Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 106, No. 5, October 1994, pp. 1203–1212.
© Printed in India.

Preparatively useful transformations of steroids and morphine alkaloids by *Mucor piriformis*

K M MADYASTHA
Department of Organic Chemistry, Indian Institute of Science, Bangalore 560012, India

Abstract. A versatile fungus isolated in our laboratory and identified as *Mucor piriformis* has been shown to effect novel and preparatively useful transformations in steroids and morphine alkaloids. The organism very effectively carries out hydroxylation of various C_{19} and C_{21} steroids at 7 and 14-positions. Although the organism is capable of catalysing hydroxylation at 6β and 11α-positions, these are not the major activities. The 14α-hydroxylase appears to have a broad substrate specificity. However, steroids with a bulky substitution at C-17 α-position or without the 4-en-3-one group are not accepted as substrates by the 14α-hydroxylase system. Studies have demonstrated how various C_{19} and C_{21} steroids can be modified to yield new structures which are either difficult to prepare by traditional methods or hitherto unknown. The organism also very efficiently and selectively carries out the N-dealkylation of thebaine and its N-variants. Interestingly, the nor-compound formed does not get further metabolized. Since thebaine is very often used as a starting material to synthesize various morphine agonists as well as antagonists, and one of the steps involved in their preparation is the N-dealkylation reaction, the microbial process could certainly offer an alternative approach.

Keywords. Steroids; morphine alkaloids; *Mucor piriformis*; hydroxylation; N-dealkylation; transformation.

1. Introduction

In recent years, the most significant development that has taken place in the field of synthetic chemistry has been the application of biological systems to chemical reactions. One of the biological systems which appears to have the greatest potential in synthetic organic chemistry is microorganisms or enzymes isolated from them. Modification of natural products using microorganisms has been a very useful method in synthetic organic chemistry. Reactions catalyzed by microbes often offer significant advantages including those of efficiency, regiospecificity, stereoselectivity, etc. The conditions under which microbial reactions take place are mild and hence compounds sensitive to heat, acids and bases can be easily subjected to such transformations. Microbes and microbial enzymes are being used as reagents in various organic synthesis (Rosazza 1982; Yamada and Shimizu 1988; Crout and Christen 1989; Davies *et al* 1989). The scope of microbial reactions is widened considerably due to the finding that these reactions can also be carried out in organic solvents or in emulsions of water and immiscible organic solvents. Today, chemists are exploring the possibility of using microbes or enzymes isolated from them in the synthesis of different chiral synthons and physiologically as well as commercially important compounds.

The field of microbial transformation of steroids got a tremendous boost after the successful 11α-hydroxylation of progesterone by the fungus, *Rhizopus arrhizus*

(Peterson and Murray 1952). Transformation of steroids by various fungi have gained industrial importance since these methods can be used in the synthesis of steroidal hormones and their analogs. Hydroxylation at specific positions of a steroid molecule can be efficiently carried out using microorganisms. In fact, significant amount of work has already been carried out on the microbial steroid transformations and excellent reviews have appeared in the literature on this topic (Charney and Herzog 1967; Iizuka and Naito 1981; Mahato *et al* 1989).

In our efforts to find suitable microorganisms which can effect novel and useful transformations in some of the representative steroids, we isolated a fungal strain identified as *Mucor piriformis* which has been shown to effect novel and preparatively useful transformations of some of the steroids and morphine alkaloids. The present paper is confined to various transformations mediated by this versatile microorganism and the scope of its synthetic applicability. However, complete potential of *Mucor piriformis* as a tool in organic synthesis is yet to be established.

2. Transformations of C_{21} and C_{19} steroids by *Mucor piriformis*

Fungi belonging to the genus *Mucor* have been reported to mainly effect hydroxylation at 14α, 11α, 7α and 6β-positions of various steroids (Tamm *et al* 1963; Vezina and Singh 1975; Holland and Riemland 1985; Madyastha and Srivatsan 1987; Krishnan *et al* 1991). However, much information has been covered by patents (Murray and Peterson 1957; Dodson and Tweitt 1960; Charney and Herzog 1967). *Mucor piriformis* isolated in our laboratory has been shown to be versatile in effecting transformations in both C_{21} and C_{19} steroids. As far as we know only two reports have appeared in the literature on the transformations of steroids; viz. 21-hydroxy-4-pregnene-3,20-dione (Murray and Peterson 1957) and 17α-21-dihydroxy-4-pregnene-3,20-dione (Eroshin 1962) by *Mucor piriformis*. These reports indicate the ability of this organism to introduce hydroxyl function at C-9, C-11 and C-6 positions. However, *Mucor piriformis* isolated in our laboratory is known to hydroxylate mostly at 7 and 14 positions in various C_{19} and C_{21} steroids.

2.1 *Transformations of progesterone (1)*

Mucor piriformis has been shown to transform progesterone (1) predominantly into 14α-hydroxyprogesterone (2) which further gets hydroxylated at 6β, 7α or 7β positions, thereby yielding the corresponding dihydroxyprogesterones (figure 1) (Madyastha and Srivatsan 1987). The organism also produces 5β, 14α-dihydroxypregnane-3,20-dione (6) as a minor metabolite. Time course experiments have clearly demonstrated that 14α-hydroxylation is the first step involved in the transformation sequence (figure 1). In fact in 12 h, nearly 75% of progesterone (1) gets metabolised mostly to 14α-hydroxyprogesterone (2) whereas prolonging the incubation to 48 h results in the formation of dihydroxyprogesterones (figure 1, compounds 3,4 and 5). Transformation of progesterone (1) into compounds 5 and 6 (figure 1) by *Mucorals* has not been reported earlier. Earlier studies have indicated that *Mucor* species have rigid stereoselectivity in their ability to hydroxylate various C_{19} and C_{21} steroids (Vezina and Singh 1975). However, it is interesting to note that *M. piriformis* isolated in our laboratory readily hydroxylates 14α-hydroxyprogesterone (2) at both 7α and 7β-positions.

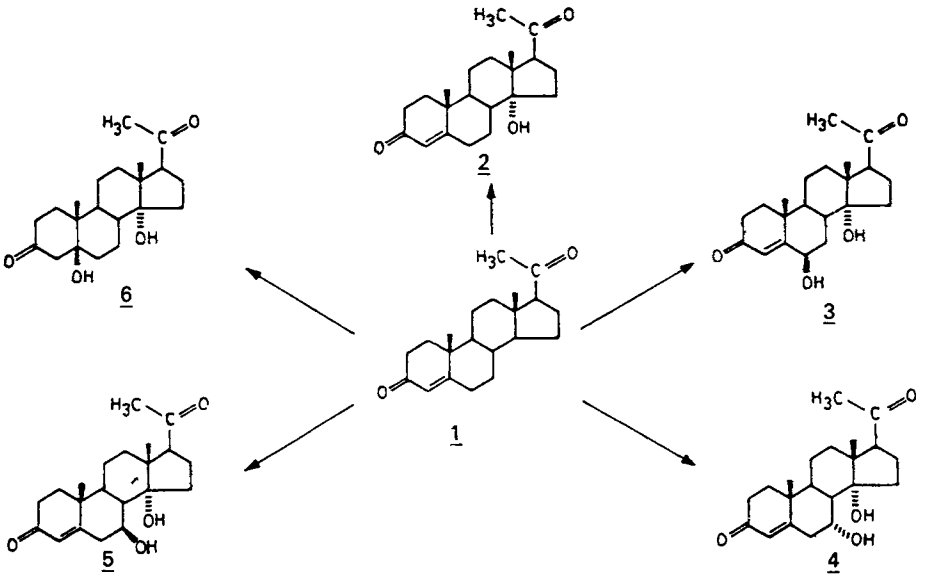


Figure 1. Transformations of progesterone (1) by *M. piriformis*.

2.2 *Transformations of 17α-hydroxyprogesterone (7)*

Mucor piriformis has not been used to study the transformations in 17α-hydroxyprogesterone (7), 16-dehydroprogesterone (12) and pregnenolone (17). Hence these studies have been carried out to establish whether or not a hydroxyl group at the 17α-position or a C-16(17) double bond in a progesterone skeleton would effect the mode of transformation by this organism. It is also of interest to find out the functional significance of 4-en-3-one group in a C_{21} steroid molecule and for this reason pregnenolone (17) which is devoid of this group has been chosen as the substrate.

The organism transforms (Madyastha and Joseph 1994) 17α-hydroxyprogesterone (7) into 17α-20α-dihydroxypregn-4-en-3-one (8), 7α,17α-dihydroxypregn-4-ene-3,20-dione (9),6β,17α,20α-trihydroxypregn-4-en-3-one(10) and 11α,17α,20α-trihydroxypregn-4-en-3-one (11) (figure 2). It is interesting to note that the presence of a hydroxyl at 17α-position sterically hinders hydroxylation at 14α-position. This is actually in accordance with the earlier report that an alkyl substitution at the 17α-position in a steroid molecule prevents hydroxylation at the 14α-position by *Mucor grisco-cyanus* (Singh *et al* 1967). The proximity of the bulky 17α-substituent probably hinders hydroxylation at 14α-position and directs it to 7α-position. However, steroids with hydroxyl group at 17β-position as in testosterone (21) is readily transformed to its 14α-hydroxy derivative (Krishnan *et al* 1991). Earlier, it has been demonstrated that incubation of 17α-hydroxyprogesterone (7) with a *Bacillus* species results in the formation of a side-chain cleaved product (Mahato and Banerjee 1986). It appears that *M. piriformis* is not capable of cleaving the C_{17} side-chain in a C_{21} steroid. Surprisingly, the organism showed its unique ability to reduce the C-20 keto group, a reaction never been reported earlier in organisms of the order *Mucorales*. However, the reduction of C-20 keto group has not been observed in the case of progesterone (1), 16-dehydroxyprogesterone (12) and pregnenolone (17) (figures 1, 3 and 4). Time

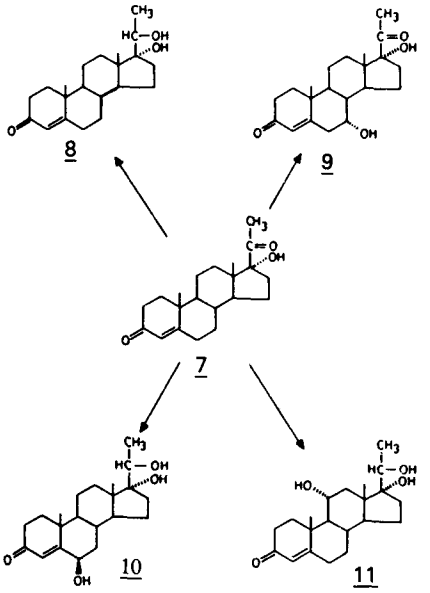


Figure 2. Transformations of 17α-hydro xyprogesterone (7) by *M. piriformis*.

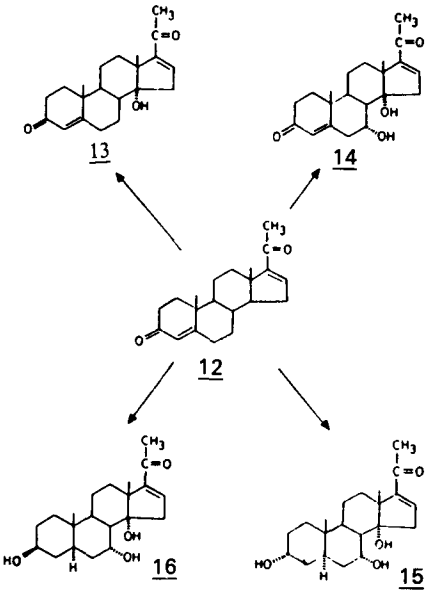


Figure 3. Transformations of 16-dehydropro gesterone (12) by *M. piriformis*.

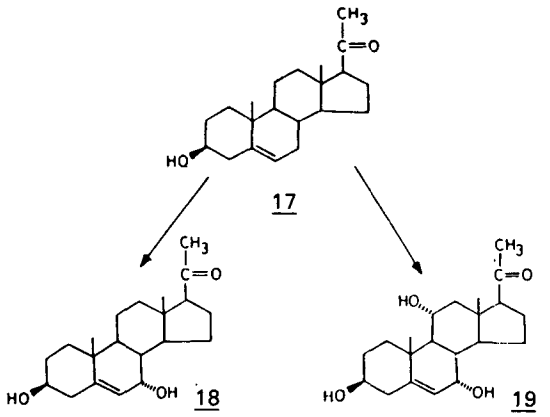


Figure 4. Transformations of pregnenolone (17) by *M. piriformis*.

course studies have indicated (Madyastha and Joseph 1994) that 17α, 20α-dihydroxypregn-4-en-3-one (8, figure 2) is the initial biotransformation product formed which then gets hydroxylated either at the 6β- or 11α-position (Madyastha and Joseph 1994).

2.3 *Transformations of 16-dehydroprogesterone (12)*

Various fungal species have been reported to transform 16-dehydroprogesterone (12) into different metabolites involving hydroxylation at 11α- and 21-positions,

dehydrogenation at C-1 position and reduction of the C-16 (17) double bond (Kita and Shull 1959; Laskin 1963; Vezina *et al* 1963). In contrast to these observations, *M. piriformis* very efficiently carries out (Madyastha and Joseph 1994) 14α-hydroxylation of 16-dehydroprogesterone (12, figure 3). Time-course studies have indicated that the organism initiates the hydroxylation at the 14α-position to yield 14α-hydroxypregna-4,16-diene-3,20-dione (13) which further gets hydroxylated at the 7α-position (14, figure 3). In fact, at the end of 48 h, nearly 80% of the substrate (12) gets transformed to 7α,14α-dihydroxypregna-4-16-diene-3,20-dione (14, figure 3) and this metabolite gets accumulated in the fermentation medium in significant levels. The reduction of the 4-en-3-one group resulting in the formation of metabolites 15 and 16 (figure 3) is not a major activity of this organism since both these metabolites are formed in very low levels even at the end of 48 h (Madyastha and Joseph 1994). However, it is interesting to note that the reduction of the 3-keto group is not stereoselective since metabolites with both 3α- and 3β-hydroxyl functions have been isolated (figure 3). It is gratifying to note that the metabolites 13, 14, 15 and 16, (figure 3), all derived from 16-dehydroprogesterone (12), appear to be hitherto unknown.

2.4 *Transformations of pregnenolone (17)*

To find out whether the organism has the ability to isomerize 5-en-3-ol to 4-en-3-one system in C_{19} and C_{21} steroids, representative steroids belonging to this category viz. pregnenolone (17) and dehydroepiandrosterone (26) have been used as substrates. These substrates have also provided an opportunity to find out the effect of a 5-en-3β-ol system on the mode of transformation by *M. piriformis*. Several reports have appeared in literature regarding the microbial conversion of pregnenolone (17) into progesterone (1) as a result of the isomerization of the 3β-hydroxy-5-ene to the 4-en-3-one system (Perlman 1952; Capek *et al* 1957). In addition to conversion of pregnenolone (17) to progesterone (1), there are reports on the further conversion of progesterone (1) to hydroxylated progesterones with hydroxyl function at 7β, 11α, 12β, or 15α-positions (Tan and Smith 1968; Namboori *et al* 1980).

The organism transforms pregnenolone (17) to 3β, 7α-dihydroxypregn-5-en-20-one (18) and 3β,7α, 11α-trihydroxypregn-5-en-20-one (19, figure 4) (Madyastha and Joseph, unpublished observation). 7α-Hydroxylation seems to be one of the characteristic features of *M. piriformis*. Hydroxylation at the 7α-position may not be due to the reactivity of the allylic position. The organism does not have the ability to cleave the C_{17} side-chain in pregnenolone (17, figure 4). Most of the earlier reports where pregnenolone (17) has been used as the substrate, progesterone (1) has been shown to be formed as a result of the isomerization of the 5-en-3β-ol system to the 4-en-3-one system (Perlman 1952; Tan and Smith 1968; Holland and Taylor 1979). *M. piriformis* appears to lack the isomerase and in this respect differs from the other organisms tested on various C_{21} steroids.

Cursory examination of the metabolites formed from different C_{21} steroids tested reveals that *M. piriformis* very efficiently carries out the 14α-hydroxylation of C_{21} steroids. However, there appears to be a rigid structural requirement for the organism to accept substrates for 14α-hydroxylation reaction. The organism readily converts steroids with a 4-en-3-one group and without bulky substitution at the 17α-position to their respective 14α-hydroxy derivatives. The organism is also known for its ability to carry out 7α-hydroxylation.

2.5 *Transformations of androstenedione (20)*

Androstenedione (20) appears to be a good substrate for *M. piriformis*. At a substrate concentration of 0·5 g per litre, virtually all the substrate added gets transformed into metabolites by the end of 24 h. The organism transforms androstenedione (20) into testosterone (21), 14α-hydroxyandrostenedione (22), 7α-hydroxyandrostenedione (23), 14α-hydroxytestosterone (24) and 7α,14α,17β-trihydroxyandrost-4-en-3-one (25) (figure 5) (Krishnan *et al* 1991; Madyastha and Joseph, unpublished observation). Time-course experiments carried out with androstenedione (20) have clearly indicated that during the early stages of incubation (24 h), hydroxylation takes place both at the 7α- and 14α-positions. However, the major metabolite formed at the end of 24 h has been shown to be 14α-hydroxytestosterone (24, figure 5, 45%). This metabolite (24) could have been formed from 22 (figure 5) in the presence of a 17-keto oxidoreductase or from testosterone (21) by 14α-hydroxylation. The major metabolite formed at the end of 24 h viz. 14α-hydroxytestosterone (24) gets further hydroxylated at the 7α-position resulting in the formation of a trihydroxy compound (25, figure 5, Madyastha and Joseph, unpublished observation). The level of this trihydroxy compound increases at the end of 48 h with concomitant decrease in the level of 24 (figure 5), suggesting a product precursor relationship.

The formation of testosterone (21) and 14α-hydroxytestosterone (24) from androstenedione (20) has been reported earlier using *Phycomyces blakesleeanus* (Smith *et al* 1989). Although the reduction of the 17-keto group has been reported in several

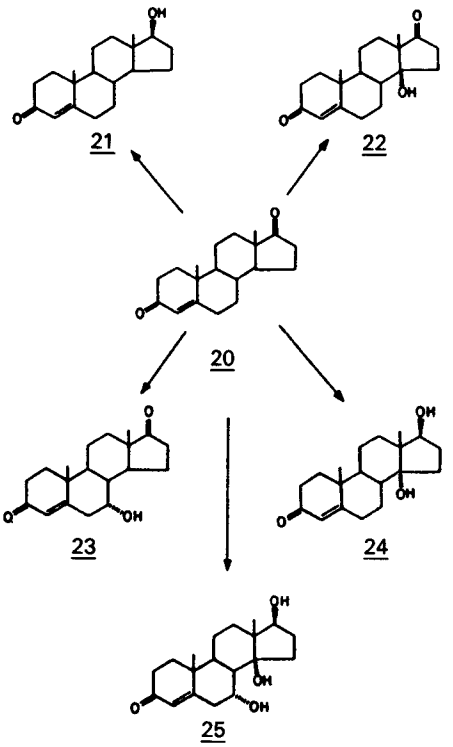


Figure 5. Transformations of androstenedione (20) by *M. piriformis*.

microbial systems, it has never been shown to be present in *M. piriformis*. Similarly, the ability of microbes to carry out 14α-hydroxylation of androstenedione (20) has been demonstrated earlier (Singh *et al* 1967; Crabb *et al* 1980). However, such an activity has never been shown in *Mucor piriformis*. The organism also accepts testosterone (21) readily as substrate and the mode of transformation is very similar to that observed with androstenedione (20). The organism readily carries out hydroxylation at both 7α- and 14α-positions. It is interesting to note that testosterone propionate is very poorly accepted as substrate (Madyastha and Joseph, unpublished observation).

2.6 *Transformations of dehydroepiandrosterone (26)*

Incubation of dehydroepiandrosterone (26) with *M. piriformis* yields mostly metabolites formed as a result of hydroxylation at the 7α-position as well as reduction of the 17-keto group (figure 6) (Madyastha and Joseph, unpublished results). The organism transforms dehydroepiandrosterone (26) into 3β,17β-dihydroxyandrost-5-ene (27), 3β-hydroxyandrost-5-ene-7,17-dione(28), 3β,17β-dihydroxyandrost-5-en-7-one (29), 3β,7α-dihydroxy-androst-5-en-17-one (30) and 3β,7α,17β-trihydroxyandrost-5-ene (31). It is interesting to note that the organism fails to carry out 14α-hydroxylation of dehydroepiandrosterone (26). Similar observation has also been made in the case of pregnenolone (17). Both these compounds (17, 26) contain 5-en-3β-ol system in their structure and it appears that one of the structural requirements needed for the 14α-hydroxylase to accept a steroid molecule as a substrate is the presence of a

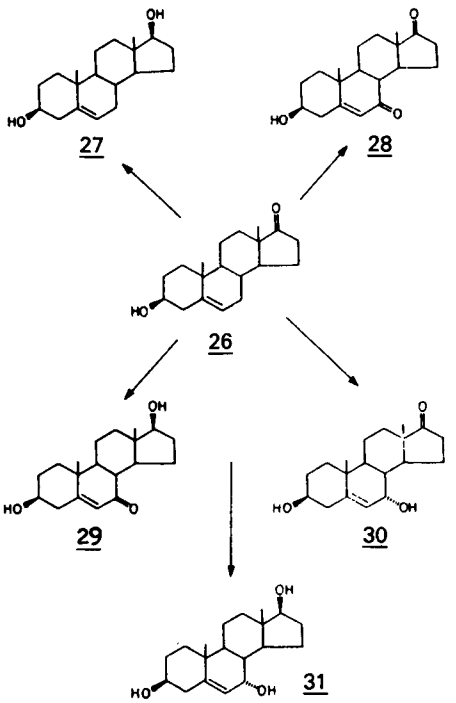


Figure 6. Transformations of dehydroepiandrosterone (26) by *M. piriformis*.

4-en-3-one group in its molecule. This is supported by the fact that *M. piriformis* has the ability to carry out hydroxylation of progesterone (1), 16-dehydroprogesterone (12), androstenedione (20) and testosterone (21) at 14α-position. The other interesting aspect is that the organism is not capable of isomerizing 5-en-3β-ol to 4-en-3-one, a reaction many organisms are capable of performing. In the case of dehydroepiandrosterone (26) which is devoid of 4-en-3-one group, the organism prefers to carry out hydroxylation at 7α-position. This 7α-hydroxylation may not be due to the reactivity of the allylic position, but rather due to the geometrical nature of the active site. Earlier it has been shown that a *Rhizopus* species transforms dehydroepiandrosterone (26) to 3β, 7α-dihydroxyandrost-5-en-17-one (30), 3β-7β-dihydroxyandrost-5-en-17-one and 3β-hydroxyandrost-5-ene-7,17-dione (28) (Dodson *et al* 1959). Thus 7-hydroxylation of dehydroepiandrosterone (26) appears to be stereospecific. *Absidia regnieri*, belonging to the order *Mucorales* has been shown to transform dehydroepiandrosterone (26) into its 7α-hydroxylated derivative (Bell *et al* 1975). The reduction of the 17-keto group of dehydroepiandrosterone (26) to 17β-hydroxyl has not been reported by fungi of the order *Mucorales*.

Studies carried out with *M. piriformis* demonstrated the versatility of this organism in introducing hydroxyl groups at the 7α- and 14α-positions in various C_{19} and C_{21} steroids. Transformation of 17α-hydroxyprogesterone (7) and 16-dehydroprogesterone (12) by this organism has resulted in the formation of metabolites (11, 13, 14, 15 and 16) which are hitherto unknown. In fact, *M. piriformis* can be used as an efficient reagent to prepare some of these novel compounds.

3. 14α-Hydroxylation by cell-free extract of *Mucor piriformis*

Mucor piriformis isolated in our laboratory has the unique ability to hydroxylate various C_{19} and C_{21} steroids (Madyastha and Srivatsan 1987; Krishnan *et al* 1991; Madyastha and Joseph 1994). Cell-free extract prepared from induced vegetative cell cultures of *M. piriformis* following the procedure developed earlier (Jayanthi *et al* 1982; Madyastha *et al* 1984; Madyastha and Joseph 1993) has been shown to contain

Table 1. Substrate specificity of the microsomal 14α-hydroxylase from *Mucor piriformis*.

Substrate	14α-hydroxylated product formed (% conversion)
Progesterone	69
Testosterone	65
Androstenedione	30
16-Dehydroprogesterone	81
1,2-Dehydrotestosterone	45
17α-Hydroxyprogesterone	—
Epitestosterone	—
17α-Ethynyl-19-nortestosterone	—
3β-Hydroxyandrost-5-ene-7,17-dione	—

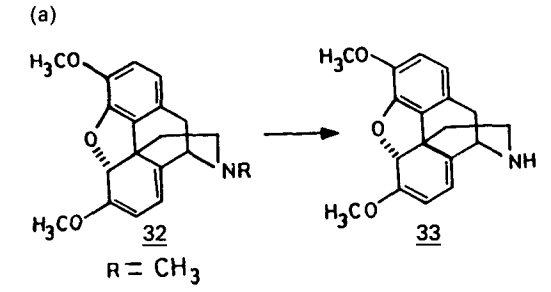
The above experiments were carried out using acetone-washed microsomes as described earlier (Madyastha and Joseph 1993).

high 14α-hydroxylase activity. Most of the 14α-hydroxylase activity is associated with the microsomes (105,000 g sediment) prepared from the active cell-free extract. Both NADPH and O_2 are necessary for the hydroxylase activity (Madyastha and Joseph 1993). Microsomes readily convert various C_{19} and C_{21} steroids to their respective 14α-hydroxy compounds (table 1) (Madyastha and Joseph 1993). Microsomes prepared from the uninduced cells are devoid of 14α-hydroxylase activity.

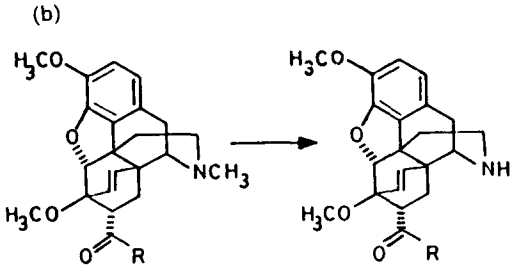
4. Transformation of thebaine and its N-variants

The work presented so far clearly demonstrates the unique ability of *M. piriformis* to carry out preparatively useful steroid transformations. To our great surprise we have now demonstrated that this organism can be used as an efficient reagent for effecting N-dealkylation of thebaine (32), an isoquinoline alkaloid, and its N-variants where the alkyl group on nitrogen is varied from methyl to ethyl, *n*-propyl, isopropyl, *n*-butyl and cyclopropylmethyl (figure 7a). The organism essentially carries out N-dealkylation resulting in the formation of norcompound (33) with high yields (~ 80) (Madyastha and Reddy 1994). It is interesting to note that the size of the alkyl group on nitrogen does not have any significant effect on the N-dealkylation reaction. The most significant part of this microbial system is that the northebaine (33) formed does not get further metabolized. The organism also carries out N-demethylation of the Diels–Alder adduct of thebaine (32) in high yields (figure 7b) (Madyastha and Reddy 1994).

Thebaine (32) is extensively used as a starting material to synthesize various morphine agonists and antagonists. One of the steps involved in their preparation



R	Yield (%)
CH_3	81
CH_2CH_3	83
$CH_2CH_2CH_3$	86
$CH(CH_3)_2$	88
$CH_2CH_2CH_2CH_3$	89
CH_2–cyclopropyl	92



R	Yield (%)
CH_3	83
OCH_3	62

Figure 7. (**a**) N-dealkylation of thebaine (32) and its N-variants by *M. piriformis*. (**b**) N-demethylation of the Diels–Alder adduct by *M. piriformis*.

is the N-dealkylation reaction which is normally carried out chemically. Since the chemical method involves harsh reaction conditions as well as hazardous and toxic reagents, microbial method could offer alternative approach. Our studies have demonstrated the suitability of *M. piriformis* as an efficient reagent to carry out N-dealkylation of thebaine (32) and its N-variants.

References

Bell A M, Jones Sir E R H, Meakin G D, Miners J O and Wilkins A L 1975 *J. Chem. Soc., Perkin I* 2040

Capek A, Hanc O and Pavlu H 1957 *Cesk. Microbiol.* **2** 168

Charney W and Herzog H L 1967 In *Microbiol. transformations of steroids: A handbook* (New York: Academic Press)

Crabb T A, Dawson P J and Williams R O 1980 *J. Chem. Soc. Perkin I* 2535

Crout D H G and Christen M 1989 In *Biotransformations in organic synthesis* (ed.) R. Schefold (Berlin: Springer) pp. 1–114

Davies H G, Green R H, Kelly D R and Roberts S M 1989 In *Biotransformations in preparative organic chemistry. The use of isolated enzymes and whole cell systems in synthesis* (London: Academic Press) p. 99

Dodson R M, Nicholson R T and Muir R D 1959 *J. Am. Chem. Soc.* **81** 6295

Dodson R M and Tweitt R C 1960 *US Patent* 2,924,611; 1960 *Chem. Abstr.* **54** 11086h

Eroshin V K 1962 *Mikrobiologiya* **31** 608

Holland H L and Riemland E 1985 *Can. J. Chem.* **63** 1121

Holland H L and Taylor G J 1979 *Phytochemistry* **18** 437

Iizuka H and Naito A 1981 In *Microbial Conversion of Steroids and Alkaloids* (University of Tokyo Press)

Jayanthi C R, Madyastha P and Madyastha K M 1982 *Biochem. Biophys. Res. Commun.* **106** 1262

Kita D A and Shull G M 1960 *US Patent* 2,903,398 (Sept. 8, 1959); 1960 *Chem. Abstr.* **54P** 3520a

Krishnan R, Madyastha K M, Seshadri T P and Viswamitra M A 1991 *Steroids* **56** 440

Laskin A I 1962 *US Patent* 3,056,730 (Oct. 2, 1962); 1963 *Chem. Abstr.* **58P** 8388f

Madyastha K M, Jayanthi C R, Madyastha P and Sumathi D 1984 *Can. J. Biochem. Cell Biol.* **62** 100

Madyastha K M and Joseph T 1963 *J. Steroid Biochem. Mol. Biol.* **45** 563

Madyastha K M and Joseph T 1994 *Appl. Microbiol. Biotechnol.* (in press)

Madyastha K M and Reddy G V B 1994 *J. Chem. Soc., Perkins I* (in press)

Madyastha K M and Srivatsan J 1987 *Can. J. Microbiol.* **33** 361

Mahato S B and Banerjee S 1986 *Biochem. J.* **239** 473

Mahato S B, Banerjee S and Poddar S 1989 *Phytochemistry* **28** 7

Murray H C and Peterson D H 1952 *US Patent* 2602,769; 1952 *Chem. Abstr.* **46** 8331f

Murray H C and Peterson D H 1957 *US Patent* 2,800,490

Namboori K, Pereira L and Merchant J R 1980 *Indian J. Biochem. Biophys.* **17** 149

Perlman D 1952 *Science* **115** 529

Peterson D H and Murray H C 1952 *J. Am. Chem. Soc.* **74** 1871

Rosazza J P 1982 In *Microbial transformations of bioactive compounds* (Boca Raton, Fl: CRC Press) vol. 1 and 2

Singh K E, Sehgal S N, Vezina C 1967 *Can. J. Microbiol.* **13** 1271

Smith K E, Latif S and Kirk D N 1989 *J. Steroid Biochem.* **32** 445

Tamm C, Gubler A, Juhasz G, Weiss-Berg E and Zurcher W 1963 *Helv. Chim. Acta.* **46** 889

Tan L and Smith L L 1968 *Biochem. Biophys. Acta.* **164** 389

Vezina C, Sehgal S N and Singh K 1963 *Appl. Microbiol.* **11** 50

Vezina C and Singh K 1975 In *The filamentous fungi* (eds) J E Smith and D R Berry (London: Edward Arnold) vol. 1, p. 158

Yamada H and Shimizu S 1988 *Angew. Chem., Int. Ed. Engl.* **27** 622