PI-083, A NEW PLATELET AGGREGATION INHIBITOR

Sir:

In the course of our screening program for platelet aggregation inhibitor from a microbial origin, we isolated a new compound, designated PI-083, from the fermentation broth of *Streptomyces matensis* A-6621. The fermentation, isolation, structure determination and biological properties are described in the present communication.

The fermentation of *S. matensis* A-6621 was carried out in a 5-liter jar fermentor containing 3 liters of medium (oatmeal 4%, meat extract 0.3%, NaCl 0.3%, CaCO₃ 0.3%, Fe₂(SO₄)₃ 0.04%, MnCl₂ 0.04%, pH 7) at 30°C under agitation (500 rpm) and aeration (3 liters/minute) for 4 days.

The fermentation broth (36 liters) was centrifuged to separate the mycelia from the supernate. Since most of the activity was associated with the mycelia, they were extracted with MeOH (5 liters), and the resulting solution was concentrated in vacuo. The concentrate was then extracted with ethyl acetate (2 liters). The brown residue (12 g) obtained by evaporation of the organic layer was chromatographed on silica gel (1 liter), using chloroform - methanol (100:0, 99:1, 98:2). The active fractions containing PI-083 were collected and were further purified by the use of reverse phase HPLC to give pure PI-083 in a red powder form (130 mg).

The physico-chemical properties of PI-083 are as follows: MP $172 \sim 175^{\circ}$ C (dec); $[\alpha]_{D}^{25} + 84.9^{\circ}$ (c 0.25, CHCl₈). PI-083 is soluble in methanol, ethanol, ethyl acetate and chloroform but insoluble in water. The UV spectrum of PI-083

showed bands at $\lambda_{\rm max}$ nm (E¹⁸_{1cm}) 218 (300), 317 (43), 425 (55) in MeOH and 217 (600), 285 (116), 390 (39), 540 (64) in 0.01 N NaOH - MeOH; IR $\nu_{\rm max}^{\rm GEO_1}$ cm⁻¹ 3450, 2950, 1729, 1700, 1640. The molecular formula was determined to be C₄₃H₅₀O₁₆ on the basis of secondary ion mass spectra (SI-MS, (M+Na)⁺ m/z 845) and elemental analysis (calcd for C₄₃H₅₀O₁₆: C 62.77, H 6.08, found: C 62.57, H 6.21).

¹⁸C NMR spectra of PI-083 are closely related to those of saquayamycin B¹³, except that a 207.6 ppm (s, C-4") is replaced by a 66.9 ppm (d, C-4") in PI-083 (Table 1). In ¹H NMR, the oxymethine signal at 3.40 ppm (1H, br d, *J*=10 Hz) due to 4"-H was observed and was shown to be equatorial through the change to a broad siglet upon the addition of D₂O. Furthermore, the connectivity from 1"-H to 6"-CH₃ can be easily traced in ¹H-¹H two-dimensional correlation spectroscopy (COSY) and homonuclear Hartmann-Hahn (HOHAHA) spectra. Based on the evidence presented above, the structure of PI-083 was determined to be as shown in Fig. 1.

The inhibitory activity against the platelet aggregation was measured by nephelometric

Table 1. NMR spectral data of the terminal sugar moiety in PI-083.

Position	$\delta_{ ext{H}}$	$\delta_{ m C}$
1''	4.91 (1H, br s)	89.3
2''	3.85 (1H, br s)	71.9
3''	2.08 (2H, m)	29.5
4"	3.40 (1H, br d, $J=10$ Hz)	66.9
4''-OH	3.75 (1H, d, J=10 Hz)	
5''	4.30 (1H, br q, $J=7$ Hz)	77.1
6''	1.26 (3H, d, $J=7$ Hz)	15.6

Spectra were taken in CDCl₃.

Fig. 1. Structure of PI-083.

Table 2. Antimicrobial activity of PI-083.

Organisms	MIC (μg/ml)
Staphylococcus aureus 209P-JC	0.39
S. epidermidis IID 866	1.56
Enterococcus faecium ATCC 8043	3.13
Bacillus cereus S 1101	12.5
B. subtilis ATCC 6633	1.56
Escherichia coli NIHJ JC-2	>100
Klebsiella pneumoniae IFO 3317	>100
Pseudomonas aeruginosa NCTC 10490	> 100

The conventional agar dilution method was used. The medium was Mueller-Hinton agar.

technique²⁾. The mixture of PI-083 and platelet rich plasma (PRP) which was prepared by the centrifugation of rabbit blood was incubated with stirring at 37°C for 3 minutes and then aggregating agent was added. Platelet aggregation was recorded by the change of the light transmittance. The inhibitory activity (IC₅₀) of PI-083, using ADP, collagen and arachidonic acid as an aggregating agent, was 30.4, 3.8 and 1.9 μ M respectively. PI-083 exhibited strong antimicrobial activity against Gram-positive bacteria, but was inactive against Gram-negative bacteria (Table

2). Furthermore, PI-083 inhibited the growth of KB cells in vitro (IC₅₀, 0.026 μ M).

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References

- UCHIDA, T.; M. IMOTO, Y. WATANABE, K. MIURA, T. DOBASHI, N. MATSUDA, T. SAWA, H. NAGANAWA, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Saquayamycins, new aquayamycin-group antibiotics. J. Antibiotics 38: 1171~1181, 1985
- Born, G. V. R. & M. J. Cross: The aggregation of blood platelets. J. Physiol. 168: 178~195, 1963