NEW PLATELET AGGREGATION INHIBITORS

Akira KAWASHIMA,* Yūko KISHIMURA, Masaharu TAMAI and Kazunori HANADA

Research Center, Taisho Pharmaceutical Co., Ltd., Ohmiya, Saitama 330, Japan

From a cultured broth of Streptomyces matensis A-6621, we isolated three new platelet aggregation inhibitors designated as PI-080, PI-085 and PI-087. The structures of these compounds were established by spectral and chemical methods.

KEYWORDS platelet aggregation inhibitor; benzanthraquinone antibiotic; microbial product; Streptomyces

In a previous paper[1] we reported the isolation of a new platelet aggregation inhibitor, PI-083, from a cultured broth of Streptomyces matensis A-6621. As a result of further investigation, three new principles which demonstrated the inhibitory activities against the platelet aggregation were isolated. Their structure determination and antiplatelet activities are described in this communication.

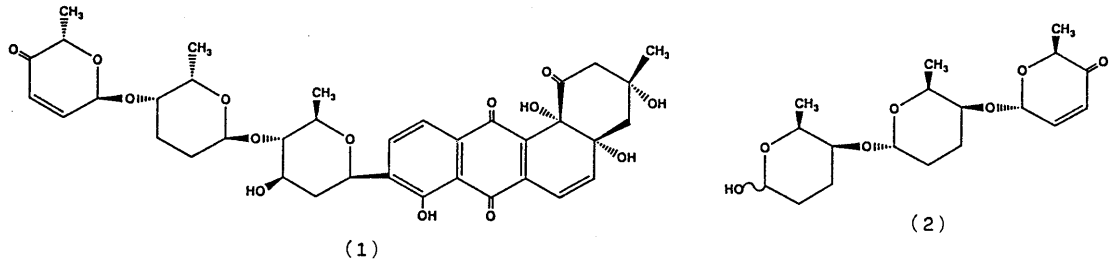
The fermentation was carried out in a 5-liter jar-fermentor containing 3 liters of medium (oat meal 4%, meat extract 0.3%, NaCl 0.3%, $CaCO_3$ 0.3%, $Fe_2(SO_4)_3$ 0.04%, $MnCl_2$ 0.04% pH 7) at 30 °C under agitation (500 rpm) and aeration (3 liters/min) for 4 days. The cultured broth (20l) was centrifuged to separate the mycelia and filtrate. The mycelia was extracted with 5 liters of MeOH and concentrated *in vacuo*. The residue was extracted twice with EtOAc (3l) and concentrated to dryness. The brown syrup thus obtained was applied on silica gel column chromatography and developed with chloroform-methanol 99:1-98:2. The fractions containing the antibiotics were collected for rechromatography by reverse phase HPLC to give pure PI-080 (36 mg), PI-085 (41 mg) and PI-087 (36 mg).

Table I. Physicochemical Properties of PI-080, PI-085 and PI-087

		PI-080	PI-085	PI-087
Nature		Red powder	Red powder	Red powder
$[\alpha]_D^{25}$ ($CHCl_3$)		+21.0° (c0.5)	+35.0° (c0.25)	+26.0° (c0.25)
mp.(°C)		191-193	162-164	165-167
Elemental analysis	found	C: 62.68	C: 62.77	C: 62.69
		H: 6.51	H: 6.40	H: 6.33
	Calcd	C: 62.98	C: 62.96	C: 62.96
		H: 6.49	H: 6.21	H: 6.21
SIMS (m/z)		1071 (M+Na)	957 (M+Na)	957 (M+Na)
Formula		$C_{55}H_{68}O_{20}$	$C_{49}H_{58}O_{18}$	$C_{49}H_{58}O_{18}$
UV λ_{max}^{nm} ($E_{1cm}^{1\%}$)	MeOH	212 (380)	230 (320)	230 (224)
		318 (30)	315 (48)	318 (48)
		430 (35)	425 (60)	425 (55)
	MeOH-NaOH	220 (260)	225 (300)	232 (238)
		280 (145)	285 (138)	290 (125)
		385 (30)	390 (34)	390 (40)
		530 (45)	540 (50)	545 (58)

Physicochemical properties are summarized in Table I. These data strongly suggest that all compounds belong to the modified benzanthraquinone antibiotics group, including the vineomycin A_1 and saquayamycins previously reported by Ohta et al.[2] and Uchida et al,[3] respectively.

Comparison of ^1H- and ^{13}C-NMR spectral data clearly indicates that PI-080, PI-085 and PI-087 are closely related to vineomycin A_1, saquayamycin B and saquayamycin A respectively, except for the sugar moiety. To identify the sugar moiety we hydrolized PI-080, PI-085 and PI-087 according to the method of Uchida et al.[3] Mild acid hydrolysis of PI-080 with acetonitrile-0.2% phosphoric acid (60:40) at room temperature for 15 min gave a red pigment (**1**) and colorless oil (**2**). (**1**) was also obtained through the hydrolysis of vineomycin A_1 under the same experimental conditions as above and had characteristic UV absorption maxima at 234, 315 and 425 nm; and FABMS showed (M+H) at m/z 711. The structure of (**1**) as shown in Fig. 1 was established by NMR spectral analysis.



(1) (2)

Fig. 1. Structures of (**1**) and (**2**)

(**2**) was found to be identical to SEN366Do[4] by comparing ^{13}C-NMR data. The connective site of this trisaccharide proved to be C-3 in (**1**), judging from the change of the chemical shift between PI-080 (δ_C 82.4) and (**1**) (δ_C 76.0). The coupling constant of the

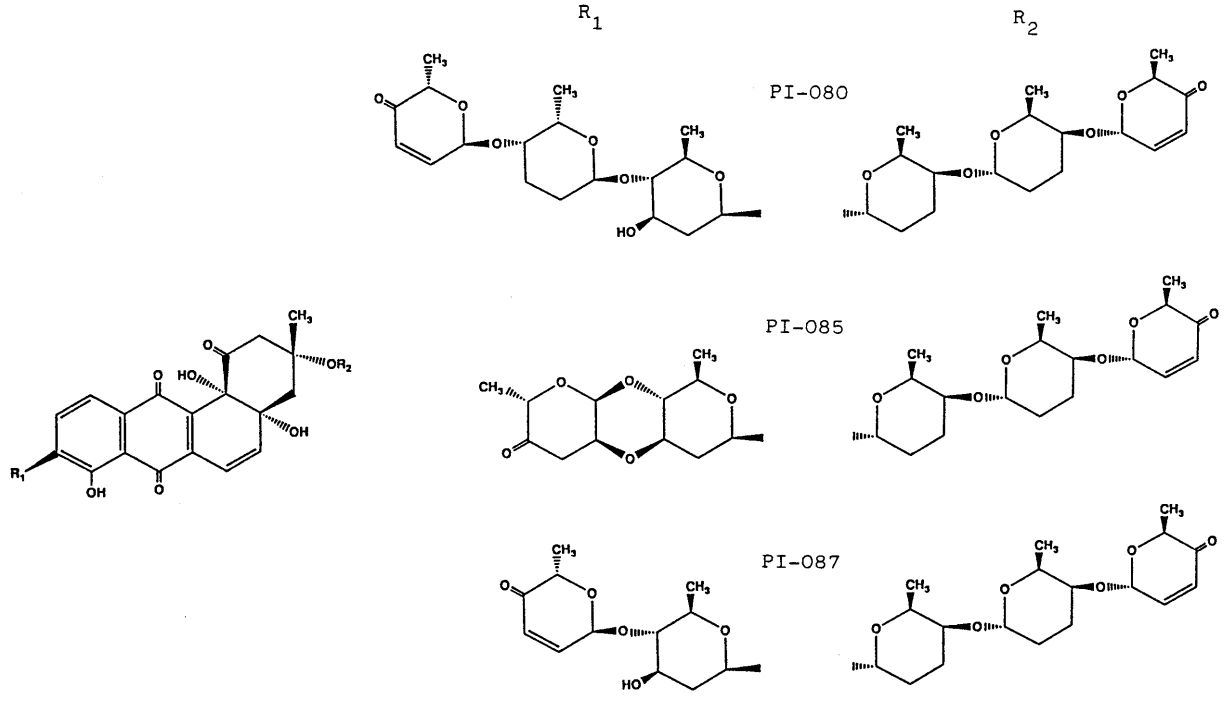


Fig. 2. Structures of PI-080, PI-085 and PI-087

anomeric proton (J=3 Hz) and the direct C-H coupling constant at C-1' (J_{C-H}=166 Hz) showed that the glycosidic linkage was α. Based on these analyses the structure of PI-080 was determined to be as shown in Fig. 2. Hydrolysis of PI-085 and PI-087 revealed that the former consisted of saquayamycin B_1 and (**2**) and the later saquqyamycin B_2 and the same saccharide. NMR spectral data established the mode of the glycoside linkage of (**2**) and its position. Thus, the structures of PI-085 and PI-087 were established, as shown in Fig. 2.

PI-080, PI-085 and PI-087 strongly inhibited rabbit platelet aggregation induced by ADP, collagen or arachidonic acid (Table II). The inhibitory activities were superior to those of OM-4842[5] which also belonged to the benzanthraquinone antibiotic group. Taking these results into account, it appeared that the sugar moiety played an important role in the antiplatelet activities.

Antimicrobial and antitumor activities are presently under investigation.

Table II. Inhibitory Activities of PI-080, PI-085 and PI-087 against Rabbit Platelet Aggregation

Sample	IC_{50} value (μg/ml) (*in vitro*)		
	ADP	Collagen	Arachidonic acid
PI-080	12.5	1.56	6.25
PI-085	3.13	3.13	3.13
PI-087	3.13	3.13	3.13

REFERENCES

1) A. Kawashima, Y. Yoshimura, J. Goto, S. Nakaike, T. Mizutani, K. Hanada and S. Omura, J. Antibiot., 41, 1913 (1988).
2) K. Ohta, E. Mizuta, H. Okazaki and T. Kishi, Chem. Pharm. Bull. 32, 4350 (1984).
3) T. Uchida, M. Imoto, Y. Watanabe, K. Miura, T. Daboshi, N. Matsuda, T. Sawa, H. Nakagawa, M. Hamada, T. Takeuchi and H. Umezawa, J. Antibiot., 38, 1171 (1985).
4) N. Kojima, K. Konno, T. Seto, H. Yamashita, Y. Ezure, T. Ozaki, Y. Yoshikuni, M. Sugiyama, T. Yasuda and T. Nakamura, Abstract of Papers, the 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, Apr. 1987.
5) S. Ōmura, A. Nakagawa, N. Fukąmachi, S. Miura, Y. Takahashi, K. Komiyama and B. Kobayashi J. Antibiot., 41, 812 (1988).

(Received October 9, 1989)