Stereochemistry of Dehydrogenation Catalysed by the Amine Oxidase from Pea Seedlings (E.C.1.4.3.5.)

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Summary (*R*)- and (*S*)-[*methylene*-$^3H_1$]Benzylamines are synthesised with configurational purities greater than 95%; they are used to prove that pea-seedling amine oxidase abstracts the *pro-S* hydrogen from the methylene group of benzylamine.

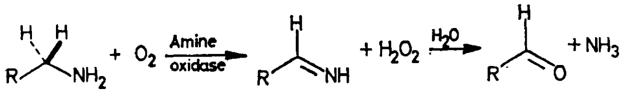
ONE LINE of research in this laboratory is concerned with the stereochemistry of enzymic processes which generate or transform prochiral centres adjacent to a nitrogen atom (starred centre in **1**). This work depends on syntheses of stereospecifically labelled (or stereoselectively labelled[1]) amines carrying ^2H or ^3H at the α-position to nitrogen (*e.g.* **5** and **10**). Labelled alkylamines, benzylamines, and arylethylamines are all important for future work. In order to assay configurational purities of labelled amines from our syntheses, we sought a stable amine oxidase which would

TABLE

[*methylene*-$^3H_1$, ^{14}C]Benzylamine	^{3}H : ^{14}C Ratio	Benzyl alcohol formed ^{3}H : ^{14}C ratio	^{3}H Retention (%)
(*R*) (10)	11·0 ± 0·3	11·0 ± 0·3	100 ± 3
(*S*) (11)	9·6 ± 0·3	0·2 ± 0·05	2 ± 0·5
(*RS*)[a]	16·3 ± 0·3	8·4 ± 0·2	52 ± 2

[a] Prepared as above from (*RS*)-[*methylene*-$^3H_1$]benzyl alcohol available from benzaldehyde by reduction with borotritiide.

dehydrogenate many amines in these classes as in the Scheme. The oxidase from pea seedlings[2] meets these requirements but the stereochemistry of its action was unknown; it has now been determined.

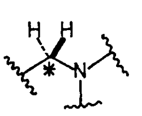


SCHEME

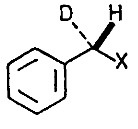
The *p*-tolylsulphonyl ester (**3**) of (+)-(*S*)-[*methylene*-$^2H_1$]benzyl alcohol[3,4] (**2**) of established absolute configuration[3,4] was converted under S_N2 conditions into the azide (**4**) which was reduced to (*R*)-[*methylene*-$^2H_1$]benzylamine (**5**). The absolute configuration expected for this product[5] was confirmed by conversion of its *N*-acetyl derivative *via* (**6**) and (**7**) into the phenol (**8**) which was degraded (ozone, peracid) to *N*-acetyl[2-$^2H_1$]glycine.† The *N*-acetyl group was cleaved with acylase-I and the configuration of the product, (+)-(*R*)-[2-$^2H_1$]glycine (**9**), was determined by o.r.d.,[7] $[\alpha]_{238}^{20}$ + 38·8° (*c*, 44·09 mg/ml in H_2O); ref. 6 records $[\alpha]_{238}$ + 36·5°.

With the configurational base secure, (*R*)-[*methylene*-$^3H_1$]-benzylamine (**10**) was prepared exactly as for the ^{2}H-analogue. A complementary sample of (*S*)-[*methylene*-$^3H_1$]-benzylamine (**11**) was synthesised by reducing benzaldehyde with liver alcohol dehydrogenase and NAD$^+$ using [1-^{3}H]ethanol as the source of tritium. The resultant (*R*)-[*methylene*-$^3H_1$]benzyl alcohol was converted as before into the (*S*)-amine (**11**).

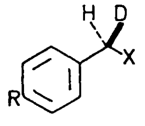
After addition of [*methylene*-^{14}C]benzylamine as internal standard to the (*R*)- and (*S*)-$^3H_1$-amines (**10**) and (**11**), they were treated separately with amine oxidase isolated[2] from pea seedlings. Liver alcohol dehydrogenase, NAD$^+$, and cyclopentanol were included in the reaction mixture to trap the product benzaldehyde by reduction; the benzyl alcohol formed was isolated as its 2,4-dinitrobenzoate. The results in the Table show that (*a*) the amine oxidase stereospecifically removes the *pro-S* hydrogen atom in the oxidation of benzylamine (**12**) to benzalimine and so to benzaldehyde and (*b*) the benzylamines prepared are of high configurational purity.



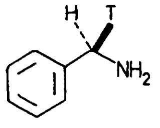
(1)



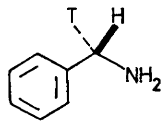
(2) X = OH
(3) X = $OSO_2C_6H_4Me$



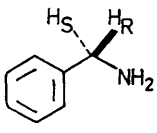
(4) R = H, X = N_3
(5) R = H, X = NH_2
(6) R = NO_2, X = NHAc
(7) R = NH_2, X = NHAc
(8) R = OH, X = NHAc



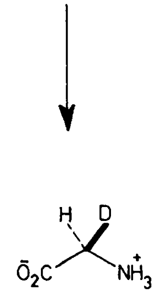
(10) (*R*)-Amine



(11) (*S*)-Amine



(12)



(9)

The amine oxidase from pea seedlings has already proved its value for configurational studies[8] on α-labelled 2-phenethylamines and tryptamines.

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† Professor D. Arigoni has kindly told us that (*S*)-[*methylene*-$^2H_1$]benzylamine of 62% configurational purity has been similarly correlated with (−)-(*S*)-[2-$^2H_1$]glycine; unpublished work in ref. 6.

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