Communications to the Editor

THIAZOSTATIN A AND THIAZOSTATIN B, NEW ANTIOXIDANTS PRODUCED BY *STREPTOMYCES TOLUROSUS*

Sir:

During screening for antioxidative substances, which are expected to be useful for chemotherapy of arteriosclerosis and ischemia, we have isolated new potent substances thiazostatins A and B from a culture of an actinomycete, *Streptomyces tolurosus* 1368-MT1, which was isolated from a soil sample collected at a field in Yamaguchi-city, Japan. Thiazostatin was formerly called AG 55[1] and its producing organism has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, with an accession No. of FERM P-9144. The fermentation, isolation, structure determination and biological properties of thiazostatin are described in the present communication.

The antioxidant activity was measured by inhibitory effect against erythrocyte hemolysis caused by a radical producing agent, 2,2'-azobis-(amidinopropane)dihydrochloride (AAPH)[2].

The fermentation medium consisted of sucrose 20 g, molasses 10 g, casein 5 g, Polypeptone 1 g, and $CaCO_3$ 4 g in 1 liter water and the pH was adjusted to 7.2. The fermentation was carried out in a 50-liter fermenter containing 30 liters of the medium at 27°C for 4 days.

After removal of the mycelium, the supernatant was applied to a column of Diaion HP-20. The column was washed with 50% MeOH and active materials were eluted with MeOH. The eluate was concentrated *in vacuo* and extracted with EtOAc at pH 2. The extract was evaporated to dryness and subjected to silica gel column chromatography using $CHCl_3$-MeOH (100:1). The combined active fractions were evaporated to dryness. The residue was rechromatographed on a silica gel column with $CHCl_3$-EtOAc (1:1) to yield 120 mg of thiazostatin A and 75 mg of thiazostatin B. Thiazostatins A and B, obtained as pale yellow powders, showed the properties as summarized in Table 1.

The molecular formula of thiazostatin A was determined to be $C_{15}H_{18}N_2O_3S_2$ by MS (fast atom

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

	Thiazostatin A		Thiazostatin B	
Appearance	Pale yellow needles		Pale yellow needles	
MP (°C, dec)	69~72		75~78	
$[\alpha]_D^{25}$ (*c* 1, MeOH)	+29.1°		−80.7°	
Molecular formula	$C_{15}H_{18}N_2O_3S_2$		$C_{15}H_{18}N_2O_3S_2$	
MW	338		338	
FAB-MS *m/z* $(M+H)^+$	339		339	
Analysis	Calcd:	Found:	Calcd:	Found:
C	53.25	53.18	53.25	53.67
H	5.33	5.55	5.33	5.84
N	8.28	8.28	8.28	7.92
O	14.20	14.63	14.20	14.51
S	18.93	18.62	18.93	18.06
UV λ_{max}^{MeOH} nm (ε)				
0.01 N HCl-MeOH	211 (18,700), 270 (11,800), 341 (4,700)		209 (18,000), 270 (11,700), 340 (4,600)	
0.01 N NaOH-MeOH	213 (23,800), 248 (12,200), 312 (5,400)		210 (24,300), 250 (12,800), 312 (5,300)	
IR ν_{max}^{KBr} cm^{-1}	3400, 1705, 1620, 1590, 1480, 1220		3400, 1720, 1620, 1590, 1480, 1220	
Rf value[a]	0.71		0.58	
Soluble	MeOH, EtOH, acetone, DMSO, EtOAc, $CHCl_3$		MeOH, EtOH, acetone, DMSO, EtOAc, $CHCl_3$	
Insoluble	H_2O, hexane		H_2O, hexane	

[a] Solvent system: $CHCl_3$-MeOH (10:1), Kieselgel 60 F_{254}.

Table 2. The 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR spectral data of thiazostatins A and B[a].

Position	Thiazostatin A		Thiazostatin B	
	δ_H	δ_C	δ_H	δ_C
1		117.2		117.5
2		159.7		160.1
3	6.92 (dd, 0.9, 8.5)[b]	117.5	6.93 (dd, 0.9, 8.5)	117.8
4	7.35 (ddd, 1.5, 7.8, 8.5)	133.6	7.35 (ddd, 1.6, 7.8, 8.5)	134.1
5	6.88 (ddd, 0.9, 7.8, 7.8)	119.6	6.88 (ddd, 0.9, 7.8, 7.8)	120.0
6	7.39 (dd, 1.5, 7.8)	131.0	7.41 (dd, 1.6, 7.8)	131.6
2′		172.7		174.0
4′	5.14 (dt, 3.2, 9.1)	80.8	5.23 (dt, 3.4, 9.0)	80.0
5′	3.28 (dd, 9.1, 11.0), 3.54 (dd, 9.1, 11.0)	33.6	3.41 (dd, 9.0, 11.2), 3.53 (dd, 9.1, 11.2)	32.4
2″	4.68 (d, 3.2)	74.3	4.68 (d, 3.4)	74.7
4″		74.8		74.7
5″	2.85 (d, 10.6), 3.17 (d, 10.6)	40.4	2.79 (d, 12.0), 3.34 (d, 12.0)	40.4
6″		176.5		176.5
7″	2.63 (s, CH_3)	34.4	2.50 (s, CH_3)	35.5
8″	1.50 (s, CH_3)	23.1	1.45 (s, CH_3)	15.0

[a] Taken in CD_3OD. [b] Coupling constants in J=Hz.

bombardment (FAB)-MS, m/z 339 $(M+H)^+$) and elemental analysis. Treatment of thiazostatin A in CH_2Cl_2 with ethereal diazomethane gave a monomethyl ester ($C_{16}H_{20}N_2O_3S_2$, field desorption (FD)-MS, m/z 352 $(M)^+$, δ_H 3.74 (OCH_3)) suggesting the presence of one carboxylic acid residue.

The ^{1}H NMR spectrum of thiazostatin A in CD_3OD showed 16 proton signals (Table 2) and suggested the presence of the following partial structures; $CHCHCH_2$, CCH_3, NCH_3 and *ortho*-substituted benzene. In addition, the ^{1}H NMR spectrum taken in DMSO-d_6 revealed a phenolic hydroxy proton at 12.9 ppm. It should be noted here that all the oxygen atoms of thiazostatin A have been exhausted to form a carboxylic acid and a phenolic hydroxy function.

The ^{13}C NMR spectrum of thiazostatin A showed 15 carbon signals, which were attributed to two methyls (23.1 and 34.4 ppm), two methylenes (33.6 and 40.4 ppm), two methines (74.3 and 80.8 ppm), one quaternary sp^3 carbon (74.8 ppm), six aromatic carbons (117.2~159.7 ppm), and two sp^2 carbons (172.7 and 176.5 ppm) linked to heteroatoms (Table 2). Since one of these sp^2 carbons is due to a carboxylic acid (*vide supra*), and since thiazostatin A possessing one phenolic hydroxy group contains only three oxygens, the remaining sp^2 carbon must be combined to nitrogen (and sulfur).

Heteronuclear multiple bond correlation (HMBC)[3] experiments on thiazostatin A proved the long range coupling of 6-H (aromatic proton) to C-2′ (172.7 ppm) (Fig. 1). Since this carbon could not be the carboxylic acid, the remaining sp^2 carbon at 176.5 ppm was assigned to the carboxylic acid by elimination. In addition, hydroperoxide oxidation of thiazostatin A gave salicylamide, suggesting that C-2′ must be combined to two heteroatoms (which must be nitrogen or sulfur) at least with one being nitrogen. The HMBC experiment also proved the long range couplings of 4′-H (CH) and 5′-H (CH_2) to C-2′ as shown in Fig. 1.

Since the C-5′ signal was observed at a higher

Fig. 1. Partial structure of thiazostatin A.

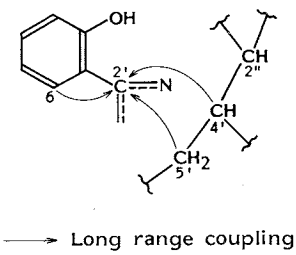


Fig. 2. The total structure of thiazostatin A.

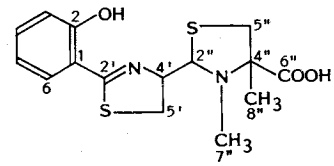
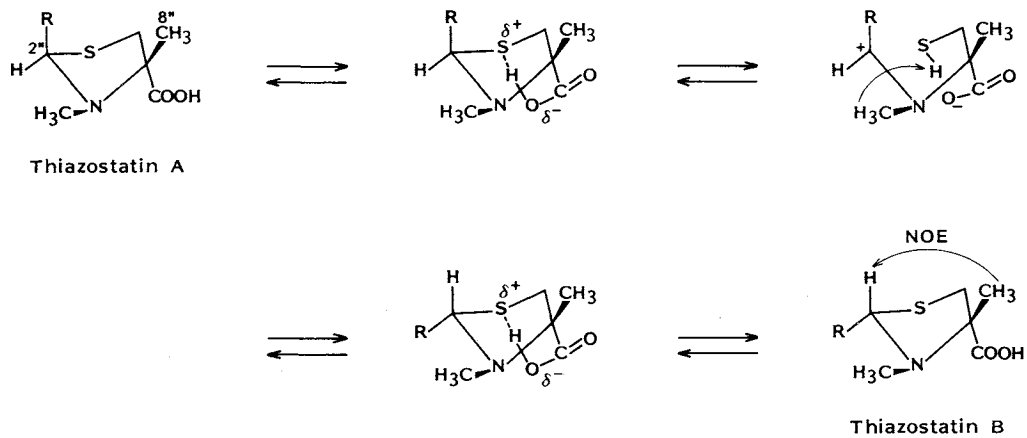


Fig. 3. Plausible reaction mechanism explaining interconversion of thiazostatins A and B.



field (33.6 ppm), and since 5′-H must be located within three bonds from C-2′, it was reasonably assumed that C-5′ was linked to C-2′ through a sulfur atom. The chemical shift of C-4′ (80.8 ppm) required it to be combined to nitrogen resulting in the formation of a thiazoline ring containing C-2′, C-4′ and C-5′. This ring structure was supported by comparison with model compounds[4].

The HMBC experiment also proved the long range couplings of 8″-H (CH_3), 5″-H (CH_2) to C-6″ (carboxylic acid) and to C-2″ or C-4″. The almost identical chemical shifts of the last two carbons precluded to identify the carbon coupled to 8″-H and 5″-H. However, since 8″-H and 5″-H were not coupled to each other, C-5″, C-8″ and C-6″ must be connected *via* the only remaining quatenary carbon C-4″.

Long range selective proton decoupling (LSPD) experiments on thiazostatin A† proved long range couplings of 7″-H (NCH_3) to C-2″ and C-4″. Since the chemical shifts of C-2″ and C-4″ are 74.3 and 74.8 ppm, respectively, it is reasonable to assume that nitrogen is located between C-2″ and C-4″. Furthermore, the LSPD experiments proved long range coupling of 5″-H to C-2″. Since sulfur is the only remaining heteroatom, it must be present between C-2″ and C-5″. Thus the planar structure of thiazostatin A has been determined as shown in Fig. 2. Thiazostatin is a 4″-demethyl derivative of pyochelin[5,6], which was isolated as an iron chelator[7] from *Pseudomonas aeruginosa*.

The molecular formula of thiazostatin B was determined to be the same as that of thiazostatin A by FAB-MS and elemental analysis (Table 1). Thiazostatins A and B were interchangeable to each other in MeOH at 50°C (*ca.* 1 : 1 mixture within 30 minutes). Nuclear Overhauser effect (NOE) observed between 8″-CH_3 and 2″-H with thiazostatin B, but not with thiazostatin A suggested that they were stereoisomers at C-2″ or C-4″ to each other. Although no confirmatory evidence is in our hand, it is presently assumed that thiazostatin B is the stereoisomer of thiazostatin A at C-2″ based on the mechanism explaining the interconversion between them (Fig. 3). This mechanism may be supported by an experimental result that the interconversion did not take place with methyl esters of thiazostatins.

Thiazostatins A and B inhibited erythrocyte hemolysis caused by AAPH. Fifty % inhibitory concentration (IC_{50}) values of thiazostatins A and B were 3 μM and slightly less active than vitamin E (IC_{50} 1 μM). Tested so far, thiazostatins A and B showed no antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi at 100 μg/ml.

Acknowledgments

We thank Prof. K. L. RINEHART for a sample of pyochelin. We also thank Dr. M. SAKAKIBARA of Kirin Brewery Co. for his helpful suggestions. This work was supported in part by a Grant-in-Aid for Cancer Research, The Ministry of Education, Science and Culture, Japan.

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† In the LSPD spectrum of thiazostatin, C-2″ and C-4″ were observed as well separated signals, since C-2″ was split to a doublet due to the direct coupling with 2″-H.

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(Received June 15, 1989)

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