14-Hydroxylation of Opiates: Catalytic Direct Autoxidation of Codeinone to 14-Hydroxycodine

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Biotransformation Profile

Time course of biotransformation of codeine (0.5 g/L, □) to codeinone (●), 14-hydroxycodeinone (○), 14-hydroxycodeine (■), and 7,8-dihydrocodeine (▲) by M. neoaurum.

Lag Phase

Time course of 14-hydroxylation of codeinone (2 mM) in the presence of thiosulfate (2.5 mM) and MnSO₄ (7 µM (■) or 42 µM (●)) in phosphate buffer (75 mM, pH 8.0).

Experimental Procedures

HPLC Analysis.
Analytical HPLC was performed on a Shimadzu system using an Alltech Alltima C8 column (7.0 x 53 mm, 3 µ) with a CH3CN–water mobile phase containing 0.1% trifluoroacetic acid at a flow rate of 1.0 mL/min and ambient temperature. Detection was at 284 nm with a runtime of 21 min. Gradient program: 100% water at 0 minutes, linear gradient to 20% organic in 8.5 minutes, linear gradient to 50% organic at 13.5 min, linear gradient to 80% organic at 14.5 min, hold at 80% organic until 16.5 min, back to 100% water at 17 min, re-equilibrate at 100% water for 4 min. Codeinone has a retention time of 11.94 min and 14-hydroxycodeinone of 11.45 min.

14-Hydroxylation of Codeinone by Manganese(II) Sulfate and Sodium Thiosulfate
To a 50 mL plastic centrifuge tube were added 4.8 mL of phosphate buffer (75 mM, pH 8.0), 100 µL of codeinone (100 mM in DMF, 10 µmole), 20 µL of sodium thiosulfate (640 mM in water, 25 µmole), and 40 µL of MnSO4 (0.88 mM in water, 0.035 µmole). This reaction mixture was kept in a rotary shaker at 29 °C and 300 rpm for 3 h. A sample of this mixture (0.5 mL) was extracted with chloroform (0.5 mL), and the organic phase was separated and evaporated to dryness. The residue was dissolved in 100 µL of DMF for HPLC analysis. The result indicated ~85% of codeinone was converted to 14-hydroxycodeinone. The identity of the 14-hydroxycodeinone was supported by an HPLC retention time match to that from a standard sample, and confirmed by LCMS, which showed an m/z 314 [M+H]+ in positive mode. The remaining reaction mixture was also extracted with chloroform, and the organic extract was evaporated to dryness. The residue showed a 1H NMR spectrum match to that of a standard sample. This was also the general procedure.

14-Hydroxylation of Codeinone Using Potassium Permanganate and Sodium Thiosulfate.
To a 2 L glass reactor were added 500 mL of potassium phosphate buffer (50 mM, pH 6.6) and codeinone (0.5 g, 1.7 mmole) with stirring. After the codeinone had dissolved, the pH of the solution was adjusted to 8.0 with 45% KOH, and maintained automatically using 1 N NaOH and 1 N HCl. To this solution were added 5 mL of sodium thiosulfate (1 M in water, 5.0 mmole) and 0.3 mL of potassium permanganate (95 mM in water, 0.03 mmole). The reaction mixture was sparged with air and stirred at room temperature. After 2.5 h the pH of the solution was adjusted to 12.0 by the addition of 1 M NaOH and the product was extracted using methylene chloride (3 x 200 mL). The combined organic phase was dried (MgSO4) and evaporated to give crude 14-hydroxycodeinone (357 mg) as an off-white solid. Crystallization from chloroform/petroleum ether (40/60) gave 14-hydroxycodeinone (300 mg, 57%) with purity greater than 99% by HPLC. 1H NMR (500 MHz) spectrum in DMSO-d6 was identical to that of a standard sample.