# *Notes*

## Pseudopyronines A and B, α-Pyrones Produced by a Marine *Pseudomonas* sp. F92S91, and Evidence for the Conversion of 4-Hydroxy-α-pyrone to 3-Furanone

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In our search for inhibitors of bacterial fatty acid biosynthesis, two new α-pyrones, pseudopyronines A (**1**) and B (**2**), were isolated from a marine *Pseudomonas* sp. F92S91. The naturally occurring α-pyrones appeared to be unstable, evidenced by the conversion of pseudopyronine B into an oxidation product, 3-furanone (**3**). Structural elucidations were made by spectroscopic analyses including 2D-NMR data.

In the course of screening of our microbial natural products library for new inhibitors of bacterial fatty acid biosynthesis (FAB), a new species of *Pseudomonas*, designated F92S91, was found to produce the α-pyrone antibiotics pseudopyronines A (**1**) and B (**2**).[1] In this paper, we present the isolation and structure elucidation of the α-pyrone antibiotics and the degradation product **3**, as well as a proposal for the conversion of 4-hydroxy-α-pyrone to 3-furanone. Taxonomy, fermentation of the producing organism, and the biological activity were reported in our previous paper.[1b]

The strain F92S91, a new species of *Pseudomonas*, was isolated from a marine sponge sample collected in Fiji. The culture was grown under previously described conditions,[1] harvested after 4 days, and frozen for later processing. The frozen culture was thawed and centrifuged to separate the cell mass and supernatant. The supernatant was extracted with ethyl acetate, and the cell pellet was extracted with methanol. Both organic extracts were combined, concentrated, and then chromatographed to give two active fractions. The active fractions were further purified by semipreparative HPLC to yield pyrone antibiotics, pseudopyronines A (**1**) and B (**2**), in order of increasing C-18 column affinity.

Pseudopyronine A (**1**) was isolated as an amorphous powder. The UV spectrum of **1** suggested it contained a typical α-pyrone chromophore with maximum absorption at 293 nm (Figure 2).[2] Its molecular formula was determined to be $C_{16}H_{26}O_3$ by high-resolution FT-ICR mass spectrometry [measured 267.19570, calcd 267.19547 for $(M + H)^+$]. The number of carbon atoms was also evident in the $^{13}C$ NMR spectrum of **1** that displayed all 16 individual carbon signals. The $^{13}C$ chemical shift values indicated the presence of five $sp^2$ and 11 $sp^3$ carbons. The DEPT experiments revealed that they were two methyls, nine methylenes, one methine, and four quaternary carbons. The HMQC experiment established the proton−carbon connectivities. Analysis of the $^1H$ NMR spectrum in conjunction with COSY and TOCSY data led to the identification of two linear fatty chains C3a to C3f and C6a to C6e. The pyrone core was established on the basis of the UV spectrum and HMBC data. In the HMBC spectrum, the olefinic proton at $\delta$ 6.10 (H5) showed four cross-peaks to three $sp^2$ carbon signals at $\delta$ 103.9 (C3), 165.2 (C6), and 167.8 (C4) and to one aliphatic carbon at $\delta$ 34.3 that was assigned to the appendage C6a. The attached methylene protons H6a at $\delta$ 2.46 in turn showed a two-bond HMBC correlation to the quaternary carbon C6 and three-bond coupling to C5 at $\delta$ 101.0. The HMBC correlations observed from the other appendage methylene protons H3a at $\delta$ 2.36 to the remaining $sp^2$ carbons C2 ($\delta$ 168.8), C3, and C4 completed the pyrone substructure assignment. An allylic coupling observed between H5 and H6a in the COSY spectrum was consistent with the assignment. The hydroxyl group was located at C4, as its proton resonance recorded in deuterated DMSO exhibited the expected two-bond HMBC correlation to C4 as well as three-bond correlations to C3 and C5.

Pseudopyronine B (**2**) was obtained as an amorphous powder. It had the same UV spectrum as pseudopyronine A, indicating the same α-pyrone chromophore. Its molecular formula was established as $C_{18}H_{30}O_3$ by high-resolution FT-ICR mass spectrometry, differing from the molecular formula of pseudopyronine A by the gain of $C_2H_4$. The $^1H$ NMR spectrum of **2** was almost identical to that recorded for **1**, indicating that the two compounds were closely related. Detailed analyses of the $^{13}C$ NMR, COSY, TOCSY, HSQC, and HMBC data led to conclusion that it had the same structural features except for two additional methylene units in the C6a-to-C6g chain (Figure 1). Coincidently, this compound, designated Sch 419560, was also isolated from a different organism, *P. fluorescens*, by Chu and co-workers at Schering-Plough.[3]

Pseudopyronine B was not stable. It was almost completely oxidized in deuterated chloroform by air after standing in an NMR tube for 10 days, although the freshly prepared NMR sample was stable enough for overnight data acquisition. HPLC purification of the oxidation product yielded the furanone derivative **3**. Compound **3** presented a totally different UV spectrum with a maximum absorption at 273 nm (Figure 2). The high-resolution FT-ICR mass spectrometry indicated that it had a molecular formula of $C_{17}H_{30}O_3$, differing from the natural product by one less carbon atom. Detailed analyses of the 1D and 2D NMR data quickly revealed that compound **3** contained the

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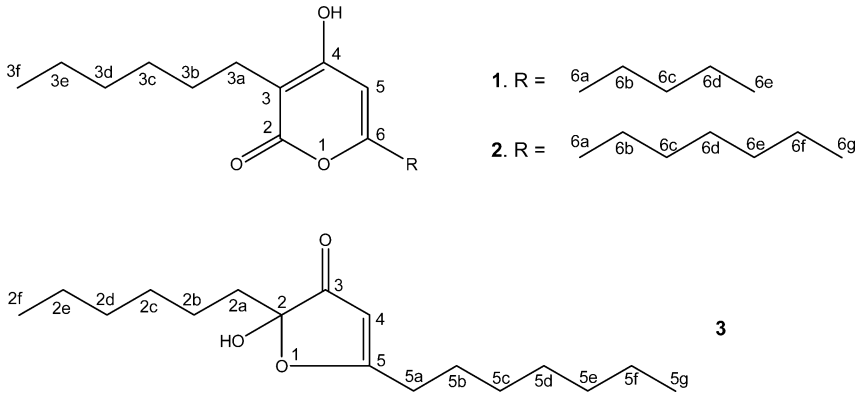


**Figure 1.** Structures of pseudopyronines A (**1**) and B (**2**) and furanone derivative **3**.

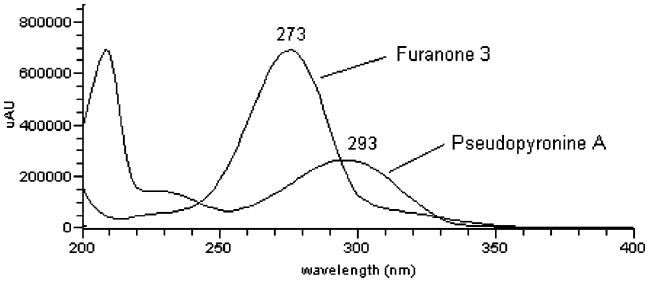


**Figure 2.** UV spectra of pseudopyronine A (**1**) and furanone **3**.

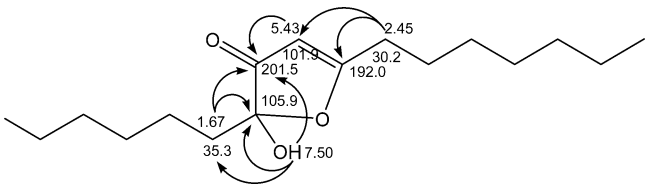


**Figure 3.** Key HMBC correlations ($^1$H ↷ $^{13}$C) for the furanone core of **3**.

same *n*-hexyl and *n*-heptyl chains. Although the chemical shifts assigned to the heptyl group were almost identical to those observed in **2**, the resonances assigned to C2a and C2b as well as their associated protons in the hexyl chain were quite different. Thus, the appendage methylene carbon C2a of **3** resonated at $\delta$ 35.3 and the attached protons H2a appeared at 1.67, while the corresponding resonances for **2** were at $\delta$ 27.5 (C3a) and 2.23 (H3a), respectively, indicating the loss of allylic character. In the HMBC spectrum, H2a showed a two-bond correlation to a low-field aliphatic carbon resonance at $\delta$ 105.9 (C2) that was assigned as a hemiketal carbon on the basis of its chemical shift.[4] This assignment was confirmed by an HMBC correlation between C2 and a proton singlet at $\delta$ 7.50 (2-OH) that must bond to an oxygen atom, as it did not show coupling to any carbons in the HSQC experiment. Additional HMBC correlations observed from the hydroxyl proton to C2a and C3 at $\delta$ 201.5 and from H5a to C4 at $\delta$ 101.0 and C5 at $\delta$ 192.0 completed the furanone ring. This was further supported by HMBC correlations from the olefinic proton H4 at $\delta$ 5.43 to C2, C3, C5, and C5a, as shown in Figure 3.

Furanone **3** is a degradation product of pseudopyronine B. A possible mechanism for formation of a 3-furanone from an α-pyrone is outlined in Figure 4. The pyrone is first oxidized by oxygen from the air to form peroxide **4**. In fact, the NMR data reacquired on the pseudopyronine B sample standing in an NMR tube for 10 days suggested the presence of **4**. For example, the olefinic proton H5 ($\delta$ 5.71) and the methylene protons H3a ($\delta$ 1.93) showed HMBC correlations to C3, now resonating at $\delta$ 91.5 instead of 103.8 in **2**, appropriate for an oxygenated quaternary carbon. Additional HMBC correlations from H3a to two low-field carbon signals at $\delta$ 167.6 (C2) and 192.1 (C4) corroborated the oxidation at C3. Whereas the chemical shift of C2 was almost identical to that in **2** ($\delta$ 168.1), the downfield shift of C4 from $\delta$ 166.6 in **2** to $\delta$ 192.1 in **4** is expected for a change from an enol to a keto form that occurs concomitant with the oxidation. (See Supporting Information for NMR spectra of **4**.) The unstable peroxy intermediate **4** is presumably hydrolyzed to **5** during HPLC purification, which then undergoes oxidative decarboxylation, yielding hydroxydione **6**. Final attack of the hydroxyl to the

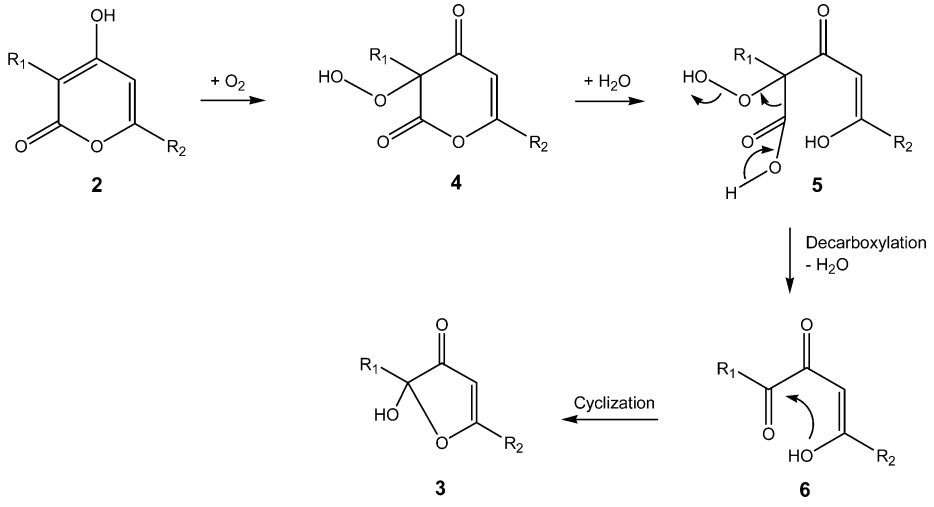


**Figure 4.** Proposed mechanism for the formation of furanone **3** from pseudopyronine B (**2**).

**Table 1.** $^1$H and $^{13}$C NMR Assignments for Pseudopyronines A (**1**) and B (**2**) and Furanone 3

| **1** (MeOH-$d_4$)[a] | | | **2** (DMSO-$d_6$)[a] | | | **3** (DMSO-$d_6$)[a] | | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta$ $^{13}$C[c] | $\delta$ $^1$H[b] ($J$ = Hz) | position | $\delta$ $^{13}$C[c] | $\delta$ $^1$H[b] ($J$ = Hz) | position | $\delta$ $^{13}$C[c] | $\delta$ $^1$H[b] ($J$ = Hz) |
| 2 | 168.8 | | 2 | 164.9 | | 2 | 105.9 | |
| | | | | | | 2-OH | | 7.50 (s) |
| 3 | 103.9 | | 3 | 102.4 | | 3 | 201.5 | |
| 4 | 167.8 | | 4 | 164.8 | | 4 | 101.9 | 5.43 (s) |
| 4-OH | | | 4-OH | | 11.02 | | | |
| 5 | 101.0 | 5.97 (s) | 5 | 99.2 | 5.95 | 5 | 192.0 | |
| 6 | 165.2 | | 6 | 162.8 | | | | |
| 3a | 23.9 | 2.36 (t, 7.5) | 3a | 22.6 | 2.23 (t, 7.5) | 2a | 35.3 | 1.67 (m) |
| 3b | 29.0 | 1.44 (quin, 7.4) | 3b | 27.5 | 1.34 (quin, 6.7) | 2b | 21.9 | 1.27 |
| 3c | 30.2 | 1.32 | 3c | 28.5 | 1.23 | 2c | 28.3 | 1.30 |
| 3d | 32.9 | 1.31 | 3d | 31.1 | 1.23 | 2d | 31.1 | 1.24 |
| 3e | 23.7 | 1.31 | 3e | 22.0 | 1.25 | 2e | 21.9 | 1.25 |
| 3f | 14.4 | 0.89 (t, 6.9) | 3f | 13.9 | 0.83 (t, 6.7) | 2f | 13.9 | 0.83 (t, 7.0) |
| 6a | 34.3 | 2.46 (t, 7.6) | 6a | 32.5 | 2.38, (t, 7.5) | 5a | 30.2 | 2.45 (m) |
| 6b | 27.6 | 1.64 (quin, 7.3) | 6b | 26.2 | 1.51 (quin, 7.1) | 5b | 25.4 | 1.57 (m) |
| 6c | 32.2 | 1.34 | 6c | 28.2 | 1.27 | 5c | 28.6 | 1.24 |
| 6d | 23.4 | 1.35 | 6d | 28.3 | 1.27 | 5d | 28.3 | 1.28 |
| 6e | 14.3 | 0.91 (t, 6.9) | 6e | 31.1 | 1.23 | 5e | 31.1 | 1.25 |
| | | | 6f | 22.0 | 1.25 | 5f | 22.0 | 1.26 |
| | | | 6g | 13.9 | 0.84 (t, 6.8) | 5g | 13.9 | 0.85 (t, 6.7) |

[a] Chemical shifts were referenced to residual solvent signals [$\delta$ 3.30 ($^1$H) and 49.0 ($^{13}$C) for MeOH-$d_4$; $\delta$ 2.49 ($^1$H) and 39.5 ($^{13}$C) for DMSO-$d_6$]. [b] Recorded at 400 MHz. [c]Recorded at 100 MHz.

$\delta$-positioned keto group, forming a hemiketal functionality, generates the degradation product **3**.

Pseudopyronine A is a new member of the naturally occurring pyranone antibiotics.[5] Pseudopyronine B is apparently identical to Sch 419560.[3] To date, over 100 α-pyrones with substitution patterns similar to pseudopyronines have been documented.[6] By contrast, there are only very few natural products with a furanone moiety in the literature, these include siphonarienfuranone,[7] aglajne 2,[8] AS 183,[9] and 2-hydroxy-2,4-dimethyl-5-propenyl-3-furanone.[10] Interestingly, all organisms that reportedly produce 3-furanone metabolites also produce 4-hydroxy-α-pyrones. On the basis of our observations, there is a reason to believe that the furanone metabolites are actually derived from the α-pyrones. In all cases, the furanones were isolated involving chloroform as extraction or silica gel chromatography solvent, which as shown in our case facilitated the degradation of pseudopyronine B. We speculate that the acidity of chloroform might be catalyzing the oxidation. Thus, the skeletal relationship of pseudopyronine B with its counterpart **3** can be perceived in pairs of pectinatone[11] and siphonarienfuranone,[7] aglajne-3 and aglajne-2,[8] and norpectinatone and its furanone version.[12] The facile oxidative decarboxylation of α-pyrones to yield furanones as demonstrated in our study puts forward that many of the furanone metabolites might indeed be artifacts.

Antibacterial activities and mechanistic activities of pseudopyronines A and B and the furanone **3** were reported earlier.[1b] These compounds exhibited moderate to poor antibacterial activities against Gram-positive bacteria, but they were inactive against Gram-negative bacteria and yeast.

## Experimental Section

**General Experimental Procedures.** UV data were obtained on an Agilent 1100 HPLC system equipped with DAD. All 1D and 2D NMR spectra were taken on a Bruker AVANCE-400 spectrometer at 400 and 100 MHz for $^1$H and $^{13}$C, respectively, using a 3 mm broadband probe. $^1$H and $^{13}$C chemical shifts were recorded in parts per million relative to partially deuterated solvent peaks [$\delta$ 3.30 ($^1$H) and 49.0 ($^{13}$C) for MeOH-$d_4$; $\delta$ 2.49 ($^1$H) and 39.5 ($^{13}$C) for DMSO-$d_6$; $\delta$ 7.26 ($^1$H) and 77.0 ($^{13}$C) for $CDCl_3$]. $^1$H−$^1$H coupling constants were measured from a 1D proton spectrum and are given in hertz. Proton-detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{C-H}$ = 140 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8.3 Hz) pulse sequences. A typical NMR data set measured for a compound included carbon, DEPT, proton, COSY, TOCSY (40 ms mixing time), HSQC, and HMBC spectra. All 2D experiments were run nonspinning. LRMS data were made on a Finnigan LCQ-DECA ion trap mass spectrometer. High-resolution mass spectra (HRMS) were recorded on a Bruker Apex-II FT-ICR mass spectrometer equipped with a 9.4 T superconducting magnet and an Apollo electrospray source.

**HPLC Systems.** An Agilent 1100 HPLC system employing a variety of columns with diode array detection was used for the analysis of fractions and for semipreparative purification of samples isolated by open column or preparative HPLC. Preparative HPLC separations were accomplished on a RANIN Dynamax system (SD-300) with UV detection at 290 nm. Fractions from preparative columns were generally collected using an ISCO Foxy fractional collector. Samples were frequently analyzed by LC/MS using a model HP1100 LC system with tandem photodiode array and a Finnigan LCQ-DECA ion trap mass spectrometer. Compounds were resolved on a YMC ODS-A HPLC column (100 × 3 mm, S-3) using a linear gradient from 5 to 95% mobile phase B [0.025% formic acid (FA) in MeCN] in mobile phase A (0.025% FA in water) over 20 min with a flow rate of 0.3 mL/min, followed by a 5 min isocratic run. All HPLC solvents were EM Omnisolv quality and used without further purification.

**Isolation.** The frozen fermentation broth (1 L) of F92S91 was thawed and centrifuged. The supernatant was extracted with EtOAc (700 mL × 2), and the mycelium mass was extracted with MeOH (100 mL × 2). Both EtOAc and MeOH extracts were combined and concentrated under reduced pressure to dryness. The dried residue was redissolved in 15 mL of DMSO−MeOH−$H_2O$ (1:2:1) and loaded onto a reversed-phase HPLC column (MetaChem ODS-3, 250 × 20 mm, 4 injections). The column was eluted with a step gradient of water with 0.02% TFA (A) and acetonitrile (B) at a flow rate of 6 mL/min, beginning with a linear gradient from 35% to 80% mobile phase B over 30 min, followed by an isocratic run of 20 min, then gradient again with B from 80 to 95% over 10 min and isocratic for 25 min. The antibiotics **1** and **2** were eluted at 51 and 68 min, respectively. Both compounds were further purified using semipreparative HPLC and methanol−water with a 0.02% TFA mobile phase system to yield pure **1** (3.5 mg) and **2** (16.8 mg).

The oxidized pseudopyronine B sample was rechromatographed using a MetaChem ODS-3 column (250 × 10 mm)

with a linear gradient of 80 to 90% MeOH and 0.025% TFA buffer over 20 min at a flow rate of 4 mL/min with UV detection at 250 nm to give only one peak at 19.9 min representing furanone **3**.

**Pseudopyronine A (1):** white powder (lyophilization); $^1$H and $^{13}$C NMR in MeOH-$d_4$ (Table 1); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 11.09 (1H, bs), 5.95 (1H, s), 2.38 (2H, t, $J$ = 7.5 Hz), 2.23 (2H, t, $J$ = 7.3 Hz), 1.51 (2H, quin, $J$ = 7.2 Hz), 1.34 (2H, m), 1.17−1.30 (10H), 0.85 (3H, t, $J$ = 6.7 Hz), 0.84 (3H, t, $J$ = 6.5 Hz); LRESMS *m/z* 267 $[M + H]^+$, 265 $[M - H]^-$; HRMS (FT-ICR) *m/z* 267.19570 (calcd for $C_{16}H_{27}O_3^+$, 267.19547).

**Pseudopyronine B (2):** white powder; $^1$H and $^{13}$C NMR in DMSO-$d_6$ (Table 1); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 9.18 (1H, bs), 6.10 (1H, s), 2.43 (4H, t, $J$ = 7.6 Hz), 1.62 (2H, quin $J$ = 7.0 Hz), 1.50 (2H, quin, $J$ = 7.5 Hz), 1.22−1.38 (14H), 0.87 (1H, t, $J$ = 6.5 Hz), 0.86 (3H, t, $J$ = 6.9 Hz); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 168.1, 166.6, 164.0, 103.8, 100.8, 33.9, 32.2, 32.0, 29.7, 29.4, 29.3, 28.4, 27.2, 23.5, 23.04, 23.00, 14.5, 14.4; LRESMS *m/z* 295 $[M + H]^+$, 293 $[M - H]^-$; HRMS (FT-ICR) *m/z* 295.22680 (calcd for $C_{18}H_{31}O_3^+$, 295.22677).

**Furanone 3:** off-white powder; $^1$H and $^{13}$C NMR in DMSO-$d_6$ (Table 1); LRESMS *m/z* 283 $[M + H]^+$; HRMS (FT-ICR) *m/z* 283.22709 (calcd for $C_{17}H_{31}O_3^+$, 283.22677).

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**Supporting Information Available:** NMR spectra of **1** to **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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