Communication to the editor

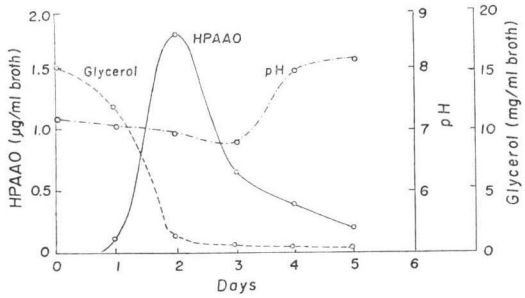
p-HYDROXYPHENYLACETALDOXIME, AN INHIBITOR OF *β*-GALACTOSIDASE, PRODUCED BY ACTINOMYCETES

Sir:

As reported previously, such inhibitors of glycosidases as panosialin, siastatin, pyridindolol and isoflavone rhamnosides have been found in culture filtrates of actinomycetes. Panosialin[1,2] and siastatin[3] inhibit sialidase, pyridindolol[4~6] and isoflavone rhamnosides[7] inhibit *β*-galactosidase. *β*-Galactosidase (EC 3.2.1.23) is a glycosidase, hydrolysing *β*-galactoside bond in glycoproteins, glycolipids and polysaccharides to yield terminal galactose. This enzyme is widely distributed among microorganisms, plants and animal tissues but its biological significance is still uncertain. In this communication, we describe the isolation and identification of *p*-hydroxyphenylacetaldoxime (HPAAO) which inhibits *β*-galactosidase.

HPAAO was found in the culture filtrate of *Streptomyces nigellus* MD824-CG2 which was isolated from a soil sample collected in Saitama Prefecture, Japan, in 1973. In order to obtain quantitative values of anti-*β*-galactosidase activity, the method of DAHLQVIST *et al.*[8] modified as follows was used: to 0.05 ml of 0.05 M *p*-nitrophenyl *β*-D-galactopyranoside (B.D.H. Chemical Ltd., England) in 0.05 M phosphate buffer (pH 7.0), 0.4 ml of the same buffer and 0.04 ml of water with or without a test material were added. After 3 minutes at 37°C, 0.01 ml of *β*-galactosidase from bovine liver in the same buffer (1 mg/ml, Sigma Chemical Co., U.S.A.) was added and the reaction mixture was incubated for 15 minutes at 37°C. At the end of the reaction, 2 ml of 0.4 M glycine-sodium hydroxide buffer (pH 10.5) was added, and the amount of liberated *p*-nitrophenol was measured at 400 nm. The same reaction was also carried out in the absence of the enzyme solution to obtain the blank value. The concentration of the inhibitor at 50% inhibition was calculated as described in a previous paper[9].

Fig. 1. Production of *p*-hydroxyphenylacetaldoxime by MD824-CG2



HPAAO was produced by shaking culture of the strain MD824-CG2 in a media containing 1.5% glycerol, 1.5% cotton seed meal, 0.3% NaCl and 0.2% L-asparagine, and pH of the medium was adjusted to 7.2 with 2 N sodium hydroxide. The time course of production of HPAAO, pH and residual glycerol in the medium are shown in Fig. 1. The purification procedures are shown in Fig. 2.

HPAAO was crystallized as colorless needles from chloroform. It melts at 110~111°C. It is soluble in methanol, acetone, ethyl acetate and ethyl ether, less soluble in water and chloroform, and almost insoluble in petroleum ether and *n*-hexane. The elemental analysis was as follows: Calcd. for $C_8H_9NO_2$: C 63.56, H 6.00, N 9.27, O 21.17; found C 63.34, H 5.88, N 9.50, O 21.17.

Fig. 2. Isolation and purification of HPAAO from MD824-CG2

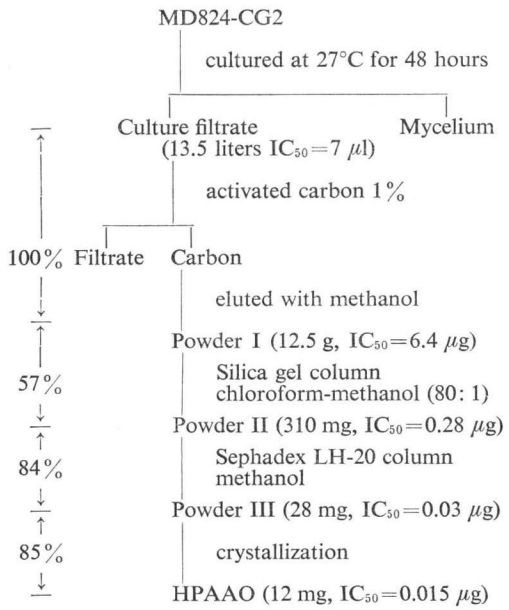
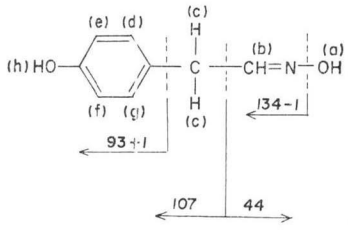


Fig. 3. The results of NMR and MS spectra of HPAAO



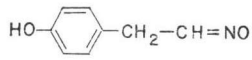
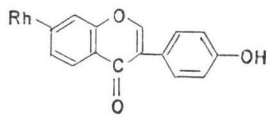
	anti-	syn-
(a)	8.15	9.63
(b)	6.78	7.41
(c)	3.59	3.38
(d), (g)	7.08	7.08
(e), (f)	6.78	6.78
(h)	10.05	10.05
J_{bc}	5.5 Hz	6.5 Hz

It gave positive cupric chloride (for oxime) and GIBBS reagent (for phenol) reactions and negative ninhydrin and RYDON-SMITH reactions. The UV spectrum in 95% methanol showed maxima at 226 (log ε 3.89) and 279 (3.29) nm and they shifted in alkaline to 243 (3.99) and 297 (3.40) nm. The ^{1}H-NMR spectrum of HPAAO in deuterochloroform showed complicated spectrum due to the mixture of stereoisomers. The results of ^{1}H-NMR and high resolution MS spectra are shown in Fig. 3. The structure of HPAAO was confirmed by the comparison of their IR, NMR, MS, TLC and activity with the synthetic one which was derived from tyramine according to the procedure by KAHR *et al.*[10] In the ^{1}H-NMR spectrum, the intensities of methylene signals at δ3.59 and 3.38 indicated that the ratio of the *anti* and *syn* isomers was 7: 3.

KINDL *et al.*[11] reported that the same compound was isolated from the root of a plant (*Sinapia alba*) as a biosynthetic intermediate of a glycoside, sinalbin. However, it is interesting to note that this compound was obtained from the culture filtrate of the Actinomycetes.

The activities of HPAAO, pyridindolol and isoflavone rhamnoside to inhibit β-galactosidase are shown in Table 1. The results shown in Table 1 indicate that HPAAO is a potent inhibitor of β-galactosidase. It did not show any inhibition against sialidase. HPAAO at 100 μg/ml showed no antibacterial and no antifungal activities. It had low toxicity and did not cause death by intraperitoneal injection of 500 mg/kg to mice. The relationships between the structure of the oxime and the inhibitory activity against β-galactosidase will be reported in the next paper.

Table 1. The inhibitory activity of HPAAO, pyridindolol and isoflavone rhamnoside against β-galactosidase

Compound	Structure	Inhibitory activity (IC_{50} μg/ml) pH4.2	pH7.0
HPAAO		0.06	0.015
Pyridindolol		2.0	>250
Isoflavone rhamnoside		14.0	3.6

Acknowledgements

This work was partly supported by a contract research fund from the Division of Cancer Treatment, the National Cancer Institute, U.S.A., NO1-CM-57009 and a grant-in-aid for Cancer Research (51–11) from the Ministry of Health and Welfare, Japan.

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(Received May 27, 1978)

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