ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 2 (1996) 33–42

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Synthesis of substituted oxindoles by chloroperoxidase catalyzed oxidation of indoles

M.P.J. van Deurzen, F. van Rantwijk, R.A. Sheldon *

Department of Organic chemistry and Catalysis, Delft University of Technology, Julianalaan 136, 2628 BL Delft, Netherlands

Received 27 November 1995; revised 9 February 1996; accepted 26 February 1996

Abstract

Chloroperoxidase catalyzed oxidation of substituted indoles yields the corresponding oxindoles in virtually quantitative yield. These include 5-chloro-oxindole, a precursor for the anti-inflammatory agent Tenidap, which was obtained in 95% yield. The reactivity of the substituted indoles depends on the nature and the position of the substituent. Both electronic and steric effects of substituents appear to be important. All of the oxygen in the product is derived from hydrogen peroxide. A mechanism is proposed for the hydroxylation which is consistent with these observations.

Keywords: Chloroperoxidase; Selective oxidation; Indole; Oxindole; *tert*-Butyl alcohol

1. Introduction

Oxindoles and aza-oxindoles are of interest due to their biological properties [1–7]. Furthermore, some derivatives are pharmaceutical intermediates, for example 5-chloro-oxindole is an intermediate in the synthesis of Tenidap (1-carbamoyl-5-chloro-3-[hydroxy(2-thienyl)methylene]indol-2-(3*H*)-one) [8], an anti-inflammatory drug. There are several known procedures to synthesize (substituted) oxindoles, for example nucleophilic substitution of halonitrobenzenes by dimethyl malonate and subsequent ring closure [9], Friedel–Crafts alkylation of α-chloro acetanilides [10], cyclization of *N*-acyl phenylhydrazines by CaH_2 [11], Gassman cyclization of azasulfonium salts [12], addition of ketene silyl radicals to nitrobenzenes [13], functionalization of nitrotoluenes [14], photo-induced cyclization of mono- and dianions of *N*-acyl-*o*-chloroanilines [15], acid-catalyzed cyclization of α-hydroxy-acetanilides [7], photo-induced cyclization of 2-(*N*-methylanilino)-acetoacetates followed by oxidative rearrangement [16], cyclization of *N*-(*o*-bromo-phenyl)-acrylamides with Pd(II) [17] and Wolff–Kishner type reduction of isatin derivatives by hydrazine [18].

Methods for the synthesis of aza-oxindoles are less numerous. These include oxidation of 7-aza-indole to 3,3-dibromo-oxindoles followed by reduction to the corresponding oxindole [19], synthesis from 2-aminonicotinic acid, diazomethane and *N*,*N*-dimethylaniline [20] and thermally induced cyclization of aminopyridine-acetic acid [21].

Most of these methods comprise multi-step procedures for the preparation of the oxindoles.

* Corresponding author.

PII S1381-1177(96)00008-2

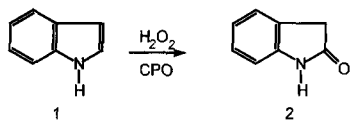

Fig. 1. Oxidation of indole to oxindole.

Often harsh conditions are necessary or low yields are obtained. Furthermore, starting materials with the substituent meta relative to the reactive group often lead to more than one product. Straightforward oxidation of indoles generally takes place at the electron-rich 3-position, unless this position is substituted [22]. Oxidation of indole at the 3-position leads to indoxyl, an intermediate for the manufacture of indigo [23]. However, the direct oxidation of indole (**1**) to oxindole (**2**) is known to be catalyzed by the enzyme chloroperoxidase from *Caldariomyces fumago* [24] (Fig. 1). Isolated yields were moderate however, and as the reaction was performed in water the reactant concentration was limited. Furthermore, no indole derivatives with substituents in the benzene ring were tested whilst these are the most interesting ones for pharmaceutical application.

Chloroperoxidase is a versatile catalyst which, in addition to its ability to chlorinate organic substrates [25], can catalyze other useful reactions such as the enantioselective oxidation of sulfides to sulfoxides [26] and alkenes to epoxides [27,28]. We have recently found [29] that chloroperoxidase performs well in *tert*-butyl alcohol–water mixtures containing up to 70% *tert*-butyl alcohol (v/v). Therefore, we have investigated the oxidation of indole in this medium and explored the scope of chloroperoxidase catalyzed oxidation of substituted indoles as a general synthetic method for the synthesis of the corresponding oxindoles.

2. Experiental

2.1. Materials and analytical methods

Chloroperoxidase from *Caldariomyces fumago* was isolated and purified as described in the literature [29]. The enzyme preparation contained 8000 U/ml according to the method of Morris et al. [30], with a purity of $R_z = 1.3$. Vanadium chloroperoxidase from *Curvularia inaequalis* was received as a gift from the University of Amsterdam and contained 21 U/ml according to the method of Morris et al. [30]. Microperoxidase-11 was purchased from Sigma. *Coprinus cinereus* peroxidase SP 502 (10 kPU/ml) and SP 676 (2263 kPU/g) were received as a gift from NOVO Nordisk. 1 peroxidase unit (PU) is the amount of enzyme which catalyzes the conversion of 1 μmol of H_2O_2 per minute in a system in which 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) is oxidized under standard conditions (pH = 7, $[H_2O_2] = 0.88$ mM, [ABTS] = 1.67 mM, 30°C).

Hydrogen peroxide 35% was obtained from Merck. All indole derivatives, pyrrole, quinoline, benzofuran, benzothiophene, and nicotinic acid were purchased from Aldrich Chemical Company. Labelled $H_2{}^{18}O_2$ (2.7% solution) was obtained from Campro Scientific.

2.1.1. HPLC analysis

Samples were diluted with methanol/water (50:50, v/v) saturated with Na_2SO_3. After filtration through a 0.4 μ membrane filter the samples were analyzed by reversed phase HPLC. A custom-packed 8 × 100 mm 10 μ Nucleosil C_{18} Waters Radial Pak cartridge (quinoline and nicotinic acid) and a 8 × 100 mm 4 μ Novapak C_{18} cartridge (all other reactants) contained in a Waters RCM 8 × 10 compression unit were used. Eluents (flow 1.0 ml/min) used: for indole derivatives, benzofuran and benzothiophene: methanol/water (50:50, v/v); for pyrrole: methanol/water (10:90, v/v), for quinoline: methanol/0.1 M aqueous ammonium formiate buffer pH 3 (40:60, v/v), which contains 2.0 g/l pentadecafluorooctanic acid, for nicotinic acid: 0.1 M aqueous ammonium formiate buffer pH 3. Dual-channel detection was performed using a Shimadzu SPD-6a UV spectrophotometer at 254 nm and an Erma ERC-7510

RI detector. *tert*-Butyl alcohol was used as internal standard.

NMR spectra were recorded on a Varian VXR-400S spectrometer, using TMS as an internal standard and $CDCl_3$ or DMSO-d_6 as the solvent.

Mass spectra were obtained using a VG 70-SE spectrometer. GC–MS was performed on a CP-SIL5CB-MS column (25 m × 0.25 mm).

2.2. General experimental

At room temperature 0.5 mmol of reactant (indole derivative, quinoline, nicotinic acid, benzofuran, benzothiophene or pyrrole) was dissolved in 50 ml of solvent, consisting of *tert*-butyl alcohol/0.1 M aqueous acetate buffer pH 4 (50:50, v/v). 400 U chloroperoxidase was added to the reaction mixture, followed by 5 min of stirring. The reaction was started by the continuous addition of 1.66 M H_2O_2 over 60 min until 1 equiv. of H_2O_2 had been added. The reactions were monitored by removing aliquots and analyzing by HPLC. Reactions with indole and pyrrole were repeated with no enzyme present.

The reaction with indole was repeated at the same conditions as above with microperoxidase (0.25 mg). Reactions with peroxidase from *Coprinus cinereus* (SP502 4 kPU, SP676 20 kPU) were similarly performed in *tert*-butyl alcohol/0.1 M aqueous phosphate buffer pH 6.5 (50:50, v/v). Reactions with vanadium chloroperoxidase (6.3 U) were performed in 5 ml *tert*-butyl alcohol/0.1 M aqueous acetate buffer pH 6 (50:50, v/v).

2.3. Preparative scale

2.3.1. Indole derivatives

1.25 mmol of indole derivative was dissolved in 25 ml of solvent, consisting of *tert*-butyl alcohol/0.1 M aqueous acetate buffer pH 4 (50:50, v/v). To the reaction mixture was added chloroperoxidase (1–6 kU) and the reaction was started by continuous addition of 1.66 M H_2O_2; 1.1 equivalent of H_2O_2 was added in 160 min. Subsequently the reaction was stopped by addition of Na_2SO_3 and the products were extracted with 3 × 25 ml of ethyl acetate. The combined fractions were dried over Na_2SO_4, filtered and concentrated in vacuo. All products contained trace amounts (< 2%) of coloured side products, probably due to oligomerization of the reaction product.

2.3.2. Pyrrole

5 mmol (330 mg) of pyrrole was dissolved in 50 ml of solvent, consisting of *tert*-butyl alcohol/0.1 M aqueous acetate buffer pH 4 (50:50, v/v). To the reaction mixture was added chloroperoxidase (4 kU) and the reaction was started by continuous addition of 1.66 M H_2O_2; 1.1 equivalent of H_2O_2 was added in 16 h. Subsequently the reaction was stopped by addition of Na_2SO_3, the solvent was evaporated in vacuo and the residue was dissolved in dioxane. After removing the remaining salts by filtration dioxane was removed by evaporation in vacuo and a yellow syrup was obtained.

The conversion and purity of the isolated products were determined by HPLC. All isolated products are known compounds and have been described in literature [9,12,14,19,31].

2.3.3. Oxindole

Yield of oxindole in the preparative synthesis: 166 mg, purity (HPLC): 96%, yield 96%. ^{13}C NMR (400 MHz, $CDCl_3$): δ 36.26 (C-3), 109.79 (C-7), 122.31 (C-5), 124,56 (C-4), 125,27 (C-3a), 127.91 (C-6), 142.55 (C-7a), 177,98 (C-2). ^1H NMR (400 MHz, $CDCl_3$): δ 3.55 (s, 2H, H-3), 6.90 (d, $J_{4,5} = 7.9$ Hz, 1H, H-4), 7.01 (dd, $J_{4,5} = J_{5,6} = 7.2$ Hz, 1H, H-5), 7.21 (dd, $J_{5,6} = J_{6,7} = 7.5$ Hz, 1H, H-6), 7.23 (d, $J_{6,7} = 7.5$ Hz, 1H, H-7), 9.09 (s, 1H, H-1). MS: *m/e* 133 (C_8H_7NO).

2.3.4. 7-Aza-oxindole

Yield of 7-aza-oxindole in the preparative synthesis: 164 mg, purity (HPLC): 99%, yield 97%. ^{13}C NMR (400 MHz, $CDCl_3$): δ 35.56 (C-3), 117.94 (C-5), 120.08 (C-3a), 132.33 (C-

4), 146.27 (C-6), 157.86 (C-7a), 175.67 (C-2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.60 (s, 2H, H-3), 6.95 (dd, $J_{4,5} = 7$, $J_{5,6} = 5$ Hz, 1H, H-5), 7.50 (dm, $J_{4,5} = 7$ Hz, 1H, H-4), 8.19 (dm, $J_{5,6} = 5$ Hz, 1H, H-6), 10.50 (s, 1H, H-1). MS: $m/e =$ 134 ($C_7H_6N_2O$).

2.3.5. 4-Chloro-oxindole

Yield of 4-chloro-oxindole in the preparative synthesis: 191 mg, purity (HPLC) 76%, yield 70%. ^{13}C NMR (400 MHz, DMSO-d_6): δ 35.15 (C-3), 107.83 (C-7), 120.81 (C-5), 124,05 (C-3a), 128.84 (C-6), 129.25 (C-4), 144.98 (C-7a), 175.03 (C-2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.49 (s, 2H, H-3), 6.79 (d, $J_{5,6} = 8$ Hz, 1H, H-5), 6.98 (d, $J_{6,7} = 8$ Hz, 1H, H-7), 7.2 (dd, $J_{5,6} = J_{6,7} = 8$ Hz, 1H, H-6), 10.60 (s, 1H, H-1). MS: $m/e = 167$ (C_8H_6NOCl).

2.3.6. 5-Chloro-oxindole

Yield of 5-chloro-oxindole in the preparative synthesis: 208 mg, purity (HPLC) 99%, yield 99%. ^{13}C NMR (400 MHz, DMSO-d_6): δ 36.16 (C-3), 110.55 (C-7), 125.09 (C-4), 126.90 (C-3a), 127.76 (C-5), 127.96 (C-6), 140.91 (C-7a), 176.94 (C-2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.50 (s, 2H, H-3), 6.80 (d, $J_{6,7} = 8$ Hz, 1H, H-7), 7.20 (dm, $J_{6,7} = 8$ Hz, 1H, H-6), 7.26 (sm, 1H, H-4), 10.50 (s, 1H, H-1). MS: $m/e =$ 167 (C_8H_6NOCl).

2.3.7. 6-Chloro-oxindole

Yield of 6-chloro-oxindole in the preparative synthesis: 200 mg, purity (HPLC) 99%, yield 96%. ^{13}C NMR (400 MHz, $CDCl_3$): δ 35.74 (C-3), 110.36 (C-7), 122.34 (C-5), 123.51 (C-3a), 125.49 (C-4), 133.65 (C-6), 143.49 (C-7a), 177.50 (C-2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.52 (s, 2H, H-3), 6.91 (d, $J_{5,7} = 2$ Hz, 1H, H-7), 7.00 (dd, $J_{4,5} = 8$ Hz, $J_{5,7} = 2$ Hz, 1H, H-5), 7.14 (d, $J_{4,5} = 8$ Hz, 1H, H-4), 8.78 (s, 1H, H-1). MS: $m/e = 167$ (C_8H_6NOCl).

2.3.8. 5-Bromo-oxindole

Yield of 5-bromo-oxindole in the preparative synthesis: 240 mg, purity (HPLC) 95%, yield 86%. ^{13}C NMR (400 MHz, DMSO-d_6): δ 35.66 (C-3), 110.77 (C-5), 112.69 (C-7), 127.12 (C-4), 128.41 (C-3a), 127.91 (C-6), 142.92 (C-7a), 175.77 (C-2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.51 (s, 2H, H-3), 6.76 (d, $J_{6,7} = 8$ Hz, 1H, H-7), 7.34 (dm, $J_{6,7} = 8$ Hz, 1H, H-6), 7.38 (sm, 1H, H-4), 10.50 (s, 1H, H-1). MS: $m/e =$ 211 (C_8H_6NOBr).

2.3.9. 5-Methyl-oxindole

Yield of 5-methyl-oxindole in the preparative synthesis: 183 mg, purity (HPLC) 94%, yield 93%. ^{13}C NMR (400 MHz, DMSO-d_6): δ 20.60 (CH_3), 35.71 (C-3), 108.78 (C-7), 125.02 (C-4), 125.77 (C-3a), 127.53 (C-6), 129.83 (C-5), 141.16 (C-7a), 176.24 (C-2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (sm, 3H, CH_3), 2.28 (s, 2H, H-3), 5.57 (d, $J_{6,7} = 8$ Hz, 1H, H-7), 5.83 (dm, $J_{6,7} = 8$ Hz, 1H, H-6), 5.88 (s, 1H, H-4), 9.14 (s, 1H, H-1). MS: $m/e = 147$ (C_9H_9NO).

2.3.10. 5-Methoxy-oxindole

Yield of 5-methoxy-oxindole in the preparative synthesis: 197 mg, purity (HPLC) 95%, yield 92%. ^{13}C NMR (400 MHz, $CDCl_3$): δ 36.71 (C-3), 55.81 (OCH_3), 110.02 (C-4), 111.81 (C-7), 112.54 (C-6), 126.65 (C-3a), 136.01 (C-7a), 155.72 (C-5), 177.70 (C-2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.52 (s, 2H, H-3), 3.78 (s, 3H, OCH_3), 6.74 (dd, $J_{6,7} = 8$ Hz, $J_{4,6} = 2.6$ Hz, 1H, H-6), 6.79 (d, $J_{6,7} = 8$ Hz, 1H, H-7), 6.84 (sm, 1H, H-4), 8.93(s, 1H, H-1). MS: $m/e = 163$ ($C_9H_9NO_2$).

2.3.11. 2,5-Dihydropyrrol-2-one

Conversion of pyrrole: 61% (HPLC). Yield of 2,5-dihydropyrrol-2-one in the preparative synthesis: 226 mg, purity (HPLC) 92%, yield 50%. ^{13}C NMR (400 MHz, DMSO-d_6): δ 48.20 (C-4), 127.08 (C-3), 146.81 (C-2), 173.65 (C-1). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.95 (m, $J_{3,5} = J_{4,5} = 1.7$ Hz, 2H, H-5), 6.04 (m, $J_{3,4} =$ 5.7 Hz, $J_{3,5} = 1.9$ Hz, $J_{1,3} = 1.9$ Hz, 1H, H-3), 7.29 (m, $J_{3,4} = 5.7$ Hz, $J_{4,5} = 1.9$ Hz, $J_{1,4} = 1.9$, 1H, H-4), 8.18 (s, 1H, H-1).

2.4. H_2O_2 dependence

The dependence of the reaction velocity on the hydrogen peroxide concentration was measured at room temperature in 5 ml *tert*-butyl alcohol/0.1 M aqueous acetate buffer pH 4 (50:50, v/v) containing 5 mM indole and 11 U chloroperoxidase. The hydrogen peroxide concentration was varied between 0.2 and 2 mM. The reaction was monitored for 90 s. Samples were analyzed on reversed phase HPLC as described under analysis and initial reaction rates were calculated.

2.5. Kinetic experiments

The kinetic experiments were carried out at 400 μM H_2O_2 concentration at 20°C. *tert*-Butyl alcohol/0.1 M aqueous acetate buffer pH 4 (50:50, v/v) was used as solvent. The substrate concentration was varied between 2 and 400 mM. 1 to 4 U/ml chloroperoxidase was added to start the reaction. Every 30 s a sample was taken which was added to a methanol/water solution containing Na_2SO_3 to stop the reaction immediately. The reaction was monitored for 90 s. Samples were analyzed on reversed phase HPLC as described under analysis. All experiments were carried out in triplicate. Initial reaction rates were calculated and kinetic parameters were determined using the Scientist program. Enzyme concentrations were determined using a molar extinction coefficient of 91200 M^{-1} cm^{-1} at 400 nm [30].

2.6. $H_2{}^{18}O_2$ studies

To a 8 mM solution of indole (2.5 ml acetate buffer, 0.1 M, pH 4) was added chloroperoxidase (100 U). Oxidation was started by the stepwise addition of $H_2{}^{18}O_2$ (2.7%). Every minute, 5 μl of $H_2{}^{18}O_2$ was added to a total of 50 μl. 5 min after the last addition the reaction mixture was extracted with dichloromethane and analyzed with GC–MS.

3. Results and discussion

3.1. General experiments

The reactivity of a number of substituted indoles was compared in small-scale experiments which were run at a low reactant concentration (10 mM) with 400 U of chloroperoxidase (Table 1).

It should be noted that chloroperoxidase is inactivated by hydrogen peroxide in the micromolar range, therefore its concentration should be kept low, preferably rate-limiting, by continuous addition [32]. Even then slow deactivation of the enzyme still takes place due to non-perfect mixing of the reaction mixture which leads to local high concentrations of hydrogen peroxide. Furthermore, in the course of a batch reaction the reactant concentration declines as the reaction proceeds and in the first order region the reaction rate will decrease proportionally. As soon as a surplus of hydrogen peroxide builds up in the solution it will rapidly deactivate the catalyst and the reaction will stop.

Table 1
Conversion of substituted indoles to the oxindoles [a]

Indole derivative	Conversion (%) after:	
	15 min	60 min
indole	25	96
7-aza-indole	25	83
3-COOH	< 1	< 1
3-CH_3	[b]	
3-CH_2-COOH	[b]	
4-CH_3	1	2
4-OCH_3	1	1
4-Cl	8	9
5-CH_3	7	11
5-OCH_3	9	10
5-Cl	19	47
5-Br	9	19
6-CH_3	< 1	< 1
6-Cl	19	46
7-CH_3	< 1	< 1

[a] Indole derivative 0.5 mmol, *tert*-butyl alcohol–0.1 M acetate buffer pH 4 (50:50, v/v), 50 ml; CPO 400 U; H_2O_2 (1.66 M in 0.1 M acetate buffer pH 4) 0.5 mmol over 60 min.
[b] A complex mixture of reaction products was observed.

Indole and 7-aza-indole proved to be the most reactive substrates. Their conversion into oxindole and 7-aza-oxindole, respectively, nearly kept pace with the addition of hydrogen peroxide (Table 1). Indoles bearing a substituent at the 3-position either did not react with chloroperoxidase (3-COOH) or gave rise to a complex mixture of products which probably result from non-selective radical reactions (3-CH_3 and 3-CH_2COOH). This deviation from the general pattern is ascribed to steric hindrance of the 2-position by substituents at the 3-position and, in the case of 3-carboxy-indole, its deactivation by the electron withdrawal.

Indoles which were substituted at the benzene ring were less reactive than indole itself; their conversion into the corresponding oxindole ranged from less than 1% to 47% (Table 1). This is mainly ascribed to unfavourable steric interactions of the reactants with the restricted active site of chloroperoxidase. In a similar way the enantioselective sulfoxidation of alkyl aryl sulfides is negatively influenced by the size of the alkyl group: increasing the size of the substrate from *p*-methylphenyl methyl sulfide to *p*-methylphenyl ethyl sulfide and *p*-methylphenyl propyl sulfide gives a large decrease in enantiomeric excess of the (*R*)-sulfoxide [26]. Also the enantioselective epoxidation of *cis*-2-alkenes is limited to smaller alkenes ($< C_9$) [28]. In order to study the effects of the substituents on binding and catalysis, kinetic experiments were performed.

3.2. Kinetic experiments

By analogy with the oxidation of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine by chloroperoxidase the oxidation of indole presumably proceeds through a ping-pong mechanism when the reactant concentration exceeds the hydrogen peroxide concentration [33]. If the concentration of the oxidant is not significantly lower than the concentration of the reactant, enzyme catalyzed hydrogen peroxide decomposition to molecular oxygen and water also occurs [33].

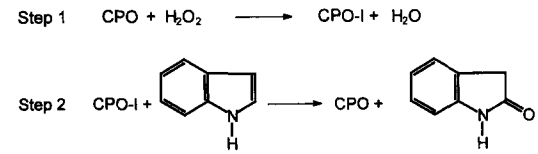

Fig. 2. Ping-pong mechanism of chloroperoxidase.

In the first step, the enzyme is oxidized by hydrogen peroxide to compound I, which is two oxidation equivalents above the resting state of the enzyme. In step 2 compound I is reduced by reactant to the free enzyme and the oxidized product is obtained (Fig. 2.).

A reliable measurement of the kinetic parameters of the indole oxidation requires that the experiments are carried out in an oxidant concentration range within which the enzyme remains saturated with hydrogen peroxide. A high oxidant concentration (millimolar range) is undesirable, because then the catalase activity, deactivation of CPO and formation of compound III interfere and the oxidation rate of indole will decrease [24]. The dissociation constant of CPO for hydrogen peroxide is reported to be very low (in the micromolar range [33]), therefore a high hydrogen peroxide concentration would seem unnecessary. Preliminary experiments confirmed that the oxidation rate of indole is independent of the hydrogen peroxide concentration between 200 μM and 400 μM (Table 2). Hence kinetic experiments were carried out in this concentration range (Table 3).

Table 2
Effect of $[H_2O_2]$ [a] on the reaction rate

$[H_2O_2]$ (μM)	$V_{initial}$ (μmol/mg min)
2000	30
1000	38
500	57
400	62
200	62

[a] For details see Experimental section.

Table 3
Kinetic parameters of substituted indoles [a]

Indole derivative	V_{max} (μmol/mg min)	k_{cat} (1/s)	K_m (mM)	k_{cat}/K_m (1/mM s)
indole	360	250	22	11.4
4-Cl	220	160	200	0.8
5-Cl	125	84	24	3.5
5-OCH_3	680	475	400	1.2
6-Cl	375	263	75	3.5

[a] For details see Experimental section.

Table 4
Preparative scale synthesis of oxindole derivatives [a]

Indole derivative	CPO (kU)	Yield (%)	Purity (%)
indole	1	96	96
7-aza-indole	2	97	99
4-Cl	6	70	76
5-Cl	2	99	99
5-Br	3	86	95
5-CH_3	6	92	94
5-OCH_3	6	93	95
6-Cl	2	96	99

[a] For details see Experimental section.

The kinetic data confirm our observation that indole is the most reactive substrate. An electron donating substituent at the 5-position (5-methoxy-indole) increases the catalytic turnover frequency (k_{cat}), whilst an electron withdrawing substituent (5-chloro-indole) at this position decreases the turnover frequency. The electron withdrawing property of the 6-chloro substituent does not affect the turnover frequency.

For all substituents the K_m value is increased. This strongly suggests that the binding of the indole derivative to the enzyme is negatively influenced by substituents. This effect is very pronounced at the 4-position where the K_m value for chloro-substituted indole is highest. 5-Chloro-indole is still bound well (in the same range as indole) whilst 5-methoxy-indole binds much weaker. 6-Chloro-indole also has a larger K_m value than indole, suggesting that the binding of this reactant to the enzyme is weaker than for indole. The observed low reactivity of 7-CH_3-indole (Table 1) similarly suggests that a substituent at this position exerts an important steric effect.

3.3. Preparative scale

Preparative scale experiments were carried out with indoles which showed sufficient reactivity (Table 1). The amount of chloroperoxidase was adjusted to the reactivity of the substrate. Nearly all oxindoles were obtained in quantitative yield with a high purity (Table 4). Only in the case of 4-chloro-indole did the reaction stop before completion resulting in contamination of the product with starting material. All products were coloured due to the presence of trace amounts (< 2%) of oligomeric products.

3.4. Other substrates

Other hetero-aromatic compounds were also tested as substrates in chloroperoxidase-mediated oxidations with hydrogen peroxide. Nicotinic acid and quinoline were unreactive, consistent with the lower reactivity of the pyridine ring compared to the pyrrole ring. This is also consistent with the results of the oxidation of 7-aza-indole, where oxidation at the pyridine moiety was not observed.

Benzothiophene and benzofuran were not oxidized by CPO, suggesting that the presence of a nitrogen atom is essential for reactivity. Pyrrole (**3**) was selectively oxidized at the 2-position to yield 2,5-dihydropyrrol-2-one (**4** in Fig. 3, the most stable tautomer [34]). In contrast with the oxindoles it was not possible to obtain **4** in quantitative yield (maximum conversion 67%).

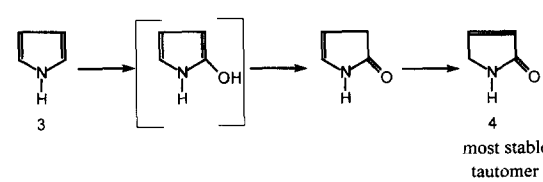

Fig. 3. Oxidation of pyrrole by chloroperoxidase.

3.5. Other enzymes

Vanadium chloroperoxidase from *Curvularia inaequalis* did not oxidize indole to oxindole. Recently, the crystal structure of vanadium chloroperoxidase has been elucidated [35]. It was shown that this enzyme has a narrow channel (5 Å) at the active site entrance. Hence, direct oxidation is restricted to small molecules such as chloride.

As known from the literature, horseradish peroxidase oxidizes indole to a mixture of products, with a trimer as the major product and only traces of oxindole [36]. *Coprinus cinereus* peroxidase which is known to catalyze the same kind of reactions as horseradish peroxidase showed no activity for the formation of oxindole. However, a very slow reaction occurred when a large amount of *Coprinus cinereus* peroxidase (20 kPU of SP676, conversion 17%) was used and unidentified products were obtained, probably due to radical reactions at the δ-heme edge comparable to the reaction of indole with horseradish peroxidase. Microperoxidase-11, which is an undecapeptide containing a heme group, showed no activity for indole oxidation.

3.6. Proposed mechanism

To investigate whether the oxygen in oxindole is derived from H_2O_2 a reaction was performed with labeled $H_2{}^{18}O_2$. The reaction mixture was analyzed with GC–MS. The results were compared to a reaction mixture in which $H_2{}^{16}O_2$ was used as the oxidant. It was shown that all oxygen (> 97%) in oxindole is derived from hydrogen peroxide. This implies that the reaction is a two electron oxygen transfer reaction proceeding via a concerted mechanism or via two fast consecutive one-electron transfer reactions. The chloroperoxidase catalyzed sulfoxidation [37] and epoxidation reactions [38] as well as the oxidation of aryl amines to *N*-oxides [39] similarly appear to involve direct oxygen transfer.

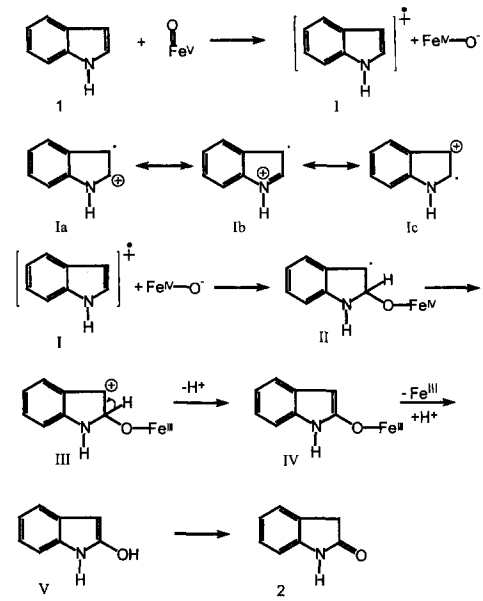

Fig. 4. Putative mechanism for the oxidation of indole to oxindole by chloroperoxidase.

We propose the mechanism in Fig. 4 for the observed hydroxylation. The first step is a one electron transfer between oxoiron(V) and indole (**1**) affording an indole radical cation (**I**) together with an oxoiron(IV) species analogous to the traditional peroxidase reaction mechanism. The resonance structures (**Ia**) and (**Ib**) presumably are the most stable ones, because the positive charge in **Ia** is stabilized by the adjacent nitrogen atom and because **Ia** and **Ib** represent a benzylic radical. Furthermore, this mechanism explains the positive influence of an electron donating group at the 5-position on the catalytic turnover frequency. This electron donating group stabilizes the intermediate radical cation.

A possible explanation for the observation that the oxidations of benzofuran and benzothiophene are not catalyzed by chloroperoxidase and 1-methyl-indole is oxidized at a much lower rate than indole [24], is that proton abstraction by the enzyme would stabilize the positive charge on the nitrogen.

In the second step of the oxidation the 2-position of the indole radical cation is attacked by the oxy anion, followed by an electron transfer

to Fe^{IV} to give Fe^{III}. After deprotonation of (**III**) at the 2-position and substitution of Fe^{III} by a proton, 2-hydroxy-indole is obtained which spontaneously converts to the more stable oxindole (**2**).

4. Conclusions

Chloroperoxidase efficiently catalyzes the selective oxidation of substituted indoles to oxindoles and of pyrrole to 2,5-dihydropyrrol-2-one. High yields of substituted oxindoles can be obtained in *tert*-butyl alcohol/water mixtures, in which a high concentration of reactant is possible.

The reactivity of substituted indoles depends on the position and the nature of the substituent. A substantial variation of the substituent is possible at the 5-position: electron withdrawing substituents have a negative effect on the reaction velocity as does the bulkiness of the substituent. It is possible to obtain 5-chloro-oxindole, a precursor for the drug Tenidap, in high yield using this method. Reactions with 4- and 6-substituted indoles are also possible, however, these positions are more sterically hindered so these substrates are weakly bound to the enzyme. The oxidation of indole to oxindole involves direct oxygen transfer from hydrogen peroxide to the substrate. An initially formed radical cation is proposed to afford the oxindole in a subsequent oxygen rebound reaction with the oxoiron(IV) anion.

Acknowledgements

We thank Dr. R. Wever of the University of Amsterdam, The Netherlands and his co-workers for the kind gift of vanadium chloroperoxidase from *Curvularia inaequalis*. A donation of *Coprinus cinereus* peroxidase by Novo Nordisk A/S, Bagsvaerd, Denmark is gratefully acknowledged.

References

[1] R.J. Sundberg, The Chemistry of Indoles, Academic Press, New York, 1970.
[2] W.J. Houlihan, Indoles, in The Chemistry of Heterocyclic Compounds, Vol. 14, Wiley-Interscience, New York, 1972.
[3] P. Kutney, in J.P. Ap Simon (Editor), Total Synthesis of Natural Products, Vol. 3, Wiley-Interscience, New York, 1977.
[4] E.W. Wiseman, J. Chaini and J.M. McManus, J. Med. Chem., 16 (1973) 131
[5] B.B. Molloy, US Patent 3882236 (1975), C.A. 83: 79075.
[6] S.B. Kadin, US Patent 4556672 (1984), C.A. 105: 24187.
[7] P.A. Petyunin, M.S. Mashevskaya and V.S. Zalesov, Khim.-Farm. Zh., 6 (1972) 11, C.A. 78: 43182.
[8] A.J. Lewis, N.S. Doherty and N.R. Ackerman, Therapeutic Approaches to Inflammatory Diseases, Elsevier, New York, 1989, p. 229.
[9] G.J. Quallich and P.M. Morrisey, Synthesis, (1993) 51.
[10] R.A. Abramovitch and D.H. Hey, J. Chem Soc., (1954) 1697.
[11] A.S. Endler and E.I Becher, Org. Synth., Collect. Vol. IV (1963) 657.
[12] P.G. Gassman and T.J. van Bergen, J. Am. Chem. Soc., 96 (1974) 5508.
[13] T.J. RajanBabu, B.L. Chenard and M.A. Petti, J. Org. Chem., 51 (1986) 1704.
[14] A.H. Becket, R.W. Daisley and J. Walker, Tetrahedron, 24 (1968) 6093.
[15] R.R. Goehring, Y.P. Sachdeva, J.S. Pisipati, M.C. Sleevi and J.F. Wolfe, J. Am. Chem. Soc., 107 (1985) 435.
[16] A.G. Schulz and W.K. Hagmann, J. Org. Chem., 43 (1978) 4231.
[17] M. Mori and Y. Ban, Tetrahedron Lett., 13 (1979) 1133.
[18] C.Crestine and R. Swaladino, Synth. Commun., 24 (1994) 2835.
[19] A. Marfat and M.P. Carta, Tetrahedron Lett., 28 (1987) 4027.
[20] H. Kägi, Helv. Chem. Acta 24 (1941) 141E.
[21] S. Okuda and M.M. Robison, J. Am. Chem. Soc., 81 (1959) 740.
[22] K. Szabó-Pusztay and L. Szabó, Synth. Commun., (1979) 276.
[23] Y. Inoue, Y. Yamamoto, H. Suzuki and U. Takaki in V. Corés Corberán and S. Vic Bellón (Ed.), Developments in Selective Oxidations II, Elsevier, Amsterdam, 1994, p. 615.
[24] M.D. Corbett and B.R. Chipko, Biochem. J., 183 (1979) 269.
[25] M.C.R. Franssen and H.C. van der Plas, Adv. Appl. Microbiol., 37 (1992) 41.
[26] S. Colonna, N. Gaggero, L. Casella, G. Carrea and P. Pasta, Tetrahedron Asymm., 3 (1992) 95.
[27] S. Colonna, N. Gaggero, L. Casella, G. Carrea and P. Pasta, Tetrahedron Asymm., 4, (1993) 1325.
[28] E.J. Allain, L.P. Hager, L. Deng and E.N. Jacobsen, J. Am. Chem. Soc., 115 (1993) 4415.
[29] M.P.J. van Deurzen, B.W. Groen, F. van Rantwijk and R.A. Sheldon, Biocatalysis, 10 (1994) 247.
[30] D.R. Morris and L.P. Hager, J. Biol. Chem., 241 (1966) 1763.

[31] V. Bocchi, L. Chierici, G.P. Gardini and R. Mondelli, Tetrahedron, 26 (1970) 4073.
[32] M.P.J. van Deurzen, K. Seelbach, U. Kragl, F. van Rantwijk and R.A. Sheldon, Biocat. Biotrans, submitted for publication.
[33] W. Sun, T.A. Kadima, M.A. Pickard and H. Dunford, Biochem. Cell. Biol., 72 (1994) 321.
[34] N. Bodor, M.J.S. Dewar and A.J. Harget, J. Am. Chem. Soc., 92 (1970) 2929.
[35] A. Messerschmidt, presented at the B.C.P. Jansen Institute, University of Amsterdam, The Netherlands, 3 April 1995; see also A. Messerschmidt and R. Wever, Proc. Natl. Acad. Sci., 93 (1996) 392.
[36] A.G. Holmes-Siedle and B.C. Saunders, Chem. Ind. (London), (1957) 265.
[37] S. Kobayashi, M. Nakano, T. Goto, T. Kimura and A.P. Schaap, Biochem. Biophys. Res. Commun., 135 (1986) 166.
[38] P.R. Ortiz de Montellano, Y.S. Choe, G. DePillis and C.E. Catalano, J. Biol. Chem., 262 (1987) 11641.
[39] D.R. Doerge and M.D. Corbett, Chem. Res. Toxicol., 4 (1991) 556.