# Labelling of Aromatic Amino-acids Stereoselectively with Tritium in the β-Methylene Group: the Stereochemistry of Hydroxylation in the Biosynthesis of Haemanthamine

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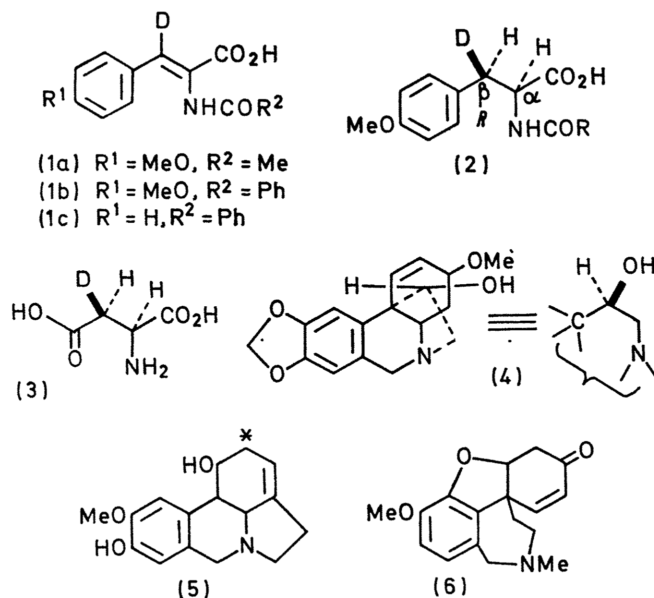
*Summary* Catalytic hydrogenation of acylaminocinnamic acids proceeds *cis* with high stereoselectivity and provides a route to aromatic amino-acids labelled stereoselectively with deuterium or tritium in the *β*-methylene groups; [*βR*-$^3$H]- and [*βS*-$^3$H]-tyrosine, prepared in this way, were used to show that a hydroxylation step in the biosynthesis of haemanthamine proceeded with retention of configuration.

AROMATIC amino-acids undergo a variety of biological transformations involving removal of a hydrogen atom from the *β*-methylene group. Determination of the stereochemistry of these processes requires amino-acids, substituted in this position with deuterium or tritium, of defined absolute stereochemistry. We report a general method for the preparation of the desired, labelled compounds.

4-Methoxy-[*formyl*-$^2$H]benzaldehyde, prepared by the morpholinonitrile technique,[1] was condensed with *N*-acetylglycine to give the corresponding oxazolone[2] which was opened with alkali to yield the acylaminocinnamic acid (**1a**) of known[3] geometrical configuration. Catalytic hydrogenation with 10% palladium–carbon in ethanol gave the racemic tyrosine derivative (**2**; R = Me). The n.m.r. spectrum of the corresponding undeuteriated material showed (see Figure) an ABX system for the *β*- and *α*-side-chain protons, the signals from the diastereotopic methylene protons appearing well separated in chemical shift. The deuterio-derivative (**2**; R = Me) showed an AX quartet, $J_{AX}$ 4·6 Hz, indicating the presence of only one diastereoisomer. In confirmation, epimerisation[4] of the *α*-centre gave a mixture of diastereoisomers having a spectrum consisting of superimposed AX and BX quartets, $J_{AX}$ 4·9, $J_{BX}$ 8·7 Hz. Comparison of the spectra showed that hydrogenation of (**1a**) has proceeded with at least 95% stereoselectivity. Analogous results were obtained with (**1b**). Catalytic hydrogenation of (**1c**), either heterogeneously (Pd–C) in ethanol or homogeneously[5] [$(Ph_3P)_3$-RhCl] in ethanol–benzene, gave *N*-benzoyl-[*β*-$^2$H]phenylalanine, again showing an AX spectrum, $J_{AX}$ 4·8 Hz. The steric course of hydrogenation was therefore insensitive to variation of substituents and was appropriate for the preparation of labelled tyrosine, phenylalanine, and presumably other aromatic amino-acids.

Highly stereoselective hydrogenation of an acyclic olefin would, *a priori*, be expected to proceed *cis*. This was shown unambiguously to be so in the present series by degradation of the racemate (**2**; R = Ph). Cleavage with concentrated hydrobromic acid under reflux gave, without loss of deuterium or epimerisation of the *α*-centre, an equimolar mixture of L-[*βR*-$^2$H]- and D-[*βS*-$^2$H]-tyrosine.† Ozonolysis[6] in formic acid at 0° followed by treatment with hydrogen peroxide gave the deuteriated DL-aspartic acid (**3**) (17%). The relative configuration of the hydrogens in (**3**) followed from the observed value of 4·0 Hz for the



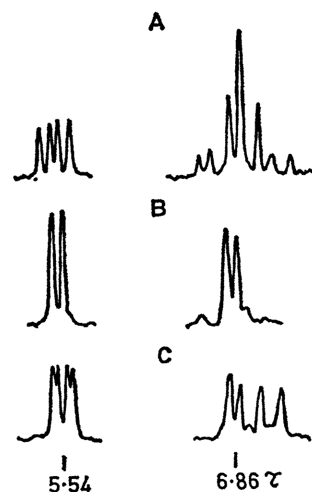




FIGURE. *N.m.r. spectra (in DαO/DO⁻) of*: A, DL-N-*acetyl-4-methoxyphenylalanine*; B, DL-(**2**; R = Me); C, DL-(**2**; R = Me) *after epimerisation of the α-centre.*

† For clarity the traditional symbols D and L are used to denote configuration at the α-centre: formulae (**2**) and (**3**) represent L-amino-acids.

*Incorporation of* DL-*tyrosine into alkaloids of the "Texas" daffodil*

| Labelling pattern | Precursor | (5) | (6) | (4) | Epihaemanthamine |
|---|---|---|---|---|---|
| | | | $^3H/^{14}C$ ratios | | |
| [α-$^{14}$C, βR-$^3$H] | 6·98 | 7·14 | 6·90 | 1·19 | <0·03 |
| [α-$^{14}$C, βS-$^3$H] | 7·22 | 7·44 | 7·42 | 6·29 | <0·02 |
| | | | Incorporations (%) | | |
| [α-$^{14}$C, βR-$^3$H] | | 0·63 | 0·032 | 0·068 | |
| [α-$^{14}$C, βS-$^3$H] | | 0·45 | 0·034 | 0·073 | |

αβ-coupling constant since L-[βS-$^2$H]aspartic acid (*erythro*-deuterioaspartate) is reported[7] to have $J_{\alpha\beta}$ 8·3 Hz. The racemic mixture of L-[βR-$^3$H]- and D-[βS-$^3$H]-tyrosine was then prepared by a strictly analogous route. This tritiated material was resolved by a well established method.[8] Acetylation with chloroacetic anhydride and aqueous sodium hydroxide gave the corresponding racemic *N*-chloroacetyl derivative which was incubated with carboxypeptidase. L-[βR-$^3$H]Tyrosine crystallised directly from the reaction mixture and the D-[βS-$^3$H]-form was obtained after chemical hydrolysis of the remaining *N*-chloroacetyl-D-tyrosine. Both samples of tyrosine were recrystallised to ensure optical purity. Epimerisation of the α-centre in each sample, by heating in 10N-hydrochloric acid at 180°, gave the diastereoisomeric mixtures, DL-[βR-$^3$H]- and DL-[βS-$^3$H]-tyrosine. A second resolution step at this stage would give separately all four monotritiated stereoisomers; this was not necessary for the present work.‡ The two tritiated forms were each mixed with DL-[α-$^{14}$C]-tyrosine to provide doubly labelled specimens for the following biosynthetic study.

Tyrosine provides biosynthetically the C-8–C-2, hydroaromatic but not the C-6–C-1, aromatic unit of the *Amaryllidaceae* alkaloid, haemanthamine[10] (4). The hydroxy-group has the absolute configuration shown[10] and the stereochemistry of hydroxylation, at what was originally the β-carbon of tyrosine, was determined as follows. DL-[α-$^{14}$C,βR-$^3$H]- and DL-[α-$^{14}$C,βS-$^3$H]-Tyrosine were fed separately to "Texas" daffodils. After one week, radioactive haemanthamine (4), norpluviine (5), and narwedine (6) were isolated from the alkaloidal mixture and rigorously purified. The last two alkaloids provided useful internal standards since they retain intact the methylene group of the precursor, tyrosine. The results are tabulated.§ Comparison of structures (2) and (4) shows that hydroxylation with retention of configuration should remove all tritium from [βR-$^3$H]tyrosine and none from the enantiomeric form. The observed (see Table) tritium loss [83% (βR)] and retention [87% (βS)] values for haemanthamine clearly showed that hydroxylation with predominant retention of configuration had occurred. The site of the residual tritium was established by conversion[11] ($CrO_3$–pyridine) of haemanthamine into haemanthaminone and thence, for convenience of crystallisation, into epihaemanthamine.¶ Essentially all tritium was removed in this process.

The observed stereochemistry of hydroxylation is important since the only apparent exception to the rule, that biological hydroxylation at saturated carbon occurs with retention of configuration, came from studies on the *Amaryllidaceae* alkaloids. It was shown[12] that, during conversion of norpluviine (5) *via* caranine into lycorine, a process involving hydroxylation of a methylene group [asterisk in (5)], hydroxylation with inversion occurred. It now seems certain that this "abnormal" process involves a fundamentally different mechanism, possibly requiring one or more intermediates.

The lack of complete retention, or loss, of tritium during the conversion of tyrosine into haemanthamine might have been caused by the biological process not being completely stereospecific but, more probably, the precursors were only *ca.* 85% stereochemically pure. The hydrogenation step appeared at least 95% stereoselective. However, partial epimerisation of the α-centre before resolution into the D- and L-forms (for example, during formation of the *N*-chloroacetyl derivatives) would explain the observed result. Each relevant step was checked in advance by control experiments with α-tritiated tyrosine. In no case did significant (> 3%) loss of tritium occur but epimerisation involving cleavage of a C–T bond would have taken place more slowly than cleavage of C–H. The stereochemical purity actually achieved was more than adequate for the present investigation. Resolution of tyrosine derivatives *via* brucine salts or by dilution with optically pure, inactive material would overcome this problem. In work with biological systems specific for L- or D-amino-acids, resolution would not of course even be necessary.

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‡ The first resolution was necessary since we had earlier shown[9] that both D- and L-tyrosine are incorporated with similar efficiency into *Amaryllidaceae* alkaloids.

§ This work appears[10] to provide the first formal proof that tyrosine is not incorporated into the C-6–C-1 unit of the galanthamine (narwedine) group of alkaloids.

¶ Reduction of haemanthaminone with sodium borohydride in methanol gave both epihaemanthamine and haemanthamine, the former predominating.

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