NOTES

A New Cytotoxic Phenylthiazoline, 4-Methylaeruginoic Acid, from *Streptomyces* sp. KCTC 9303

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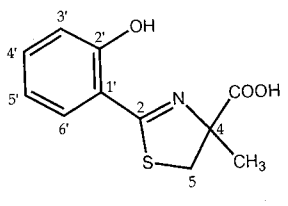
In the course of our screening for new antitumor antibiotics from microbial metabolites, a new cytotoxic 2-*o*-hydroxyphenylthiazoline derivative, 4-methylaeruginoic acid (**1**) (Fig. 1), was isolated from the culture broth of *Streptomyces* sp. KCTC 9303. Several members of phenylthiazoline group have been isolated from microbial metabolites. They include aeruginoic acid[1], pyochelin[2], (+)-(*S*)-dihydroaeruginoic acid[3], and several 2-phenylthiazole derivatives[4] from *Pseudomonas*, and thiazostatins A, B[5] and ferrithiocin[6] from *Streptomyces*. These compounds have been known as iron-chelating growth promoter[2], antibiotic[3] or antioxidant[5]. 4-Methylaeruginoic acid is an additional member of this group with antitumor antibiotic activity. This paper describes the production, isolation, structure elucidation and cytotoxicity against several human tumor cell lines of this compound.

The producing strain was identified as *Streptomyces* by means of ISP method[7] and has been deposited in Korean Collection for Type Cultures, KCTC 9303. The spore suspension of producing strain from agar slant culture was inoculated into 500-ml baffled Erlenmeyer flask containing 100 ml of culture medium (glucose 2%, soluble starch 1%, meat extract 0.1%, yeast extract 0.4%, soybean meal 2.5%, NaCl 0.2% and K_2HPO_4 0.005%, adjusted to pH 7.3 before autoclaving). The inoculated flask was shaken on a rotary shaker at 160 rpm for 48 hours at 28°C. The cultured seed (60 ml) was transferred to a 5-liter jar fermenter containing 3 liters of the culture medium. The fermentation was carried out for 72 hours at 28°C under the following condition; 200 rpm stirring speed and 20 liters/minute aeration rate.

Fig. 1. Structure of 4-methylaeruginoic acid (**1**).



The cultured whole broth was centrifuged at 6,000*g* and the supernatant (2.8 liters) was passed through a Diaion HP-20 (Nippon Rensui, Japan) column with increased proportion of MeOH. The 30% MeOH eluent was concentrated and the residue was partitioned between *n*-BuOH and water. The organic layer was purified by Sephadex LH-20 column chromatography (Pharmacia, 2.5 × 70 cm, 20% MeOH). Re-chromatography of an active fraction using MCI-gel (CHP 20P, Mitsubishi, 2 × 30 cm, 0 to 100% aqueous MeOH) afforded 8 mg of an active substance (**1**).

The physico-chemical properties of **1** are summarized in Table 1. The $[M]^+$ ion peak at *m/z* 237 and a relatively intense $[M+2]^+$ peak at *m/z* 239 in EI-MS indicated the presence of one nitrogen and one sulfur, respectively. The molecular formula of the compound was determined to be $C_{11}H_{11}NO_3S$ by molecular ion peak at *m/z* 237.0460 in HR-EI-MS, which was supported by ^{13}C and ^1H NMR data (Table 2). Its IR spectrum suggested the presence of hydroxyl (3200 cm^{-1}) and carbonyl (1737 cm^{-1}) groups.

All the ^1H and ^{13}C NMR signals were assigned as shown in Table 2. In the ^1H NMR, four resonances at δ 6.94 (H-3'), 7.35 (H-4'), 6.88 (H-5') and 7.44 (H-6') were characteristic to *ortho*-disubstituted benzene. Two doublet signals of isolated methylene protons were observed at δ 3.85 and 3.33 with typical geminal coupling,

Table 1. Physico-chemical properties of 4-methylaeruginoic acid (**1**).

Appearance	Yellow powder
Molecular formula	$C_{11}H_{11}NO_3S$
EI-MS (*m/z*)	237 (M^+)
HREI-MS (*m/z*)	
Found	237.0460
Calcd.	237.0459
UV λ_{max}^{MeOH} nm (ε)	215 (27,900), 250 (14,250), 312 (5,378)
IR υ_{max} (KBr) cm^{-1}	3300 (OH), 2980 (aliphatic CH), 1737 (C=O)
CD υ_{max}^{MeOH} nm (*c* 0.01)	$[\theta]_{206}$ 9326, $[\theta]_{218}$ 4268, $[\theta]_{260}$ -10058, $[\theta]_{280}$ -7416, $[\theta]_{321}$ 2631
Solubility	
Soluble	MeOH, EtOAc, Me_2CO, H_2O
Insoluble	*n*-hexane, $CHCl_3$
Rf value	0.2[a]

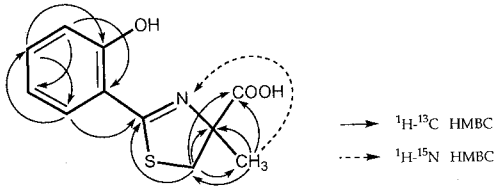
[a] Merck, Kieselgel 60 F_{254}; BuOH-MeOH-H_2O (15:1:1).

Table 2. ^{13}C and ^{1}H NMR data of 4-methylaeruginoic acid (**1**).[a]

Position	δ_C	δ_H
2	172.69 (s)	
4	84.85 (s)	
4-CH_3	24.68 (q)	1.65 (3H, s)
4-COOH	176.03 (s)	
5	40.69 (t)	3.85 (1H, d, 12.0)[b]
		3.33 (1H, d, 12.0)
1'	117.33 (s)	
2'	160.23 (s)	
3'	117.96 (d)	6.94 (1H, dd, 0.8, 8.3)
4'	134.46 (d)	7.35 (1H, ddd, 1.5, 8.0, 8.3)
5'	120.08 (d)	6.88 (1H, ddd, 0.8, 8.0, 8.0)
6'	131.49 (d)	7.44 (1H, dd, 1.5, 8.0)

[a] ^{13}C NMR (150 MHz) and ^{1}H NMR (600 MHz) were measured in CD_3OD.
[b] Proton number, multiplicity and coupling constants in Hz are given in parentheses

Fig. 2. PFG-HMBC data of 4-methylaeruginoic acid (**1**).



and a methyl singlet appeared at δ 1.65. In the ^{13}C NMR, four aromatic methines, one methylene at δ 40.69 and one methyl carbon at δ 24.68 were observed and the assignments were confirmed by pulse field gradient (PFG)-HMQC[8] spectrum. In the PFG-HMBC[8] spectral data (Fig. 2), several long range correlations were observed for five quaternary carbons at δ 84.85, 117.33, 160.23, 172.69 and 176.03. Two carbon signals at δ 117.3 (C-1') and 160.3 (C-2') were assigned to be carbons of phenyl group, and the chemical shift of the latter indicated the presence of a phenolic hydroxyl group. Other long range correlations in HMBC spectrum and comparison of NMR data with dihydroaeruginoic acid[3] and desferri-ferrithiocin[6] suggested the presence of 4-methyl-2-thiazoline-4-carboxylic acid as a partial structure. From the chemical shifts and HMBC data, the carbons at δ 172.69 and 176.03 were assigned to be C-2 of thiazoline ring and a carbonyl carbon of carboxylic acid, respectively. The sp^3 quaternary carbon at δ 84.85 (C-4 of thiazoline ring) correlated with the methyl and methylene protons in HMBC spectrum. ^{15}N NMR signal observed at δ 267.8 (NH_4NO_3 in DMSO-d_6 at 0 ppm for external reference) in ^{1}H-^{15}N PFG-HMBC spectrum (Fig. 3) through three bonds coupling from the methyl protons confirmed the 4-methylthiazoline structure. The fragment ion peaks at m/z 120 $[HOPh-CN+H]^+$ and 72 $[HOOC-C-CH_3]^+$ in EI-MS spectrum confirmed

Fig. 3. ^{1}H-^{15}N PFG-HMBC spectrum of 4-methylaeruginoic acid (**1**).

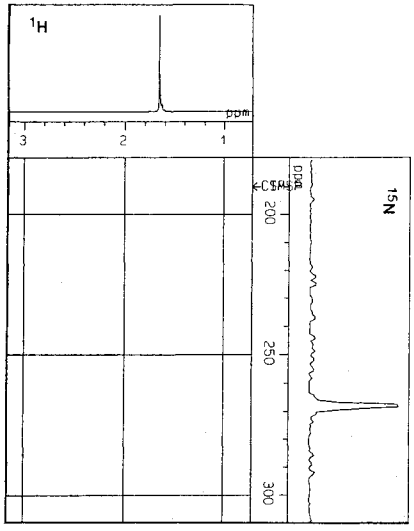


Table 3. Cytotoxicity (ED_{50}) of 4-methylaeruginoic acid (**1**) against human tumor cell lines.

	CRL 1579 (skin)	SNB-75 (CNS)[a]	MOLT-4F (leukemia)	NCI-H522 (lung)	PC-3 (prostate)
1	0.89[b]	0.02	0.10	8.68	0.04
Adriamycin	0.16	0.62	0.02	0.14	1.09

[a] Central nervous system.
[b] ED_{50}s are presented as $\mu g\ ml^{-1}$.

also the typical fragmentation patterns of a hydroxyphenylthiazoline skeleton.[10] From these spectral evidences, the structure of **1** has been determined as 2-(*o*-hydroxyphenyl)-4-methyl-2-thiazoline-4-carboxylic acid which is a 4-methyl derivative of aeruginoic acid.

The cytotoxicity of this compound against several human tumor cell lines was examined by SRB method[9] (Table 3). Compared with adriamycin, it showed higher cytotoxicity against SNB-75 and PC-3 with ED_{50}s of 0.02 and 0.04 $\mu g/ml$, respectively.

Acknowledgments

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