Mucor piriformis, An Efficient *N*-Dealkylating Reagent for Thebaine and its *N*-Variants

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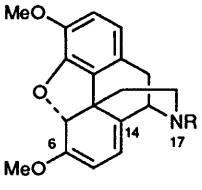
Mucor piriformis very efficiently *N*-dealkylates thebaine, its *N*-substituted variants and Diels–Alder adducts.

Although microorganisms have been successfully utilized to carry out critical steps in the synthesis of a number of pharmaceutically important steroids,[1] their use in the preparation of non-steroidal compounds such as alkaloids has been less. *N*-Dealkylation of the isoquinoline alkaloid thebaine **1a**, a crucial step in its use for the synthesis of various morphine agonists and antagonists,[2,3] is usually carried out either by the CNBr method[4] or by treatment with vinyl chloroformate.[5] These reactions, however, involve harsh reaction conditions as well as hazardous, toxic and expensive reagents and microbial methods could offer a promising and alternative approach.

Although microbiol conversion of **1a** into 14-hydroxycodeinone and 14-hydroxycodeinone *N*-oxide has been reported,[6] when the 14-position in **1a** is blocked by an ethenobridge, as in the case of its Diels–Alder adduct, the *N*-demethylation gives a very poor yield of product (3–10%).[7] Earlier we isolated a versatile fungal strain, identified as *Mucor piriformis*, which was shown to induce useful steroid transformations.[8,9] We have now demonstrated that this organism efficiently effects *N*-dealkylation of **1a** and its *N*-alkyl variants (alkyl = Et, Pr, Pr^i, Bu c-PrCH$_2$; Scheme 1, **1a–f**) to give the



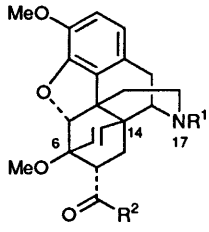
1 R = Alkyl
2 R = H

1	R	Yield (%) of 2
1a	Me	81
1b	Et	83
1c	Pr	86
1d	Pr^i	88
1e	Bu	89
1f	$CH_2CHCH_2CH_2$	92

Scheme 1

nor compound in 80–90% yield; some 5% of the substrate was converted into other metabolites. In fact, the yield of the nor compound (northebaine) was increased (~95%) by a prolonged incubation period. It is significant that the microbial process induces no further change in the northebaine produced. Thus, by monitoring the disappearance of the substrate from the fermentation medium, it is possible to convert nearly 95% of the added substrate to its nor compound. As indicated in Scheme 1 the size of the *N*-alkyl had no significant effect on the *N*-dealkylation.

Similarly, 6,14-*endo*-ethenotetrahydrothebaine alkaloids **3** (Scheme 2) were transformed into the corresponding nor



3 R^1 = Me
4 R^1 = H

3	R^2	Yield (%) of 4
a	Me	83
b	OMe	62

Scheme 2

compound **4** in high yields. Contrary to earlier reports,[7] the organism induces only *N*-demethylation of the Diels–Alder adduct and the yields of the nor compound can be further improved by a prolonged incubation period.

Experimental

N-Variants[10] and Diels–Alder adducts[11] of **1a** were prepared according to the reported procedures.

Mucor piriformis was maintained on Czapek-Dox agar slants. The slants were inoculated under aseptic conditions from stock culture and incubated at 30 °C for 5 days to ensure good sporulation. The slants were then stored at 4 °C until further used. Sterile water (5 cm^3) was added to the sporulated slant and the spores were loosened by scraping the surface gently. The spore suspension (1 cm^3, 2.7 × 10^7 spore count cm^{-3}) was added to each 500 cm^3 Erlenmayer flask containing 100 cm^3 of sterile modified Czapek-Dox medium[8] (pH 6–6.3). Substrates (**1a–f**, **3a**, **b**) were added at 1 mmol dm^{-3} concentration. The flasks were incubated on a rotary shaker (220 rpm) at 29–30 °C. At the end of the incubation period (4 days) the contents of the flasks were extracted with $CHCl_3$–MeOH (2:1). From the organic layer metabolites (nor compounds in Schemes 1 and 2) were purified by silica gel column chromatography using 2–10% MeOH in $CHCl_3$. TLC was carried out on silica gel G plates (0.25 mm) developed with 4% MeOH in $CHCl_3$. The amount of *N*-dealkylated compound formed was estimated by HPLC on a Water Associates ALC/GPC L44 series instrument using microporasil normal phase column and $CHCl_3$–MeOH (4:1, v/v) containing 0.025% NH_4OH as mobile phase. Eluents were monitored by UV detector at 254 nm. The chromatographically pure compounds were characterised by conventional spectroscopic methods.

The present study has clearly demonstrated the suitability of *Mucor piriformis* as an efficient reagent to carry out *N*-dealkylation of **1a** and its *N*-variants.

The spectral data of compounds **2** corresponded well to that reported for northebaine earlier;[12] *J* values are given in Hz and [α] values in 10^{-1} deg mol^{-1} cm^{-1}.

Compound **4a** $[\alpha]_D^{23}$ −240 (*c* 0.1, $CHCl_3$); ν_{max}(film)/cm^{-1} 3300; δ_H(90 MHz, $CDCl_3$) 6.64 (1 H, d, *J* 7.2, 1-H), 6.52 (1 H, d, *J* 7.2, 2-H), 5.9 (1 H, d, *J* 10.8, 18-H), 5.54 (1 H, d, *J* 10.8, 17-H), 4.56 (1 H, s, 5-H), 3.76 (3 H, s, 3-OMe), 3.02 (3 H, s, 6-OMe) and 2.18 (3 H, s, 20-H); *m/z* (70 eV) 367 (M^+, 100%) [Found: *m/z* (HRMS) 367.1778. $C_{22}H_{25}NO_4$ requires 367.1784]; δ_C(90 MHz, $CDCl_3$) 208.52, 147.96, 141.89, 135.39, 133.55, 127.59, 126.18, 119.35, 113.72, 95.62, 81.00, 56.51, 53.48, 52.72, 50.44, 47.95, 42.10, 37.01, 33.76, 32.57, 30.61 and 29.42.

Compound **4b** $[\alpha]_D^{24}$ −180 (*c* 0.1, $CHCl_3$); ν_{max}(film)/cm^{-1} 3300; δ_H(90 MHz, $CDCl_3$) 6.64 (1 H, d, *J* 7.2, 1-H), 6.52 (1 H, d, *J* 7.2, 2-H), 5.86 (1 H, d, *J* 10.8, 18-H), 5.58 (1 H, d, *J* 10.8, 17-H), 4.56 (1 H, s, 5-H), 3.84 (3 H, s, 3-OMe), 3.68 (3 H, s, 20-H), 3.02 (3 H, s, 6-OMe); *m/z* (70 eV) 383 (M^+, 100%) [Found: *m/z* (HRMS) 383.1728. $C_{22}H_{25}NO_5$ requires 383.1733] δ_C(90 MHz, $CDCl_3$) 174.00, 148.54, 142.37, 135.65, 134.35, 128.39, 127.31, 119.94, 114.09, 94.80, 81.15, 56.99, 53.30, 52.33, 48.54, 43.77, 42.58, 37.81, 34.61, 33.48 and 31.10.

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