BIOTRANSFORMATIONS OF MORPHINE ALKALOIDS BY FUNGI: *N*-DEMETHYLATIONS, OXIDATIONS, AND REDUCTIONS

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Dedicated to Dr. Alfred Bader on the occasion of his 85th birthday.

Morphine alkaloids and some of its derivatives (morphine, codeine, thebaine, oripavine, hydrocodone, and oxycodone) were subjected to fermentations with six fungal strains. The alkaloids were transformed to a variety of products via biological oxidations, reductions, and oxidative demethylations. The strain *Cunninghamella echinulata* proved to be the most effective for demethylations of all of the above compounds, except for morphine. The time profile of the conversion of 3-[$^{14}CH_3$]thebaine to 3-[$^{14}CH_3$]northebaine by *C. echinulata* cultures was also determined.

Keywords: Morphine alkaloids; Fungal biotransformations; Demethylation; Oxidation; Reduction.

The morphine alkaloids and their semisynthetic derivatives, some of which are shown in Fig. 1, make up a sizable portion of the market for analgesia, anesthesia, and addiction control[1]. Commercial derivatives may contain a hydroxy group at C-14, *N*-allyl, *N*-(cyclopropylmethyl), other *N*-alkyl groups, or a combination of these functionalities. Oxidation at C-14 or the replacement of the *N*-methyl group with other alkyl moieties are not trivial operations and usually require several chemical steps. For example, the transformations required for the synthesis of naloxone and naltrexone usually involve reactions of morphinans with cyanogen bromide[2] or chloroformates[3], or required the preparation of *N*-oxides[4], the use of photochemical demethylation[5], and, most recently, palladium-catalyzed

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demethylation–acylation protocols[6]. A summary of mechanistic options for these processes is shown in Fig. 2. Although these methods are efficient, with some used in commercial manufacturing, the use of alternative biological methods to accomplish such tasks would be of great benefit. Ideally such methods could reduce the chemical operations to one step, conducted in aqueous environment.

Biotransformations of morphine alkaloids by a variety of strains were reported in the early 1960s. A compilation of these has been published[7]. Biological oxidation of thebaine to 14-hydroxycodeinone with the spores of *Trametes sanguinea* was reported by Iizuka[8]; a variety of thebaine-transforming strains of *Trametes* were investigated by Groger and Schmauder[9]. Mitscher and coworkers discovered that incubation of 7α-acetyl-6,14-ethenotetrahydrothebaine (**9**) with *Cunninghamella echinulata* resulted in demethylation (Fig. 3)[10]. Kieslich[7] remarks in his compilation that "*Microbial dealkylation of these compounds is superior to any chemical method*".

Encouraged by these reports, we decided to investigate demethylations of other morphinans, especially as we had observed that palladium-catalyzed demethylations seemed to be limited to hydrocodone and a few tropane-type alkaloids. In this paper we report the outcome of biotransformations of several morphine alkaloids with a variety of fungal strains.

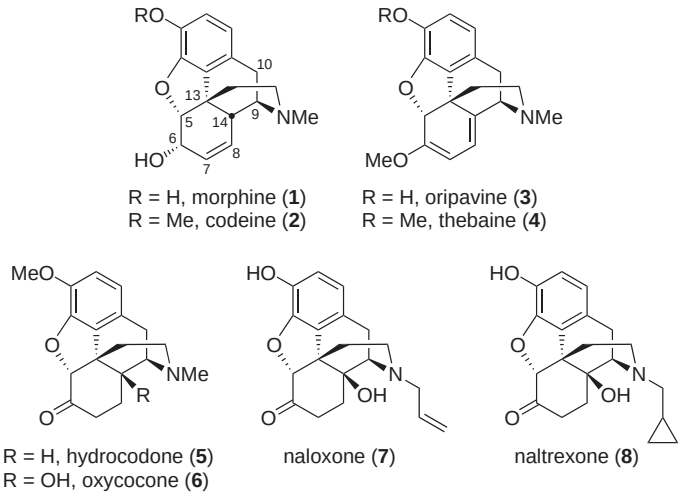


FIG. 1
Morphine alkaloids and some of their semisynthetic derivatives

DISCUSSION

In 1960 Iizuka and coworkers described the conversion of thebaine to 14-hydroxycodeine and 14-hydroxycodeinone by the basidiomycete *Trametes sanguinea*[8a]. Two years later the same group showed that *T. sanguinea* transformed codeinone to codeine, 14-hydroxycodeine, and 14-hydroxycodeinone[8b,8c]. In 1969, Groger found that *T. cinnabarina* converted thebaine to 14-hydroxycodeine, 14-hydroxycodeinone, and 14-hydroxycodeinone *N*-oxide[9].

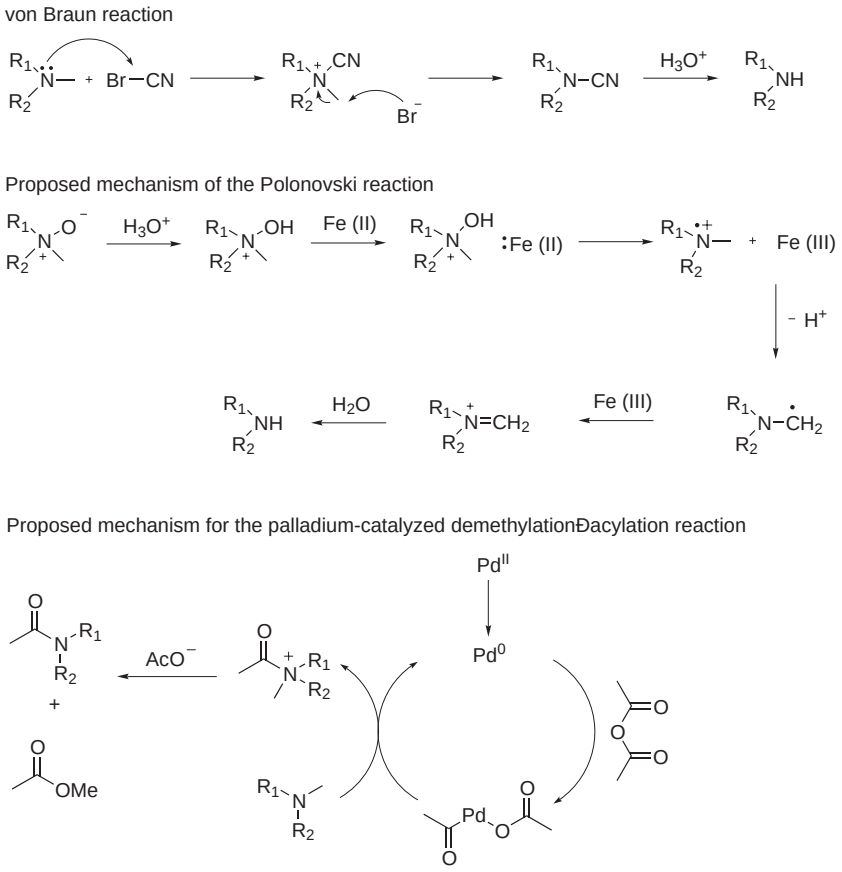


FIG. 2
Mechanistic representation of various demethylation protocols applicable to morphine alkaloids and derivatives

Sewell[11] and coworkers discovered in 1984 that several species of the fungal genus *Cunninghamella*, especially *C. bainieri*, could *N*-demethylate codeine to norcodeine. In 1994 Madyastha[12] found that the fungus *Mucor piriformis* transformed thebaine to northebaine in about 77% yield. Selective *N*- and *O*-demethylation of buprenorphine intermediates by *C. echincelata* (NRRL 2384) was reported by Carnell[13] in 2002. The *N*-demethylated buprenorphine intermediate **12** (Fig. 3) was formed in 39% yield.

We examined six fungal strains for biotransformations of six morphine alkaloids. The choice of the particular strains – *Cunninghamella echinulata* (American Type Culture Collection, ATCC 9244), *Thammostylum (helicostylum) piriforme* (ATCC 8992), *Trametes (Pycnoporus) sanguinea* (ATCC 14622), *Trametes (Pycnoporus) cinnabarina* (ATCC 14623), *Sporotrichum sulfurescens* (ATCC 7159), and *Curvularia lunata* (ATCC 12017) – was based on previous reports in the literature with respect to specific biotransformations. For example, *T. (helicostylum) piriforme* (ATCC 8992) is known for 14α-hydroxylations of steroids[14] and *S. sulfurescens* was used to hydroxylate monocyclic alcohols[15], perhydroquinolines[16], and *N*-acyladamantanamines[17]. In 1959 Holmlund and coworkers[18] observed hydroxylation of tetracycline derivatives by *C. lunata*, which is also known for hydroxylations of steroids[19].

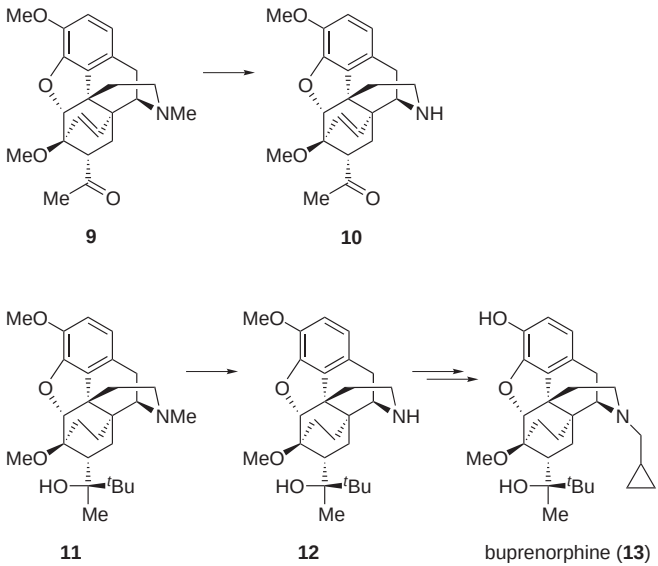
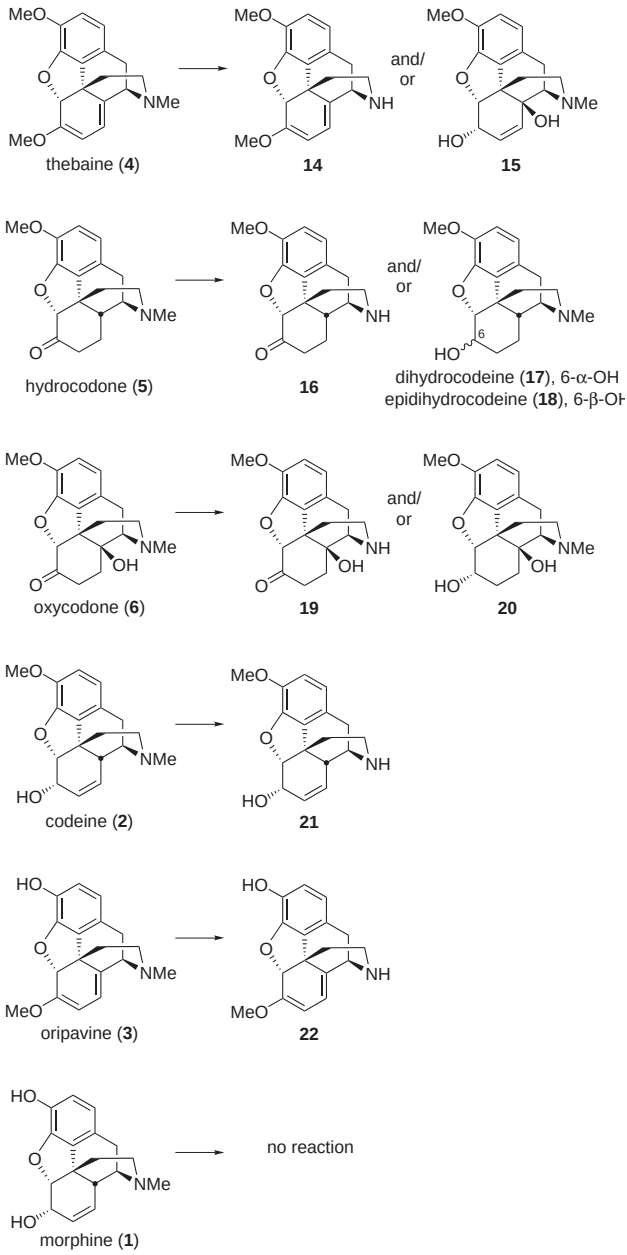


FIG. 3
N-Demethylation of 7α-acetyl-6,14-*endo*-ethenotetrahydrothebaine and buprenorphine intermediates

Each strain was obtained from the ATCC and cultivated according to standard protocols (see Experimental for description of maintenance of cultures). There was a significant difference in the conversion rates of morphine alkaloids depending on the morphology of the fungi. When Erlenmeyer flasks were used for biotransformations, the fungi produced a thick mycelium that aggregated to form a single huge biomass resulting in a low conversion rate. In baffled flasks, the fungi grew as small pellets, as shown in Fig. 4. The larger surface area of the pellet-like mycelium led to better rates of biotransformation.

Cunninghamella echinulata (ATCC 9244) was found to be the most effective for *N*-demethylation of thebaine, hydrocodone, codeine, oxycodone, and oripavine. We also observed C-14 oxidation as well as C-6 reduction with some of the strains. The results of biotransformations and the structures of all products are shown in Scheme 1. The profiles of biotransformations of the six different fungal strains and the specific products obtained with different strains are summarized in Table I. Thus thebaine (**4**) was transformed to either northebaine (**14**) or 14-hydroxycodeine (**15**) whereas oripavine (**3**) yielded only nororipavine (**22**). Hydrocodone (**5**) was *N*-demethylated to **16** or reduced at C-6 to dihydrocodeine (**17**) and epidihydrocodeine (**18**); oxycodone (**6**) yielded noroxycodone (**19**) or suffered C-6 reduction to 14-hydroxydihydrocodeine (**20**). Codeine (**2**) was *N*-demethylated to **21**. Morphine (**1**) proved completely inert to biotransformations by any of the six strains under investigation.

FIG. 4
Appearance of *Cunninghamella echinulata* when grown in a baffled flask



SCHEME 1
Products of biotransformations of morphine alkaloids with several fungal strains

The maximum accumulation of the demethylated products was observed when thebaine was used to induce the cultures. The yield of northebaine from thebaine varied between 35 and 50%. Hydrocodone, codeine, and oripavine were converted to the corresponding *N*-demethylated products norhydrocodone, norcodeine, and nororipavine, respectively, in yields of about 10%. The yield of noroxycodone from oxycodone was about 15%. To our knowledge, this is the first example of microbial *N*-demethylation of the morphine derivative hydrocodone. Neither morphine nor oxymorphone was found to be a substrate for any of the strains tested; both alkaloids were recovered unchanged from the culture media.

The *N*-demethylations of oripavine, hydrocodone, and oxycodone were noteworthy and hold considerable commercial potential if the conversions and yields can be improved. To this end, we have performed preliminary experiments designed to localize the enzyme(s) responsible for the demethylation.

Previous enzymatic studies with cell-free extracts of *Cunninghamella bainieri* suggested that *N*-demethylation of codeine occurs through the membrane-associated cytochrome-P450-dependent monooxygenase. Although cultures of *C. echinulata* were extracted by means of identical protocols, none of the conditions used by Gibson[20] produced northebaine as a reaction product when analyzed by HPLC or by thin-layer chromato-

TABLE I
Summary of biotransformations of morphine alkaloids by fungi

Substrate	*Cunninghamella echinulata* yield	*Helicostylum piriforme* yield	*Trametes sanguinea* yield	*Trametes cinnabarina* yield	*Sporotrichum sulfurescens*	*Curvularia lunata* yield
Thebaine (**4**)	*N*-demethylation 35–50%	*N*-demethylation 40%	14-hydroxycodeine 9–40%	14-hydroxycodeine 15%	No biotransformation	No biotransformation
Hydrocodone (**5**)	*N*-demethylation 10%	C-6 reduction 40%	C-6 reduction 12%	C-6 reduction 35%	No biotransformation	C-6 reduction 40%
Oxycodone (**6**)	*N*-demethylation 15%	C-6 reduction 10–15%	C-6 reduction 19%	No biotransformation	No biotransformation	C-6 reduction 63%
Codeine (**2**)	*N*-demethylation 10%	No biotransformation	No biotransformation	No biotransformation	No biotransformation	No biotransformation
Oripavine (**3**)	*N*-demethylation 9–21%	No biotransformation	No biotransformation	No biotransformation	No biotransformation	Not tested
Morphine (**1**)	No biotransformation	No biotransformation	No biotransformation	Not tested	No biotransformation	No biotransformation

graphy (TLC). In order to better reproduce the results of Gibson, a novel highly sensitive assay using 3-[$^{14}CH_3$]thebaine was also developed for detection of labeled northebaine in cell-free extracts of *C. echinulata*. While 3-[$^{14}CH_3$]thebaine was converted with time essentially quantitatively to 3-[$^{14}CH_3$]northebaine in biotransformation studies (Fig. 5), cell-free extracts assayed as described by Gibson were totally ineffective in producing this compound. An alternative mechanism postulated by Abel[21] suggested that *N*-demethylation may proceed by transfer of the *N*-methyl group to the phenolic OH group on C-3 by a regiospecific *N*- to *O*-methyltransferase. In biotransformation studies with 3-[$^{14}CH_3$]northebaine, the mechanism proposed by Abel should have involved loss of the label for regiospecific *N*- to *O*-methyltransferase activity to occur. Since 3-[$^{14}CH_3$]northebaine was almost quantitatively recovered during this biotransformation of 3-[$^{14}CH_3$]thebaine, it seems unlikely that *N*-demethylation would occur by this mechanism. Nevertheless, the availability of 3-[$^{14}CH_3$]thebaine will be extremely important to eventually develop an assay for this reaction and to verify what type of biochemical reaction is responsible for this biotransformation in *C. echinulata*.

In summary, we have demonstrated that *C. echinulata* cultures produce *N*-demethylated products from several morphine alkaloids and their derivatives. Future endeavors in this area will focus on improving the yields in order to achieve the long-term objectives of elucidating the mechanism of demethylation and identifying the responsible enzyme(s).

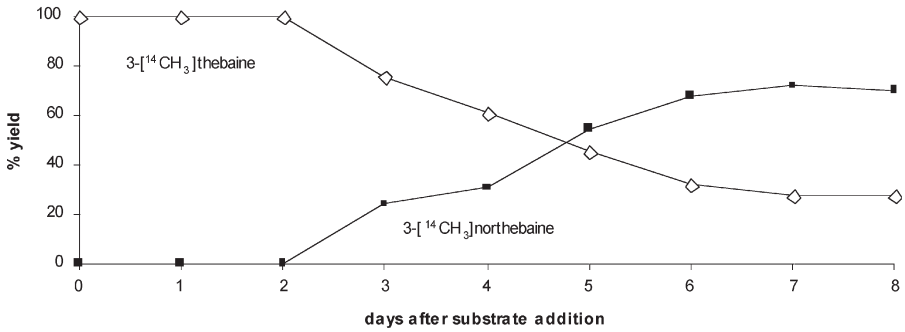


FIG. 5
Conversion of 3-[$^{14}CH_3$]thebaine to 3-[$^{14}CH_3$]northebaine by *C. echinulata* cultures

EXPERIMENTAL

Liquid reagents were distilled prior to use. Commercial solids were used as supplied. Analytical thin-layer chromatography was performed on Silicycle 60 Å 250 μm TLC plates with F-254 indicator. Flash-column chromatography was performed on Natland 200–400 mesh silica gel. Melting points were recorded on a Hoover Unimelt apparatus and are uncorrected. IR spectra (ν_{max}, cm^{-1}) were obtained on a Perkin–Elmer One FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were recorded on a Bruker spectrometer (300 or 600 MHz). All chemical shifts are referenced to TMS or to residual undeuterated solvent ($CHCl_3$ or dichloromethane (DCM)). Separation by HPLC was either performed on a Hitachi L-6000 chromatograph with a Hitachi L-4000H UV detector (254 nm) on a Phenomenex primespher 5 C18 HC 250 × 10 mm column (conditions: 2 ml/min flow; 5 mM KH_2PO_4, 0.1% NEt_3, pH 2.8 adjusted with 2 M HCl/MeOH (80:20)) or performed on an Agilent 1100 series chromatograph using a Phenomenex primesphere 5 C18 HC, 150 × 4.6 mm column and 1.3 ml/min flow. Compounds were detected at 280 nm and opioids were eluted with a gradient of 5 mM KH_2PO_4, 0.1% NEt_3, pH 2.8 adjusted with 2 M HCl/MeOH. Mass spectra were recorded on Kratos/MsI Concept 1S mass spectrometer at Brock University.

General Procedure for Biotransformations

Biotransformations were carried out in 200 ml of fermentation medium in 1-l reaction vessels. The medium was inoculated with 0.5 ml of concentrated spore suspension made up in sterile distilled water (typically 3 ml for 1 slant). The substrates were thebaine, hydrocodone, codeine, morphine, oxycodone or oripavine (100 mg/ml dissolved in 1 M HCl, except in the case of codeine). Codeine phosphate, 150 mg/ml dissolved in distilled water, was added to the fermentation medium to give a final concentration of 0.5 mg/ml after a specific time period (varies for each fungi) of post-inoculation. The progress of the biotransformation was followed by TLC, as described below.

After completion of the biotransformation (fermentation times and conditions are stated for each fungi below), the broth was separated from fungi by centrifugation, followed by filtration. The broth was alkalinized to pH 10 using concentrated NH_4OH (except in the case of morphine and oripavine for which the pH was adjusted to 8) and extracted with chloroform (3 × 250 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo. The crude residue was separated by flash column chromatography on silica gel with $CHCl_3$ and MeOH. The products were identified by ^{13}C and ^{1}H NMR, IR, MS and HPLC, and matched to literature data when possible.

TLC and Sample Preparation

The progress of the alkaloid demethylation reaction was monitored by TLC in a solvent system comprising DCM/MeOH/NH_4OH (92:8:1, v/v/v). Samples for analysis were prepared by mini work-up. This was performed as follows: 2 ml of broth was centrifuged, the supernatant was basified using concentrated aqueous NH_4OH and then extracted with DCM.

Propagation of Cultures

Cunninghamella echinulata, ATCC 9244, was propagated on potato dextrose agar (ATCC medium 336) slants at 26 °C. Mature slants (older that 7 days) were used for the preparation

of spore suspensions. Batch fermentations were carried out in GCN medium (glucose, 2%; corn steep liquor, 5%; Difco nutrient broth, 1%; pH 5.0) at 26 °C with vigorous aeration (150 rpm in baffled flasks on an orbital shaker). The substrate was added to the fermentation medium 24–48 h after inoculation and the fermentation was run for up to 15 days.

Trametes sanguinea, ATCC 14622, was propagated on yeast mold agar (ATCC medium 200) slants at 26 °C. Mature slants (older than 7 days) were used for the preparation of spore suspensions. Batch fermentations were carried in M-2 medium (glucose, 1%; peptone, 0.2%; beef/meat extract, 0.1%; yeast extract, 0.1%; corn steep liquor, 0.3%; pH 5.0) out at 30 °C with vigorous aeration (160 rpm on an orbital shaker). The substrate was added to the fermentation medium 4 days after inoculation and the fermentation was run for up to 22 days.

Trametes cinnabarina, ATCC 14623, was propagated in yeast mold broth (ATCC medium 200) at 26 °C. Mature cultures (older than 7 days) were used for the inoculation of the biotransformation medium. The culture was maintained on yeast mold agar slants. Batch fermentations were carried in nutrient-rich medium (NRM): (glucose, 0.5%; KH_2PO_4, 0.05%; L-asparagine, 0.052%; yeast extract, 0.05 g; KCl, 0.05 g; $MgSO_4 \cdot 7H_2O$, 0.05%; $FeSO_4$, 0.001%; dissolved in 950 ml distilled water; 50 ml mineral salt solution was added; pH 4.5; mineral salt solution: $Mn(CH_3COO)_2 \cdot 4H_2O$, 8 mg; $CuSO_4 \cdot 5H_2O$, 3 mg; $ZnSO_4 \cdot 7H_2O$, 2 mg; $Ca(NO_3)_2 \cdot 4H_2O$, 50 mg; deionized water, 50 ml) out at 30 °C with vigorous aeration (160 rpm on an orbital shaker). The substrate was added to the fermentation medium 7 days after inoculation and the fermentation was run for up to 9 days.

Helicostylum piriforme, ATCC 8992, was propagated on cornmeal yeast glucose agar (CMYG) (ATCC medium 310) slants at 24 °C. Mature slants (older than 7 days) were used for the preparation of spore suspensions. Batch fermentations were carried out in glucose–corn steep liquor–salt (GCS) medium (glucose, 3%; corn steep liquor, 1%; K_2HPO_4, 0.2%; KH_2PO_4, 0.1%; $NaNO_3$, 0.2%; KCl, 0.05%, $MgSO_4 \cdot 7H_2O$, 0.05%, $FeSO_4 \cdot 7H_2O$, 0.002%) at 26 °C with vigorous aeration (125 rpm in baffled flasks on an orbital shaker). The substrate was added to the fermentation medium 24 h after inoculation and the fermentation was run for up to 15 days.

Curvularia lunata, ATCC 12017, was propagated on rabbit food agar (ATCC medium 340) slants at 24 °C. Mature slants (older than 14 days) were used for the preparation of spore suspensions. Batch fermentations were carried out in Beef extract medium (BEM) (glucose, 0.5%; peptone, 0.1%; yeast extract, 0.1%; beef extract, 0.1%) at 27 °C with vigorous aeration (150 rpm in baffled flasks on an orbital shaker). The substrate was added to the fermentation medium 48 h after inoculation and the fermentation was run for up to 10 days.

Sporotrichum sulfurescens/Beauveria bassiana, ATCC 7159, was propagated on potato dextrose agar (PDA) (ATCC medium 336) slants at 24 °C. Mature slants (older than 7 days) were used for the preparation of spore suspensions. Batch fermentations were carried out in glucose–corn steep liquor medium (glucose, 1%; corn steep liquor, 2% in tap water) at 28 °C with vigorous aeration (150 rpm in baffled flasks on an orbital shaker). The substrate was added to the fermentation medium 4 days after inoculation and the fermentation was run for up to 7 days.

Purity of 3-[$^{14}CH_3$]thebaine

The 3-[$^{14}CH_3$]thebaine (specific activity 2.035 GBq/mmol) was produced by American Radiolabeled Chemicals (St. Louis, MO). The purity of 3-[$^{14}CH_3$]thebaine was checked by co-chromatography of the radioactive compound with pure thebaine using TLC in 2 separate solvents (toluene/ethyl acetate/diethylamine in the ratio 70:20:10 and DCM/MeOH/NH_4OH

(92:8:1). Autoradiography of these TLCs revealed that the sample contained a single radioactive spot corresponding to pure thebaine.

Time Profile of Biotransformations with 3-[$^{14}CH_3$]thebaine

C. echinulata was grown in 25 ml GCN broth for 48 h at 26 °C and 125 rpm to produce pellet form of fungal cells in baffled flasks (Fig. 4). At this point the culture was inoculated with 0.5 μCi 3-[$^{14}CH_3$]thebaine together with 12.5 mg unlabeled thebaine. Samples (1 ml) were harvested for analysis every 24 h for biotransformation product formation. Samples were titrated with NaOH to pH ~10, extracted for alkaloids into EtOAc and the EtOAc solution was evaporated to dryness. Samples were dissolved in 10 ml of methanol and subjected to TLC analysis. The spots corresponding to 3-[$^{14}CH_3$]thebaine (R_F 0.5) and 3-[$^{14}CH_3$]northebaine (R_F 0.25) were harvested from the TLC plates and quantified by counting in a liquid scintillation counter.

Analytical and Spectral Data

Northebaine (**14**). The title compound **14** was isolated as colorless solid following the protocol for biotransformations using *Cunninghamella echinulata* (ATCC 9244) and *Helicostylum piriforme* (ATCC 8992) in 35–50 and 39% yields, respectively. Data for **14** were identical to those published in the literature[22]. M.p. 209–210 °C (MeOH/diethyl ether); R_F 0.59 (DCM/MeOH/NH_4OH, 98:2:1); $[\alpha]_D^{20}$ –197.9 (*c* 0.31, 5% MeOH in $CHCl_3$) (lit.[23] $[\alpha]_D^{23}$ –200 (*c* 0.1, 5% MeOH in $CHCl_3$); lit.[24] (+)-northebaine: $[\alpha]_D^{28}$ +235.2 (*c* 0.108, $CHCl_3$)). IR (film): 3295, 3004, 2931, 2837, 1668, 1607, 1505, 1447, 1234, 1019. ^{1}H NMR (MeOH-d_4, 600 MHz): 6.74 (d, *J* = 8.2, 1 H), 6.64 (d, *J* = 8.2, 1 H), 5.63 (d, *J* = 6.5, 1 H), 5.27 (s, 1 H), 5.15 (d, *J* = 6.5, 1 H), 4.00 (d, *J* = 6.4, 1 H), 3.84 (s, 3 H), 3.62 (s, 3 H), 3.07–3.19 (m, 3 H), 2.94 (dd, *J* = 13.4, 4.5, 1 H), 2.14 (dt, *J* = 12.9, 5.0, 1 H), 1.79 (dd, *J* = 13.2, 2.8, 1 H). ^{13}C NMR (MeOH-d_4, 150 MHz): 152.9, 144.8, 142.8, 133.2, 131.6, 127.4, 119.2, 113.7, 111.7, 95.5, 88.7, 55.8, 54.0, 53.4, 46.1, 38.4, 37.5, 36.2. MS (EI), *m/z* (%): 298 (3), 297 (13), 88 (19), 86 (65), 84 (100), 49 (16), 47 (21), 45 (40). HRMS (EI): for $C_{18}H_{19}NO_3$ calculated 297.1365, found 297.1368.

14-Hydroxycodeine (**15**). The title compound was isolated following the protocol for biotransformations using *Trametes sanguinea* (ATCC 14622) and *Trametes cinabarina* (ATCC 14623) in 35–50% yield as colorless solid. M.p. 109–111 °C (MeOH/diethyl ether); R_F 0.25 (DCM/MeOH/NH_4OH, 98:2:1); $[\alpha]_D^{20}$ –127.9 (*c* 0.3, $CHCl_3$) (lit.[25] $[\alpha]_D^{20}$ –129.5 (*c* 0.175, $CHCl_3$)). ^{1}H NMR ($CDCl_3$, 600 MHz): 6.65 (d, *J* = 8.2, 1 H), 6.57 (d, *J* = 8.2, 1 H), 5.91 (d, *J* = 9.8, 1 H), 5.49 (dd, *J* = 9.9, 2.9, 1 H), 4.87 (d, *J* = 6.5, 1 H), 4.60–4.65 (m, 1 H), 3.83 (s, 3 H), 3.18 (d, *J* = 18.5, 1 H), 3.03 (d, *J* = 6.4, 1 H), 2.98 (d, *J* = 10.2, 1 H), 2.45–2.57 (m, 2 H), 2.41 (s, 3 H), 2.35–2.39 (m, 2 H), 1.72–1.79 (m, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz): 145.5, 142.5, 138.1, 132.3, 128.8, 125.8, 119.3, 113.1, 90.0, 68.7, 65.2, 64.0, 56.3, 46.7, 45.2, 42.8, 31.7, 22.2. MS (EI), *m/z* (%): 316 (20), 315 (100), 313 (12), 230 (19), 229 (31), 188 (16), 175 (11), 143 (31), 141 (12), 140 (21), 131 (14), 150 (12), 70 (56), 58 (17), 55 (11), 44 (58). HRMS (EI): for $C_{18}H_{21}NO_4$ calculated 315.1471, found 315.1470.

Norhydrocodone (**16**). The title compound was isolated following the protocol for biotransformations using *Cunninghamella echinulata* (ATCC 9244) in 10% yield as colorless solid. Data for **16** matched those published in the literature[26]. M.p. 149–151 °C (MeOH/diethyl ether); R_F 0.25 (DCM/MeOH/NH_4OH, 98:2:1); $[\alpha]_D^{20}$ –83.9 (*c* 0.215, $CHCl_3$) (lit.[27] $[\alpha]_D^{11}$ –4.0 (*c* 1.0, MeOH)). IR (film): 3369, 2928, 1725, 1636, 1609, 1504, 1439, 1274, 1061. ^{1}H NMR

($CDCl_3$, 600 MHz): 6.74 (d, *J* = 8.2, 1 H), 6.67 (d, *J* = 8.2, 1 H), 4.66 (s, 1 H), 3.93 (s, 3 H), 3.48–3.52 (m, 1 H), 2.85–2.95 (m, 2 H), 2.79 (d, *J* = 18.5, 1 H), 2.71–2.77 (m, 1 H), 2.55 (dt, *J* = 12.6, 3.2, 1 H), 2.45 (dt, *J* = 13.8, 4.6, 1 H), 2.40 (td, *J* = 13.8, 4.6, 1 H), 1.97 (td, *J* = 12.3, 4.8, 1 H), 1.82–1.91 (m, 2 H), 1.22 (qd, *J* = 13.3, 3.2, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz): 207.6, 145.5, 142.9, 127.3, 126.3, 119.9, 114.7, 91.6, 56.8, 52.4, 47.7, 43.0, 40.3, 39.0, 36.0, 30.8, 25.8. MS (EI), *m/z* (%): 285 (9), 87 (11), 86 (21), 85 (65), 84 (35), 83 (100), 49 (14), 48 (13), 47 (33). HRMS (EI): for $C_{17}H_{19}NO_3$ calculated 285.1365, found 285.1364.

Dihydrocodeine (**17**) *and epidihydrocodeine* (**18**). A mixture of alcohols **17** and **18** (5:4) was isolated following the protocol for biotransformations using *Curvularia lunata* (ATCC 12017) in 40% combined yield as colorless oil. Careful purification by flash-column chromatography ($CHCl_3$/MeOH) allowed for the preparation of analytically pure samples. Data for dihydrocodeine (**17**) matched those published in the literature[28]. R_F 0.55 (DCM/MeOH/NH_4OH, 98:2:1) (lit.[29] $[\alpha]_D^{21}$ –210 (*c* 0.246, 1,4-dioxane)). ^{1}H NMR ($CDCl_3$, 600 MHz): 6.74 (d, *J* = 8.1, 1 H), 6.65 (d, *J* = 8.2, 1 H), 4.62 (d, *J* = 5.7, 1 H), 4.03–4.08 (m, 1 H), 3.89 (s, 3 H), 3.08–3.12 (m, 1 H), 3.02 (d, *J* = 18.3, 1 H), 2.53 (dd, *J* = 12.0, 4.6, 1 H), 2.36–2.44 (m, 4 H), 2.20–2.29 (m, 2 H), 1.90 (td, *J* = 12.3, 5.0, 1 H), 1.69–1.75 (m, 1 H), 1.55–1.62 (m, 1 H) 1.41–1.53 (m, 2 H), 1.10–1.19 (m, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz): 146.2, 141.6, 130.4, 127.0, 119.2, 113.1, 90.5, 67.2, 59.7, 56.4, 46.8, 43.0, 42.2, 40.7, 37.5, 27.2, 20.0, 19.2. MS (EI), *m/z* (%): 302 (20), 301 (100), 300 (23), 299 (32), 244 (14), 243 (11), 242 (19), 185 (12), 164 (16), 115 (10), 70 (19), 59 (24), 44 (23). HRMS (EI): for $C_{18}H_{23}NO_3$ calculated 301.1678, found 301.1678.

Data for epidihydrocodeine (**18**) matched those published in the literature[28,30]. R_F 0.51 (DCM/MeOH/NH_4OH, 98:2:1) (lit.[30a] $[\alpha]_D^{28}$ –132 (*c* 0.32, $CHCl_3$)). ^{1}H NMR ($CDCl_3$, 600 MHz): 6.73 (d, *J* = 8.2, 1 H), 6.65 (d, *J* = 8.2, 1 H), 4.37 (d, *J* = 6.5, 1 H), 3.89 (s, 3 H), 3.43–3.50 (m, 1 H), 3.09 (m, 1 H), 3.03 (d, *J* = 18.3, 1 H), 2.53 (dd, *J* = 11.9, 4.5, 1 H), 2.42 (s, 3 H), 2.36 (dd, *J* = 18.3, 5.3, 1 H), 2.12–1.23 (m, 2 H), 1.80–1.90 (m, 2 H), 1.69–1.74 (m, 1 H), 1.56–1.63 (m, 1 H), 1.38 (q, *J* = 12.9, 1 H), 1.00 (q, *J* = 12.9, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz): 144.1, 143.5, 130.5, 126.7, 119.0, 113.3, 97.2, 73.3, 59.4, 56.5, 47.0, 43.2, 43.0, 42.9, 35.6, 30.0, 23.7, 20.1. MS (EI), *m/z* (%): 302 (20), 301 (100), 300 (20), 299 (16), 286 (11), 244 (26), 242 (12), 185 (12), 86 (50), 84 (73), 70 (21), 59 (31), 57 (32), 56 (21), 55 (11), 49 (12), 47 (14), 44 (23). HRMS (EI): for $C_{18}H_{23}NO_3$ calculated 301.1678, found 301.1672.

Noroxycodone (**19**). The title compound was isolated following the protocol for biotransformations using *Cunninghamella echinulata* (ATCC 9244) in 15% yield as colorless oil in 80% purity. Data for **19** matched closely to those published in the literature[31]. R_F 0.21 (DCM/MeOH/NH_4OH, 98:2:1); $[\alpha]_D^{20}$ –102.7 (*c* 0.875, MeOH) (lit.[31a] $[\alpha]_D^{20}$ –232 (*c* 0.2); lit.[24] (+)-noroxycodone: $[\alpha]_D^{25}$ +100.4 (*c* 0.55, MeOH)). IR (film): 3399, 3350, 3007, 2932, 2838, 1725, 1636, 1608, 1505, 1440, 1277, 1051. ^{1}H NMR ($CDCl_3$, 600 MHz): 6.73 (d, *J* = 8.2, 1 H), 6.66 (d, *J* = 8.2, 1 H), 4.68 (s, 1 H), 3.92 (s, 3 H), 2.95–3.28 (m, 3 H), 2.68–2.86 (m, 2 H), 2.22–2.50 (m, 3 H), 1.85–1.97 (m, 1 H), 1.47–1.74 (m, 2 H). MS (EI), *m/z* (%): 301 (5), 88 (11), 86 (65), 84 (100), 83 (12), 49 (18), 47 (23). HRMS (EI): for $C_{17}H_{19}NO_4$ calculated 301.1314, found 301.1316.

14-Hydroxydihydrocodeine (**20**). The title compound was isolated following the protocol for biotransformations using *Trametes sanguinea* (ATCC 14622) in 10–15% yield as colorless oil. Data (^{1}H NMR and ^{13}C NMR) for compound **20** were found to be identical to the major isomer obtained from the reduction of oxycodone with sodium borohydride in methanol. R_F 0.35 (DCM/MeOH/NH_4OH, 98:2:1); $[\alpha]_D^{20}$ –93.1 (*c* 0.65, $CHCl_3$) (lit.[25] $[\alpha]_D$ –169 (*c* 0.6, $CHCl_3$); –138 (*c* 1.8, 10% HOAc)). ^{1}H NMR ($CDCl_3$, 600 MHz): 6.78 (d, *J* = 8.2, 1 H), 6.62 (d, *J* = 8.2, 1 H), 4.78 (d, *J* = 6.5, 1 H), 4.12–4.29 (m, 1 H), 3.88 (s, 3 H), 3.14 (d, *J* = 18.5, 1 H),

2.80 (d, *J* = 5.5, 1 H), 2.60 (dd, *J* = 18.5, 5.6, 1 H), 2.40–2.51 (m, 1 H), 2.38 (s, 3 H), 2.19–2.31 (m, 2 H), 1.74–1.89 (m, 1 H), 1.38–1.70 (m, 3 H), 1.09–1.28 (m, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz): 146.5, 141.7, 131.4, 126.1, 118.9, 113.8, 90.8, 70.0, 66.7, 64.7, 56.5, 46.3, 45.0, 43.1, 33.2, 28.2, 23.7, 22.1.

Norcodeine (**21**). The title compound was isolated following the protocol for biotransformations using *Cunninghamella echinulata* (ATCC 9244) in 10% yield as colorless solid. Data for **21** matched those published in the literature[22]. M.p. 182–183 °C (MeOH/diethyl ether); R_F 0.22 (DCM/MeOH/NH_4OH, 98:2:1); $[\alpha]_D^{20}$ –90.9 (*c* 0.224, $CHCl_3$) (lit.[24] for (+)-norcodeine: $[\alpha]_D^{28}$ +115.2 (*c* 1.09, $CHCl_3$)). IR (film): 3309, 3000, 2935, 2837, 1635, 1603, 1504, 1453, 1282, 1127, 943. ^{1}H NMR ($CDCl_3$, 600 MHz): 6.70 (d, *J* = 8.2, 1 H), 6.60 (d, *J* = 8.2, 1 H), 5.72–5.76 (m, 1 H), 5.28 (dt, *J* = 9.9, 2.5, 1 H), 4.89 (d, *J* = 6.4, 1 H), 4.17–4.22 (m, 1 H), 3.87 (s, 3 H), 3.65–3.69 (m, 2 H), 2.98 (td, *J* = 12.1, 4.4, 1 H), 2.86–2.93 (m, 2 H), 2.83 (d, *J* = 18.7, 1 H), 2.59–2.63 (m, 1 H), 1.88–1.98 (m, 2 H). ^{13}C NMR ($CDCl_3$, 150 MHz): 146.4, 142.2, 133.7, 131.1, 128.2, 127.4, 119.6, 112.8, 91.9, 66.3, 56.3, 52.0, 43.9, 41.3, 38.5, 36.6, 31.4. MS (EI), *m/z* (%): 285 (39), 87 (13), 86 (17), 85 (70), 84 (25), 83 (100), 82 (10), 59 (34), 49 (11), 47 (25), 45 (13), 44 (29), 43 (19), 42 (12). HRMS (EI): for $C_{17}H_{19}NO_3$ calculated 285.1365, found 285.1368.

Nororipavine (**22**). The title compound **22** was isolated following the protocol for biotransformations using *Cunninghamella echinulata* (ATCC 9244) in 9–21% yield as colorless oil. R_F 0.15 (DCM/MeOH/NH_4OH, 98:2:1); $[\alpha]_D^{20}$ –69.4 (*c* 0.475, MeOH) (lit.[32] $[\alpha]_D$ –90 (*c* 0.27, MeOH)). ^{1}H NMR (MeOH-d_4, 600 MHz): 6.66 (d, *J* = 8.2, 1 H), 6.63 (d, *J* = 8.2, 1 H), 5.94 (d, *J* = 6.4, 1 H), 5.39 (s, 1 H), 5.23 (d, *J* = 6.4, 1 H), 4.57 (d, *J* = 6.8, 1 H), 3.67 (s, 3 H), 3.21–3.38 (m, 6 H), 2.25–2.36 (m, 1 H), 2.02 (d, *J* = 13.5, 1 H). ^{13}C NMR ($CDCl_3$, MeOH-d_4, 150 MHz): 154.5, 143.6, 140.1, 131.7, 124.7, 122.9, 119.7, 117.1, 116.9, 94.8, 87.5, 54.4, 53.6, 44.9, 37.2, 34.2, 33.3. MS (EI), *m/z* (%): 284 (17), 283 (55), 282 (29), 265 (15), 229 (16), 228 (13), 227 (10), 149 (23), 97 (11), 84 (11), 83 (15), 82 (11), 71 (17), 70 (15), 69 (18), 57 (30), 56 (13), 55 (23), 46 (20), 45 (100), 44 (45). HRMS (EI): for $C_{17}H_{17}NO_3$ calculated 283.1208, found 283.1214.

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