AURANTININ B, A NEW ANTIMICROBIAL ANTIBIOTIC FROM BACTERIAL ORIGIN

Sir:

During the course of the screening program for new antibiotics from true bacteria, we have found that *Bacillus aurantinus* Masuma and Ōmura sp. nov. (*B. aurantinus* KM-214) produced a novel antibiotic, aurantinin A which exhibits biological activity against Gram-positive bacteria and have reported its characterization and biological activity in the previous paper.[1,2] Recently, we found that the organism produced a new component, aurantinin B prior to the production of aurantinin A during the fermentation. In this communication, we wish to report the isolation, characterization and biological properties of aurantinin B.

Fermentation was carried out by the same procedure used for aurantinin A.[1,2] The culture broth (70 liters) of *B. aurantinus* sp. nov. fermented in a 100-liter tank using a medium consisting of glycerol 1%, starch 1%, soybean meal 2%, dried yeast 0.3%, NaCl 0.5%, $(NH_4)_2SO_4$ 0.2%, K_2HPO_4 0.1%, $CaCO_3$ 0.3% at pH 7.0 was adjusted to pH 2.0 and centrifuged. The antibiotic was extracted with *n*-butyl acetate from the filtrate. The organic layer was concentrated *in vacuo* to afford an oily residue which was precipitated with *n*-hexane to give a crude powder (15 g). The powder was chromatographed on a Sephadex LH-20 column eluted with MeOH and the combined active fractions were concentrated to dryness to afford a crude powder (5.5 g). This powder was then applied to centrifugal liquid chromatography in $CHCl_3$ - MeOH (30 : 1) followed by preparative silica gel TLC using $CHCl_3$ - MeOH (10 : 1) to obtain active substance (1.3 g). Aurantinins A and B show Rf values 0.13 and 0.10, respectively, on silica gel TLC in $CHCl_3$ - MeOH (10 : 1). Each component was finally rechromatographed on Sephadex LH-20 to obtain pure aurantinins A (200 mg) and B (118 mg), as yellowish powders. The antibiotics were detected as red spots on silica gel plate after spraying with conc sulfuric acid and also by orange fluorescence on UV irradiation. The physicochemical properties of aurantinin B were as follows: MP 98°C; $[\alpha]_D^{25}$ +124° (*c* 0.33, MeOH); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 268 (433), 278 (541), 287 (480), 320 (218); IR (KBr) cm^{-1} 3400 (OH), 1765 (COO), 1725 (CO, COOH, COO), 1082, 1035 (COC). The molecular formula, $C_{35}H_{54}O_9$ for aurantinin A was proposed, from electron impact mass spectral (EI-MS) data in a previous paper.[2] However, subsequent data, field desorption mass spectra (FD-MS) (*m/z* 636, M^+) and EI-MS (*m/z* 618.358, M^+-H_2O, calcd for $C_{38}H_{50}O_7$, 618.356) for aurantinin A and FD-MS (*m/z* 762, M^+) and EI-MS (*m/z* 762.396, M^+, calcd for $C_{44}H_{58}O_{11}$, 762.397) for triacetyl aurantinin A, led to the revised molecular formula, $C_{35}H_{54}O_9$ for the molecular formula for aurantinin A. The molecular formula, $C_{44}H_{60}O_{12}$ of component B was deduced from the FD-MS data of aurantinin B (*m/z* 780, M^+), a tetraacetate (*m/z* 948, M^+) and monomethyl ester (*m/z* 794, M^+) and its ^{13}C NMR spectrum.

Comparisons of ^1H and ^{13}C NMR spectral analyses of aurantinins A and B showed that component B was similar structurally to A. The ^{13}C NMR spectrum (100 MHz, in acetone-d_6) of component B showed the existence of eight methyls, five methylenes, eight methines, seven oxymethines, twelve olefinic carbons, two anhydride carbons, and one carboxyl and one ketone carbonyl carbon, as shown in Fig. 1. The difference ^{13}C spectrum of both components indicated that aurantinin B was a glycoside which is constituted from aurantinin A as an aglycone and a keto sugar possessing the formula, $C_6H_{10}O_5$ (a ketone carbonyl at δ 206.5, an anomeric carbon at δ 105.1, three oxygenated carbons at δ 78.0, 77.8 and 72.8, and a methyl carbon at δ 18.4).

Methanolysis of compound B afforded the same product, a monomethyl ester, (mp 94~96°; $[\alpha]_D^{25}$ +148° (*c* 0.35, MeOH); FD-MS *m/z* 650 (M^+)) as obtained by methylation of A with CH_2N_2. This means that the aglycone moiety of B is identical with A. The structure elucidation of aurantinins by NMR analysis and biosynthetic means using ^{13}C-labeled precursor deduced a novel polyketide structure with four rings containing a triene and an acid anhydride moiety for aurantinin A which corresponds to the aglycone part of aurantinin B. The structure and biosynthesis of aurantinins will be reported soon in a separate paper. The occurrence of polyketide antibiotic containing a triene as a secondary metabolite of true bacteria is extremely rare. The only reported examples are pro-

Fig. 1. ^{13}C NMR spectra of aurantinins A and B (100 MHz, acetone-d_6).

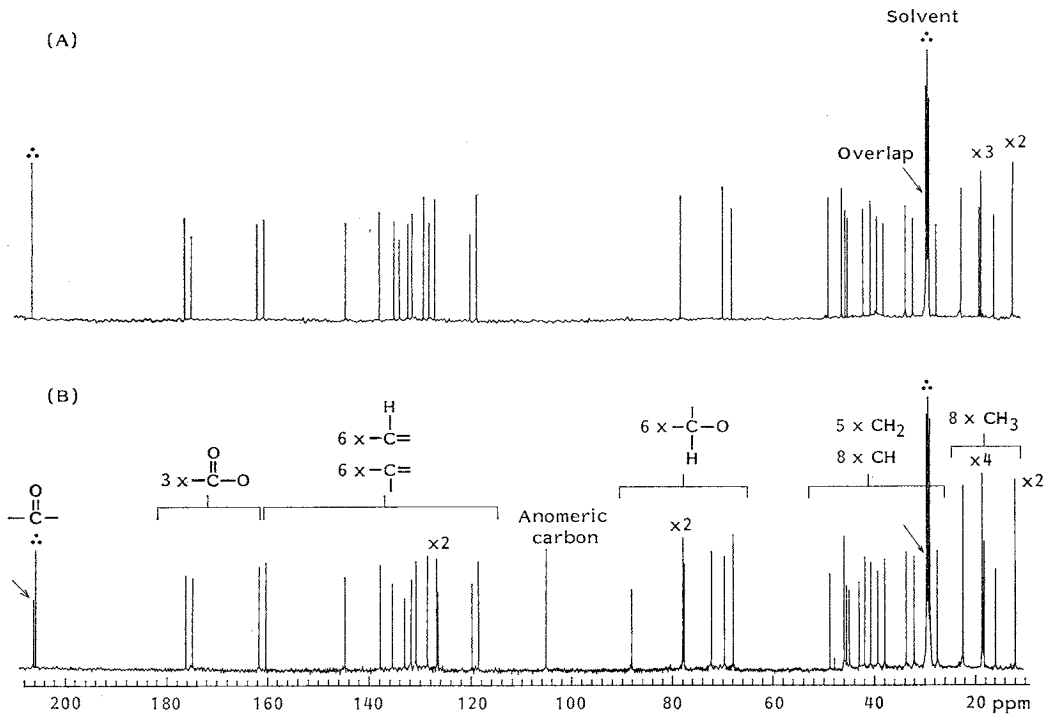

Table 1. Antibacterial activity of aurantinin B against selected aerobes and anaerobes.

Organism	MIC (μg/ml)*		
	Aurantinin B	Erythromycin gluceptate	Cefoxitin
Staphylococcus aureus Pen^S	4.0	0.06	
S. aureus A	1.0	0.06	
Streptococcus pyogenes E^S	8.0	<0.008	
S. pyogenes E^R	4.0	>128.0	
Corynebacterium pyogenes A	4.0	<0.008	
Pasteurella haemolytica A	8.0	1.0	
P. multocida A	4.0	0.5	
Clostridium perfringens	≤0.06	NT	0.25
C. ramosum	0.5	1.0	32.0
C. difficile Clin^R	0.5	8.0	4.0
Peptostreptococcus anaerobius	0.5	NT	1.0
Bacteroides fragilis	>128.0	32.0	8.0
Fusobacterium mortiferum	>128.0	16.0	32.0

* Agar dilution assay.
Pen: Penicillin, A: animal origin, E: erythromycin, Clin: clindamycin, ^{S}: sensitive, ^{R}: resistant.
NT: Not tested.

ticin[3,4] and difficidin[5] and its analog.

Aurantinins exhibit a broad antimicrobial activity against Gram-positive bacteria but not against Gram-negative bacteria, filamentous fungi and yeast. Aurantinin B is more potent than A in antimicrobial activity. The antimicrobial activities of aurantinin B against selected aerobes and anaerobes are shown in Table 1. The most interesting activities attributable to aurantinin B are those directed against anaerobic bacteria, especially *Clostridium perfringens*, *C. ramosum* and *C. difficile* in which the activity was

higher than cefoxitin. Aurantinin B was considerably less active than erythromycin against sensitive *Streptococcus pyogenes*, but showed good activity against an erythromycin-resistant strain.

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YAEKO KONDA
AKIRA NAKAGAWA
YOSHIHIRO HARIGAYA
MASAYUKI ONDA

School of Pharmaceutical Sciences,
Kitasato University,
Minato-ku, Tokyo 108, Japan

ROKURŌ MASUMA
SATOSHI ŌMURA*

The Kitasato Institute,
Minato-ku, Tokyo 108, Japan

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