OM-4842, A NEW PLATELET AGGREGATION INHIBITOR FROM *STREPTOMYCES*

Sir:

During the course of screening for novel antibiotics showing inhibition of platelet aggregation, a new isotetracenone antibiotic, OM-4842 was isolated from the cultured broth of *Streptomyces*. In this paper, we wish to describe the fermentation, isolation, structure elucidation and biological activity of OM-4842. The platelet aggregation inhibitor was found from screening 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 aggregating agent, as reported in previous papers.[1~3] The strain producing OM-4842 substance was isolated from a soil sample collected at Chiba Prefecture, Japan. It was identified as *Streptomyces* sp. OM-4842 by morphological, cultural and physiological characteristics. Fermentation was carried out in a 50-liter jar fermentor containing 30 liters of a medium (oatmeal 2%, pH 7.0 prior to sterilization) at 27°C with agitation (250 rpm) and aeration (15 liters/minute). The broth (27 liters) of 4-day culture was extracted with EtOAc. After evaporation of the extract, the residue was chromatographed on silica gel with $CHCl_3$ - MeOH (3 : 1). The crude powder (135 mg) obtained from active fractions was rechromatographed on Sephadex LH-20 (MeOH as the eluant) to afford 83 mg of pure OM-4842 as orange needles, mp 178~182°C; $[\alpha]_D^{25}$ +60° (*c* 0.2, CH_3OH). Elemental analysis, fast atom bombardment (FAB)-MS (*m/z* 715 $(M+H)^+$ and ^{13}C NMR spectral data established the molecular formula of OM-4842 as $C_{37}H_{46}O_{14}$. The UV absorption maxima, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 220 (356.7), 321.5 (63.2), 427.5 (83.2) and ^1H and ^{13}C NMR spectral data indicated that the inhibitor possesses an isotetracenone skeleton and is closely similar to the kerriamycin and urdamycin structures, which have been elucidated by ŌTAKE *et al.*,[4~6] and ROHR and ZEECK,[7] respectively. Hydrolysis of OM-4842 with 2 N HCl, in addition to the comparative ^{13}C and ^1H NMR analysis with kerriamycins A, B and C, indicated that the antibiotic consists of the aglycone aquayamycin[8] and two *O*-glycosidically bonded rhodinoses. The connectivity of two rhodinoses to the aquayamycin part was elucidated by nuclear Overhauser effect (NOE) experiments. Irradiation of the anomeric proton (1‴-H, δ 5.39) of rhodinose caused the NOE enhancement on the carbon (C-12b, δ 80.2) of the aglycone of aquayamycin. The NOE was furthermore observed between the anomeric proton (1″-H, δ 5.04) of the other rhodinose and the proton (3′-H, δ 3.72) of the sugar moiety of aquayamycin. From these observations, OM-4842 was assigned the structure which of kerriamycins A and B, lacking kerriose and olivose, respectively, as shown in Fig. 1. ^{13}C Chemical shift assignments of OM-4842 are shown in Table 1. Other components possessing anti-

Fig. 1. Structure of OM-4842.

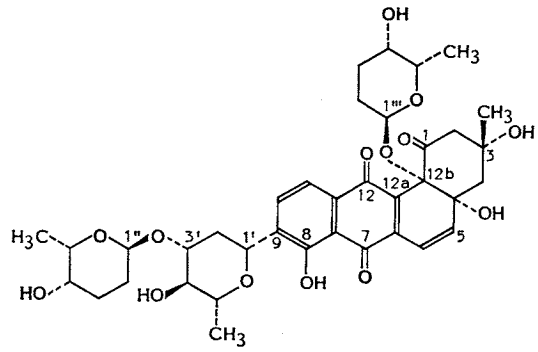

Table 1. ^{13}C Chemical shift assignments of OM-4842 (100 MHz, in $CDCl_3$).

Carbon No.	δ_C(ppm)	Carbon No.	δ_C(ppm)
C-1	201.6	C-1′	71.1
C-2	53.9	C-2′	37.5
C-3	75.3	C-3′	81.8
3-CH_3	29.8	C-4′	76.0
C-4	43.2	C-5′	76.1
C-4a	81.8	5′-CH_3	18.4
C-5	144.8	C-1″	97.4
C-6	116.8	C-2″	25.1
C-6a	137.3	C-3″	24.5
C-7	187.8	C-4″	67.6
C-7a	113.5	C-5″	67.1
C-8	158.0	5″-CH_3	17.1
C-9	138.6	C-1‴	94.6
C-10	133.9	C-2‴	23.0
C-11	120.2	C-3‴	25.3
C-11a	130.1	C-4‴	67.1
C-12	182.3	C-5‴	67.6
C-12a	138.7	5‴-CH_3	16.5
C-12b	80.2		

Table 2. The inhibitory effect of OM-4842 on aggregation induced by platelet aggregating agents.

Platelet aggregating agents	MIC (μg/ml)
ADP (5 μM)	12.5
Arachidonic acid (100 μM)	5.0
PAF (5×10^{-8} M)	25.0
Collagen (100 μg/ml)	>25.0

Platelet rich plasma (PRP) was collected from Japanese white rabbit. The number of platelets in the PRP was adjusted to 1×10^{6}/ml using autologous plasma. The PRP (0.4 ml) was then transferred to 24-well plate and incubated with aggregating agents at 37°C for 5 minutes. The degree of aggregation was measured with the naked eye.

platelet activity that were isolated from the cultured broth together with OM-4842 were identified as kerriamycins B and C from their physicochemical properties.

The inhibitory effect of OM-4842 on platelet aggregation induced by various aggregating agents (ADP, arachidonic acid, platelet activating factor (PAF) or collagen) is shown in Table 2. OM-4842 markedly inhibited platelet aggregation by arachidonic acid, ADP or PAF, however, no inhibition was observed on collagen induced aggregation at a dose of 25 μg/ml of the antibiotic. The inhibitory activity of OM-4842 is greater than that of kerriamycins B and C.

OM-4842 exhibited growth inhibition against doxorubicin-resistant cells of P388 mouse leukemia at a dose of 1.5 μg/ml when the cells were exposed to the antibiotic for 72 hours *in vitro*. Biological activity of OM-4842 is of interest since members of this class have demonstrated antitumor activity on Ehrlich ascites carcinoma *in vivo*.[4,5] The acute toxicity (LD_{50}) of OM-4842 in *dd*Y mice was approximately 200 μg/kg by intravenous injection.

We like to name OM-4842 aggreticin.

Acknowledgments

The authors wish to thank Prof. H. SETO of Institute of Applied Microbiology, The University of Tokyo for providing us with an authentic sample of kerriamycins. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

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(Received December 28, 1987)

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