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# First asymmetric oxidation of tertiary amines by cyclohexanone monooxygenase

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**Abstract**

Cyclohexanone monooxygenase catalyzes the asymmetric oxidation of some tertiary amines to amine *N*-oxides. The structure of the amine markedly influences the enantiomeric excess of products. 

*Keywords:* amines; enzymes; enzyme reactions; *N*-oxides; stereoselection.

Tertiary amine *N*-oxides are widely used as reagents for the oxidation of alkenes and alcohols.[1] They are also useful intermediates in the functionalization of amines via C–H or C–C fragmentation (Polonovski reaction), and in the production of alkenes via β C–H elimination (Cope reaction).[1] Among these compounds, tertiary amine *N*-oxides, which are chiral either because of the presence of a stereocenter at nitrogen or because of the existence of planar axial chirality, are becoming increasingly popular among the endless list of chiral catalysts. In the field of asymmetric synthesis they have been used in the Pauson–Khand cyclization,[2] in the allylation of aldeydes,[3] in the reduction of ketones[4] and in the synthesis of thiols via a thione rearrangement.[5] These applications take advantage of the notable electron pair donor properties of the amine *N*-oxides oxygen which make them capable of forming complexes with metals, silicon atoms or to act as nucleophiles. Beside their use as catalysts, chiral amine *N*-oxides have been proposed and synthesized to give locally high definite conformation to oligomeric peptides by means of intramolecular hydrogen bond networks.[6]

Tertiary amine *N*-oxides are also found in nature, which produces this class of compounds for various purposes; for example: metabolism of alkaloids in humans,[7] detoxification of plant alkaloids in insects[8] or as osmoregulating agents in fishes.[9]

The abiotic synthesis of amine *N*-oxides is based on the classical reaction of amines with $H_2O_2$ or peracids, whereas in vivo the enzymatic oxidation is catalyzed by monooxygenases. Among this

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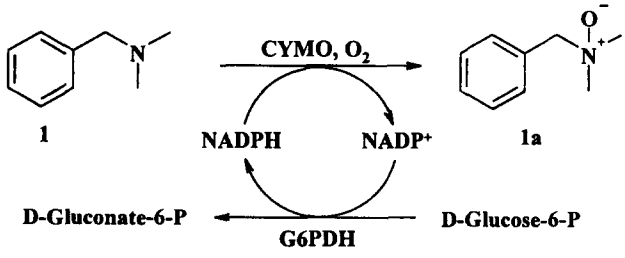
class of enzymes the flavine monooxygenases (FMO) have been widely studied in vivo,[9,10] but very few reports have appeared on the use of isolated enzymes.[7,10a,11] Cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 (CYMO), which was purified and characterized in 1976 by Trudgill and co-workers,[12] is an FMO that is gaining attention among organic chemists for its versatility and synthetic potentialities. CYMO, in fact, can catalyze the oxidation of sulfides to sulfoxides[13] and of cyclic ketones to lactones[14] (Baeyer–Villiger oxidation), generally with good enantioselectivity and high chemical yields. Furthermore, CYMO can catalyze the oxidation of heteroatoms such as N, P and Se.[15] Concerning *N*-oxidation, the only example described so far refers to *N,N*-dimethyl benzylamine, where kinetic studies would indicate *N*-oxide formation but the product was not isolated and characterized.[15]

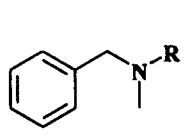
In an ongoing study within our group on the suitability of CYMO for application in organic synthesis, we decided to confirm the reported *N,N*-dimethyl benzylamine **1** oxidation[15] by means of isolated CYMO[16] and to test its ability to perform chemo-, diastero- and enantioselective *N*-oxidations. The oxidation of **1** (and of the other substrates) was coupled to a second enzymatic reaction, based on the glucose-6-phosphate/glucose-6-phosphate dehydrogenase (G6PDH) system, to regenerate the coenzyme NADPH (Scheme 1). The reaction was almost complete in 2 days[17] and the isolated product was confirmed as *N,N*-dimethyl benzylamine *N*-oxide **1a** by HPLC comparison with an authentic sample obtained by chemical oxidation with 30% $H_2O_2$.[18] Thus, it was unambiguously demonstrated that CYMO is able to catalyze the formation of amine *N*-oxides.




Scheme 1. CYMO catalyzed oxidation of *N,N*-dimethyl benzylamine with in situ coenzyme regeneration

One of the major drawbacks in the study of amine *N*-oxides is that they can undergo spontaneous degradation (i.e. Cope elimination reaction) even under the mild conditions of enzymatic reaction in vivo.[19] To address this problem and to also study the capability of CYMO to catalyze enantioselective *N*-oxidations, we investigated a series of *N*-methyl *N*-substituted benzylamines (Fig. 1). To prevent the Cope elimination reaction, substrates such as **3**, **5** and **6**, devoid of a hydrogen in the β position in respect to the nitrogen atom, were chosen. For compounds **2** and **4**, the hydroxyl group present in the β position should ensure stability due to the formation of an intramolecular hydrogen bond with the oxygen of the *N*-oxide group.[4,6] The results obtained indicate that the majority (compounds **2**–**4**) of the *N*-methyl *N*-substituted benzylamines were substrates for CYMO and gave the corresponding *N*-oxides: **2a**[20] conv. 58%, ee 32%; **3a**[21] conv. 94%, ee 30%; **4a**[22] conv 36%, de 24%. As for compounds **5** and **6**,[23] it should be emphasized that their resistance to oxidation could be due, at least in part, to their very low solubility in the aqueous reaction medium. No attempt has yet been made to determine the absolute configuration of these *N*-oxides.

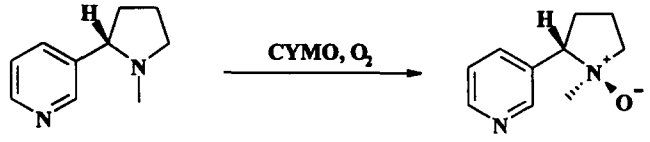
Finally, (*S*)-(−)-nicotine (**7**), the most studied substrate for *N*-oxidation by FMOs in vivo, was subjected to in vitro oxidation with CYMO (Scheme 2).[24] The enzymatic oxidation of **7** was both highly chemo- and diastereoselective, the only product of the reaction being the *cis*-(*S*)-(−)-nicotine *N*-1′-oxide (*cis*-**7a**). The chemical oxidation of **7** in 30% $H_2O_2$ afforded the *cis*-**7a** together with the *trans*-**7a** in 29:71 ratio, in agreement with the literature.[25] The chemically produced **7a** was utilized as reference for the



2 R = $CH_2$-$CH_2$-OH
3 R = $CH_2$-C≡CH
4 R = (*R*)-CHPh-$CH_2$-OH
5 R = $CH_2$-Ph-pCl
6 R = $CH_2$-Ph-p$CF_3$

Figure 1.

*cis*:*trans* ratio attribution, carried out by chiral HPLC, of the enzymatically prepared **7a**.[24] The selectivity, expressed as *cis*:*trans* ratio, of CYMO for **7** was very high and peculiar if compared with that of other FMOs (human liver (form 3) 0:100,[7] porcine liver 51:49,[26] guinea pig liver 79:21,[26]), where the products were only the *trans* or a mixture of *cis*/*trans*-isomers.




7 *cis*-7a

Scheme 2. CYMO-catalyzed oxidation of (*S*)-(−)-nictone to *cis*-(*S*)-(−)-nicotine *N*-1′-oxide

In conclusion, the present work has demonstrated that CYMO can catalyze, with various degrees of selectivity, the oxidation of tertiary amines to chiral amine *N*-oxides.

## Acknowledgements

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17. *N,N*-Dimethyl benzylamine *N*-oxide. Compound **1a**. Enzymatic synthesis of **1a**. Compound **1** (22 μmol) was magnetically stirred for 2 days in 500 μL of 0.05 M Tris–HCl buffer, pH 8.6, containing 0.6 μmol NADP, 58 μmol glucose-6-phosphate, 2 units of CYMO and 18 units of G6PDH. Conversion 93%. HPLC: column PartiSphere $C_8$-RP Watman, $\lambda_{254nm}$, flow rate 1 mL/min; gradient: buffer A (25 mM potassium phosphate pH 7), buffer B (25 mM potassium phosphate pH 4), 100% A for 5 min then to 100% B in 35 min, $R_t$: 9 min for **1**, 13.5 min for **1a**.
18. Chemical synthesis of **1a**. Yield 95%. TLC: AcOEt:MeOH:25% $NH_3$ 5:4:1, developed with $I_2$, $R_f$=0.47. $^1$H NMR (300 MHz, $CDCl_3$) $\delta_{H(ppm)}$: 3.15 (6H, s, N-$CH_3$), 4.45 (2H, s, N-$CH_2$-Ph), 7.45 (5H, m, Ph).
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20. *N*-Benzyl *N*-methyl ethanolamine *N*-oxide. Compound **2a**. Enzymatic synthesis of **2a**: conversion 58%, ee 32% by chiral HPLC (second eluted peak more abundant). HPLC: column Chiracel OD Diacel, $\lambda_{220nm}$, flow rate 1 mL/min; eluent: 98% light petroleum ether, 2% absolute EtOH, 0.05% TFA, $R_t$: 94 min for **2**, 121 min and 127 min for **2a** enantiomers. Chemical synthesis of **2a**. Yield 72%. TLC: AcOEt:MeOH:25%$NH_3$ 7:3:1, developed with $I_2$, $R_f$=0.46. $^1$H NMR (300 MHz, $CDCl_3$) $\delta_{H(ppm)}$: 3.08 (3H, s, N-$CH_3$), 3.20 (1H, ddd, $J$=12, 6, 3 Hz, N-C$H_A$$H_B$-$CH_2$OH), 3.46 (1H, ddd, $J$=12, 7.5, 3.5 Hz, N-$CH_A$$H_B$-$CH_2$OH), 4.10 (2H, m, $CH_2$-OH), 4.40 and 4.48 (each 1H, AB system, $J$=12 Hz, Ph-$CH_2$-N), 7.45 (5H, m, Ph).
21. *N*-Methyl *N*-propargyl benzylamine *N*-oxide. Compound **3a**. Enzymatic synthesis of **3a**: conversion 94%, ee 30% by chiral HPLC (first eluted peak more abundant). HPLC: column Chiracel OD Diacel, $\lambda_{220nm}$, flow rate 1 mL/min; eluent: 98.5% light petroleum ether, 1.5% absolute EtOH, 0.007% trifluoroacetic acid, $R_t$: 28 min for **3**, 161 and 173 min for **3a** enantiomers. Chemical synthesis of **3a**. Yield 54%. TLC: AcOEt:MeOH 7:3, developed with $I_2$, $R_f$=0.15. $^1$H NMR (300 MHz, $CDCl_3$) $\delta_{H(ppm)}$: 2.72 (1H, t, $J$=2.5 Hz, C≡CH), 3.25 (3H, s, N-$CH_3$), 3.83 and 3.90 (each 1H, AB part of ABX system, $J$=12.5, 2.5, N-$CH_2$-C≡), 4.44 and 4.49 (each 1H, AB system, $J$=13 Hz, Ph-$CH_2$-N), 7.5 (5H, m, Ph).
22. *N*-Benzyl *N*-methyl (*R*)-2-phenylglycinol *N*-oxide. Compound **4a**. Enzymatic synthesis of **4a**: conversion 36%, de 24% (second eluted peak more abundant). HPLC: column Cyclobond I (β) CD Astec, $\lambda_{254nm}$, flow rate 0.5 mL/min; eluent 1% triethylamine-AcOH, pH 4.3, $R_t$: 11 min for **4**, 17 and 19 min for **4a** diastereoisomers.Chemical synthesis as for **1a**. Yield 65%, de 29% (second eluted peak more abundant). TLC: $CHCl_3$:MeOH 95:5, developed with $I_2$, $R_f$=0.54 for **4**, $R_f$=0.10 for **4a**. $^1$H NMR (300 MHz, $C_6D_6$) $\delta_{H(ppm)}$: 2.47 (3H, s, N-$CH_3$, major abundant), 2.54 (3H, s, N-$CH_3$, minor abundant), 3.72 (1H, d, $J$=12 Hz, $PhCH_2N$, minor), 3.93–3.98 (1H, m, CH, minor), 3.93–3.98 (1H, m, CH, major), 3.95 (1H, d, $J$=12 Hz, $PhCH_2N$, major), 4.12 (1H, d, $J$=12 Hz, $PhCH_2N$, major), 4.31 (1H, d, $J$=12 Hz, $PhCH_2N$, minor), 4.39 (1H, dd, $J$=8, 3.7 Hz, C$H_A$$H_B$OH, major), 4.41 (1H, dd, $J$=8, 3.7 Hz, C$H_A$$H_B$OH, minor), 4.51 (1H, t, $J$=8 Hz, $CH_A$$H_B$OH, minor), 4.54 (1H, t, $J$=8 Hz, $CH_A$$H_B$OH, major), 7.0–7.4 (m, Ph, minor and major).
23. Compounds **5** and **6** were synthesized from benzylmethylamine and *p*-Cl and *p*-$CF_3$ benzaldehyde followed by $NaBH_3CN$ reduction.
24. (*S*)-(−)-Nicotine *N*-1′-oxide. Compound **7a**. Enzymatic synthesis of *cis*-**7a**, conversion 32%, de>99%. HPLC: column Cyclobond I (β) CD Astec, $\lambda_{260nm}$, flow rate 0.5 mL/min; eluent: 1% triethylamine-AcOH, 5% MeOH, pH 5.4, $R_t$: 9 min for **7**, 16 min (*trans N*-oxide **7a**), 17 min (*cis N*-oxide **7a**). Chemical synthesis of **7a**. Yield 93%, de 43.2% (*cis*:*trans* 29:71). TLC: AcOEt:MeOH:25%$NH_3$ 5:4:1, $R_f$=0.51. $^1$H NMR (300 MHz, $CDCl_3$) $\delta_{H(ppm)}$: 2.82 (3H, s, N-$CH_3$ *cis*), 3.08 (3H, s, N-$CH_3$ *trans*), 7.32 (1H, m, $H_5$ *trans*), 7.36 (1H, m, $H_5$ *cis*), 7.91 (1H, db, $J$=8.5 Hz, $H_4$ *cis*), 8.12 (1H, db, $J$=8.5 Hz, $H_4$ *trans*), 8.58 (1H, s, $H_6$ *trans*), 8.61 (1H, s, $H_2$ *trans*), 8.68 (1H, db, $J$=5.1 Hz, $H_6$ *cis*), 8.71 (1H, db, $J$=1.7 Hz, $H_2$ *cis*).
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