Mupirocin W, a Novel Pseudomonic Acid Produced by Targeted Mutation of the Mupirocin Biosynthetic Gene Cluster.

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Electronic Supplementary Information

Bacterial strain and culture conditions. Pseudomonas fluorescens NCIMB 10586 was used as the wild-type of pseudomonic acid production. The bacterial strain and its mutants were grown in L-broth which consisted of 1% tryptone, 0.5% yeast extract, 1% NaCl, 0.1% glucose pH 7.0 and L-agar (L-broth containing 1.5% agar) at 25°C and 250 rpm for 24 hours. Secondary Stage Media (SSM) 100 ml (2.0% soya flour, 0.25% spray dried corn steep liquor, 10.0 % glucose, 0.25% NaH2PO4, 0.9% (NH4)2SO4, 0.05% MgSO4 7H2O, 2.0 % CaCO3 in 20 x 500 ml baffle flasks were inoculated with 5 % volume of seed culture and grown at 22°C, 250 rpm for 50 hours. After 50 hours, the cultures were harvested by centrifugation at 10,000 rpm, 4°C for 15 minutes. The supernatant was acidified to pH 4.5 with dilute HCl, saturated with sodium chloride, extracted with half amount of ethyl acetate three times. The organic phases were combined, dried over magnesium sulphate and evaporated to dryness. The crude extract was taken up in methanol.

Purification of compounds. The crude extract was purified by HPLC (System Gold, Beckmann) using a Luna C18 reverse-phase column (4.6 x 250 mm, 5 μ, Phenomenex). H2O (A, containing 0.05 % formic acid) and acetonitrile (B, containing 0.005% formic acid) as mobile phase, UV detection at 233 nm with 1 ml min⁻¹ of flow rate and 480 μl injection. Programme: 0-1min, 5%B; 1-2min ramp to 37%B; 2-4min ramp to 99%B; 20-23min 99%B; 23-25min, ramp to 5%B; 25-29min, 5%B. The separated fractions were collected. The acetonitrile was removed by evaporation. The aqueous layer was reextracted with ethyl acetate three times, dried over magnesium sulphate and evaporated to dryness, to give mupirocin W (2), 5mg from 20 baffle flasks, corresponding to 2.5mgL⁻¹. 1H NMR (400MHz, CDCl3, TMS): δ = 0.75 (3H, d, J = 7.0, H-16), 1.01 (3H, d, J = 6, H-17), 1.07 (3H, d, J = 6.5, H-14), 1.25-1.27 (8H, m, H-4'-7'), 1.45-1.50 (5H, m, H-5', H-8', H-8), 1.96 (2H, m, H-9), 2.10 (3H, s, H-15), 2.13 (1H, d, J = 12, H-4), 2.18 (2H, t, J = 7.0, H-2'), 2.24 (1H, m, H-12), 2.50 (1H, d, J= 6.0, H-4), 3.40 (1H, t, J = 2.4, H-6), 3.50-3.58 (2H, m, H-7, H-11), 3.78 (1H, dd, J = 3.5, J = 2.9, J = 1.2 H-5), 3.89 (1H, dq, J = 2.5, J = 5.1, H-13), 3.98 (2H, t, J = 2.8, H-9'), 4.00 (1H, m, H-10), 5.67 (1H, s, H-2). 13C NMR (100MHz, CDOD): δ = 10.2 (C-17), 18.0 (C-15),18.2 (C-2', C-14 and C-16), 25.1 (C-3'), 26.1 (C-7'), 28.8 (C-8'), 29.15, 29.2, 29.3 (C-4', C-5', C-6'), 34.0 (C-2'), 35.6 (C-9), 37.6 (C-8), 43.3 (C-12), 43.9 (C-3), 63.8 (C-9'), 69.2 (C-6), 71.1 (C-7), 74.9 (C-13), 77.0 (C-5), 80.4 (C-10), 86.2 (C-11), 117.5 (C-2), 158.5 (C-3), 167.5 (C-1), 176.8 (C-1').

Preparation of methyl ester 3. The purified compound (5 mg) in methanol (3 ml) was treated with an excess of diazomethane on ice and stirred for 15 minutes. To eliminate the excess of diazomethane, a few drops of acetic acid were added. The solution was evaporated to dryness. 1H NMR (600MHz, CDCl3, TMS): δ = 0.79 (3H, d, J = 7, H-17), 1.17 (3H, d, J = 6, H-16), 1.19 (3H, d, J = 6, H-14), 1.30 - 1.42 (12H, m, H-3' – 8'H-), 1.48 (1H, ddq, J = 9, 8, 6, H-12), 1.63 (2H, m, H-8'), 1.68 (1H, dt, J = 12, 11, HH-9), 2.01 (1H, ddd, J = 7, 12, 5, HH-9), 2.14 (1H, d, J = 4, 6-0H), 2.23 (3H, d, J = 1, H-15), 2.29 (1H, dd, J = 14, 9, HH-4), 2.30 (2H, t, J = 7.5, H-2'), 2.37 (1H, dqq, J = 11, 7, 7, H-8), 2.43 (1H, d, J = 3, 5-0H), 2.59 (1H, dd, J = 14, 2, HH-4), 2.85 (1H, s, 11-OH), 3.62 (1H, dd, J = 7, 6, 4, H-6), 3.64 (1H, dd, J = 7, 7, H-7), 3.67 (3H, s, OCH3), 3.76 (1H, dd, J = 9, 3, H-11), 3.84 (1H, dq, J = 8, 6, H-13), 3.90 (1H, s, 13-OH), 3.95 (1H, dddd, J = 13, 4, 3, 2, H-5), 4.08 (2H, t, J = 7, H-9'), 4.12 (1H, dddd, J = 11, 5, 3, H-10), 5.79 (1H, bs, H-2). 13C NMR (100MHz, CDCl3, TMS): δ = 12.4 (C-17), 18.3 (C-16), 19.0 (C-15), 20.1 (C-14), 24.9 (C-3'), 25.9 (C-7'), 28.6 (C-8'), 29.0 (C-4'), 29.1 (2C, C-5', C-6'), 33.7 (C-9), 34.1 (C-2'), 37.6 (C-2), 42.3 (C-12), 43.9 (C-4), 51.5 (CO2Me), 63.9 (C-9'), 70.6 (C-5), 72.0 (C-13), 76.1 (C-7), 76.7 (C-11), 80.5 (C-10), 86.2 (C-6), 118.5 (C-2), 156.2 (C-3), 166.5 (C-1), 174.4 (C-1'). MS (EI): m/z (%) 503 ([M]+, 0.5).

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Preparation of tetraacetate 4. The methyl ester (5mg) was dissolved in dichloromethane (2 ml). Pyridine (0.5 ml) and acetic anhydride (0.25 ml) were added to the solution which was stirred at room temperature overnight. To remove the pyridine, dichloromethane 2 ml was added, the solution was extracted with 1 N HCl three times, washed with brine, dried over magnesium sulphate and evaporated to dryness. $^1$H NMR (600MHz, CDCl$_3$, TMS): $\delta$ = 0.97 (3H, d, $J$ = 7, H$_3$-17), 1.05 (3H, d, $J$ = 6, H$_3$-16), 1.16 (3H, d, $J$ = 7, H$_3$-14), 1.30 – 1.40 (8H, m, H$_3$-4’-7’), 1.50 (1H, ddq, $J$ = 12, 10, 8, H-12), 1.60 (4H, m, H$_2$-3’, H$_2$-8’). 1.62 (1H, dd, $J$ = 14, 7, HH-9), 1.98 (3H, s, OCOCH$_3$), 2.03 (3H, s, OCOCH$_3$), 2.08 (3H, s, OCOCH$_3$), 2.09 (3H, s, OCOCH$_3$), 2.10 (1H, dd, $J$ = 6, 2, HH4). 2.16 (3H, d, $J$ = 1 , H$_3$-15), 2.30 (2H, t, $J$ = 7.5, H$_2$-2’), 2.44 (1H, ddd, $J$ = 14, 11, 11, H-8), 2.51 (1H, dd, $J$ = 14, 3, HH4), 3.50 (1H, dd, $J$ = 8, 4, H-7), 3.62 (3H, s, OCOCH$_3$), 4.06 (2H, t, $J$ = 7, H$_2$-9’), 4.15 (1H, ddd, $J$ = 10, 6, 4, H-10), 5.00 (1H, dd, $J$ = 11, 4, H-11), 5.01 (1H, d, $J$ = 10, 6, H-13), 5.08 (1H, dd, $J$ = 8, 3, H-6), 5.35 (1H, ddd, $J$ = 11, 6, 3, H-5), 5.70 (1H, s, H-2). $^{13}$C NMR (100MHz, CDCl$_3$, TMS): $\delta$ = 10.8 (C-17), 15.7 (C-14), 17.8 (C-16), 18.4 (C-15), 20.9, 20.9, 21.1, 21.4 (COCOCH$_3$), 24.9 (C-3’), 25.9 (C-7’), 28.7 (C-8’), 29.1, 29.7 (C-4’, C-5’, C-6’), 34.1 (C-2’), 36.6 (C-9), 38.5 (C-12), 39.4 (C-8), 40.0 (C-4) 51.8 (CO$_2$Me), 63.8 (C-9’), 70.6 (C-5), 70.8 (C-11), 74.9 (C-13), 75.2 (C-6), 78.2 (C-10), 83.1 (C-7), 118.5 (C-2), 155.0 (C-3), 166.5 (C-1), 170.6 (2C, 2 × OCOCH$_3$), 170.7 (OCOCH$_3$), 170.8 (OCOCH$_3$), 174.3 (C-1’). MS (Cl): m/z (%) 685 ([M]H$^+$, 18.7), 653 ([M-OCOCH$_3$]$^+$, 4), 625 ([M-OCOCH$_3$]$^+$, 30.7), 565 ([M - (CH$_2$)$_2$CO-OCH$_3$ - H$_2$O]$^+$, 33.3), 497 ([M - O(CH$_2$)$_3$CO-Me]$^+$, 4.7). HRMS (Cl) [M]H$^+$ caleed for C$_{35}$H$_{56}$O$_{13}$ 685.3799, found 685.3810.