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Enzyme Catalysed Oxidation of Nazlinin and Nazlinin derivatives. Characterisation of the Reaction Products.

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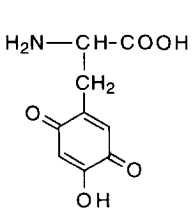
Abstract: Porcine Kidney Diamine Oxidase (PKDO) was used to catalyse the oxidative transformation of *Nitraria* alkaloids. Nazlinin yielded indoloquinolizidine **4**, an alkaloid which has been obtained as a natural product from *Nitraria* plant extracts. Oxidative deamination of 1-(4-butylamino)-β-carboline **10** with PKDO yielded an unstable aldehyde (**11**), which cyclised to a new, arborescidine-type azepine (**12**). Addition of alcohol dehydrogenase during the oxidative deamination reduced the aldehyde *in situ* to the corresponding alcohol **13**. Topaquinone-analogue **9** was effective as a stoichiometric oxidant for these primary amines. Characterisation of the enzyme reaction products was done by comparison with the chemically synthesised compounds.

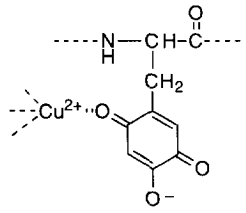
Introduction

Oxidative deamination of amines plays a key role in nitrogen metabolism in mammals, plants and micro-organisms.[1] This process receives an increasing amount of attention since recently it was shown that these copper containing amine oxidising enzymes all are topaquinone dependent.[2] Studies with bovine serum amine oxidase (BSAO) by Klinman and co-workers disclosed the structure of the covalently bound topaquinone cofactor and provided more insight in the trans-amination mechanism of these enzyme catalysed reactions.[3]

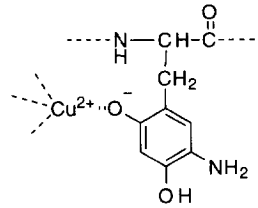
$$R\text{-}CH_2\text{-}NH_2 + H_2O + O_2 \longrightarrow R\text{-}CH{=}O + H_2O_2 + NH_3 \qquad (1)$$



Topaquinone



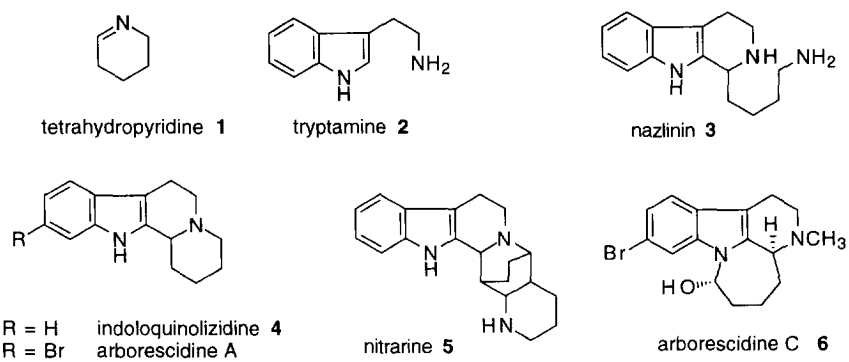
enzyme-Cu^{2+}-cofactor complex



reduced cofactor

To demonstrate the biosynthetic relationship between several indole alkaloids we examined the oxidative deamination of nazlinin (**3**), a serotonergic alkaloid obtained from *Nitraria* plant extracts.[4,5] The biosynthesis of the *Nitraria* alkaloids has not been elucidated, but the repeating C_5N structural units they consist of, makes

it most likely that these alkaloids are derived from lysine.[6] From labelling studies in Lupine plants it has become clear that lysine, via decarboxylation followed by oxidative deamination of the intermediate 1,5-diaminopentane (cadaverine), is transformed into dehydropiperidine (**1**). Nazlinin for instance can be seen as the biosynthetic condensation product of dehydropiperidine (**1**) and tryptamine, a process which has also been performed efficiently in our laboratory.[5]



Scheme 1

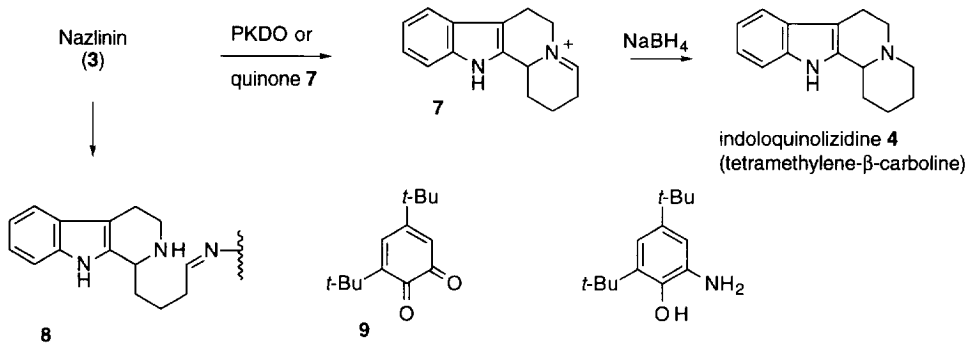
Further transformation reactions of nazlinin will also have synthetic value, since oxidative deamination of nazlinin will lead to the *Nitraria* alkaloid indoloquinolizidine **4**, and eventually to nitrarine (**5**)[7,8] via condensation reactions with a second equivalent of **1**.

A related group of brominated indole alkaloids, obtained from the tunicate *Pseudodistoma Arborescens*, is also interesting from biosynthetic point of view, since a common, nazlinin-like precursor results in cyclisation on N-2 or on N-9, leading to arborescidine A and arborescidine C respectively.

In our previous work[9] we studied the kinetics of the interaction of the diamine nazlinin **3**, a natural product, and its aromatised analogue 1-(4-butylamino)-β-carboline (**10**) with PKDO. PKDO catalyses the oxidative deamination of diamines like cadaverine (1,5-diaminopentane), putrescine (1,4-diaminobutane) and histamine according to the trans-amination shown in equation 1. We found that nazlinin interacts with PKDO as a poor substrate and an inhibitor while β-carboline **10** behaved as a good substrate. In this paper we describe the isolation of the products of these enzymatic reactions. The reaction products were characterised by their biomimetic and chemical synthesis.

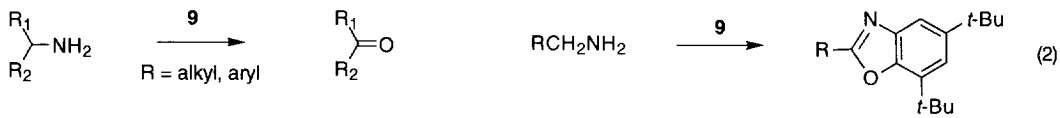
Results and Discussion

A. Nazlinin 3 as substrate. Under the experimental conditions, the enzyme catalysed reaction of nazlinin (**3**) with PKDO resulted in the formation of the expected products **7**, in poor yield (0.5%). Reduction of **7** with $NaBH_4$ gave 1,2,3,4,6,7,12,12b-octahydroindolo-(2,3-a)-quinolizidine **4**, which has been obtained as a natural product from *Nitraria* species.[10] Variation of some parameters in the PKDO reaction, such as the concentration of the substrate (up to 100 mM) and the pH (from 6 to 10) did not change the final yield of **4**.



Scheme 2

An interesting alternative for the enzymatic deamination is the use of quinones as stoichiometric oxidants. Although several analogues of the topaquinone cofactor are in development,[3] the commercially available ortho-quinone **9** has shown its value in oxidative deamination reactions. According to Corey and Achiwa[11] ketones are obtained in high yield from **9** with primary, α-branched amines such as cyclohexylamine. Primary amines containing an α-unbranched substituent (e.g. benzylamine) are not suitable as substrate in the oxidative



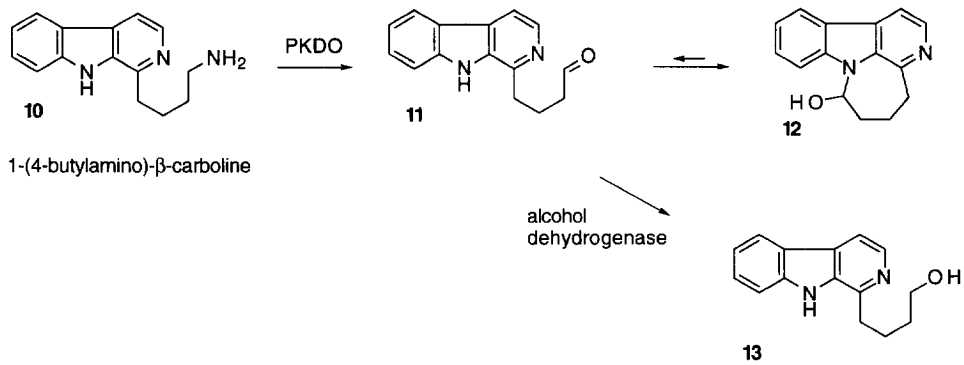
deamination with **9** since via a second quinone catalysed oxidation step, benzoxazoles are formed instead of aldehydes. In our substrate over-oxidation to the corresponding benzoxazole is prevented by the presence of a second amino group, which takes over the imino carbon atom initially formed (**8**) and leads to a 6-membered cyclic iminium salt **7**. Oxidation of nazlinin with **9** in methanol gave a 51% yield of **4** after $NaBH_4$ reduction.

B. 1-(4-butylamino)-β-carboline (10) as substrate. With 1-(4-butylamino)-β-carboline (**10**) as substrate in the PKDO oxidation, an aldehyde was formed, which did not cyclise easily under the reaction conditions. The presence of a second amino group in nazlinin has the advantage that the intermediate Schiff base **8** can cyclise directly to indoloquinolizidine **7** (Scheme 2). When this second nucleophilic amino group is not present in the substrate, the enzyme reaction is complicated by condensation of the aldehyde formed with the starting material to the corresponding imine.

Acid/base workup of the reaction yielded the azepine (**12**), which in $CDCl_3$-solution slowly reached an 75 : 25 equilibrium with the ring opened aldehyde **11**, as was observed in ^{1}H-NMR spectra (see experimental).

In order to obtain a more stable product from the PKDO catalysed oxidation of **10**, we added alcohol dehydrogenase (ADH) to the reaction mixture which reduced the intermediate aldehyde *in situ* to the corresponding alcohol (**13**), which could easily be isolated.

Biomimetic oxidation of **10** with quinone **9** was not successful, due to the benzoxazole formation mentioned in eq 2.

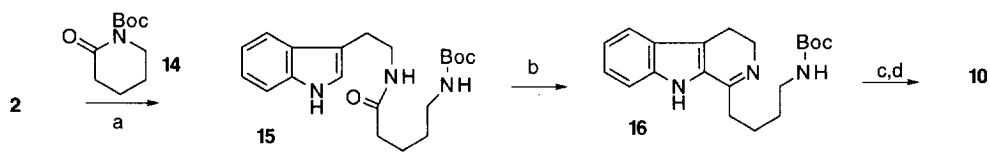


Scheme 3

It should be noted that both 10-bromo-indoloquinolizidine (arborescidine A) and the azepine alkaloid arborescidine C (Scheme 1) were isolated from the tunicate *Pseudodistoma Arborescens.*[12] Possibly the direction of the ring closure *in vivo*, which apparently occurs spontaneously, is depending on the substitution of the β-carboline-nitrogen atom.

Synthesis of substrates and enzyme reaction products.

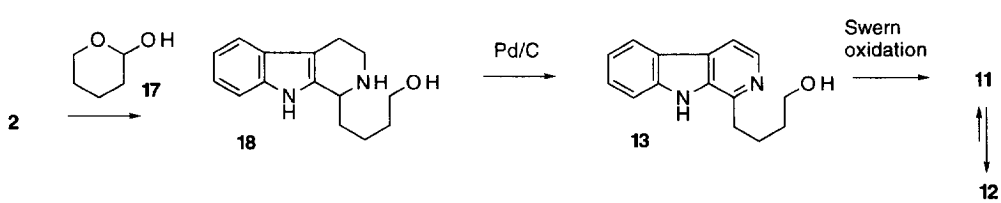
The preparation of nazlinin **3** has been described before.[5] Since direct aromatisation of nazlinin to 1-(4-butylamino)-β-carboline **10** was not possible, this compound was prepared in 4 steps from tryptamine and N-Boc-piperidinone (**14**) as is shown in scheme 4.



a) THF, EtOH, rT, 80%; b) PPh_3, CCl_4, K_2CO_3, DCM, 52%; c) Pd/C, xylene, Δ; d) HCl, 64%, 2 steps.

Scheme 4

To establish unequivocally the structures of aldehyde **11**, azepine **12** and 1-(4-hydroxybutyl)-β-carboline **13** respectively, these compounds were synthesised according to scheme 5. Tetrahydro-1-(4-hydroxybutyl)-β-carboline **18** was obtained from a Pictet Spengler reaction between tryptamine and 2-hydroxypyran.



Scheme 5

Aromatisation of **18** with Pd/C gave the corresponding β-carboline **13**. Further oxidation of **13** under Swern conditions gave the azepine **12**. The NMR spectrum showed the expected equilibrium with the aldehyde **11**, as was mentioned before (Scheme 3). All the synthetic products were identical to the PKDO reaction products.

Conclusions.

Although nazlinin is a poor substrate for PKDO, the reaction product could be isolated and characterised. The aromatised analogue of nazlinin was a better substrate for oxidative deamination by PKDO, leading to a new, arborescidine type of alkaloid, the structure of which was determined via chemical synthesis.
The amine oxidase derived topaquinone analogue **9**, which is not suitable for the oxidation of primary amines that contain an α-unbranched substituent, shows promising activity in the oxidative deamination of 1,5-diamines such as nazlinin.[13]

EXPERIMENTAL

General information. Infrared spectra were obtained from a Perkin-Elmer 1310 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained from Bruker AMX 300 (300 MHz) and Bruker ARX 400 (400 MHz) spectrophotometers. These machines were also used for ^{13}C NMR (APT) spectra at 75 resp. 100 MHz. Mass spectra were recorded on a VG Micromass ZAB-HFqQ instrument or on a JEOL JMS-SX/SX102A Tandem Mass Spectrometer. A resolving power of 10,000 (10% valley definition) for high resolution electron impact or FAB mass spectrometry was used. Thin layer chromatography (TLC) was performed on silica gel-coated plastic sheets (Merck silica gel 60 F_{254}). Chromatography refers to flash chromatography, using Janssen Chimica silica gel (0.030 - 0.075 mm). When ammonia containing eluents were used, the silica gel was pre-treated with this solvent.
Porcine Kidney Diamine Oxidase [EC 1.4.3.6., 0.06 units/mg prot.] and catalase [EC 1.11.1.6., 2000 units/mg prot.] were purchased from Sigma, alcohol dehydrogenase (ADH) from yeast [EC 1.1.1.1. ca 400 units/mg prot.] (lyophilised) was from Boehringer Mannheim GmbH and β-nicotinamide adenine dinucleotide reduced disodium salt trihydrate (NADH. $Na_2.3H_2O$) from Fluka. All the enzymes were used without further purification and the activities shown are those given by each firm.

General Procedure for the enzymatic reactions.
A solution containing the substrate dissolved in 0.1 M phosphate buffer pH 7.4 was prepared and carefully mixed with a solution containing the other reagents and enzymes dissolved in the same buffer. The reaction mixture was stirred at 36 °C and followed by TLC. The reaction was saturated with NaCl and extracted with diethyl ether, dried over Na_2SO_4 and purified by flash chromatography.

PKDO catalysed synthesis of 1,2,3,4,6,7,12,12b-octahydroindolo-(2,3-a)-quinolizine **4**.
100 mLof a solution containing PKDO (0.14 g, 8.4 units) and catalase (0.5 mg, 800 units) was added to a solution of nazlinin (5.3 mM, 100 mL). The mixture was stirred at 36 °C during 48 h. After cooling in ice the reaction was treated with $NaBH_4$ (0.38 g, 10 mmol) and stirred at room temperature for one night. The mixture was saturated with NaCl, extracted with diethyl ether and dried over Na_2SO_4. After evaporation of the solvent and chromatography (DCM/MeOH/NH_4OH 97/3/0.3) of the resulting oil, **4** (0.5 mg, 0.5%) was obtained as a solid: mp 144 - 146 °C (ether); ^{1}H NMR ($CDCl_3$) δ 7.84 (bs, 1H), 7.63 - 7.08 (m, 4H), 3.25 (bd, J = 10.8, 1H), 3.07 - 2.99 (m, 3H), 2.76 - 2.62 (m, 2H), 2.40 (ddd, J = 3.9, 11.3, 11.3, 1H), 2.04 (m, 1H), 1.89 (m, 1H), 1.78 - 1.73 (m, 2H), 1.60 (m, J = 12.6, J = 3.6, 1H), 1.5 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 136.0, 134.9, 127.5, 121.3, 119.3, 118.1, 110.7, 108.1, 60.20, 55.64, 53.49, 29.89, 25.63, 24.22, 21.49, IR ($CHCl_3$) 3480 cm^{-1}; HRMS (EI) obs. mass 226.1463, calcd. for $C_{15}H_{18}N_2$ 226.1496. According to its Rf-value and MS and NMR spectra this product was identical to synthetic **4**[14] and to the natural product.[15]

PKDO catalysed synthesis of **12**.
5 mL of a solution containing PKDO (50 mg, 3 units) and catalase (0.5 mg, 800 units) was added to a solution of 1-(4-butylamino)-β-carboline **10** (8 mM, 5 mL). After stirring at 36 °C for 24 h the reaction was treated with 1M HCl to hydrolyse the formed imine. K_2CO_3-workup and chromatography (EtOAc/MeOH/Et_3N 85/10/5) gave **12** (1.2 mg, 18%) as a solid (mp 137 - 139 °C).
12 is in a time- and solvent dependant equilibrium with the ring opened aldehyde **11**. After dissolving **12** in $CDCl_3$ an initial ratio (after 15 min) of **11** : **12** = 95:5 is observed, which stabilises at a ratio of **11** : **12** = 75:25 after standing in $CDCl_3$ during 24 h. In DMSO-d6 only the cyclised form is present, while in CD_3OD the ring opened form dominates (**11** : **12** = 22 : 78).
12: ^{1}H NMR ($CDCl_3$) δ 8.26 - 7.17 (m, 6H), 6.42 (m, 1H), 3.56 (ddd, *J* = 16.7, 5.3, 3.7, 1H), 3.14 (m, 1H), 2.98 (m, 1H), 2.64 (m, 1H), 2.43 (m, 1H), 2.25 (m, 1H), 2.03 (m, 1H); ^{1}H NMR (DMSO-d6) δ 8.30 - 7.23 (m, 6H), 6.42 (m, 1H), 6.23 (m, 1H), 3.56 (ddd, *J* = 16.7, 5.3, 3.7, 1H), 3.14 (m, 1H), 2.39 (m, 2H), 2.19 (m, 1H), 1.95 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 20.6, 33.4, 43.1, 111.5, 113.1, 119.9, 121.7, 128.2, 134.4, 138.3, 140.3, 144.8, 203.5.
11: ^{1}H NMR ($CDCl_3$) δ 9.85 (s, 1H), 9.65 (bs, 1H), 8.26 - 7.17 (m, 6H), 3.56 (ddd, *J* = 16.7, 5.3, 3.7, 1H), 3.14 (m, 1H), 2.98 (m, 1H), 2.64 (m, 1H), 2.43 (m, 1H), 2.25 (m, 1H), 2.03 (m, 1H), ^{13}C NMR ($CDCl_3$) δ 18.0, 34.9, 38.8, 78.0, 110.7, 112.3, 120.7, 121.5, 122.5, 128.5, 130.9, 134.9, 140.0, 141.8, 147.2; IR (KBr) ν 3213, 2934, 1240 cm^{-1}; HRMS (EI) obs. mass 238.1125, calcd. for $C_{15}H_{14}N_2O$ 238.1208.

PKDO catalysed synthesis of 1-(4-butanol)-β-carboline **13**.
5 mL of a solution containing PKDO (50 mg, 3 units), catalase (0.5 mg, 800 units) ADH (14 mg) and NADH (12 mM) was added to a solution of 1-(4-butylamino)-β-carboline **10** (8 mM, 5 mL). The pH was adjusted to 8.5 with 1 M HCl and the reaction mixture was stirred at 36 °C for 24 h. The reaction mixture was saturated with K_2CO_3, extracted with diethyl ether, and dried over Na_2SO_4. The solvent was eliminated and chromatography (DCM/MeOH/NH_4OH 90/10/1) gave **13** (1 mg, 10 %), which was identical to synthetic **13**: mp 167 - 168 °C; ^{1}H NMR ($CDCl_3$ + 2 drops of CD_3OD) δ 8.15 - 7.05 (m, 6H), 3.51 (td, *J* = 6.1, 2.8, 2H), 2.97 (m, 2H), 1.82 (m, 1H), 1.75 (m, 1H) 1.52 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 26.6, 33.4, 34.4, 62.7, 112.9, 114.1, 119.7, 120.7, 122.6, 122.7, 129.4, 130.2, 135.9, 138.0, 147.1; IR (KBr) ν 3164, 2858, 1626, 1325, 1190 cm^{-1}; MS (EI) obs. mass 240.1264, calcd. for $C_{15}H_{18}N_2O$ 240.1270.

Reaction of tryptamine with N-Boc-piperidone.
N-Boc-piperidone **14** (1.99 g, 10 mmol) and tryptamine (1.6 g, 10 mmol) were dissolved in a mixture of THF (20 mL) and ethanol (1 mL) and stirred at rT during 48 h. Evaporation of solvents, addition of ethyl acetate (20 mL) and stirring at 0 °C (seeding may be required) yielded amide **15** (2.875 g, 8.0 mmol, 80%): mp 100 - 101.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.52 (bs, 1H), 7.6 - 7.0 (m, 5H), 5.7 (bs, 1H), 4.65 (bs, 1H), 3.57 (m, 2H), 3.06 (m, 2H), 2.96 (t, *J* = 6.6 Hz, 2H), 2.08 (t, *J* = 7.4 Hz, 2H), 1.57 (m, 2H), 1.44 (s, 9H), 1.4 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 172.8, 156.1, 136.5, 127.4, 122.2, 120.0, 119.3, 118.6, 112.7, 111.3, 79.18, 39.98, 39.69, 36.03, 29.51, 28.44, 25.28, 22.75; IR ($CHCl_3$) 3420, 3360, 1690, 1660, 1630 cm^{-1}; HRMS (EI) obs. mass (M+1) 360.2267, calcd. for $C_{20}H_{30}N_3O_3$ 360.2323.

1,2-dihydro-β-carboline **16**.
Triphenylphosphine (3.29 g, 12.5 mmol) was added in 3 portions during 5 h to a vigorous stirred suspension of amide **15** (1.795 g, 5 mmol), CCl_4 (4.84 mL, 50 mmol) and powdered, anhydrous K_2CO_3 (0.50 g, 10 mmol) in DCM (50 mL, dest. over P_2O_5) at rT. After stirring for an additional 3 h, the reaction mixture was extracted with diluted Na_2CO_3 solution. The water layer was extracted with DCM and the combined extracts were dried (Na_2SO_4) and evaporated. Chromatography (DCM/MeOH/NH_4OH 90/9/1) gave **16** as a foam (0.887 g, 52%): ^{1}H NMR ($CDCl_3$) δ 9.83 (bs, 1H), 7.6 - 7.1 (m, 4H), 4.87 (bs, 1H), 3.86 (m, 2H), 3.27 (m, 2H), 2.85 (m, 2H),

2.71 (m, 2H), 1.75 (m, 2H), 1.60 (m, 2H), 1.47 (s, 9H), 1.4 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 161.5, 157.1, 136.8, 128.8, 125.4, 124.1, 119.9, 119.8, 116.3, 112.3, 79.54, 48.25, 38.78, 34.95, 30.67, 28.47, 24.29, 19.35.

Synthesis of 1-(4-butylamino)-β-carboline **10**.
A mixture of 1,2-dihydro-β-carboline **16** (0.78 g, 2.29 mmol), 10% Pd on carbon (0.1 g) and anhydrous Na_2CO_3 (10 mg) in xylenes (mixture of isomers, 6 mL)was refluxed during 4h. The reaction mixture was filtrated over Celite with ethanol, evaporated and dissolved in a mixture of ethanol/H_2O/concd HCl = 6/3/2. After one night at rT the solution was diluted with water (ca 20 mL), and the water layer was extracted 3 times with ether. The ether layers were washed once with water and then the combined aqueous layers were made alkaline with a large excess of solid K_2CO_3. The aqueous layer was extracted 5 times with ether containing 2% ethanol, and the residue after drying (Na_2SO_4) and evaporation was purified by chromatography (DCM/MeOH/NH_4OH 75/25/2.5). The slowly crystallising product (0.349 g, 64%) was isolated as a solid by stirring with a small amount of anhydrous ether: mp 135 - 139 °C; ^1H NMR ($CDCl_3$) δ 10.7 (bs, 1H), 8.33 (d, J = 5.4, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 5.4 Hz, 1H), 7.47 (m, 2H), 7.25 (m, 1H), 3.13 (m, 2H), 2.92 (t, J = 6.4 Hz, 2H), 1.98 (m, 2H), 1.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 146.2, 140.4, 138.3, 134.6, 128.3, 127.8, 121.9, 121.6, 119.5, 112.8, 111.5, 40.90, 33.42, 31.12, 25.17; IR: 3470 cm^{-1}; HRMS (EI) obs. mass 240.1496, calcd. for $C_{15}H_{18}N_3$ 240.1518.

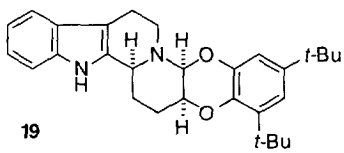
1-(4-Hydroxybutyl)-1,2,3,4-tetrahydro-β-carboline (**18**).[16]
A solution of 2-hydroxypentanal (0.41 g, 4 mmol) in water (2 mL) was added to a solution of tryptamine hydrochloride (0.393 g, 2 mmol) in water (5 mL). The solution was kept at 45°C for 3 days and then basified to pH 13 with 20% NaOH solution. The water layer and the separated yellow gum were extracted with ether (6x5 mL). After drying and evaporation of the ether, the remaining solid was purified by chromatography (EtOAc/MeOH/Et_3N 60/35/5) yielding **18** (0.334 g, 68 %) as a yellow solid: mp 193 °C; ^1H NMR ($CDCl_3$, + 2 drops of CD_3OD) δ 7.46 – 7.03 (m, 4H), 4.05 (m, 1H), 3.63 (m, 2H), 3.32 (ddd, J = 12.6, 4.5, 4.5, Hz, 1H), 2.99 (ddd, J = 13.2, 7.9, 5.6, 1H), 2.74 (m, 2H), 1.92 (m, 1H), 1.68 (m, 1H), 1.58 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 21.8, 22.3, 31.9, 34.0, 42.2, 52.3, 61.7, 108.3, 10.7, 117.8, 119.0, 121.3, 127.1, 135.6; IR (KBr) ν 3250, 2935, 2863, 1045 cm^{-1}; HRMS (EI) obs. mass 244.1591, calcd. for $C_{15}H_{20}N_2O$ 244.1643.

1-(4-Hydroxybutyl)-carboline **13**.
To a solution of **18** (0.098 g, 0.4 mmol) in xylene (3 mL, mixture of isomers) palladium on carbon (0.02 g, 10%) was added and the mixture was refluxed under nitrogen for 90 minutes. MeOH (3 mL) was added and the mixture was filtrated over Celite. The residue was washed with MeOH (6x5 mL). The filtrate was evaporated, affording a white solid which was recrystallised from methanol yielding **13** as white crystals (0.083 g, 86%). This product was identical to the PKDO reaction product.

4,5,6,7-Tetrahydro-cyclohept-(3,7-a)-fluoren-7-ol **12**.
A solution of dry DMSO (0.53 mL) in dry dichloromethane (4 mL) was added dropwise to a solution of oxalylchloride (0.26 mL) in dichloromethane (8 mL) at -78 °C. Then a solution of **13** (0.40 g, 1.66 mmol) in dichloromethane (3 mL) and DMSO (2 mL) was added during 15 minutes. The mixture was stirred for 3 h. Et_3N (0.93 mL) was added and the mixture was stirred at -78 °C for an additional 5 minutes before it was allowed to warm to rT. After stirring for 45 minutes water (5 mL) was added, the layers were separated and the water layer was extracted with dichloromethane (6x3 mL). The combined organic layers were washed with brine (2x10 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was evaporated three times with ether. The remaining solid was purified by chromatography (EtOAc/MeOH/Et_3N 85/10/5) yielding **12** as a yellow solid (0.118 g, 30%), which was identical to the enzyme reaction product.

Oxidation of nazlinin with ortho-quinone **9**.

A solution of quinone **9** (77 mg, 0.35 mmol) in THF (0.5 mL) was added in 1 portion to a solution of nazlinin **3** (49 mg, 0.2 mmol) in MeOH (1 mL). After stirring the reaction mixture during 40 min at room temperature, $NaBH_4$ (38 mg, 1 mmol) was added and stirring was continued for 3 h. Workup with 10% NaOH, ether extraction and chromatography (DCM/MeOH/NH_4OH 97/3/0.3) gave **4** (23 mg, 51%). Prolonged reaction times and the use of 2 or more equivalents of quinone **9** resulted in the formation of substantial amounts of cycloaddition product **19** which directly crystallised from the reaction mixture: mp > 300 °C; ^1H NMR ($CDCl_3$) δ 7.73 (bs, 1H), 7.51 - 7.08 (m, 4H), 6.73 (d, *J* = 2.2 Hz, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 5.01 (d, *J* = 1.6 Hz, 1H), 4.28 (bd, *J* = 11.3 Hz, 1H), 3.57 (m, 1H), 3.47 (ddd, *J* = 11.8, 11.8, 4.2, 1H), 3.22 (m, 1H), 2.99 (m, 1H), 2.80 (m, 1H), 2.18 (m, 1H), 2.00 (m, 1H), 1.85 (m, 1H), 1.71 (m, 1H), 1.39 (s, 9H), 1.28 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 143.0, 137.2, 136.0, 134.8, 130.3, 121.4, 119.5, 119.1, 118.1, 117.2, 113.5, 110.7, 110.6, 108.3, 87.68, 71.06, 50.77, 50.62, 49.58, 35.10, 31.56, 29.87, 29.20, 27.02, 22.13; IR ($CHCl_3$) ν 3478, 1570 cm^{-1}; HRMS obs. mass 444.2764, calcd for $C_{29}H_{36}N_2O_2$ 444.2792.



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