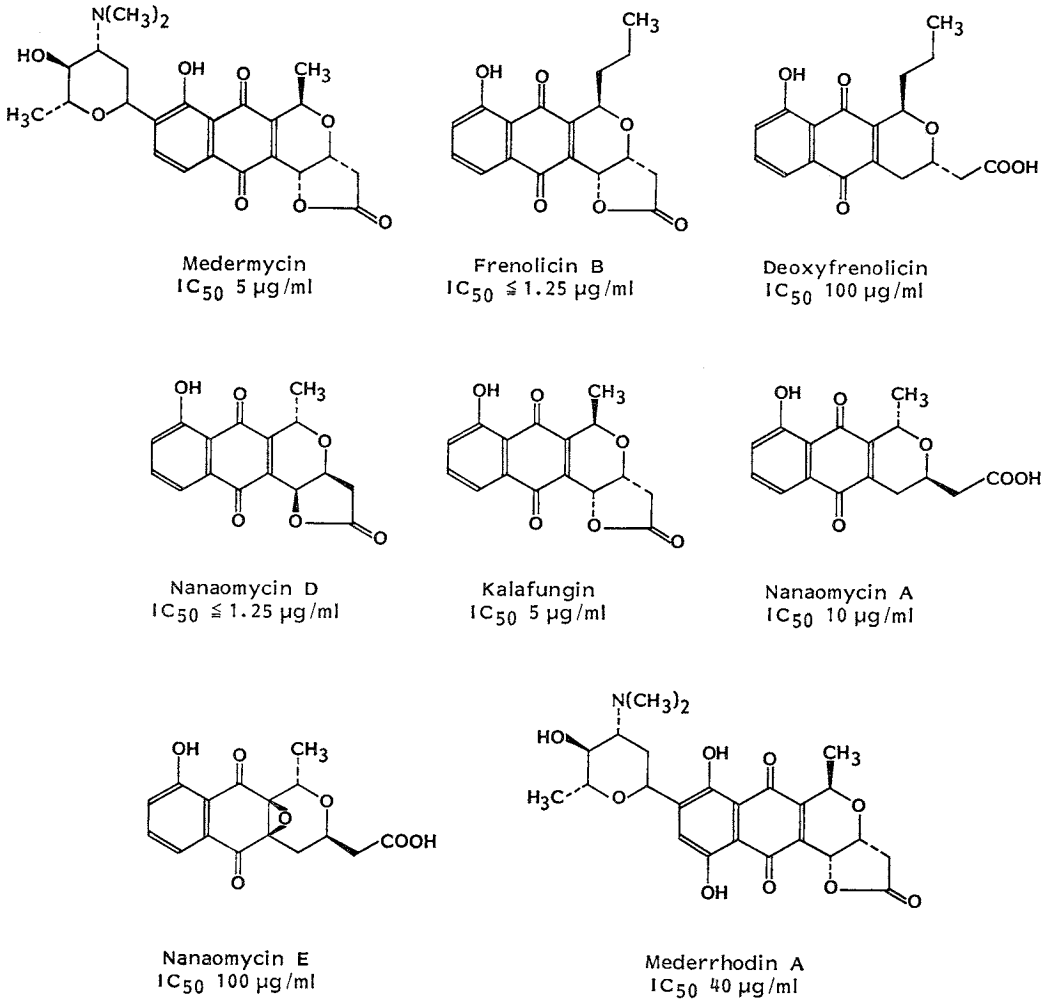
INHIBITION OF PLATELET AGGREGATION BY MEDERMYCIN AND IT'S RELATED ISOCHROMANEQUINONE ANTIBIOTICS

Sir:

During the course of screening inhibitors from microorganisms for platelet aggregation, we found that an isochromanequinone antibiotic medermycin isolated from the cultured broth of *Streptomyces* sp. KM-11749 possessed a potent anti-platelet activity. In this paper, we wish to describe the fermentation, isolation, and *in vitro* and *in vivo* anti-platelet activities of medermycin and its related antibiotics such as frenolicins, nanaomycins, kalafungin and mederrhodin.

The platelet aggregation inhibitor was screened by incubation of platelet rich plasma (PRP) from rabbit blood with fermentation broth in a 24-well plate using thrombin or adenosine diphosphate (ADP) as a platelet aggregation agent, as reported in a previous paper.[1,2] The seed culture of *Streptomyces* sp. KM-11749 was inoculated in a Sakaguchi flask containing 100 ml of production medium (starch 2.0%, soybean meal 1.0%, NaCl 0.3%, $CaCO_3$ 0.3%, pH 7.0 prior to sterilization) and then cultured for 72 hours at 27°C with agitation (250 rpm). The cultured broth (3 liters) was extracted twice with EtOAc. After evaporation of the extract, the residue (190 mg) was chromatographed on a silica gel column (developer: $CHCl_3$ - MeOH, 50 : 1 to 10 : 1) to obtain a crude powder. The powder

Fig. 1. The structures and the inhibitory activities (IC_{50}) of isochromanequinone antibiotics.



(30 mg) was further purified on preparative silica gel TLC (developer: $CHCl_3$ - MeOH, 5 : 1, Merck Kieselgel 60 F_{254}) to obtain an orange crystalline powder (13 mg). The active substance was identified as medermycin from the following spectral data, mass (M^+ m/z 457), UV (λ_{max}^{EtOH} nm 258, 269 and 431) and IR (ν_{max}^{KBr} cm^{-1} 2980, 1790, 1660, 1645, 1620, 1430 and 1245). Medermycin has been reported as antibacterial antibiotic from *Streptomyces bikiniensis* by TAKANO *et al.*[3] ADP (5 μM), thrombin (1 U/ml) and arachidonic acid (100 μM)-induced aggregation of PRP were inhibited by medermycin at the IC_{50} value of 5 μg/ml, 6 μg/ml and 100 μg/ml, respectively. Medermycin was less effective in inhibiting platelet aggregation when washed platelets were used instead of PRP. The inhibitory effect on ADP-induced aggregation was investigated for the isochromanequinone antibiotics, frenolicin B, deoxyfrenolicin, nanaomycins A, D and E, kalafungin and mederrhodin A. As shown in Fig. 1, frenolicin B and nanaomycin D indicated a strong inhibitory effect (IC_{50}: 1.25 μg/ml and 1.25 μg/ml, respectively) on ADP-induced aggregation. On the other hand, deoxyfrenolicin and nanaomycin A in which the five membered lactone ring has been cleavaged exhibited lower inhibition activity (IC_{50}: 100 μg/ml and 10 μg/ml, respectively) than each mother antibiotic. The level of the inhibition activity (IC_{50}: 5 μg/ml) of kalafungin which is an enantiomer of nanaomycin D implies that isochromanequinone skeletone is essential but the configuration is not important for the inhibition of platelet aggregation. Further, as indicated in mederrhodin A, the introduction of the hydroxyl group to the benzoquinone skeleton resulted in decrease of the activity. This finding that isochromanequinone antibiotics possess a potent anti-platelet activity, has not been observed before.

The antithrombotic effect of frenolicin B was examined in an *in vivo* mouse model,[4] using intravenous injection of sodium arachidonate. Frenolicin B, 30 mg/kg, as a suspension in 0.5% sodium carboxymethylcellulose was injected, intraperitoneally, into *dd*Y mice (male, body weight of 21~25 g) 30 minutes prior to the intravenous injection of sodium arachidonate, 80 mg/kg. The control group received the same volume of vehicle. The time lapses until death of mice in both groups were compared. Frenolicin B significantly prolonged the time to 172 $\pm$25 seconds (n=7) compared with 56$\pm$7 seconds (n=7) for the control.

The effect of nanaomycin D on the systemic blood pressure of the anesthetized rat was examined as follows; male Sprague-Dawley rats, 8 weeks old, were anesthetized with pentobarbital sodium, 30 mg/kg, and the systemic blood pressures were monitored by a transducer (Nihon Kohden Polygraph, AP-621G) *via* a cannula inserted into the femoral artery. Nanaomycin D showed hypotensive effect of about 30~60 mmHg decrease of systemic blood pressure in 30~45 minutes, when rats were injected intraperitoneally with a dose of 30 mg/kg.

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