Lack of Stereoselectivity in the Enzymatic Conversion of *N*-Acetyldopamine into *N*-Acetylnoradrenaline in Insect Cuticle

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Enzymatic oxidation of the sclerotization substance *N*-acetyldopamine with insect cuticle leads to racemic *N*-acetylnoradrenaline.

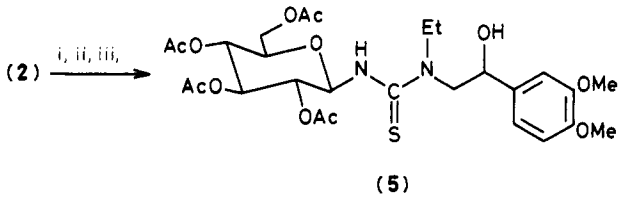
The enzymatic hydroxylation of phenylethylamines by dopamine β-mono-oxygenase (E.C. 1.14.17.1) yields stereospecifically (*R*)-1-phenylethanolamines.[1] This reaction is of fundamental importance in the biosynthesis of certain neurotransmitters, such as noradrenaline and octopamine. In insects, *N*-acetyldopamine (**1**) is required in large quantities for construction of the sclerotized exoskeleton.[2] Enzymatic oxidation of (**1**) in the cuticle of insects leads to mostly polymeric products which interact with other biopolymers in such a way that the macromolecular assembly becomes insoluble.[3] Several low molecular weight side chain oxidation products of (**1**), among them *N*-acetylnoradrenaline (**2**), have

been found after chemical degradation of sclerotized cuticle (see ref. 4 for a review). In order to see whether the oxidation of (**1**) in insect cuticle shows stereoselectivity, we have determined the enantiomeric composition of (**2**).

Radiolabelled (**1**) was added to a suspension of finely ground insect cuticle (see Table 1). After 48 h, (*R*)-(**2**) [prepared from (*R*)-(−)-noradrenaline (Serva)] was added and subsequently reisolated.[5] Transformation of (**2**) into a pair of diastereoisomers (**5**) was performed as outlined in Scheme 1. The diastereoisomeric tetra-acetylglucose isothiocyanate (GITC) derivatives (**5**) of (**4**) were separated by h.p.l.c.[6] (Figure 1). Table 1 shows that the ^3H as well as the ^{14}C radioactivity was distributed nearly equally between both diastereoisomers. Integration of the peak areas from u.v. detection, however, reveals that the faster eluting diastereoisomer which is derived from the (*R*)-enantiomer of (**2**) is predominant. Thus, the optically active, radioinactive (**2**) which was added as a carrier, is not racemized detectably during the derivatization procedure, whereas the radiolabelled (**2**) is racemic within the accuracy of the determination. This result is confirmed by the following two experiments. Firstly, when racemic (**2**) is added to the incubation mixture, h.p.l.c. separation shows that the radioactivity and u.v. absorption cochromatograph, with two equally sized peaks for the diastereoisomers of (**5**). Secondly, when labelled dopamine is oxidized by means of dopamine-β-monooxygenase from bovine adrenal medulla and subsequently diluted with racemic unlabelled noradrenaline, the GITC derivative shows two u.v. absorbing peaks for the diastereoisomers, but the radioactivity cochromatographs with the first one only, which is derived from (*R*)-noradrenaline (*cf.* ref. 6).

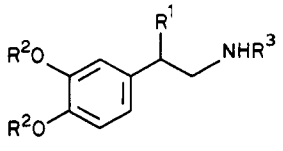
The yield of the enzymatic reaction, as estimated from the amount of ^{14}C radioactivity cochromatographing with (**2**) on Sephadex LH-20 after oxidation of (**1**) with insect cuticle, was 8.6—10.2%. When (**1**) was shaken in buffer without added insect cuticle but otherwise identical conditions, the amount of ^{14}C radioactivity cochromatographing with (**2**) on Sephadex LH-20 was ≤1%. The difference in the yield reflects the enzymatic activity of the cuticle.

The results of this study prove that there is no stereoselectivity in the conversion of (**1**) into (**2**) by an enzyme or an enzyme system that is present in insect cuticle. Several mechanistic possibilities could account for the stereochemical result. A semiquinone radical may be the primary oxidation product which on further oxidation yields a semiquinone methide. Spontaneous 1,6-addition of H_2O then will yield racemic (**2**). The semiquinone and the quinone methide may



(**5**)

Scheme 1. *Reagents:* i, CH_2N_2–MeOH–Et_2O; ii, $LiAlH_4$–tetrahydrofuran; iii, GITC–dimethylformamide.



(**1**) $R^1 = R^2 = H$, $R^3 = Ac$
(**2**) $R^1 = OH$, $R^2 = H$, $R^3 = Ac$
(**3**) $R^1 = OH$, $R^2 = Me$, $R^3 = Ac$
(**4**) $R^1 = OH$, $R^2 = Me$, $R^3 = Et$.

Table 1. Preparation of diastereoisomers (**5**) and h.p.l.c. separation.[a]

	^{14}C d.p.m.	$^3H/^{14}C$
(**2**)	1.81×10^5	5.6
(**3**)	1.07×10^5	6.3
(**4**)	3.10×10^4	6.2
(**5**)[b]	2.69×10^3	4.7
(**5**)[c]	2.30×10^3	4.6

[a] The oxidation mixture contained a suspension of 0.75 equiv. of the cuticle from one pupa of the tobacco hornworm, *Manduca sexta*, in 3 ml 50 mM potassium phosphate buffer pH 6.8, and 0.27 mg [7-^3H, 8-^{14}C]-(**1**) [2.1×10^6 disintegrations per minute (d.p.m.) ^{14}C; $^3H/^{14}C$ = 7.8]. After an incubation time of 48 h at 27 ± 2 °C in a shaking water bath, 1 mg (*R*)-(**2**) was added. Centrifugation was followed by chromatographic resolution of the supernatant on Sephadex LH-20 with H_2O as the eluant (ref. 5). [b] Diastereoisomer from (*R*)-(**2**). [c] Diastereoisomer from (*S*)-(**2**).

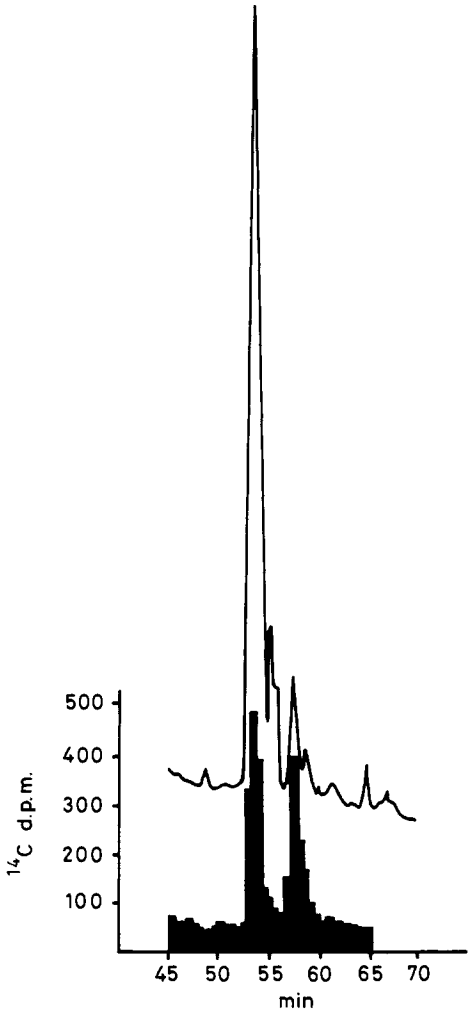


Figure 1. H.p.l.c.separation of diastereoisomers (**5**) on a 0.8 × 25 cm 5μ C_{18}-Nucleosil column. Elution conditions: 1.0 ml min^{-1}, 25 mM $CF_3CO_2NH_4$, pH 3.0, 40% MeOH, 5 min isocratic, then linear gradient up to 95% MeOH within 90 min. Upper line: u.v. detector response at 280 nm; lower bars: ^{14}C radioactivity in 0.5 ml fractions. These results are from an experiment using ^{14}C labelled (**1**) as the substrate.

also be important in the mechanism of oxidative polymerization in the main reaction. An alternative pathway, namely stereoselective β-hydroxylation followed by an enzymatic racemization with considerable retention of tritium may also be reasonable. Table 1 shows that the oxidation occurs with a small but detectable isotope effect. Finally, a third possibility would be the direct non-stereoselective oxygenation by analogy with a reaction which is catalysed by a fungal lignin degrading enzyme.[7]

Racemic noradrenaline has been found to occur naturally in banana plant tissue.[8] An enzyme differing in cofactor requirement from animal dopamine-β-mono-oxygenase has been detected in the same source. Our results add a second example of an unusual non-stereoselective formation of a β-hydroxycatecholamine which in this case is *N*-acetylated. Since the formation of a sclerotized exoskeleton through oxidation of (**1**) is apparently unique to insects, this process may eventually become a novel target for the development of new insecticides.

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