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Stereoselective Benzylic Deprotonation in the Enzymatic Rearrangement of *N*-Acetyldopamine Derived *o*-Quinone to the *p*-Quinone Methide

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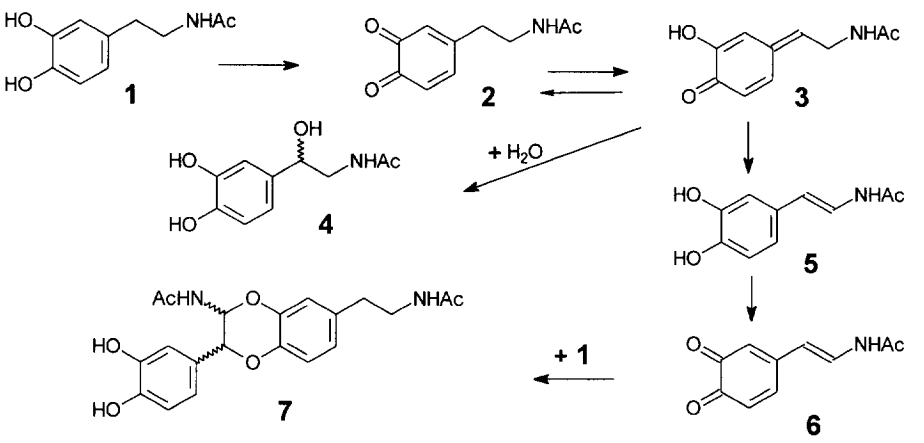
Abstract: (*R*)-*N*-acetyl-[β-$^2H_1$]-dopamine {(*R*)-**1**} undergoes an enzyme catalyzed oxidative tautomerization to quinone methide **3** with loss of the benzylic H_{Si}, as proven by isolation of deuteriated *rac*. *N*-acetylnoradrenaline (**4**), while (*S*)-[β-$^2H_1$]-**1** loses the deuterium label. Thus, the *o*-quinone-*p*-quinone methide isomerization is a stereoselective enzymatic reaction.

Enzymatic deprotonation at benzylic positions is a key reaction in the shikimate pathway leading to the biosynthesis of many aromatic natural products. [1] Elimination of ammonia from *e.g.* L-phenylalanine by phenylalanine-ammonia-lyase (PAL; EC 4.3.1.5) is a crucial step in the biosynthesis of cinnamic acid and hence lignins as well as many alkaloids and other aromatic secondary metabolites. The PAL catalyzed *anti*-elimination of ammonia from L-phenylalanine results in exclusive loss of the benzylic H_{Si}.[2] Recently, two novel enzymatic benzylic deprotonations were described to occur in the isomerization of an *o*-quinone to a *p*-quinone methide. One of the enzymes, named dopachrome conversion factor, [3] catalyzes a fundamental step in the biosynthesis of melanin pigments in most organisms, namely the rearrangement of 5,6-dioxoindoline-2-carboxylic acid (dopachrome) to 5,6-dihydroxyindole-2-carboxylic acid or to 5,6-dihydroxyindole, respectively, [4] which may also occur spontaneously. [5] The other enzyme, named simply *o*-quinone-*p*-quinone methide isomerase, [6] occurs in insects which use *N*-acetyldopamine (**1**) and other *N*-acylcatecholamines for tanning and hardening of the exoskeleton. A complex sequence of reactions, some principles of which are sketched in Scheme 1 (for reviews, see [7]), is initiated by the diphenoloxidase catalyzed conversion of **1** to *o*-quinone **2**. The latter rearranges with participation of the isomerase to quinone methide **3**. Non-enzymatic addition of water to **3** yields *rac*. *N*-acetylnoradrenaline (**4**) [8] while addition of N^{α}-acetyl-L-histidine results in a diastereoisomeric mixture of the corresponding imidazole conjugates. [9] Further rearrangement of **3** into **5** and its oxidative coupling with **1** leads to dihydrobenzodioxine dimers of type **7**. [10] This reaction scheme resembles the early steps in the biosynthesis of lignins. However, a fundamental difference will be recognized in the fact that, in the latter case, the quinone methide is actually a resonance structure of the semiquinone radical which is derived from a *p*-hydroxy vinylbenzene (*i.e.* hydroxycinnamyl alcohol) unit.

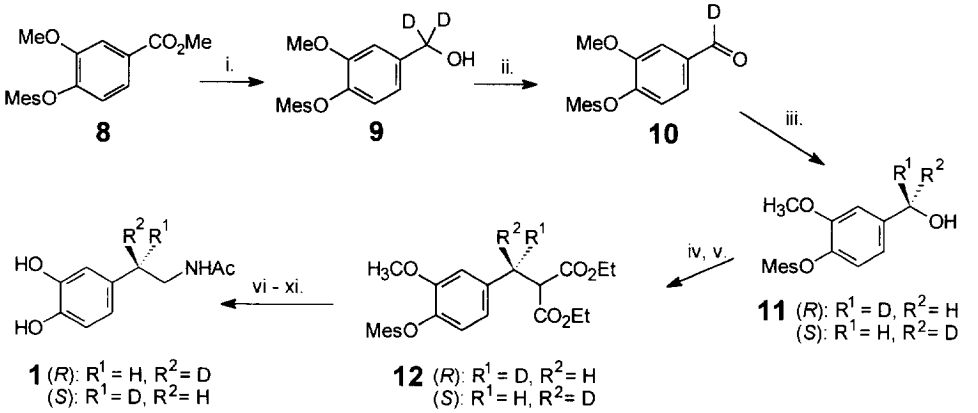
The stereochemical course of the quinone methide isomerization has not yet been reported for any of the enzymes. Within the framework of our work on the biotransformations of *N*-acetyldopamine, [7,8,9] we have synthesized both enantiomers of [β-$^2H_1$]-**1** and found that the benzylic deprotonation of **1** (or **2**, respectively) occurs indeed with a remarkable stereoselectivity.



Scheme 1

Synthesis of stereospecifically deuterium-labelled *N*-acetyldopamine

Asymmetric synthesis of chiral deuterium labeled benzyl alcohols has been achieved earlier in a chemoenzymatic approach by *Battersby* et al.. [2] Following generally known procedures (see Scheme 2), we have substituted the enzymatic step with a stereospecific reduction of benzaldehyde **10** by means of alpine boranes that were prepared from highly purified (+)- or (-)- α-pinene (e.e. > 99%), respectively.



i. $LiAlD_4/Et_2O$ (75%); ii. PDC/CH_2Cl_2 (68%); iii. (*R*)-alpine borane/THF {80% of (*S*)-**11**}, or (*S*)-alpine borane/THF {92% of (*R*)-**11**}; iv. $MesCl/CH_2Cl_2$ (74%); v. diethyl malonate, NaH/THF (94%); vi. 5 N HCl, reflux (88%); vii. NaOH (88%); viii. BnCl, $Me_4N^+I^-$, K_2CO_3/acetone (52%); ix. Et_3N, $ClCO_2Et$/acetone; x. NaN_3/acetone, then reflux in toluene, then MeMgCl (70%); xi. BBr_3/CH_2Cl_2 (88%).

Scheme 2

Briefly, methyl 4-mesylvanillate **8** is reduced with $LiAlD_4$ to give benzyl alcohol **9**. *Corey* oxidation [11] of **9** affords highly deuterium enriched benzaldehyde **10** which is reduced to benzyl alcohol (*S*)-**11** [12] (e.e. ≥ 98% [13]) by means of (*R*)-alpine borane. Likewise, (*R*)-**11** is obtained by using (*S*)-alpine borane. Chain extension to (*S*)-**12** is achieved by reaction of diethylmalonate with the mesylate of (*S*)-**11**. [14] Conversion of (*S*)-**12** into the corresponding phenylpropionic acid is followed by *Curtius* degradation, *Grignard* reaction with the corresponding isocyanate, and deprotection to give (*R*)-**1**. [15]

Enzymatic reaction and analysis of *N*-acetylnoradrenaline

(*R*)-**1** or (*S*)-**1**, respectively, is mixed with an aqueous extract of the cuticle from 5th instar larvae of the tobacco hornworm, *Manduca sexta*. Analysis of the reaction mixture by means of HPLC [16] reveals rapid disappearance of **1** with concomitant formation of several products, *inter alia* **4**. The reaction is terminated after 45 min and **4** is isolated in 10-12% yield by means of semipreparative HPLC.

The results of the mass spectrometric analysis (Table 1) show that **4** derived from (*R*)-**1** retains essentially all of the deuterium while, in the complimentary experiment, (*S*)-**1** looses essentially all of the deuterium upon conversion into **4**. We have not yet investigated the kinetics of the deprotonation which is expected to show an isotope effect. However, we have also found recently deuterium in a dihydrobenzodioxine dimer of type **7** which was isolated after incubation of (*R*)-**1** with an extract of locust cuticle (details will be published elsewhere). Thus, it is concluded that the *p*-quinone methide **3** undergoes further non-enzymatic reactions such as nucleophilic additions more rapidly than tautomeric equilibration between **2** and **3**.

Table 1: Mass Spectrometric Analysis [17] of **1** and **4** {(*m/z* (%)}.

peak	1	(*R*)-1	(*S*)-1	4	4 *ex* (*R*)-1	4 *ex* (*S*)-1
$[M]^+$	**195 (40)**	**196 (5)**	**196 (44)**	**211 (1.7)**	**212 (nil)**	**211 (< 0.1))**
				195 (2)		195 (1)
				194 (24)	195 (7)	194 (8)
$[M - 18]^+$				**193 (32)**	**194 (96)**	**193 (96)**
				192 (nil)	193 (2)	192 (nil)
				153 (10)		153 (8)
$[M - 59]^+$	**136 (100)**	**137 (100)**	**137 (100)**	**152 (64)**	**153 (7)**	**152 (10)**
$[M - 60]^+$		**136 (28)**	**136 (78)**	**151 (100)**	**152 (100)**	**151 (100)**
				150 (26)	151 (14)	150 (14)
					150 (4)	
HR-MS:			$[M]^+$: $C_{10}H_{12}D_1NO_3$ calcd.: 196.0958 found.: 196.0952	$[M]^+$: $C_{10}H_{13}NO_4$ calcd.: 211.0844 found.: 211.0852	$[M - 18]^+$: $C_{10}H_{10}D_1NO_3$ calcd.: 194.0801 found.: 194.0798	$[M - 18]^+$: $C_{10}H_{11}NO_3$ calcd.: 193.0739 found.: 193.0749

The fact that the enzymatic *o*-quinone-*p*-quinone methide isomerization displays the same stereochemical course of benzylic deprotonation as observed with PAL, *i.e.* loss of H_{si}, raises the intriguing question whether there is an evolutionary relationship between the enzymes catalyzing benzylic deprotonations in plants and those in insects. The substrates differ in the topicity of the benzylic protons

which are diastereotopic in L-phenylalanine but enantiotopic in **1**. Furthermore, the type of the reaction is completely different: PAL catalyzes an elimination while the quinone methide rearrangement is an isomerization. Finally, it will be most interesting in the light of our results to learn about the stereoselectivity of the enzymatic *o*-quinone-*p*-quinone methide isomerization which occurs as the first step in rearrangement of dopachrome to dioxyindoles.

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13. ^{1}H NMR {250 MHz, $CDCl_3$, 0.5 mol equiv. Eu(hfc)$_3$}: (*S*)-**11**: δ = 15,55 (0.013 H), 15.36 (0.987 H); (*R*)-**11**: δ = 15,27 (0.980 H); 15,10 (0,02 H).
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16. Column: 25 cm x 4 mm Nucleosil RP-18 (7 µ); eluent: H_2O/MeOH/TFA 600/100/7; flow: 1.5 ml · min^{-1}.
17. AEI MS-50, direct inlet, ionization energy 70 eV; ion source at 180°C; peak intensities are calculated relative to [M^+-C_2H_5NO] = 100% in the mass spectrum of **1** and [M^+-H_2O, -C_2H_2O] = 100% in the mass spectrum of **4**.

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