Communication to the Editor

HARZIANIC ACID, A NEW ANTIMICROBIAL ANTIBIOTIC FROM A FUNGUS

Sir:

In the course of our screening for antibiotics, we isolated a new antibiotic, harzianic acid (**1**) from a culture filtrate of a strain *Trichoderma harzianum*, SY-307, which was isolated from a water sample collected at Hiroshima Pref. Japan. The antibiotic shows antimicrobial activity against *Pasteurella piscicida* sp. 6395. In this communication, we report the production, isolation, physico-chemical properties, biological properties and structure of **1**.

The producing strain, SY-307 was inoculated into 500-ml Sakaguchi flasks each containing 125 ml of a producing-medium composed of malt extract (Difco) 1%, yeast extract (Difco) 1%, Polypepton (Nippon Seiyaku) 0.1% and glucose 2% (adjusted to pH 5.5 before sterilization). The fermentation was carried out at 25°C for 5 days on a reciprocating shaker.

1 was extracted with EtOAc at pH 2.0 from culture filtrate (4.5 liters, pH 6.0), and transferred to H_2O (3 liters) at pH 8.0 and reextracted with EtOAc at pH 2.0. The extract which was concentrated and dried under reduced pressure was chromatographed on a Sephadex LH-20 column using MeOH as a solvent. After rechromatography on a Sephadex LH-20 column, centrifugal partition chromatography (Sanki Engineering Limited, CPC Model NMF) using a solvent system of *n*-heptane - acetonitrile - MeOH - AcOH (4:1:1:1, v/v) gave pure 80 mg of **1** as orange powder. The antibiotic activity was assayed by a cylinder diffusion method using *Pasteurella piscicida* sp. 6395 as a test organism. The physico-chemical properties of **1** are shown in Table 1.

The molecular formula of **1** was determined as $C_{19}H_{27}NO_6$ by HRFAB-MS and elemental analysis.

In acidic methanol solution, **1** showed UV absorption maxima at 244 and 360 nm ($\log\varepsilon$ 3.96 and 4.42). In basic solution, the maxima occured at 263, 287 and 337 nm ($\log\varepsilon$ 4.15, 4.19 and 4.22). This UV spectral behavior of **1** is essentially the same as that of streptolydigin[1], indicating the presence of the dienoyltetramic acid chromophore found in the tirandamycin-streptolydigin type antibiotics[2].

The ^1H NMR in CD_3OD exhibited a doublet at 7.09 ppm (2-H, $J=15$ Hz), an olefinic proton at 7.53 ppm (3-H) and overlapped signals centered at 6.39 ppm (4-H and 5-H). In the ^1H-^1H COSY spectrum, the olefin signal at 6.39 ppm connected to a methylene at 2.23 ppm (6-H_2). The methylene (6-H_2) was adjacent to a methylene at 1.50 ppm (7-H_2), which was coupled to a methyl signal (8-H_3)

Table 1. Physico-chemical properties of harzianic acid (**1**).

Appearance	Orange powder
Molecular formula	$C_{19}H_{27}NO_6$
Elemental analysis	Calcd for $C_{19}H_{27}NO_6\cdot\frac{1}{2}H_2O$: C 60.95, H 7.54, N 3.74
	Found: C 60.54, H 7.01, N 3.87
FAB-MS $[m/z\ (M+H)]^+$	366
HRFAB-MS (m/z) Calcd:	366.1916 ($C_{19}H_{28}NO_6$)
Found:	366.1922
$[\alpha]_D^{25}$	+19.6° (c 1.06, MeOH)
UV λ_{max} nm ($\log\varepsilon$)	
in MeOH:	244 (4.04), 299 (sh, 4.02), 343 (sh, 4.37), 359 (4.44), 376 (sh, 4.37), 398 (sh, 4.02)
0.01 N HCl - 90% MeOH:	244 (3.96), 314 (sh, 4.07), 346 (sh, 4.35), 360 (4.42), 377 (sh, 437), 398 (sh, 4.03)
0.01 N NaOH - 90% MeOH:	263 (4.15), 287 (4.19), 337 (4.22)
IR ν_{max}^{KBr} cm^{-1}:	3440, 2970, 1720 (br), 1620, 1570, 1470, 1455, 1410, 1265, 1180, 1042, 1003, 890, 780, 720
TLC (Rf value)	0.32
Silica gel TLC (Merck Art No. 5715)	$CHCl_3$ - MeOH - H_2O (2:1:0.2)
Solubility	Soluble in MeOH, EtOAc, acetonitrile and acetone Slightly soluble in toluene and *n*-hexane

Fig. 1. Summary of HMBC spectrum of harzianic acid.

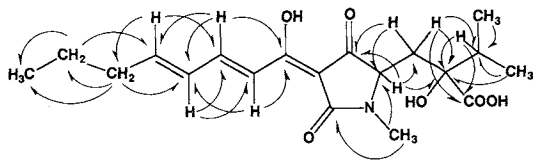

Fig. 2. Structure of harzianic acid.

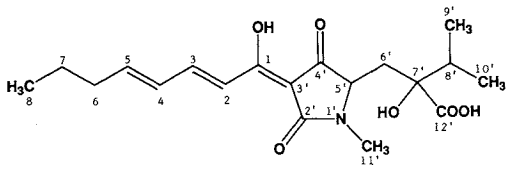

Table 2. NMR data of harzianic acid in CD_3OD.

Position	^{13}C[a]	Multi	^{1}H[b]	Multi	J (Hz)
1	175.9	s	—	—	—
2	120.4	d	7.09	d	15.0
3	147.2	d	7.53	m	—
4	131.0	d	6.39	m	—
5	149.7	d	6.39	m	—
6	36.4	t	2.23	dt	6.0, 6.0
7	22.9	t	1.50	m	—
8	14.0	q	0.95	t	7.3
2′	173.9	br s	—	—	—
3′	100.9	s	—	—	—
4′	198.9	br s	—	—	—
5′	65.5	d	3.80	dd	2.0, 9.0
6′	36.4	t	1.99	dd	9.0, 14.3
			2.33	dd	2.0, 14.3
7′	79.8	s	—	—	—
8′	37.4	d	2.02	m	—
9′	17.5	q	0.97	d	6.8
10′	16.5	q	0.94	d	6.8
11′	27.1	q	2.94	s	—
12′	178.6	s	—	—	—

[a] 100 MHz.
[b] 400 MHz.

at 0.95 ppm. Other information from the ^{1}H-^{1}H COSY spectrum was as follows: a methylene at 1.99 and 2.33 ppm (6′-H_2) connected to a methine at 3.80 ppm (5′-H), and an isopropyl group at 0.97 and 0.94 ppm (9′- and 10′-H_3) coupled with a methine at 2.02 ppm (8′-H).

The analysis of the ^{13}C NMR spectrum of **1** suggested that **1** was a tetramic acid derivative and **1** had a carboxyl group (178.6 ppm). The connectivities among moieties above described were determined by the analysis of the HMBC spectrum of **1** (Fig. 1).

Thus, the structure of **1** was proposed as shown in Fig. 2. The ^{1}H NMR spectrum of **1** in C_5D_5N indicated that the geometries of the double bonds were 2*E* and 4*E* on the basis of coupling constants (2-H, 7.71 ppm, d, J=15 Hz, 3-H, 7.60 ppm, dd, J=11 and 15 Hz, 4-H, 6.22 ppm, dd, J=11 and 15 Hz, 5-H, 6.06 ppm, td, J=8 and 15 Hz). However, other stereochemical assignments remain to be determined.

The ^{1}H and ^{13}C NMR data of **1** are shown in Table 2. **1** is related to streptolydigin by virtue of its mode of substitution in the tetramic acid moiety.

The antimicrobial activity of **1** was weak. MICs determined by agar dilution method was 12.5 and 25.0 μg/ml against *Pasteurella piscidida* sp. 6395 (1/3 BHI agar +2% NaCl, 27°C, 23.5 hours) and *Proteus mirabilis* IFM OM-9 (Mueller Hinton agar, 37°C, 17 hours), respectively.

The acute toxicity of **1** in mice (ip) was >150 mg/kg.

RYUICHI SAWA
YUMI MORI
HIRONOBU IINUMA
HIROSHI NAGANAWA
MASA HAMADA
SUSUMU YOSHIDA†
HIROAKI FURUTANI†
YOSHIO KAJIMURA†
TOHRU FUWA†
TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan
† Wakunaga Pharmaceutical Co., Ltd.,
1624 Shimokotachi, Koda-cho, Takata-gun,
Hiroshima 729-64, Japan

(Received February 2, 1994)

References

1) RINEHART, Jr. K. L.; J. R. BECK, D. B. BORDERS, T. H. KINSTLE & D. KRAUSS: Streptolydigin. III. Chromophore and structure. J. Am. Chem. Soc. 85: 4038~4039, 1963
2) LEE, V. J. & K. L. RINEHART, Jr.: ^{13}C NMR spectra of streptolydigin, tirandamycin, and related degradation products. J. Antibiotics 33: 408~415, 1980
3) BRILL, G. M.; J. B. MCALPINE & D. WHITTERN: Tirandalydigin, a novel tetramic acid of the tirandamycin-streptolydigin type. II. Isolation and structural characterization. J. Antibiotics 41: 36~44, 1988