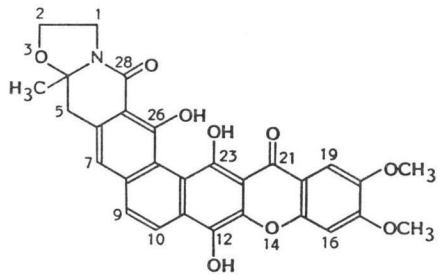
ENHANCED ANTIMICROBIAL ACTIVITY OF ACETYL DERIVATIVES OF CERVINOMYCIN

Sir:

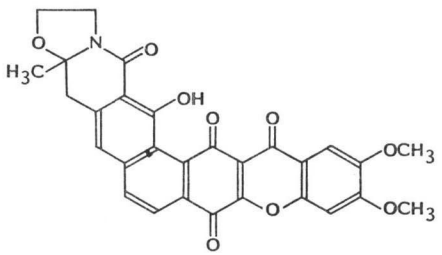
Cervinomycin is an anti-anaerobic and anti-mycoplasmal antibiotic produced by *Streptomyces cervinus* sp. nov.[1] The antibiotic contains two components, A_1 (**1**) and A_2 (**2**) which possess a polycyclic structure containing a xanthone skeleton.[2] Chemical modification of cervinomycins has been carried out in order to increase the antimicrobial activity and solubility because of these very insoluble compounds. In this communication we describe the antimicrobial activities, especially against anaerobic bacteria, of cervinomycin A_1 triacetate (**3**) and A_2 monoacetate (**4**).

The acetyl derivatives **3** and **4** were obtained as follows. Acetic anhydride (2.9 ml, 2.8×10^{-2} mol) was added to a suspension of **1** (2.5 g, 4.7×10^{-3} mol) and triethylamine (1.8 ml) as a catalyst in pyridine (50 ml). The reaction mixture was stirred for 4 days at room temperature. Triacetate **3**, mp 283~285°C, $[\alpha]_D^{26}$ −115° (*c* 0.3, $CHCl_3$), EI-MS *m/z* 655 (M^+), $C_{35}H_{29}NO_{12}$ in which an acetyl group was introduced to C-12, C-23 and C-26, was obtained as a yellowish crystalline powder in 87% yield. In a similar manner, monoacetate **4**, mp 283°C, $[\alpha]_D^{26}$ −297.5° (*c* 0.3, $CHCl_3$), EI-MS *m/z* 569 (M^+), $C_{31}H_{23}NO_{10}$ was obtained as an orange crystalline powder from **2** in 67% yield. The solubilities of **3** and **4** in methanol, ethanol, acetone, ethyl acetate and benzene were over 50 times higher than those of the mother antibiotics **1** and **2**, respectively, but hardly soluble in water.

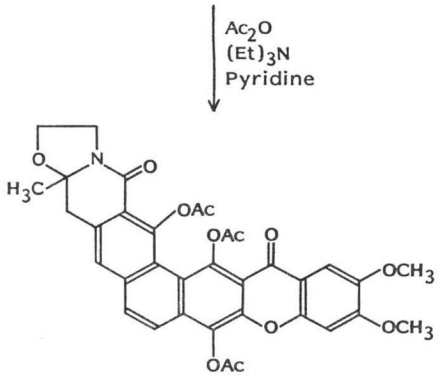
The antimicrobial activities (MIC) of **1**, **2**, **3**, **4** and clindamycin which is a clinically useful antibiotic against anaerobic bacteria are shown in Table 1. Both acetates exhibited potent antimicrobial activity against Gram-positive bacteria including anaerobic bacteria and mycoplasmas. In a separate study, compounds **3** and **4** were shown to be active against clinical isolates (4 strains and 1 strain, respectively) of erythromycin and clindamycin-resistant Staphylococci and Streptococci (the MIC values: 0.20~1.56 μg/ml). Furthermore, neither compound



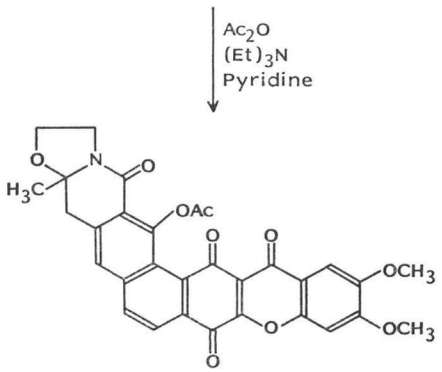
1



2



3



4

Table 1. Antibacterial activities of **1**, **2**, **3**, **4** and clindamycin.

Test organism	Medium and method	MIC (μg/ml)				
		1	**2**	**3**	**4**	Clindamycin
Staphylococcus aureus ATCC 6538P	I	0.78	1.56	<0.025	0.05	<0.025
Bacillus subtilis ATCC 6633	I	0.05	0.2	<0.025	0.05	0.40
B. cereus IFO 3001	I	0.025	0.05	<0.025	<0.025	<0.025
Micrococcus luteus ATCC 9341	I	0.39	1.56	<0.025	<0.025	<0.025
Escherichia coli NIHJ JC-2	I	>25	>25	6.25	>25	6.25
Klebsiella pneumoniae ATCC 10031	I	>25	>25	>25	>25	6.25
Proteus vulgaris IFO 3167	I	>25	>25	>25	>25	>25
Pseudomonas aeruginosa IFO 3080	I	>25	>25	>25	>25	>25
Clostridium acetobutylicum IFO 3346	II	12.5	0.20	6.25	0.10	50
C. difficile ATCC 9689	II	0.05	0.10	0.05	0.10	6.25
C. perfringens ATCC 13124	II	0.05	0.10	0.025	0.10	0.10
Eubacterium limosum ATCC 8468	II	0.10	0.10	0.05	0.10	0.20
Peptococcus prevotii ATCC 9321	II	0.20	0.20	0.10	0.39	0.05
P. variabilis ATCC 14955	II	0.05	0.39	0.025	0.02	0.39
Streptococcus mutans RK-1	II	0.05	0.39	0.025	0.20	0.05
Bacteroides fragilis ATCC 23745	II	0.78	1.56	0.10	0.78	0.025
Fusobacterium varium ATCC 8501	II	>25	>25	>25	>25	1.56
Veillonella alcalescens ATCC 17745	II	>25	>25	>25	>25	1.56
Mycoplasma gallisepticum KP-13	III	3.12	25	0.78	0.20	6.25
M. gallisepticum S-6	III	1.56	12.5	0.39	0.20	6.25
M. gallisepticum 333P*	III	3.12	12.5	0.39	0.20	>25
M. pneumoniae KB 173	III	3.12	12.5	0.39	0.20	6.25
Acholeplasma laidlawii PG8	III	1.56	12.5	0.20	0.20	6.25
A. laidlawii Bml	III	1.56	25	0.39	0.20	6.25

I: Heart infusion agar, 1×10^6 cells/ml, agar dilution method (37°C, 20 hours).
II: GAM agar, 1×10^6 cells/ml, agar dilution method (37°C, 48 hours, under anaerobic conditions).
III: PPLO agar, agar dilution method (37°C, 7 days).
* Spiramycin-resistant strain.

induced macrolide-resistance in an inducible strain, *Staphylococcus aureus* 0.126. Compounds **3** exhibited potent antimicrobial activity against various anaerobic bacteria such as *Clostridium difficile* ATCC 9689, *Clostridium perfringens* ATCC 13124, *Eubacterium limosum* ATCC 8468, *Peptococcus variabilis* ATCC 14955, *Streptococcus mutans* RK-1 and *Bacteroides fragilis* ATCC 23745. In general, it was similar to or more potent than clindamycin. The bactericidal activities of **3** against *B. fragilis* ATCC 23745 and *C. perfringens* ATCC 13124 were tested as follows. A cell suspension of the bacteria cultured in GAM medium (Nissui) at 37°C for 18 hours was inoculated into the same medium containing **3** and cultured at 37°C. Viable cells in the cultures were counted on GAM agar plates after 2, 4 and 8 hours of incubation. The minimal bacteriocidal concentrations of **3** against *B. fragilis* ATCC 23745 and *C. perfringens* ATCC 13124 were found to be 4.0 and 0.1 μg/ml, respectively. The initial viable cell count of about 1×10^7 cells/ml for both bacteria decreased less than 1×10^2 cells/ml within 2 hours after addition of **3** at the same concentration described above.

The LD_{50} value of **3** in mice was 1,620 mg/kg po and 93 mg/kg iv. Compound **3** is under development as a drug against anaerobes because of its potent antimicrobial activity and its high solubility and low toxicity.

Akira Nakagawa
Yuzuru Iwai
Hideki Shimizu†
Satoshi Ōmura

The Kitasato Institute and School of Pharmaceutical Sciences,
Kitasato University,
Minato-ku, Tokyo 108, Japan
†Asahi Chemical Industry Co., Ltd.,
Nobeoka-shi, Miyazaki 882, Japan

(Received June 24, 1986)

References

1) ŌMURA, S.; Y. IWAI, K. HINOTOZAWA, Y. TAKAHASHI, J. KATO & A. NAKAGAWA: Cervinomycin A_1 and A_2, new antibiotics active against anaerobes, produced by *Streptomyces cervinus* sp. nov. J. Antibiotics 35: 645~652, 1982

2) ŌMURA, S.; A. NAKAGAWA, K. KUSHIDA & G. LUKACS: Structure of cervinomycin, a novel antianaerobic antibiotic. J. Am. Chem. Soc. 108: 6088~6089, 1986