PYRIDINDOLOL, A NEW β-GALACTOSIDASE INHIBITOR PRODUCED BY ACTINOMYCETES

Sir:

As resported in previous papers, we have succeeded in finding protease inhibitors in culture filtrates of actinomycetes[1,2]. We also screened culture filtrates of various microorganisms for activity to inhibit the other hydrolytic enzymes.[3] In this communication, characterization of pyridindolol which inhibits β-galactosidase in acidic conditions is reported. Although inhibitory activity against acidic β-galactosidase was found in culture filtrates from various species of actinomycetes, one strain (MD401-C5) was chosen and used as the source for further production and isolation. The strain MD401-C5 was isolated from a soil sample collected in Minamisaku, Nagano Prefecture and classified as *Streptomyces alboverticillatus*.

In order to apply the method described by DAHLQVIST *et al.*[4] to measure anti-β-galactosidase activity of culture filtrates, it was modified as follows: β-galactosidase (EC3.2.1.23) from bovine liver was purchased from Sigma Chemical Co., in U.S.A.; to 0.05 ml of 0.05 M *p*-nitrophenyl-β-D-galactopyranoside (B.D.H. Chemical Ltd. England) in 0.05 M citrate-phosphate buffer (pH 4.2) was added 0.425 ml of the same buffer with or without a test material; after 3 minutes at 37°C, 0.025 ml of the enzyme solution (25 μg of β-galactosidase in the same buffer) was added and the reaction mixture incubated for 30 minutes at 37°C; after the incubation, 2 ml of 0.4 M glycine-sodium hydroxide buffer (pH 10.5) was added, and the optical density of liberated *p*-nitrophenol was measured at 400 nm. The reaction was also carried out in the reaction mixture without the enzyme solution to obtain the blank value. The concentration of the inhibitor for 50 % inhibition (ID_{50}) was calculated as described in a previous paper[5].

Pyridindolol was produced by shaking culture or tank fermentation of the strain MD401-C5 in media containing various kinds to carbon sources and nitrogen sources. For example it was produced in a medium containing 2 % glycerol, 1.5 % corn steep liquor, 0.3 % NaCl, 0.1 % K_2HPO_4, 0.1 % $MgSO_4 \cdot 7H_2O$, 0.0007 % $CuSO_4 \cdot 5H_2O$, 0.0001 % $FeSO_4 \cdot 7H_2O$, 0.0008 % $MnCl_2 \cdot 4H_2O$, and 0.0002 % $ZnSO_4 \cdot 7H_2O$ adjusted to pH 7.2 with 2 N NaOH. The maximum production was attained after 48～72 hours on a shaking machine or 48 hours in the tank fermentation and maintained for 1～2 days thereafter.

Pyridindolol in a culture filtrate was adsorbed on active carbon and eluted with 0.05 N HCl in 50 % acetone. The active eluate adjusted to pH 6.5 was evaporated under reduced pressure and the residue was extracted with ethyl acetate. The brownish oily residue thus obtained was dissolved in methanol and purified by Sephadex LH-20 column chromatography with methanol. The active fractions were combined and evaporated under reduced pressure and the residue was extracted with methanol. A pale yellowish powder thus obtained was recrystallized from methanol - ethyl acetate (1:1). The purified pyridindolol showed 50 % inhibition of β-galactosidase at 2.0 μg/ml in acidic conditions.

Pyridindolol is obtained as colorless needles, m.p. 167～168°C, $[\alpha]_D^{25}$ −49° (*c* 1.0, MeOH). It has the molecular formula $C_{14}H_{14}O_3N_2$ (M.W. 258.27). Calcd.: C 65.10, H 5.46, O 18.59, N 10.85. Found: C 65.52, H 5.56, O 18.51, N 11.15. This molecular formula is confirmed by mass spectrometry of pyridindolol and its triacetyl derivative. Found: *m/e* 258.0977 and 384.1356 Calcd.: 258.1003 and 384.1320. The UV spectrum of pyridindolol is shifted to longer waves in acidic solution (Fig. 1). The IR spectrum is shown in Fig. 2.

It is soluble in methanol, ethanol, *n*-butanol, dimethylsulfoxide, N,N-dimethyl formamide, 0.5 N hydrochloric acid, slightly soluble in water and insoluble in benzene, chloroform, ethyl ether and petroleum ether. It gives the following Rf values on silica gel thin-layer chromatography: 0.40 with *n*-butanol - *n*-butyl acetate - acetic acid - water (4:2:1:1), 0.08 with ethyl acetate - methanol (95:5). UV light (3650 Å) irradiation gives a yellowish blue fluorescence spot and the iodine vapor gives a brown spot on the silica gel thin-layer chromatography. It moves toward the cathode in formic acid - acetic acid - water (25:75:900) under 3,500 V electrophoresis for 15 minutes with an Rm value of 0.8 taking alanine as

Fig. 1. Ultraviolet absorption spectra of pyridindolol.

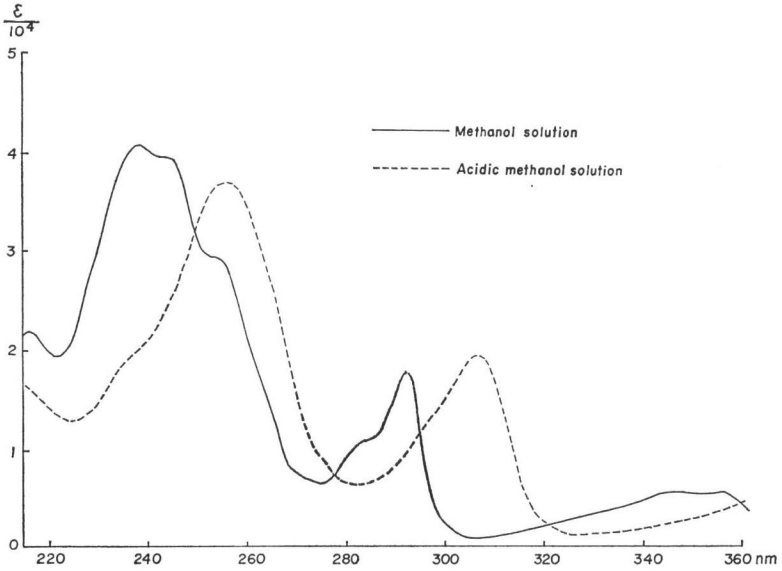

Fig. 2. Infrared spectrum of pyridindolol in KBr disk.

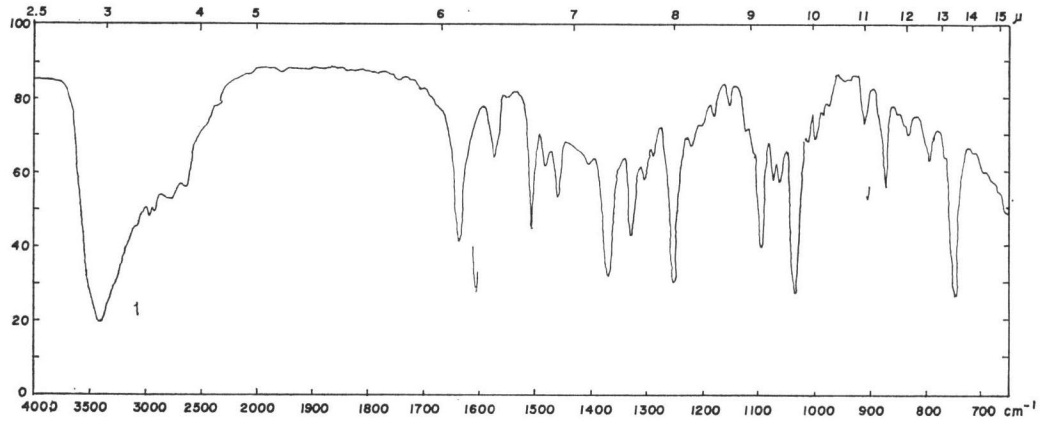

Table 1. Inhibitory activity of pyridindolol on various glucosidase.

Inhibitor	ID_{50} (μg/ml)				
	Sialidase			β-galactosidase	
	Cl. perfringens	*Streptomyces*	A/Aichi/2/68	Bovine liver	
				pH 4.2	pH 7.0
Pyridindolol	>250	>250	>250	2.0	>250

The method of purification of sialidase from *Cl. perfringens*, *Streptomyces* and A/Aichi/2/68 (H3N2) strain of influenza virus, and the assay of its activity were carried out as described previously[6,7].

1.0 It shows pKá values 5.3 in 50 % methanol (titration equivalent 258). The structure of pyridindolol was determined as 1-[1(R), 2-dihydroxyethyl]-3-hydroxymethyl-9H-pyrido[3, 4-*b*] indole. The structure study will be reported in the next paper.

The activities of pyridindolol against β-galactosidase in acidic and neutral conditions and other glucosidases are shown in Table 1. The methods employed for testing these activities have been described in previous papers[6-8]. The results shown in Table 1 indicate that pyridindolol is a specific inhibitor of β-galactosidase in acidic conditions. Pyridindolol at 100 μg/ml showed no antibacterial and no antifungal activity. It has low toxicity and the intraperitoneal injection of 500 mg/kg to mice caused no death.

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