Hydroxylation at Saturated Carbon: Haemanthamine

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Summary Specimens of *O*-methylnorbelladine (**1**) have been synthesised carrying ^{3}H-labels of known absolute configuration; they have been used to prove that the hydroxy-group at C-11 of haemanthamine (**6**) is introduced with retention of configuration.

MANY biosynthetic and metabolic reaction sequences include hydroxylation at saturated carbon ($\geq$C–H $\rightarrow$ $\geq$C–OH) as an important biochemical step. The reaction is generally carried out by a mixed function oxidase working in conjunction with oxygen and a reducing agent.[1,2] To help understand the mechanism of this reaction we are studying the stereochemistry of hydroxylation reactions occurring at sites with widely differing chemical and steric environments. The results for haemanthamine, which is of rigorously established absolute configuration[3] (**6**), are outlined here.

O-Methylnorbelladine (**1**) is an important intermediate on the biosynthetic pathway to haemanthamine[4] and recent work[5] has shown that oxocrinine (**3**) stands later in the sequence. The substrate for enzymatic hydroxylation at the prochiral centre C-11 must thus be (**3**), (**4**), or (**5**) and the stereochemical course of this reaction can be determined by synthesising *O*-methylnorbelladine (**1**) labelled randomly and stereospecifically with isotopic hydrogen at C-2. The route used in the latter case was based upon methods developed earlier[6] for other purposes in the vanillin series.

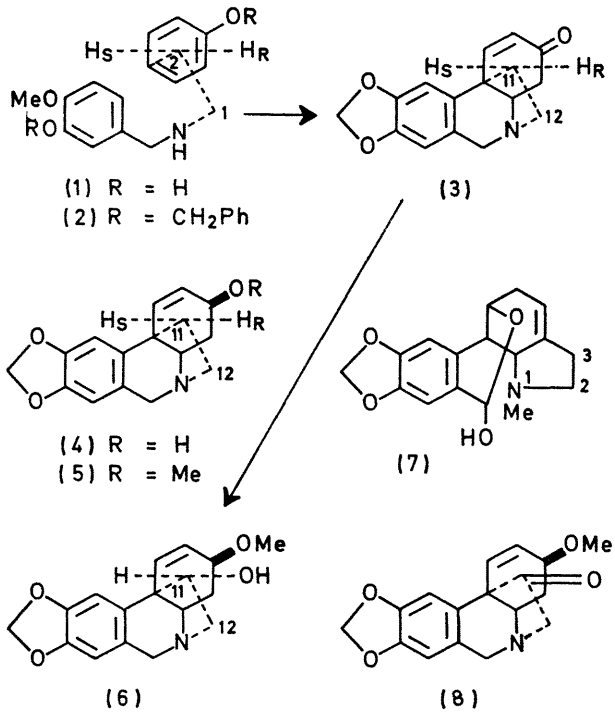
The methylene protons of the nitrile (**9**) were equilibrated against tritiated water and the product was reduced to give the randomly labelled [2-$^3H_2$]ethylamine (**10**). This was converted[4] into *OO*-dibenzyl-*O*-methyl[2-$^3H_2$]norbelladine (**2**), then mixed with [1-^{14}C]-labelled material (**2**), and debenzylated. Expt. 1 (Table) shows the results gained by feeding this precursor (**1**) to daffodils. Incorporation into haemanthamine (**6**) occurs with loss of 50% of the tritium† whereas oduline[7] (**7**) from the same plants retains essentially all the ^{3}H-label and thus acts as an internal reference; in biosynthetic terms, positions 2 and 3 of oduline (**7**) correspond, respectively, to positions 12 and 11 of haemanthamine (**6**). It follows that the enzymatic hydroxylation occurs stereospecifically. Asymmetrically labelled precursors were therefore synthesised and the absolute configuration shown for the main component in each case is established later.

Reduction of 4-benzyloxy[7-^{2}H]benzaldehyde (**11**) with liver alcohol dehydrogenase, ethanol, and NADH gave the

Tracer experiments on Narcissus pseudonarcissus (*King Alfred*)

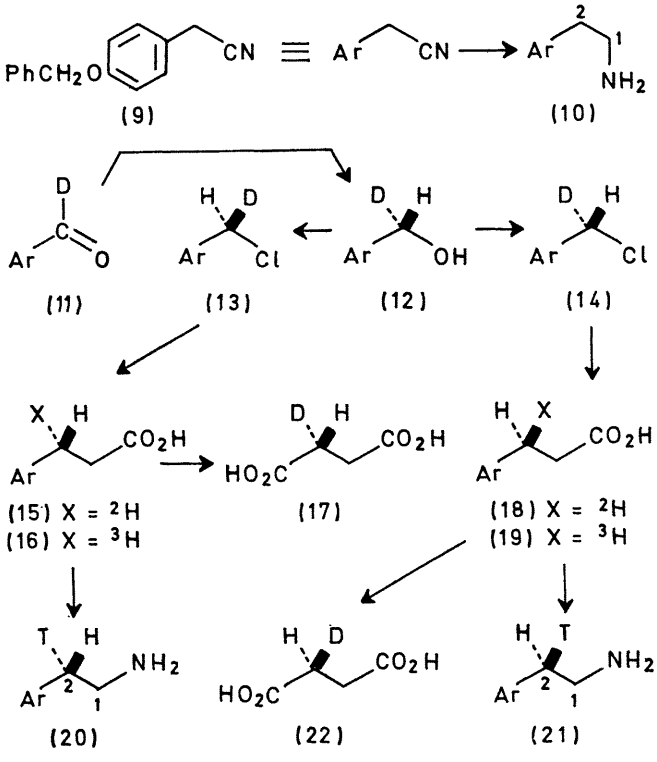
Expt. No.	Precursor	Incorporations (%) and ^{3}H retentions: Haemanthamine (**6**)	Oduline (**7**)
1	*O*-Methyl[2-$^3H_2$,1-^{14}C]norbelladine(**1**) Ratio ^{3}H:^{14}C 9·17 ± 0·15	0·01; Ratio 4·63 ± 0·1 (50 ± 2% retention ^{3}H)	0·02; Ratio 9·19 ± 0·15 (100 ± 3% retention ^{3}H)
2	*O*-Methyl-(2*S*)-[2-$^3H_1$,1-^{14}C]norbelladine[a] (**1**) Ratio ^{3}H:^{14}C 3·40 ± 0·08	0·006; Ratio 2·24 ± 0·08 (66 ± 4% retention ^{3}H)	0·016; Ratio 3·16 ± 0·08 (93 ± 4% retention ^{3}H)
3	*O*-Methyl-(2*R*)-[2-$^3H_1$,1-^{14}C]norbelladine[a] (**1**) Ratio ^{3}H:^{14}C 7·50 ± 0·15	0·085; Ratio 2·34 ± 0·08 (31 ± 2% retention ^{3}H)	0·10; Ratio 7·87 ± 0·15 (105 ± 4% retention ^{3}H)
4	*O*-Methyl[1-$^3H_2$,1-^{14}C]norbelladine (**1**) Ratio ^{3}H:^{14}C 4·60 ± 0·1	0·60; Ratio 4·27 ± 0·1 (93 ± 4% retention ^{3}H)	0·26; Ratio 4·26 ± 0·1 (93 ± 4% retention ^{3}H)

[a] The recorded configuration is that of the major enantiomer present (see text).



† The proviso "within experimental error" is to be understood throughout; the ranges recorded in the Table give the maximum spread.

alcohol (**12**) which was converted into the chloride (**13**) by triphenylphosphine–carbon tetrachloride.[8] A malonate synthesis then led to the (3*R*)-[3-$^2H_1$]propionic acid (**15**) which was debenzylated, ozonised, and oxidised to yield [$^2H_1$]succinic acid. This was shown by o.r.d. and mass spectrometry[9] to contain 72 ± 6% of the (2*R*)-isomer (**17**). The configuration at C-3 of the major enantiomer (**15**) is thus established and it is as expected (two inversions with partial racemisation).



Complementary data were obtained by preparing the chloride (**14**) from (**12**) using ethereal thionyl chloride. The foregoing steps then yielded the (3*S*)-[3-$^2H_1$]propionic acid (**18**) and the derived [$^2H_1$]succinic acid contained 68 ± 6% of the (2*S*)-isomer (**22**).

Precise repetition of the two foregoing sequences, now in the ^3H-series, afforded the (3*R*)-[3-$^3H_1$]- and (3*S*)-[3-$^3H_1$]-propionic acids (**16**) and (**19**), respectively, which were converted into the amines (**20**) and (**21**) by the amide–hypochlorite method. *O*-Methyl-(2*S*)-[2-$^3H_1$]norbelladine (**1**; ^3H at H_S) and the (2*R*)-[2-$^3H_1$]-isomer (**1**; ^3H at H_R) were then prepared as before from the amines; it must be remembered that the recorded configurations refer to the *major* enantiomer present (*ca.* 70% in each case). These products, in admixture with ^{14}C-labelled material, were used for Expts. 2 and 3. The tritium retention values, which interlock, prove that the *pro-R* proton is removed in the hydroxylation step. Jones oxidation of the haemanthamine from Expt. 2 gave oxohaemanthamine (**8**) which was devoid of tritium; suitable control experiments excluded the possibility that the ^3H label had undergone biochemical migration to C-12 and had been lost therefrom by enolisation of (**8**).

Knowing the absolute stereochemistry at C-11 of haemanthamine (**6**), the foregoing results show that hydroxylation has occurred with retention of configuration as is the case for the other examples studied so far[10] (mainly in the steroid and long-chain aliphatic series) (see ref. 12 however).

Before mechanistic conclusions can be drawn from the foregoing results, it is essential to determine whether position 12 [see (**3**), (**4**), and (**5**)] is involved in the hydroxylation process. Accordingly, the nitrile (**9**) was reduced with cobalt chloride–borotritiide reagent[12] and the randomly labelled [1-$^3H_2$]amine (**10**) so formed was converted as before into *O*-methyl[1-$^3H_2$]norbelladine (**1**). Expt. 4 shows close agreement between the ^3H : ^{14}C ratios found for haemanthamine and oduline which points against involvement of position 12 in the hydroxylation process. When this sample of haemanthamine was oxidised to oxohaemanthamine (**8**), the product retained 26% of the original ^3H (partial exchange). Mild aqueous base completely eliminated the ^3H from (**8**). All the foregoing data are in agreement with a process involving, in effect, the direct insertion of an oxygen atom (an "oxene" or "oxenoid" mechanism)[2,13] and other hydroxylation reactions under current study will broaden the stereochemical test.

Related work is reported by G. W. Kirby and J. Michael in *Com.* 2129, p. 187.

We thank the United States Public Health Service and the Nuffield Foundation for financial support.

(*Received, December 9th,* 1970; *Com.* 2127.)

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