Materials and Methods.

General.  Radiolabeled substrates including [2-\(^{14}\)C]malonylCoA, L-[U-\(^{14}\)C]lysine and L-[U-\(^{14}\)C]proline were obtained from Moravek Biochemicals (Brea, CA).  All others (DL-[1-\(^{14}\)C]ornithine, [U-\(^{14}\)C]glycine, L-[U-\(^{14}\)C]valine, DL-[1-\(^{14}\)C]glutamic acid, L-[U-\(^{14}\)C]tyrosine, L-[U-\(^{14}\)C]threonine, L-[U-\(^{14}\)C]ornithine) were acquired from American Radiolabeled Chemicals (St. Louis, MO).  TLC plates were purchased from Analtech (Newark, DE).  Co-factors (SAM, FAD, and NADPH) and all cell media and buffer components were obtained from Sigma Biochemicals unless otherwise specified.  **OPTI-Fluor** O was purchased from Perkin Elmer (Wellesley, MA).  The FAS/PKS inhibitor cerulenin was purchased from Sigma Biochemicals (St. Louis, MO) and all cytochrome P-450 enzyme inhibitors metrapone, chloramphenicol, and miconazole were available from Aldrich (Milwaukee, WI).  Radioactive products were measured on a Beckman LS5910 scintillation counter and the Bead Beater utilized in the preparation of cell-free extracts purchased from Biospec (Bartlesville, OK).  **Streptomyces sahachiroi** (NRRL 2485) was acquired from the American Type Culture Collection (ATCC; Manassas, VA).

**Culture Conditions.** *Streptomyces sahachiroi* was initially cultured on GYM plates until sporulation, typically 5-7 days at 28 °C.  GYM agar plates contained per liter: glucose monohydrate, 4 g; yeast extract (Difco), 4 g; malt extract (Difco), 10 g; CaCO\(_3\), 2 g; Bacto-agar (Difco), 12 g; and tap water; adjusted to pH 6.8 with 1 M NaOH prior to sterilization.\(^1\) A starting culture of *Streptomyces sahachiroi* in PS5 medium (100 mL) was prepared by inoculation of a loop full of spores from the GYM plates.  PS5 medium was prepared from 5 g/L of Pharmamedia (yellow cotton seed flour; Traders Protein; Memphis, TN) and 5 g/L of soluble starch, adjusted to a pH of 7.0.\(^1\) Following 24 h of growth at 30 °C, 250 rpm, 25 mL of the starting culture was utilized to inoculate 500 mL of PS5 in 2 L baffled Erlenmeyer flasks.  The cultures were grown for about 64 h (30 °C, 250 rpm).  The cells were harvested by centrifugation, the cell pellet frozen in liquid nitrogen (in aliquots), and stored at -80 °C.

**Cell-Free Extract Preparation.** The cell-free extract was prepared by combining frozen cells (13-15 g), glass beads (28 g; 0.1 mm), and cell-free extract buffer (100 mM potassium phosphate, pH 7.5; 50% glycerol, 2 mM dithiothreitol, and 1 mM EDTA; 80 mLD; 4 °C) in a bead beater equipped with an ice water jacket.  The cells were pulverized employing ten 1 min. cycles separated by 1 min. intervals to prevent warming of the protein extract and subsequently centrifuged (7,660 G, 15 min) to give the crude cell-free extract.

**Enzyme Activity Assays.** To each aliquot of the protein extract (5 mL) was added 1 µL of acetyl-CoA (1 mg/1 mL), 80 µL of co-factor solution (FAD, 1 mg; SAM, 1 mg; NADPH, 1 mg in 1 mL of deionized distilled water), and 0.25 µCi of \(^{14}\)C-radiolabeled material.  The resulting reaction mixture was incubated at 37 °C for 24 h.  The reactions were vortexed with dichloromethane (3 mL), organs were transferred to fresh tubes, and evaporated to dryness.  The organic residue was resolubilized in a minimal volume of dichloromethane (60 µL), and applied to TLC (5 : 0.3; dichloromethane : methanol; \(R_f = 0.25\), naphthoate; \(R_f = 0.37\), azinomycin B) to which was added unlabeled naphthoate or azinomycin B (isolated from *Streptomyces sahachiroi* cultures).\(^2,3\) Samples (TLC spots) with appropriate \(R_f\) values were scraped from the tlc plate, transferred to vials containing **OPTI-Fluor** O and analyzed by scintillation counting.

**HPLC Coinjection Assays.** Enzyme activity assays were performed as detailed above.  Following extraction and evaporation of the samples to dryness, the organic residue was solubilized in methanol (100 µL) to which was added unlabeled naphthoate or azinomycin B and analyzed by HPLC with a Phenomenex C8 column (250 x 4.6 mm), employing a gradient of acetonitrile/water.  Conditions are

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listed in Table 1. Retention times were 14.9 min. (naphthoate) and 16.2 min. (azinomycin B), respectively. The corresponding peaks were collected, transferred to vials containing OPTI-Fluor O and analyzed by scintillation counting.

**Protein Inhibition Assays.** To each aliquot of the protein extract (3 mL) was added 1 µL of acetyl-CoA (1 mg/1 mL), 0.25 µCi of [2-14C]malonylCoA, 80 µL of cofactor solution (FAD, 1 mg; SAM, 1 mg; NADPH, 1 mg in 1 mL of deionized distilled water) in addition to 10 µM or 100 µM inhibitor.

**Naphthoic Acid Synthesis.**

1-o-Tolyl-propan-2-ol

To a solution of 2-Bromotoluene 1 (171 mg, 1.0 mmol) in dry ethyl ether (5 mL) at -78 °C, was added butyllithium (0.46 mL, 2.5 M in hexane) dropwise. Following addition, the cold bath was removed and the reaction mixture allowed to warm to room temperature (RT). After an additional 3 h of stirring, the reaction mixture was cooled to -78 °C and a solution of propylene oxide (0.14 mL in 0.14 mL of ethyl ether) added dropwise. Stirring was continued at -78 °C for an additional hour then allowed to warm to RT. The reaction was allowed to stir overnight and subsequently quenched by the addition of water. The resulting mixture was extracted with ethyl acetate (10 mL x 3), dried over MgSO4 and concentrated. The crude product was purified by flash silica chromatography (230-400 mesh), eluting with EtOAc/hexanes (1:6), to afford 2 as a colorless oil (514 mg, yield 63%).

1-o-Tolylpropan-2-one

1H NMR (300 MHz, CDCl3) δ: 1.25 (d, 3H, J = 6.3 Hz), 2.33 (s, 3H), 2.73 (dd, 1H, J = 7.8, 13.8 Hz), 2.77(dd, 1H, J = 5.4, 13.8 Hz), 4.00 (m, 1H), 7.15 (m, 4H).

13C NMR (75 MHz, CDCl3) δ: 19.8 (CH3), 23.2 (CH3), 43.1 (CH2), 68.1 (CH), 126.2 (CH), 126.8 (CH), 130.3 (CH), 130.7 (CH), 136.8 (C), 137.0 (C).

To a solution of 1-o-Tolyl-propan-2-ol 2 (50 mg, 0.33 mmol) in ethyl acetate (2.5 mL), was added 1-hydroxy-1,2-benziodoxol-3(1H)-one-1-oxide (IBX), (280 mg, 1.0 mmol) and the reaction stirred vigorously at 80 °C for ca. 5 h. The resulting suspension was filtered and the filter cake rinsed three times with ethyl acetate. The combined filtrate was concentrated to afford the crude product, which was then purified by flash silica chromatography [ethyl acetate and hexane (1:6)] to afford 3 as a colorless oil (44.5 mg, yield 91%).

1-o-Tolyl-propan-2-ol

1H NMR (300 MHz, CDCl3) δ: 2.15 (s, 3H), 2.25 (s, 3H), 3.72 (s, 2H), 7.20 (m, 4H).

13C NMR (75 MHz, CDCl3) δ: 19.8 (CH3), 29.4 (CH3), 49.3 (CH2), 126.4 (CH), 127.6 (CH), 130.5 (CH), 130.7 (CH), 133.3 (C), 137.0 (C), 206.6 (C).

2-Hydroxy-4-oxo-5-o-tolyl-pent-2-enoic acid ethyl ester

A solution of 1-o-Tolylpropan-2-one 3 (1.9 g, 12.8 mmol) in diethyl ether (60 mL), was cooled to 0 °C to which a solution of EtONa (Na 294 mg, 12.8 mmol in 5 mL of ethanol) was added dropwise. The reaction was allowed to stir for about 30 min. (at 0 °C), followed by the dropwise addition of diethyl oxalate (1.87 g, 12.8 mmol). The reaction mixture was subsequently stirred at RT for 24 h. The resultant pale yellow solid product, was collected by filtration and washed with cold ethyl ether giving the desired product 4 as its sodium enolate in 85% yield (2.9 g). The enolate was directly utilized in the next step without further purification.

**sodium enolate salt**

1H NMR (300 MHz, CDCl3) δ: 1.15 (t, 3H, J = 7.2 Hz), 2.11 (s, 3H), 3.38 (s, 2H), 3.97 (q, 2H, J = 7.2 Hz), 5.76 (s, 1H), 6.97-7.01 (m, 4H).

3-Hydroxy-5-methyl-naphthalene-1-carboxylic acid ethyl ester

Concentrated sulfuric acid (0.5 mL) was added slowly to a chilled solution of starting material 4 (0 °C), sodium enolate (65.7 mg, 0.24 mmol) in chloroform (9 mL), with vigorous
stirring. Following addition, the reaction mixture was stirred continuously at 0 °C for about 20 min. The reaction was then warmed to RT and stirred for an additional 30 min. The reaction mixture was poured into ground ice (25 mL), extracted with methylene chloride (25 mL x 4), dried over MgSO₄ and concentrated in vacuo. The organic residue was subsequently purified by flash silica chromatography (1:5 EtOAc/Hexanes) to generate the desired product 3-hydroxy-5-methyl-naphthalene-1-carboxylic acid ethyl ester 5 (38.7 mg, 71% yield).

3-hydroxy-5-methyl-naphthalene-1-carboxylic acid ethyl ester

\[ ^1H \text{ NMR (300 MHz, CDCl}_3 \delta: 1.48 (t, 3H, } J = 7.2 \text{ Hz), } 2.63 \text{ (s, 3H), } 4.50 \text{ (q, 2H, } J = 7.2 \text{ Hz), } 5.81 \text{ (bs, 1H, OH), } 7.34-7.38 \text{ (m, 2H), } 7.54 \text{ (dd, 1H, } J = 0.75, 2.7 \text{ Hz), } 7.81 \text{ (d, 1H, } J = 2.7 \text{ Hz), } 8.61 \text{ (m, 1H).} \]

\[ ^{13}C \text{ NMR (75 MHz, CDCl}_3 \delta: 14.6 \text{ (C}_H^3 \text{), } 20.2 \text{ (CH}_3 \text{), } 61.7 \text{ (CH}_2 \text{), } 111.7 \text{ (CH), } 121.1 \text{ (CH), } 124.1 \text{ (CH), } 125.1 \text{ (CH), } 127.1 \text{ (C), } 127.8 \text{ (CH), } 130.2 \text{ (C), } 133.2 \text{ (C), } 134.8 \text{ (C), } 152.2 \text{ (C), } 168.0 \text{ (C).} \]

3-Methoxy-5-methyl-naphthalene-1-carboxylic acid

To a solution of 3-Hydroxy-5-methyl-naphthalene-1-carboxylic acid ethyl ester 5 (101 mg, 1.0 mmol) and dimethyl sulfate (142.3 mg, 1.1 mmol) in 0.5 mL of dioxane, was added a 25% solution of aqueous sodium hydroxide (1.5 mL). After stirring at RT for 30 min, the mixture was heated to ~100 °C for 6 h and cooled to RT. The resulting basic mixture was extracted with dichloromethane (3 mL x 2). The aqueous phase was acidified to pH 5 by addition of concentrated hydrochloric acid and extracted with ethyl acetate (5 mL x 3). The organics were dried over MgSO₄ and concentrated in vacuo to afford the final product 6 as a pale white solid in quantitative yield.

\[ ^1H \text{ NMR (300 MHz, CDCl}_3 \delta: 2.69 \text{ (s, 3H), } 3.99 \text{ (s, 3H), } 7.38-7.43 \text{ (m, 2H), } 7.54 \text{ (d, 1H, } J = 2.4 \text{ Hz), } 8.05 \text{ (d, 1H, } J = 2.4 \text{ Hz), } 8.30 \text{ (m, 1H).} \]

\[ ^{13}C \text{ NMR (75 MHz, CDCl}_3 \delta: 20.2 \text{ (CH}_3 \text{), } 55.5 \text{ (CH}_3 \text{), } 109.3 \text{ (CH), } 122.8 \text{ (CH), } 123.9 \text{ (CH), } 125.2 \text{ (CH), } 127.1 \text{ (C), } 127.6 \text{ (CH), } 127.8 \text{ (C), } 133.2 \text{ (C), } 134.4 \text{ (C), } 155.8 \text{ (C), } 172.9 \text{ (C).} \]

References