Stereochemical Course of Hydroxylation of Dopamine by Dopamine-β-hydroxylase (EC1.14.17.1)

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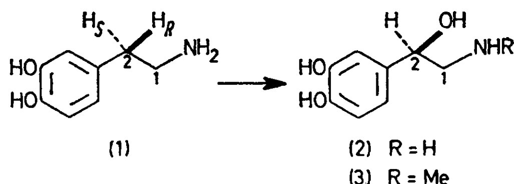
Summary (2*R*)-, and (2*S*)-[2-$^3H_1$]Dopamines are synthesised having high configurational purity; they are used to prove that hydroxylation of dopamine to yield noradrenalin occurs with retention of configuration.

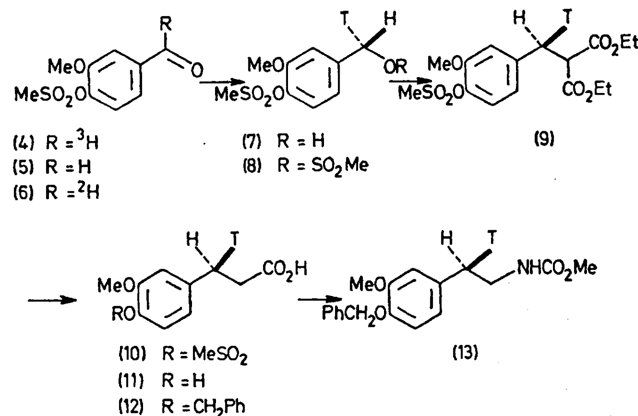
DOPAMINE β-HYDROXYLASE is a mixed function oxidase[1] which converts dopamine (**1**) into (−)-noradrenaline (**2**); the latter is the biological precursor of (−)-adrenaline (**3**) and all three catecholamines are important substances in the chemistry of the central nervous system.[2] Earlier work has established the illustrated (*R*)-configurations[3] for (−)-(**2**) and (−)-(**3**) and has shown that (a) the oxygen atom introduced at C-2 of dopamine is derived from molecular oxygen not water[4] and (b) only one hydrogen atom is removed from C-2 in the oxidative step.[5] The following studies, based on syntheses of (2*R*)-, and (2*S*)-[2-$^3H_1$] dopamines, define the stereochemistry of the hydroxylation process.



O-Methanesulphonyl[*formyl*-^{3}H]vanillin (**4**) was reduced with liver alcohol dehydrogenase, its coenzyme, and ethanol to yield the (*S*)-alcohol (**7**) (Scheme). The corresponding dimethanesulphonyl derivative (**8**) was then treated with diethyl malonate anion in the presence of excess diethyl malonate to yield the diester (**9**). Clean S_N2 inversion (see later) was achieved only when the strongly electron withdrawing aryl-*O*-methanesulphonyl group was used. Acid hydrolysis of the ester (**9**) afforded the acid (**10**) which *via* the phenol (**11**) gave the ether (**12**). Curtius degradation[6] to the urethane (**13**) and removal of all three protecting groups† first using alkali, and then HBr, gave (2*R*)-[2-$^3H_1$]-dopamine [(**1**),[3] H at H_R] isolated as its hydrochloride.

The enantiomeric product was prepared in a complementary way by reduction of unlabelled aldehyde (**5**) with alcohol dehydrogenase, NAD$^+$ and [1-^{3}H]cyclohexanol.[7] The product [enantiomer of (**7**)] was then converted into (2*S*)-[2-$^3H_1$]dopamine [(**1**), ^{3}H at H_S] by the above sequence.

The chiral sense and enantiomeric purity of the labelled dopamines was assayed by preparing the deuteriated aldehyde (**6**) which was carried through the synthesis as far



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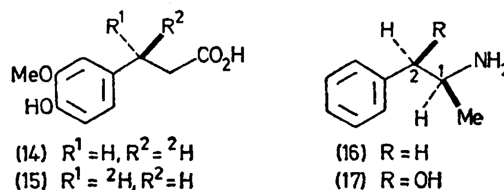
as the acid (**14**) under conditions identical with those used above. Its enantiomer (**15**) was prepared as before by transfer of deuterium in the initial enzymic step. Degradation of the acids (**14**) and (**15**) by ozone and then peracid yielded (2*S*)-, and (2*R*)-[2-$^2H_1$]succinic acid, respectively; both were shown to be enantiomerically pure, within experimental error, by mass spectrometric and o.r.d.[8] measurements. The assigned absolute configurations for the tritium labelled dopamines and their high configurational purity were thus confirmed.

TABLE. Enzymic hydroxylation of labelled dopamines

Configuration of [2-$^3H_1$]dopamine (as **1**)	^{3}H:^{14}C Ratio in dopamine	Conversion[a] (%)	^{3}H:^{14}C Ratio in (−)-noradrenaline[b]	Retention of ^{3}H (%)
(2*R*)	14·2 ± 0·6	0·8	0·07 ± 0·02	0·5
(2*RS*)	16·9 ± 0·6	0·6	8·3 ± 0·2	49 ± 2
(2*S*)	13·9 ± 0·3	0·6	13·5 ± 0·3	97 ± 3

[a] Conversions of *ca.* 1% are normal in the homogenate system. [b] Assayed as its tetraisobutyroyl derivative; multiple determinations on repeatedly purified samples.

The two chirally tritiated dopamines and also randomly labelled dopamine (*i.e.* 2*RS*) were mixed in known ratio with [1-^{14}C]dopamine; the three samples were treated separately with an homogenate from bovine adrenal glands



† The *O*-methyl group was used because labelled 3′-*O*-methyldopamines were required for another biosynthetic study.

containing ascorbic acid and proniazid (as inhibitor of amine oxidase).[9] Radio-inactive dopamine (**1**) and (−)-noradrenalin (**2**) were then added as carriers and isolated as their tri-isobutyroyl derivatives. The results in the Table show that the hydroxylation catalysed by dopamine β-hydroxylase involves stereospecific removal of the 2-*pro-R* hydrogen atom from dopamine (**1**). This corresponds to retention of configuration in an attack at a benzylic carbon.

Dopamine-β-hydroxylase will also transform several phenethylamines which are not its natural substrates; an example is (1*S*)-amphetamine (**16**) which yields (1*S*, 2*R*)-ephedrine (**17**). Taylor has recently shown[10] using (1*S*, 2*S*)-[2-$^{3}H_{1}$]amphetamine (as **16**) that the hydroxylation occurs with retention of configuration which matches our finding on dopamine and also the results for biological hydroxylations in other systems.[11]

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