Aeruginol {2-(2'-Hydroxyphenyl)-4-hydroxymethylthiazole}, a New Secondary Metabolite from Pseudomonas aeruginosa

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AERUGINOL [2-(2′-HYDROXYPHENYL)-4-HYDROXYMETHYLTHIAZOLE], A NEW SECONDARY METABOLITE FROM *PSEUDOMONAS AERUGINOSA*

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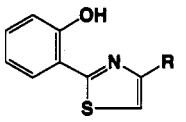
ABSTRACT.—The $CHCl_3$ extract of *Pseudomonas aeruginosa* UI 29791 cultures afforded a novel fluorescent compound, aeruginol [**1**]. The structure of **1** was elucidated by spectroscopic methods, including uv, eims, cims, hreims, and ^1H nmr. Aeruginol appears to be biosynthetically related to aeruginoic acid.

Cultures of *Pseudomonas aeruginosa* UI 29791 were grown using lard as an unusual carbon source to yield a new fluorescent metabolite, aeruginol [**1**], 2-(2′-hydroxyphenyl)-4-hydroxymethylthiazole. The structure assigned to aeruginol is related to 2-(2′-hydroxyphenyl)thiazole [**2**] and its 4-carboxaldehyde and 4-carboxylic acid (aeruginoic acid) derivatives, **3** and **4**, respectively, which have been isolated from other Pseudomonads (1–3).

A $CHCl_3$ extract of *P. aeruginosa* grown on lard as the sole source of carbon was separated by cc to yield a fraction which showed greenish yellow fluorescence by 366 nm, uv irradiation. This fraction was further purified by preparative Si gel GF_{254} tlc to yield an analytical sample for spectral analysis. The eims showed the parent peak $[M]^+$ *m/z* 207, and a fragmentation at *m/z* 189 $[M-H_2O]^+$ while cims gave $[MH]^+$ *m/z* 208. The hreims gave $[M]^+$ *m/z* 207.0355 for $C_{10}H_9O_2NS$ (calcd 207.0354). The uv spectrum exhibited a reversible blue shift upon 6 N NaOH addition, indicating the likely presence of a phenolic hydroxy group. The fluorescent nature of the compound obtained from *Pseudomonas fluorescens* and these properties suggested the relationship of the isolated metabolite to known aeruginoic acid derivatives. The ^1H-nmr spectrum ($CDCl_3$) exhibited one singlet at δ 7.17 for H-5; two doublets of doublets at δ 7.05 and δ 7.61 for protons H-6′ and H-3′; two doublets of doublets of δ 6.90 and δ 7.31 for protons H-4′ and H-5′; a singlet at δ 4.83 for-CH_2-; and a broad singlet for the hydroxyl group of CH_2OH at δ 5.34. These spectral properties are similar to those reported for aeruginoic acid [**4**] and related compounds **2** and **3**. The ^1H-nmr spectrum (DMSO) of aeruginoic acid [**4**] contains similar signals for H-5 at δ 8.43; for protons H-6′ and H-3′ δ 7.09 and δ 8.16; for protons H-4′ and H-5′ at δ 7.01 and δ 7.39; and at δ 11.36 for -COOH (3). The structure is further suggested by the apparent biosynthetic relationship of aeruginol, aeruginoic acid, and pyochelin, an endogenous growth promoter that solubilizes ferric iron (4). This is the first report of aeruginol from a *Pseudomonas* species.



1 R=CH_2OH
2 R=H
3 R=CHO
4 R=COOH

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hreims was obtained by using direct inlet sample introduction on a VG-ZAB-HF spectrometer (Midwest Center for Mass Spectrometry, University of Nebraska–Lincoln). Nmr spectra were obtained on Bruker NM-360 MHz high field spectrometer equipped with an IBM Aspect-2000 processor at 360.134 MHz using TMS (d=0)

Chemical shift values are reported in ppm, and coupling constants (J values) in Hz. Abbreviations for nmr signals are s=singlet, dd=doublet of doublets, and ddd=doublet of doublets of doublets.

Tlc was performed on 0.25 mm layers of Si gel GF_{254} (Merck) prepared on 5×20 cm or 20×20 cm glass plates with a Quikfit Industries spreader (London). Plates were air-dried and activated at 120° for 1 h prior to use. Plates were developed in a solvent mixture of petroleum ether/Et_2O/HCOOH, and developed chromatograms were visualized under 366 nm uv light to observe compound fluorescence after spraying with a solution of 0.1% 2′,7′-dichlorofluorescein in MeOH.

MICROORGANISM.—*P. aeruginosa* UI-29791 (5) was obtained from the culture collection of the University of Iowa, Department of Microbiology, and was maintained on sporulation agar (ATCC #5 medium). Medium #5 slants consisted of yeast extract 0.1%, beef extract 0.1%, tryptone 0.2%, $FeSO_4$ 1 mg/liter, glucose 1%, and agar 2%. The medium was adjusted to pH 7.2 before autoclaving.

P. aeruginosa slants were used to inoculate ten 1-liter flasks containing 200 ml of defined media consisting of the following salts (in g/liter): $NaNO_3$, 15; KCl, 1.1; NaCl, 1.1; $FeSO_4 \cdot 7H_2O$, 0.0003; $Ca(NO_3)_2 \cdot 4H_2O$, 0.01; KH_2PO_4, 3.4; K_2HPO_4, 4.4; and $MgSO_4 \cdot 7H_2O$, 0.5. A stock solution (5 ml) containing (in g/liter) $ZnSO_4 \cdot 7H_2O$, 0.29; $CaCl_2 \cdot 4H_2O$, 0.24; $CuCl_2 \cdot 6H_2O$, 0.24; $CuSO_4 \cdot 5H_2O$, 0.25; and $MnSO_4 \cdot H_2O$, 0.17 was added to 1 liter of the salt mixture described above. The entire mixture was adjusted to pH 6.5, 15 g of lard was added into each flask as the sole source of carbon, and flasks were autoclaved at 121° for 15 min. Cultures were incubated with shaking at 200 rpm at 37° on a New Brunswick Scientific G25 Gyrotory shaker for 10 days before they were pooled and centrifuged at 8000×*g* at 25°. The supernatants were extracted twice with half-volumes of $CHCl_3$, and the solvent was evaporated under vacuum. The oily extract was dissolved in $CHCl_3$, absorbed onto Si gel, applied to 40–100 mesh Si gel (Baker 3404) packed in a 1.5×40 cm glass column which was eluted with petroleum ether/Et_2O/HCOOH mixtures of increasing polarities. Fractions containing a greenish-yellow fluorescence spot by 366 nm uv irradiation were combined and further purified by flash cc (3×30 cm) over 40 mm silica eluted with hexane-EtOAc (4:1). The fluorescent fraction from the flash cc (300 mg) was subjected to further preparative tlc, and the fluorescent band (26 mg) was eluted with $CHCl_3$-EtOAc (9:1). The analytical sample of 6 mg of **1** was obtained as an off-white amorphous solid.

Aeruginol [**1**].—Hreims *m/z* $[M]^+$ 207.0355 (calcd 207.0354 for $C_{10}H_9O_2NS$); cims *m/z* $[MH]^+$ 208 (100%), eims *m/z* $[M]^+$ 207 (65%), $[M-H_2O]^+$ 189 (100%); uv [MeCN-H_2O (65:35)] λ max 324 nm, shifted to 365 nm with 1 drop 6 N NaOH; ^1H nmr (360 MHz, $CDCl_3$) 4.83 (2H, s, $-CH_2-$), 5.34 (s, broad, $-CH_2OH$), 6.90 (1H, ddd, H-4′, $J_{H6'}$=1.1 Hz, $J_{H3',5'}$=8 Hz), 7.05 (1H, dd, H-6′, $J_{H4'}$=1.1 Hz, $J_{H5'}$=8 Hz), 7.17 (1H, s, H-5), 7.31 (1H, ddd, H-5′, $J_{H3'}$=1.5 Hz, $J_{H4',6'}$=8 Hz), 7.61 (1H, dd, H-3′, $J_{H4'}$=8 Hz, $J_{H5'}$=1.5 Hz).

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