THIAZOCINS, NEW ALDOSE REDUCTASE INHIBITORS FROM *Actinosynnema* sp.

1. FERMENTATION, ISOLATION AND CHARACTERIZATION

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New antibiotics designated as thiazocins A and B were isolated from the culture broth of *Actinosynnema* sp. C-304. Thiazocins A and B exhibited inhibitory activities against an aldose reductase from human placenta.

An aldose reductase is the enzyme that catalyzes transformation of glucose to sorbitol. This enzyme is extensively present in mammalian organisms[1]. The enzyme plays important rules to the etiology of diabetes sequelae, for example, cataract[2,3] and neuropathy[4]. Aldose reductase inhibitors could repress the abnormal rise of sorbitol in various organs of diabetes patients. So aldose reductase inhibitors are noted in pharmacology as drugs for prevention of diabetes sequelae. In the course of screening to find aldose reductase inhibitors we found new antibiotics, thiazocins A and B, from the culture broth of *Actinosynnema* sp. C-304. In this article we report on the fermentation, isolation, physico-chemical and biological properties of thiazocins A and B.

Materials and Methods

Chemicals

Chemicals employed were as follows; Packed column of ODS from Yamamura Scientific Co., Ltd., Kyoto, Japan. Kiesel gel 60 and TLC plate, Silica gel 60 F_{254} (0.25 mm thickness) from E. Merck, Darmstadt, FRG. All other chemicals were of analytical grade.

Enzyme and Assay

An aldose reductase was prepared from human placenta by the method of BENDICHT and VON WARTBURG[5]. The determination of its activity was essentially conducted as described by HAYMAN and KINOSHITA[6]. The substrate used was DL-glyceraldehyde and the activity was expressed as the rate of OD 340 nm due to utilization of NADPH in a reaction shown in the equation below:

$$\text{Glyceraldehyde} + \text{NADPH} \longrightarrow \text{glycerol} + \text{NADP}$$

Fermentation

Spores of the strain of *Actinosynnema* sp. C-304 were inoculated into 60 ml of seed mediums consisted of glucose 1.0%, potato starch 2.0%, yeast extract 0.5%, Polypepton 0.5%, $CaCO_3$ 0.4% (pH 7.0) in 500-ml Erlenmeyer flasks and cultured at 28°C for 48 hours on a rotary shaker (200 rpm). The seed cultures (1,200 ml) were transferred into production medium (the same with a seed medium) in jar fermenters. Fermentation was carried out at 27°C for 90 hours with aeration (60 liters/minute) and agitation (100 rpm).

Fig. 1. Isolation of thiazocins A and B.

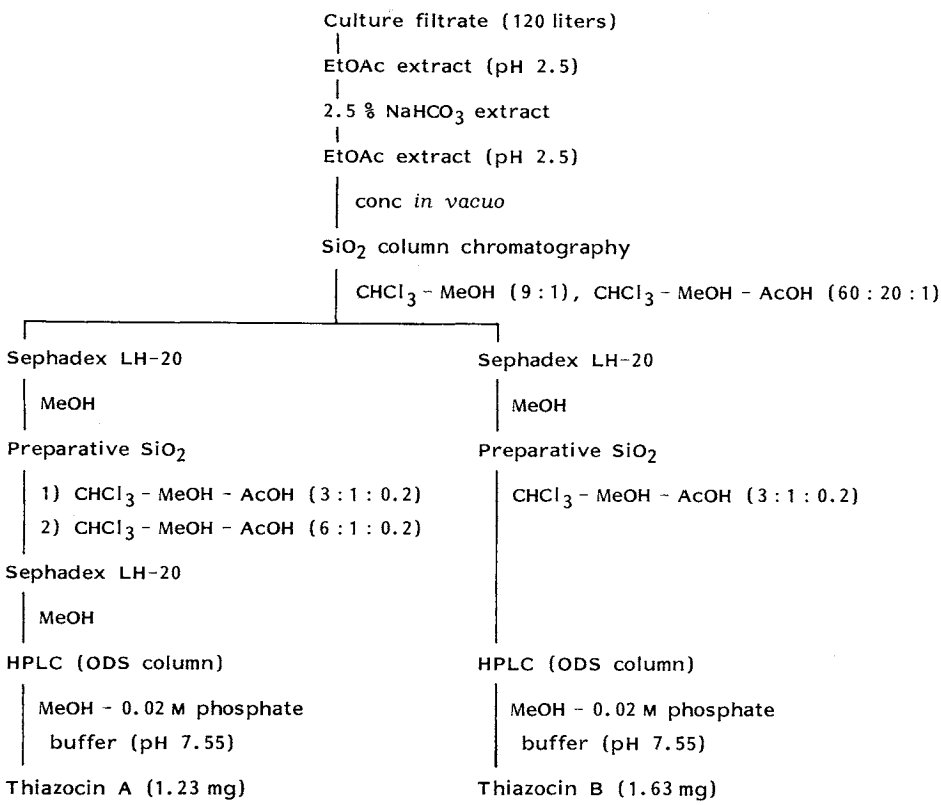


Table 1. Physico-chemical properties of thiazocins A and B.

Property	A	B
Nature	White powder	White powder
MP (°C)	181	196
Molecular formula	$C_9H_7NO_3S$	$C_{18}H_{17}N_3O_6S_2$
FAB-MS	209	435
IR (KBr) cm^{-1}	1710, 1630	1710, 1630
Rf[a]		
Solvent 1	0.66	0.28
Solvent 2	0.36	—
Solvent 3	—	0.49

[a] Silica gel 60 F_{254} TLC plate (E. Merck, Art. No. 5715): Solvent 1; $CHCl_3$ - MeOH - AcOH (3 : 1 : 0.2), solvent 2; $CHCl_3$ - MeOH - AcOH (6 : 1 : 0.2), solvent 3; $CHCl_3$ - MeOH - AcOH (2 : 1 : 0.2).

Fig. 2. UV spectrum of thiazocin A (MeOH).

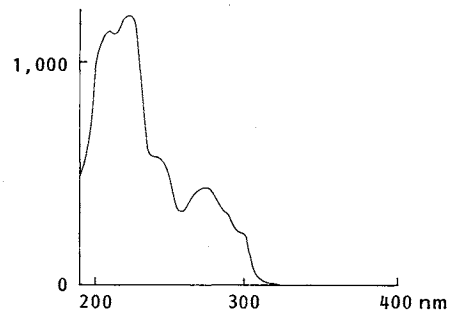
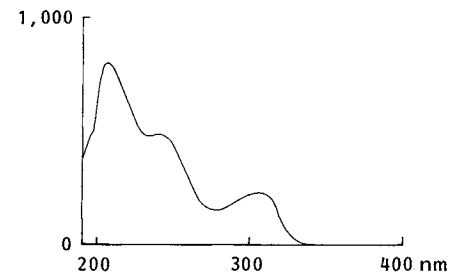


Fig. 3. UV spectrum of thiazocin B (MeOH).



Isolation

Isolation of thiazocins was carried out as outlined in Fig. 1. The antibiotics in the culture fluid (120 liters) were extracted with ethyl acetate (60 liters × 2) at pH 2.5, transferred to water layer (2.5%

$NaHCO_3$) after concentration and again extracted with ethyl acetate at pH 2.5. The ethyl acetate extract was concentrated *in vacuo* to give a brown residue. The residue was chromatographed on a column of silica gel (Wakogel C-200, Wako Pure Chemical Industries, Ltd.) using $CHCl_3$-MeOH (9:1) followed by $CHCl_3$-MeOH-acetic acid (60:20:1) as eluants. Active fractions were collected and concentrated to give crude components of thiazocins A (840 mg) and B (300 mg), respectively. The crude thiazocin A was dissolved in a small amount of methanol and subjected to a column chromatography on Sephadex LH-20

Fig. 4. IR spectrum of thiazocin A (KBr).

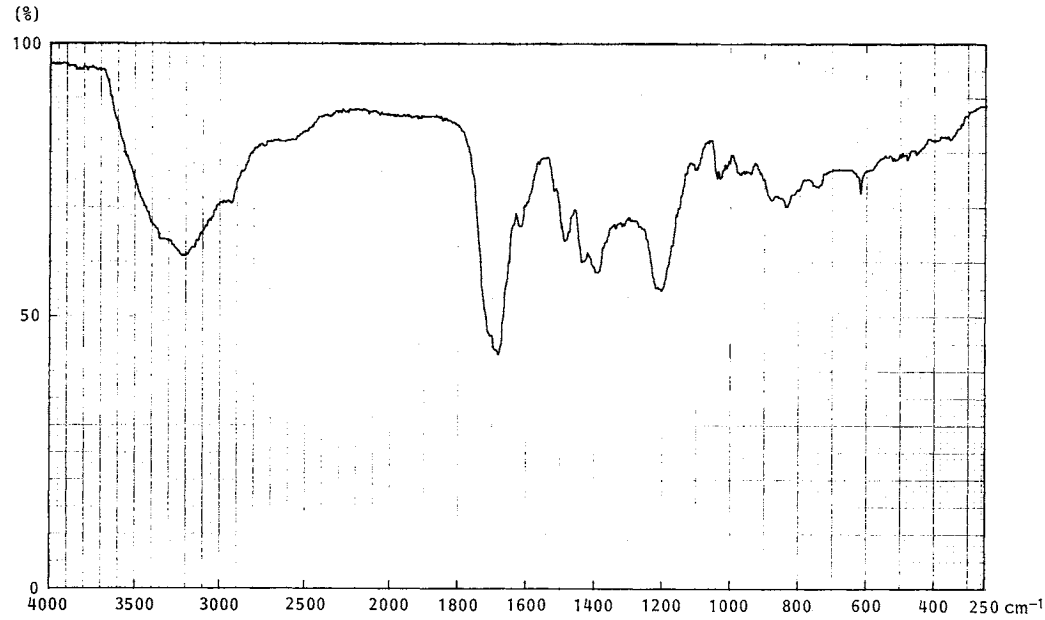
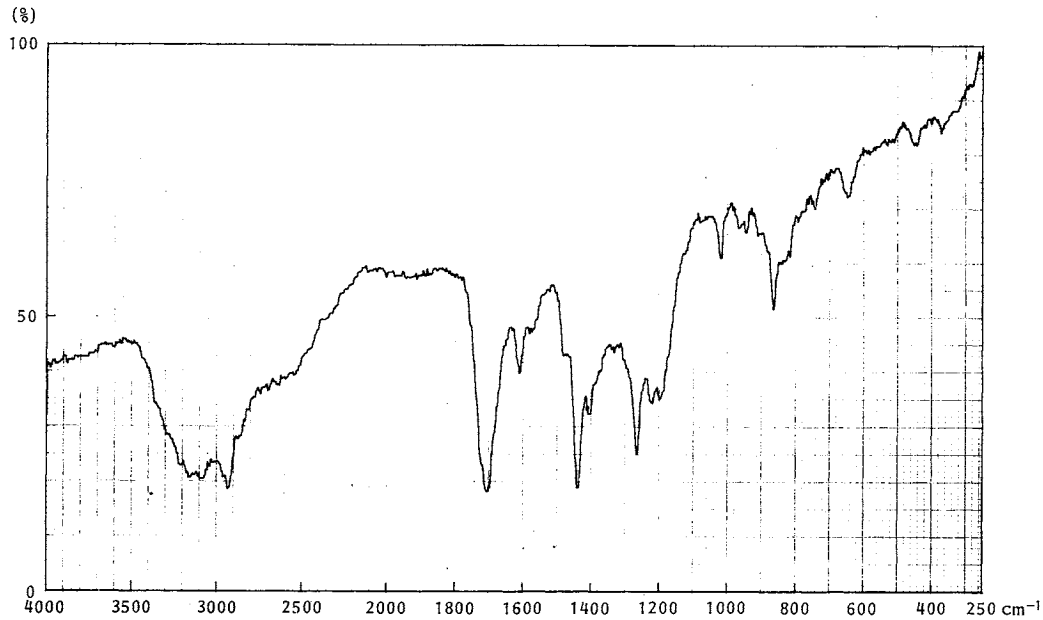


Fig. 5. IR spectrum of thiazocin B (KBr).



(Pharmacia) using methanol as an eluent. The active fractions were again chromatographed on a preparative silica gel plate (Kiesel gel 60 F_{254}) using $CHCl_3$ - MeOH - acetic acid (3 : 1 : 0.2) and furthermore active fractions were subjected to chromatographies on a preparative silica gel (Kiesel gel 60 F_{254}) ($CHCl_3$ - MeOH - acetic acid (6 : 1 : 0.2)) and a column of Sephadex LH-20 (MeOH). The active fractions were chromatographed on HPLC (ODS column; 4.6 i.d. × 250 mm, Yamamura Chemical Laboratories Co.,

Fig. 6. ^1H NMR spectrum of thiazocin A (CD_3OD).

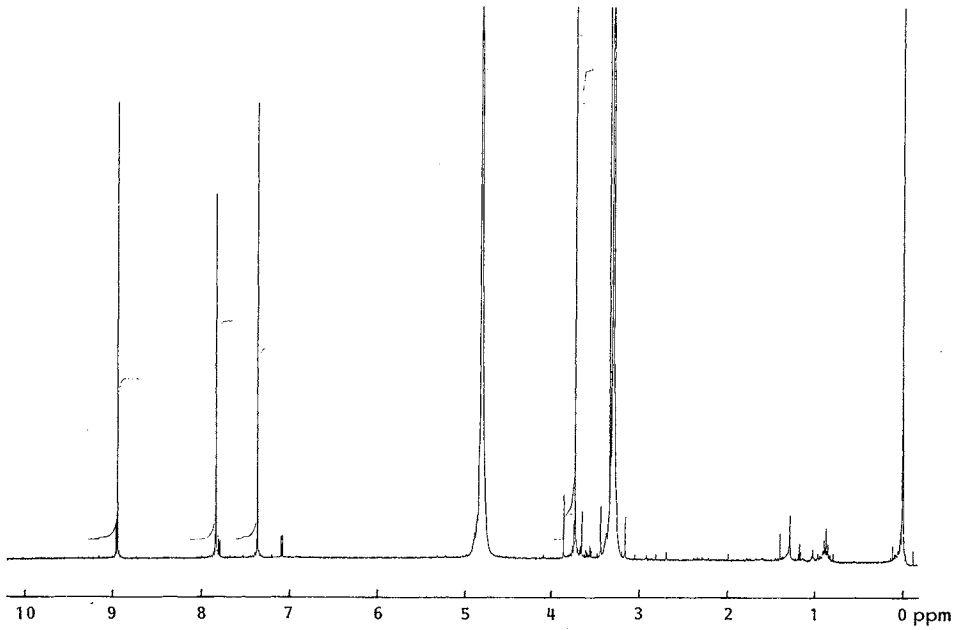
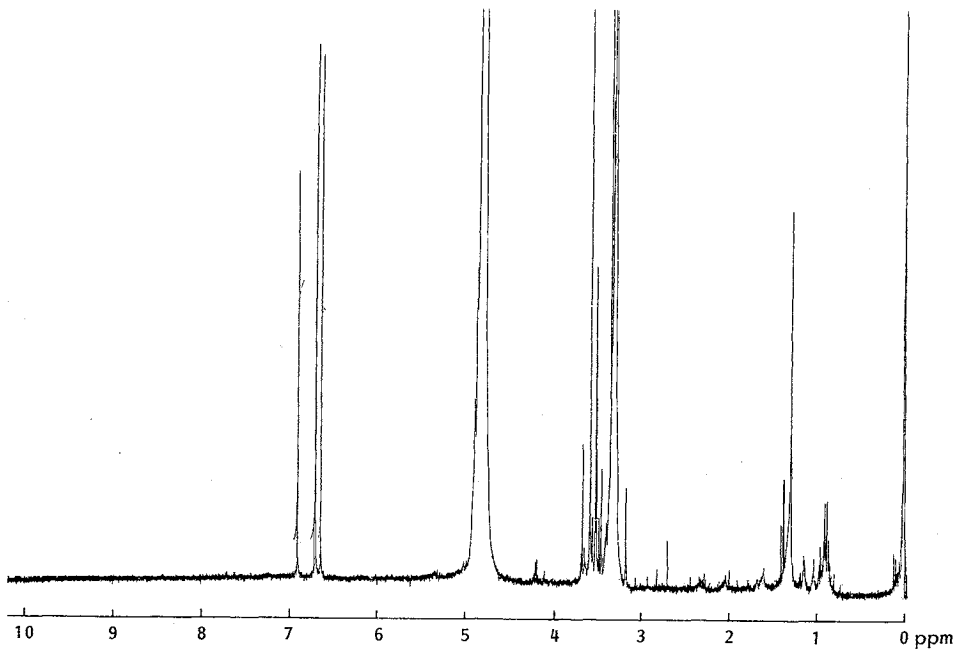


Fig. 7. ^1H NMR spectrum of thiazocin B (CD_3OD).



Ltd.) using MeOH - 0.02 M phosphate buffer (pH 7.55) to give thiazocin A (1.23 mg) as a white powder. By the almost same purification method, thiazocin B (1.63 mg) was obtained as a white powder.

Physico-chemical Properties

The physico-chemical properties of thiazocins A and B were summarized in Table 1. Thiazocins A and B were acidic, respectively. These were soluble in alkaline water, methanol and acetone, but were

Fig. 8. ^{13}C NMR spectrum of thiazocin A (CD_3OD).

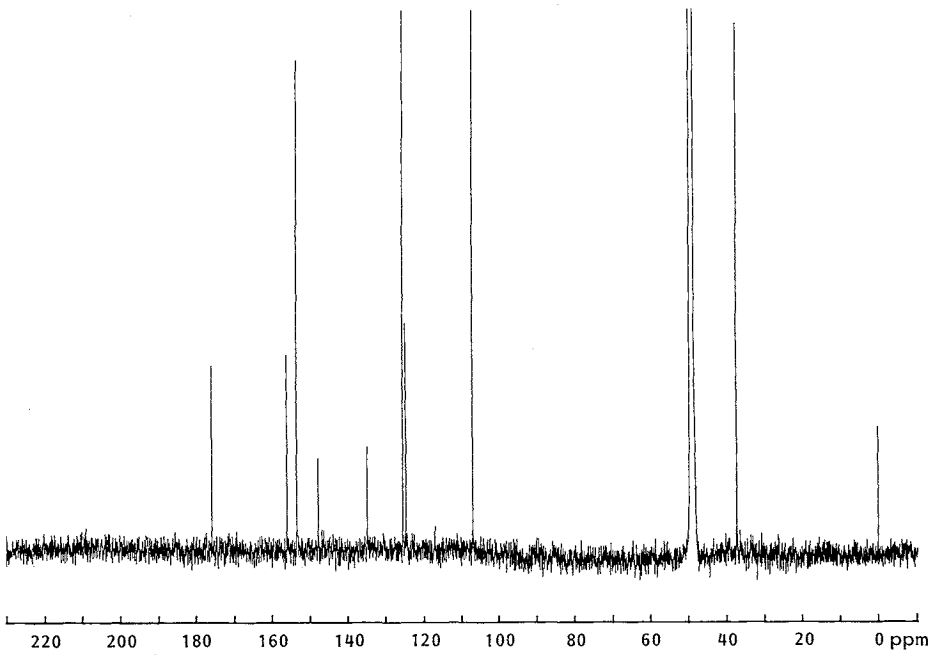
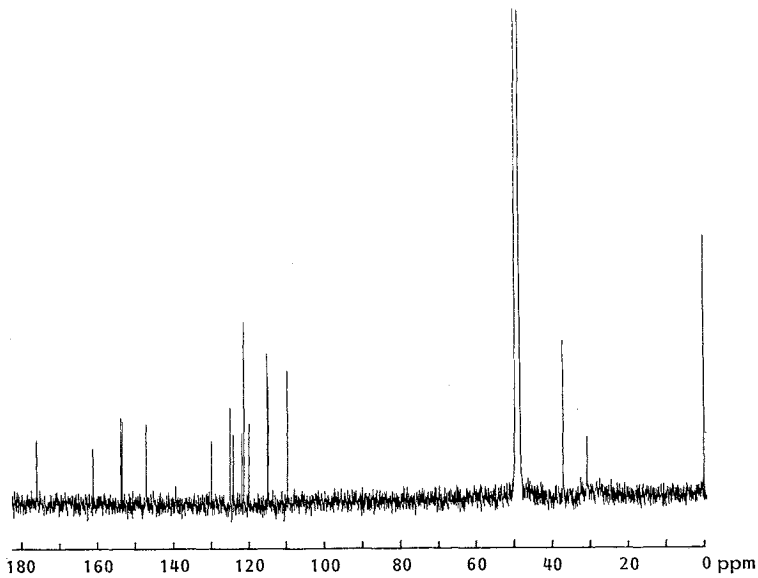


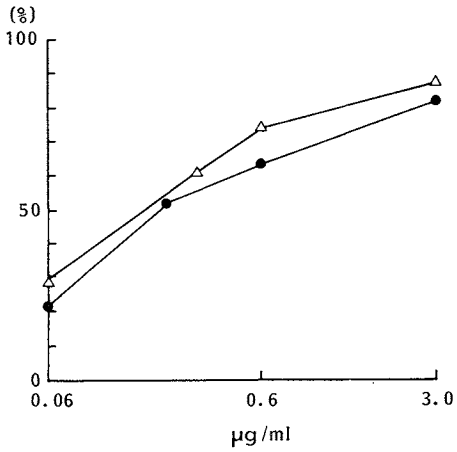
Fig. 9. ^{13}C NMR spectrum of thiazocin B (CD_3OD).



almost insoluble in water. Thiazocins A and B gave positive responses to ferric chloride, sulfuric acid, but negative responses to ninhydrin and Sakaguchi reagents. UV spectrum of thiazocins A and B was shown in Figs. 2 and 3. IR spectrum, ^{1}H NMR and ^{13}C NMR spectrum of thiazocins A and B were shown in Figs. 4, 5, 6, 7, 8 and 9. The molecular formula of thiazocins A and B was determined to be $C_9H_7NO_3S$ and $C_{18}H_{17}N_3O_6S_2$, respectively, by the MS and ^{13}C NMR spectrum. From physicochemical properties shown in Table 1, it was concluded that thiazocins A and B were new antibiotics. An aldose reductase inhibitor from microorganisms is reported by Nishikawa *et al.*[7]. Details of the structure elucidation of thiazocin A will be reported elsewhere.

Fig. 10. Aldose reductase inhibitor activities of thiazocins A and B.

● Thiazocin A, △ thiazocin B.



Biological Properties

The inhibitory activities of thiazocins A and B against an aldose reductase were shown in Fig. 10. IC_{50} of thiazocins A and B was 4.55×10^{-7} M and 2.20×10^{-7} M, respectively. These antibiotics were not effective to Gram-positive and Gram-negative bacteria and fungi.

Acknowledgments

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