMERAMYCINS: NEW MACROLIDE ANTIBIOTICS PRODUCED BY HYBRID BIOSYNTHESIS*

Sir:

Microbial conversion of antibiotically inactive macrocyclic lactones to active compounds is an important approach in the search for new macrolide antibiotics. Mutational biosynthesis originally proposed by SHIER *et al.*[1] was proved useful also for constructing new macrolide antibiotics and many new derivatives were obtained. However, these were virtually inactive,[2~4] or weakly active and acquired substantial antibiotic activity only after chemical modifications.[5] As an alternative, we have proposed a hybrid biosynthesis method which utilizes enzyme inhibitors. By means of hybrid biosynthesis, the biologically inactive tylosin aglycone, protylonolide, was converted into 5-*O*-desosaminylprotylonolide (=M-4365 G_1)[6] by a pikromycin-producing streptomycete in the presence of cerulenin, an inhibitor of *de novo* synthesis of the aglycone moiety.

Using this technique, further attempts were carried out to convert protylonolide by a spiramycin-producing strain, *Streptomyces ambofaciens* KA-448. Protylonolide was transformed into two new active macrolide antibiotics named chimeramycins A and B. This communication describes the fermentation, isolation, structure elucidation and biological properties of the chimeramycins.

S. ambofaciens KA-448 (ATCC 15154) was cultured in a spiramycin production medium (glucose 1.0%, dried yeast 1.0%, NaCl 0.5%, $CaCO_3$ 1.0%, $NaNO_3$ 0.1%, pH 7.5) in the presence of cerulenin (40 μg/ml/day). After 24 hours, 100 μg/ml of protylonolide was added into the culture and the cultivation was continued for a further 48 hours. The cultured broth was centrifuged to remove mycelia. The supernatant was extracted with an equal volume of benzene, and the organic solvent layer was concentrated to afford a dark brownish residue. The residue was subjected to silica gel column chromatography with $CHCl_3$ - MeOH - conc. NH_4OH (10: 1: 0.05) to obtain the chimeramycin complex, which was further purified by preparative TLC on aluminum oxide developed with ethyl acetate - benzene (6: 1). Thirty-seven mg of chimeramycin A (**1**) and 45 mg of chimeramycin B (**2**) were obtained as white powders from 10 liters of a cultured broth.

The chemical structures of the chimeramycins were determined by spectroscopy. The physicochemical properties of the chimeramycins are summarized in Table 1. The UV spectra (λ_{max}^{MeOH} 232 nm) indicate the presence of a conjugated double bond in the molecules. In the ^1H NMR spectrum of **1**, the signals of an aldehyde (δ 9.64 s), three olefinic protons (6.32 d H-11, 5.64 dd H-10, 5.24 d H-13), three anomeric protons (5.02 d H-1″, 4.34 d H-1‴, 4.15 d H-1′), two *N*-dimethyl groups (2.45 s, 2.19 s), an acetyl group (2.15 s) and a C-12 methyl (1.72 s) were observed. The spectrum of **2** was similar to that of **1** except for the absence of signals assignable to acetyl group. These data suggest that the chimeramycins possess the aglycone of tylosin and three sugars; one is a neutral sugar, mycarose, and the other two are the aminosugars, mycaminose and forosamine. All these three sugars are contained in the spiramycin molecule.

The ^{13}C NMR chemical shifts of **1** and **2** are listed in Table 2. Compared with the spectrum of spiramycin I, the signals corresponding to mycaminose, mycarose and forosamine were almost identical, while significant differences were observed in the aglycone moiety. The signals of carbons 1 through 23 of compound **1** agree with a

Table 1. Physicochemical properties of chimeramycins A (**1**) and B (**2**).

		1	**2**
Melting point (°C)		108~110	114~115
$[\alpha]_D^{22}$ (*c* 1.0, $CHCl_3$)		+40.0°	+14.4°
λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)		232 (288)	232 (305)
Molecular weight		910	868
Molecular formula		$C_{48}H_{82}N_2O_{14}$	$C_{46}H_{80}N_2O_{13}$
Elemental analysis	C:	63.73 (63.27)	62.45 (63.57)
(%) Found	H:	9.85 (9.07)	9.18 (9.28)
(Calcd.)	N:	2.92 (3.07)	3.25 (3.22)

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Table 2. ^{13}C NMR chemical shifts for chimeramycins A (**1**) and B (**2**).

Carbon No.	**1**	**2**	Spiramycin I[11]	Tylosin[7]
1	170.8[a]	174.9	174.1	173.9
2	38.9	39.0	37.7	39.4
3	70.1	71.6[c]	68.3	71.7
4	42.0[c]	42.1[c]	85.3	45.1
5	79.1	79.4	78.8	81.6
6	34.3	33.2	30.6	32.3[a]
7	30.7	30.5	30.7	32.9[a]
8	37.8	38.0	31.8	40.3
9	80.5	82.6	79.3	202.8
10	128.8	129.5	128.6	118.8
11	137.1	134.8	134.6	148.0
12	133.0	133.9	132.8	134.9
13	135.9	135.1	131.0	142.2
14	37.8	37.3	42.0	44.7
15	77.1[c]	77.3[c]	69.2	75.3
16	25.0	24.8	20.1	25.5
17	9.5	8.3	43.3	9.0[b]
18	10.0	9.6	202.8	9.6[b]
19	44.0	44.1	15.3	43.9
20	202.3	202.7	61.8	203.0
21	16.8	16.7	—	17.4
22	12.7	12.9	—	13.0
23	15.9	16.6	—	68.2
24	171.1[a]	—	—	—
25	21.4	—	—	—
1′	104.2	105.0	103.9	103.9
2′	71.7	71.4	71.7	69.5
3′	68.8	69.0	68.8	69.5
4′	74.8	74.6	75.0 (d)	75.3 (d)
5′	73.3	73.4	73.1	73.2
6′	19.0	19.3	19.0	19.0
7′	42.0	42.1	42.0	42.0
8′	42.0	42.1	42.0	42.0
1″	96.4	96.3	96.4	96.6
2″	41.0	41.0	40.9	41.1
3″	69.4	69.4	69.4	69.0
4″	76.4	76.5	76.4 (e)	76.5 (e)
5″	66.1	66.0	66.0	66.1
6″	18.3	18.3	18.3	18.3
7″	25.4	25.4	25.4	25.5
1‴	101.1	102.4	100.2	101.1
2‴	31.3	31.2	31.3	82.0
3‴	18.5	18.5	18.5	79.9
4‴	64.9	64.8	68.8 (f)	72.9 (g)
5‴	73.8	73.8	73.8	70.6
6‴	19.1	19.1	19.0	17.8
7‴	40.7	40.7	40.7	59.6
8‴	40.7	40.7	40.7	61.7

Chemical shifts are given im ppm relative to TMS at internal standard. [a], [b]; Assignments may be reversed. [c]; Tentatively assigned. (d) Mycaminose, (e) mycarose, (f) forosamine, (g) mycinose.

structure in which the carbon skeleton corresponds to the aglycone of tylosin with attachments at three sites. The signals of C-9 and C-23 were observed at δ 80.5 and 15.9, respectively. These upfield shifts indicate the presence of a glycosidic linkage at C-9, and the absence of mycinose which is attached at C-23 in tylosin. Another upfield shift from δ 173.9 to δ 170.8 in the signal at C-1, together with two additional signals assigned to C-24 and 25, suggests the presence of a 3-*O*-acetyl group in **1**, but not in **2**. The signal of the ester carbonyl in leucomycin A_5 (δ 173.5),[7] a C-3 hydroxyl derivative, was observed at about 4 ppm lower field than that of leucomycin A_3 (δ 169.9), a 3-*O*-acetyl substance. From these results, the chemical structures of **1** and **2** are proposed as shown in Fig. 1. The configuration at C-9 was assumed to be *R* from the *J* value ($J_{9,10}$=8.8 Hz)[5] in the ^1H NMR spectrum of **1**.

Mild acidic hydrolysis of **1** and **2** in 0.07 N HCl (pH 2.2) at 42°C for 2 hours gave demycarosylchimeramycins A (**3**) (mp 91~93°C, $[\alpha]_D^{22}$ −15.9° (*c* 0.5, $CHCl_3$), M^+ *m/z* 766) and B (**4**) (mp 98~100°C, $[\alpha]_D^{22}$ +23.2° (*c* 0.5, $CHCl_3$), M^+ *m/z* 724).

The antibacterial activities of the chimeramycins and the derivatives are shown in Table 3. The chimeramycins are as active *in vitro* as tylosin and spiramycin, whereas the demycarosylchimeramycins have stronger activity than that of chimeramycins.

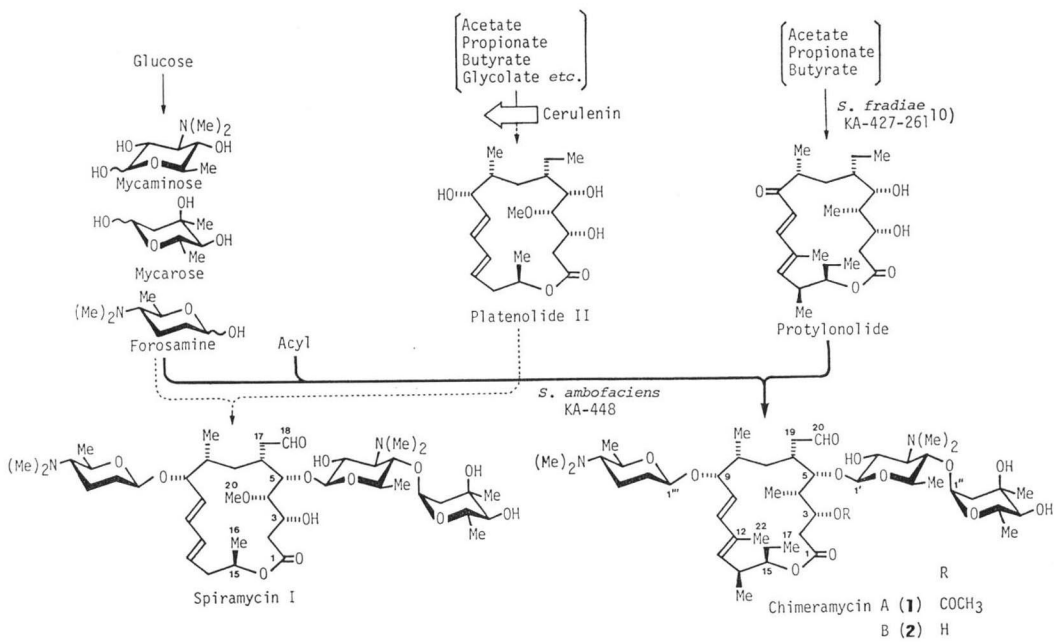
It is emphasized that chimeramycins are new hybrid macrolides derived from tylosin and spiramycin. The hybrid structures were synthesized in the presence of cerulenin by the combination of the biosynthetic capability of two microorganisms, *S. fradiae* KA-427-261 and *S. ambofaciens* KA-448. The former organism biosynthesized protylonolide. The latter one oxidized (C-20) and reduced (C-9) the lactone, and attached to it three sugars and an acetyl group, presumably utilizing the biosynthetic and assembling mechanisms which normally serve for the synthesis of its own antibiotic. The allowance of substrate specificity for a foreign intermediate may restrict the processing in each step. From this standpoint, *S. ambofaciens* KA-448 was more favorable for chimeramycin production than another strain of *S. ambofaciens*, KA-1028 which produced hybrids other than chimeramycins in a similar attempt.[9] The convenience in the use of an enzyme inhibitor permitted the hybrid biosynthesis using a variety of com-

Table 3. Antimicrobial activity of chimeramycins **A** (**1**) and **B** (**2**) and their demycarosyl derivatives (**3**, **4**).

Test organism	MIC (μg/ml)						
	1	**2**	**3**	**4**	SPM I	SPM III	TYL
Staphylococcus aureus ATCC 6538P	6.25	3.12	1.56	1.56	6.25	12.5	3.12
S. aureus FDA 209P	6.25	3.12	1.56	1.56	6.25	12.5	3.12
S. aureus KB 199 (EM^r, TC^r)	>100	>100	>100	>100	>100	>100	>100
S. aureus KB 224 (EM^r, TC^r, KM^r, SM^r)	>100	>100	>100	>100	>100	>100	>100
Bacillus subtilis PCI 219	1.56	0.78	1.56	1.56	1.56	1.56	0.78
B. cereus IFO 3001	3.12	1.56	1.56	1.56	3.12	3.12	1.56
Micrococcus luteus ATCC 9341	0.4	0.4	0.4	0.2	0.4	0.4	0.2
Streptococcus pneumoniae III KB 165	0.4	0.4	0.2	0.2	0.4	0.4	0.78
S. pyogenes KB 166	N.D.	0.4	0.4	0.4	N.D.	N.D.	0.78
Mycobacterium smegmatis ATCC 607	>100	>100	>100	>100	>100	>100	>100
Escherichia coli NIHJ	>100	>100	>100	>100	>100	>100	>100
E. coli N-33 (EM^s, LCM^s)	1.56	0.78	0.4	0.2	1.56	1.56	3.12
Klebsiella pneumoniae ATCC 10031	>100	>100	50	25	>100	>100	>100
Salmonella typhimurium KB 20	>100	>100	50	25	>100	>100	>100
Proteus vulgaris IFO 3167	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa IFO 3080	100	>100	>100	>100	>100	>100	>100

SPM I; Spiramycin I, SPM III; spiramycin III, TYL; tylosin.
N.D.; Not determined.

Fig. 1. The hybrid biosynthesis of chimeramycins by a spiramycin-producing strain *S. ambofaciens* KA-448.



binations of organisms.

Experiments are now in progress to breed hybrid organisms which produce further hybrid macrolide antibiotics, based upon these results of hybrid biosynthesis.

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