Stereoselective β-Labelling of Aromatic Amino-acids with Deuterium and Tritium

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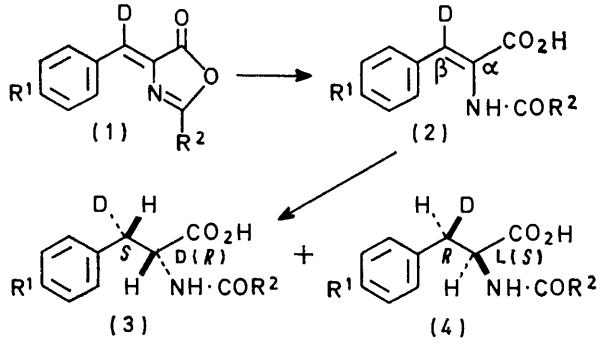
Tyrosine and phenylalanine, labelled stereoselectively with deuterium and tritium in the β-methylene groups, have been prepared *via cis*-hydrogenation of appropriate, β-labelled α-acylaminocinnamic acids obtained from the corresponding benzylideneoxazolinones. The configuration of (β*R*)-L-[β-^{2}H]tyrosine was confirmed by ozonolysis of its racemate to give the racemate of (β*S*)-L-[β-^{2}H]aspartic acid of known stereochemistry.

The incorporation of the (β*R*)-[β-^{3}H]- and (β*S*)-[β-^{3}H]-forms of tyrosine into haemanthamine in 'Texas' daffodils was studied to show that hydroxylation of the original methylene group occurred highly stereospecifically with retention of configuration.

AROMATIC amino-acids are transformed biologically into a wide variety of primary and secondary metabolites. Many of these transformations involve the removal of one of the β-methylene hydrogen atoms by, for example, β-hydroxylation, β-alkylation, αβ-elimination, or αβ-desaturation. These processes, if subject to enzymic control, should remove only one of the two stereochemically distinct β-hydrogen atoms. Moreover, metabolic transformations involving cleavage of the αβ- or β-aryl-carbon–carbon single bonds or rearrangement of the side-chain functional groups should have stereochemical consequences discernible from the fate of the original methylene hydrogen atoms. A proper understanding of the mechanisms of biochemical reactions demands a precise knowledge of the stereochemistry of each discrete step.[1] For this reason we have developed a general synthesis of aromatic amino-acids labelled stereoselectively with deuterium or tritium in the β-methylene groups.

Our approach [2] was based on the expectation that catalytic hydrogenation of an α-acylamino[β-^{2}H]cinnamic acid (2), derived from the readily accessible [3] oxazolinone (1), would proceed in *cis* fashion to give the racemic mixture [(3) + (4)]. The D(α*R*)-form † of the acylamino-acid (3) would thus contain deuterium at the β-*pro*-*S*-position and the L(α*S*)-form † (4) deuterium at the β-*pro*-*R*-position. Resolution of the mixture by



conventional methods based on the chirality of the α-centre would then provide separately the desired (β*S*)- and (β*R*)-labelled amino-acids.

Preliminary studies [4] with *o*-hydroxybenzylideneoxazolinone derivatives (5) were encouraging. The stable geometrical isomers of the oxazolinones (5; R = Ac or $PhCH_2$) were shown to have the *Z*-configuration and catalytic hydrogenation of the deuteriated cinnamic

† For clarity and concise presentation the traditional symbols D and L are retained to denote configurations at the α-centre: for all the amino-acids in this paper (D ≡ α*R* and L ≡ α*S*).

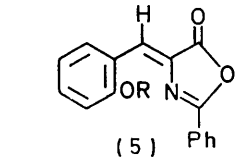
[1] R. Bentley, 'Molecular Asymmetry in Biology,' Academic Press, New York, 1969, vol. I; 1970, vol. II.

[2] G. W. Kirby and J. Michael, *Chem. Comm.*, 1971, 187, 415.

[3] R. Filler, *Adv. Heterocyclic Chem.*, 1965, **4**, 95; W. Steglich, *Fortschr. chem. Forsch.*, 1969, **12**, 77.

[4] G. W. Kirby, J. Michael, and S. Narayanaswami, *J.C.S. Perkin I*, 1972, 203.

acid (6) was found to proceed in *cis* fashion with high stereoselectivity. Recently a number of other stable benzylideneoxazolinones have been shown [5,6] to have the *Z*-configuration and this, perhaps, will prove to be a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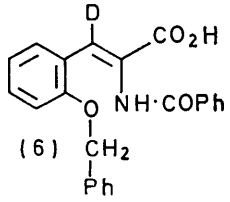
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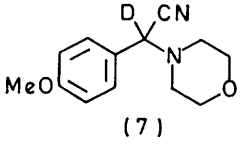
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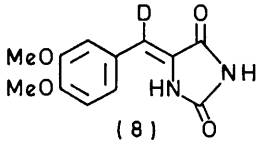
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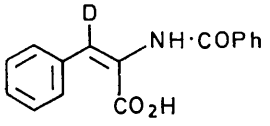
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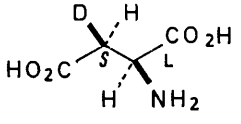
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general rule. Nevertheless independent proof of the configuration of the labelled species (3) and (4) was sought as follows.

4-Methoxy[α-^{2}H]benzaldehyde, prepared [7] by hydrolysis of the morpholinonitrile (7), was condensed,[8] in acetic anhydride containing potassium carbonate, with *N*-acetylglycine to give the oxazolinone (1; R^1 = MeO, R^2 = Me). Earlier experiments [9] on the preparation of the hydrantoin (8) from 3,4-dimethoxy[α-^{2}H]benzaldehyde showed that partial loss of deuterium from the product (8) occurred and was increased by prolonged heating of the reaction mixture. This complication was less serious (*ca.* 4% loss of ^{2}H) in our oxazolinone condensations providing reaction times were kept as brief as was consistent with acceptable yields of products. Hydrolysis of the oxazolinone (1; R^1 = MeO, R^2 = Me) with alkali gave the acid (2; R^1 = MeO; R^2 = Me), which was hydrogenated over 10% palladium–carbon in ethanol to give the racemate [(3) + (4); R^1 = MeO, R^2 = Me]. The n.m.r. spectrum of this mixture, in D_2O–DO^-, revealed an AX quartet (*J* 4·6 Hz) for the α- and β-protons, indicating the presence of only one racemic diastereoisomer. Epimerisation [10] of the α-centre gave a mixture of diastereoisomers having a spectrum consisting of superimposed AX and BX quartets (*J* 4·9 and 8·7 Hz). Comparison of the spectra * showed that hydrogenation had proceeded with at least 95% stereospecificity. Entirely analogous results were obtained with the benzoyl derivative (2; R^1 = MeO, R^2 = Ph). Furthermore, catalytic hydrogenation of (2; R^1 = H, R^2 = Ph), either heterogeneously (Pd–C) in ethanol or, more slowly, homogeneously [11] [$(Ph_3P)_3RhCl$] in ethanol–benzene, gave the racemate [(3) + (4); R^1 = H, R^2 = Ph], showing again an AX spectrum (*J* 4·8 Hz). A route to a racemate having the opposite relative configuration of the α- and β-centres was explored by Dr. S. Narayanaswami. Treatment of the oxazolinone (1; R^1 = H, R^2 = Ph) with hydrobromic acid [12] gave the unstable (*E*)-isomer without loss of deuterium. Hydrolysis with alkali yielded the (*E*)-acid (9). Catalytic (Pd–C) hydrogenation proceeded cleanly, on the first occasion, to give deuteriated *N*-benzoylphenylalanine showing the expected BX n.m.r. spectrum (*J* 8·4 Hz). However, in a second experiment a mixture of diastereoisomers was produced although the (*E*)-isomer (9) was not itself isomerised by the catalyst in the absence of hydrogen. Presumably,[13,14] isomerisation of the αβ-double bond in (9) occurred, during reduction, as a consequence of the stepwise addition of the two hydrogen atoms and, apparently, isomerisation was critically dependent on the precise reaction conditions. This route was not explored further but can be recommended for preparative purposes only if the configuration of each batch of product is monitored spectroscopically.

The results so far showed that hydrogenation of (*Z*)-α-acylaminocinnamic acids takes place highly stereoselectively.† We believed that *cis*- rather than *trans*-addition of hydrogen had occurred for the following reasons: (i) hydrogenation of acyclic olefins normally proceeds *cis*; [3] (ii) hydrogenation of the acid (6) was shown [4] unequivocably to proceed *cis*; (iii) the hydrogenation product of (6) had an n.m.r. spectrum closely resembling that of [(3) + (4)]; (iv) the predominant conformation for phenylalanine in alkaline solution has the aryl and carboxy-groups *trans*.[15] If this holds true for the racemates [(3) + (4)] then conformations having the α- and β-protons *gauche* (as drawn) would predominate in the equilibrium mixture. This is consistent with the observed small (*J* 4·8 Hz) vicinal proton coupling constant. The issue was placed beyond doubt by degradation of the racemate [(3) + (4); R^1 = MeO, R^2 = Ph]. Cleavage with concentrated hydrobromic acid under reflux gave deuteriated tyrosine without loss

* Spectra are displayed in the preliminary publication.[2]

† Current investigations by M. J. Varley have shown this to hold true also for the indolylacrylic precursors of tryptophan.

[5] A. P. Morgenstern, C. Schutij, and W. Th. Nauta, *Chem. Comm.*, 1969, 321; A. E. A. Porter and P. G. Sammes, *J. Chem. Soc.* (*C*), 1970, 2530; K. Brocklehurst, R. P. Bywater, R. A. Palmer, and R. Patrick, *Chem. Comm.*, 1971, 632.

[6] K. R. Hanson, R. H. Wightman, J. Staunton, and A. R. Battersby, *Chem. Comm.*, 1971, 185.

[7] *Cf.* D. J. Bennett, G. W. Kirby, and V. A. Moss, *J. Chem. Soc.* (*C*), 1970, 2049.

[8] J. B. Niederl and A. Ziering, *J. Amer. Chem. Soc.*, 1942, **64**, 885.

[9] V. A. Moss, Ph.D. Thesis, London, 1967, p. 77.

[10] H. Matsuo, Y. Fujimoto, and T. Tatsuno, *Tetrahedron Letters*, 1965, 3465.

[11] J. F. Young, J. A. Osborn, F. H. Jardine, and G. Wilkinson, *Chem. Comm.*, 1965, 131; R. E. Harmon, J. L. Parsons, and S. K. Gupta, *ibid.*, 1969, 1369.

[12] S. Tatsuoko and A. Morimoto, *J. Pharm. Soc. Japan*, 1950, **70**, 253; R. Filler, K. B. Rao, and Y. S. Rao, *J. Org. Chem.*, 1962, **27**, 1110.

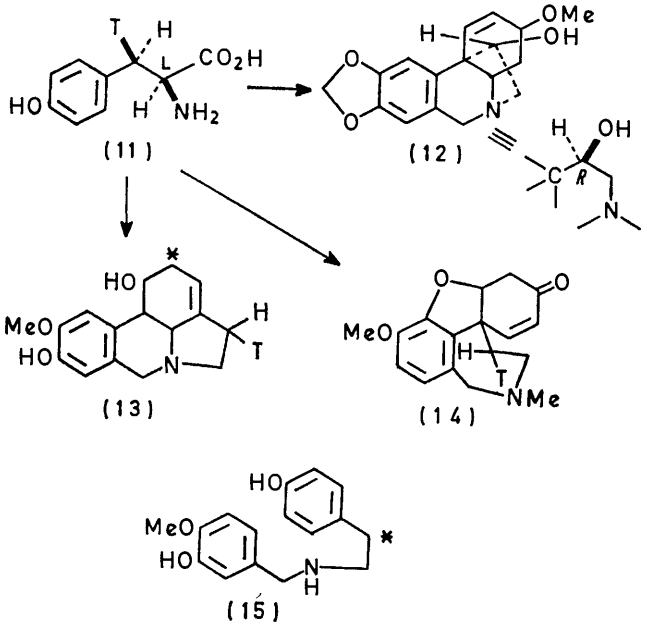
[13] *Cf.* S. Siegel, *Adv. Catalysis*, 1966, **16**, 123.

[14] L. Crombie, P. A. Jenkins, D. A. Mitchard, and J. C. Williams, *Tetrahedron Letters*, 1967, 4297.

[15] J. R. Cavanaugh, *J. Amer. Chem. Soc.*, 1967, **89**, 1558 and references cited.

of deuterium or epimerisation of the α-centre (n.m.r. control). Ozonolysis [16] in formic acid at 0 °C followed by treatment with hydrogen peroxide gave the deuteriated DL-aspartic acid (10; the L-form is displayed). The relative configuration of the α- and β-centres followed from the observed vicinal coupling constant of 4·0 Hz since (β*R*)-L-[β-^{2}H]aspartic acid, of rigorously defined configuration,[17] is reported [18] to have $J_{\alpha\beta}$ 8·3 Hz.

Specimens of stereospecifically tritiated tyrosine were next prepared for use in biosynthetic studies. A racemic mixture of (β*R*)-L-[β-^{3}H]- and (β*S*)-D-[β-^{3}H]-tyrosine was obtained by catalytic (Pd–C) hydrogenation of the acid (2; R^1 = MeO, R^2 = Ph, T in place of D) followed by removal of protecting groups from the product [(3) + (4); R^1 = MeO, R^2 = Ph, T in place of D] with hydrobromic acid. Acylation with chloroacetic anhydride and sodium hydroxide gave the corresponding, racemic *N*-chloroacetyl derivative, which was incubated with carboxypeptidase.[19] (β*R*)-L-[β-^{3}H]Tyrosine (11) crystallised directly from the reaction mixture, and (β*S*)-D-[β-^{3}H]tyrosine was obtained by isolation and chemical hydrolysis of the remaining *N*-chloroacetyl-D-tyrosine. Both labelled specimens were crystallised several times from water to ensure optical purity. Generally, D- and L-forms of amino-acids are metabolised at different rates in biological systems and often, especially with isolated enzymes, only one form is attacked. However, in daffodils, incorporation of both L- and D-tyrosine into the *Amaryllidaceae* alkaloids proceeds with similar efficiency.[20] Nevertheless it seemed desirable to conduct parallel experiments with (β*R*)- and (β*S*)-labelled materials having identical configurations at the α-centre. Accordingly, each labelled sample was epimerised by heating in 10N-hydrochloric acid at 180° to give separately the diastereoisomeric mixtures, (β*R*)-DL-[β-^{3}H]- and (β*S*)-DL-[β-^{3}H]-tyrosine. This method was selected since it involved only one operation, the recovery of tyrosine was good, and a control experiment with DL-[α-^{3}H]tyrosine showed that epimerisation was complete (>99% loss of ^{3}H). As expected, no significant loss of β-^{3}H occurred during α-epimerisation. These tritiated species were each mixed with DL-[α-^{14}C]tyrosine to produce doubly labelled specimens for the following biological studies.



Incorporation of DL-tyrosine into alkaloids of the 'Texas' daffodil

Labelling pattern	Precursor	^{3}H : ^{14}C ratios (13)	(14)	(12)	Epihaemanthamine
(β*R*)-[α-^{14}C,β-^{3}H]	6·98	7·14	6·90	1·19	<0·03
(β*S*)-[α-^{14}C,β-^{3}H]	7·22	7·44	7·42	6·29	<0·02
		Incorporations (%)			
(β*R*)-[α-^{14}C,β-^{3}H]		0·63	0·032	0·068	
(β*S*)-[α-^{14}C,β-^{3}H]		0·45	0·032	0·073	

Tyrosine provides biosynthetically the hydroaromatic (C_6–C_2) but not the aromatic (C_6–C_1) unit of the characteristic *Amaryllidaceae* alkaloids, haemanthamine (12), norpluvine (13), and narwedine (14).[21] During conversion of tyrosine into haemanthamine one β-hydrogen atom is replaced by a hydroxy-group having the *R*-configuration [22] (12). Hydroxylation must occur at a later stage of biosynthesis since the *O*-methylnorbelladine (15) is a known [23,24] precursor of the alkaloid. The stereochemistry of hydroxylation was determined as follows. (β*R*)-DL-[α-^{14}C, β-^{3}H]- and (β*S*)-DL-[α-^{14}C, β-^{3}H]Tyrosine were fed separately to flowering 'Texas' daffodils. After 1 week the alkaloids (12)—(14) were isolated and purified by chromatography and crystallisation. Comparison of the ^{3}H : ^{14}C ratios in precursors and products provided a measure of tritium loss (or retention) during biosynthesis (see Table). Norpluvine (13) and narwedine (14) served as convenient internal standards since they retain intact the original methylene group of tyrosine and, as expected, lost no significant amounts of tritium during biosynthesis. Most of the tritium (83%) in the (*R*)-[^{3}H]tyrosine (11) was lost during incorporation into haemanthamine (12). Conversely, tritium in the (*S*)-[^{3}H]-form was largely (87%) retained. Conversion [25] of the labelled haemanthamine into the corresponding ketone and thence, for convenience of crystallisation, into epihaemanthamine, caused loss of

[16] *Cf.* H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, 1955, **38**, 2038.
[17] See ref. 1, vol. II, p. 152.
[18] J. L. Bada and S. L. Miller, *J. Amer. Chem. Soc.*, 1970, **92**, 2774.
[19] S. M. Birnbaum, L. Levintow, R. B. Kingsley, and J. P. Greenstein, *J. Biol. Chem.*, 1952, **194**, 455; J. B. Gilbert, V. E. Price, and J. P. Greenstein, *ibid.*, 1949, **180**, 473.
[20] I. T. Bruce and G. W. Kirby, *Chimia* (*Switz.*), 1968, **22**, 315.
[21] W. C. Wildman, 'The Alkaloids,' ed. R. H. F. Manske, Academic Press, New York, 1968, vol. XI, p. 308.
[22] J. Clardy, F. M. Hauser, D. Dahm, R. A. Jacobson, and W. C. Wildman, *J. Amer. Chem. Soc.*, 1970, **92**, 6337.
[23] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J. Chem. Soc.*, 1963, 4545.
[24] A. R. Battersby, J. E. Kelsey, and J. Staunton, *Chem. Comm.*, 1971, 183.
[25] H. M. Fales and W. C. Wildman, *J. Amer. Chem. Soc.*, 1960, **82**, 197.

essentially all of the tritium. Degradation of [^{14}C]-haemanthamine, derived biosynthetically from [α-^{14}C]-tyrosine, had been reported earlier [26] and was not repeated. Clearly the hydroxylation process involved in the biosynthesis of haemanthamine had occurred with predominant retention of configuration. This accords with earlier stereochemical studies on the hydroxylation of, for example, steroids and fatty acids.[27] Previously it was shown [20,28] that, during the biological conversion of norpluviine (13), *via* caranine, into lycorine, hydroxylation of a methylene group [asterisk in (13)] took place with inversion of configuration. It now seems likely that this ' abnormal ' stereochemical result is not characteristic of hydroxylation reactions in the *Amaryllidaceae* but is a consequence of a fundamentally different mechanism, possibly involving one or more intermediates. Battersby *et al.* have independently shown [24] that the biosynthesis of haemanthamine involves hydroxylation with retention of configuration; they employed a different method using the *O*-methylnorbelladine (15) labelled asymmetrically with tritium in the appropriate methylene group [asterisk in (15)].

The incomplete loss, or retention, of tritium observed during the incorporation of our labelled tyrosines into haemanthamine requires comment. Either biological hydroxylation was not completely stereospecific or, more probably, the precursors were only *ca.* 85% stereochemically pure. The preliminary experiments with deuteriated materials left no doubt that hydrogenation of the α-acylaminocinnamic acids (2) was highly stereospecific. However, if partial epimerisation of the chiral α-centre in the racemate [(3) + (4)] had occurred before resolution into the two components, then the L-amino-acid would have become contaminated with *pro-S* deuterium (or tritium) and the D-amino-acid equally with *pro-R* deuterium (or tritium). A likely occasion for this to happen would be during *N*-chloroacetylation of the tyrosine prior to enzymic resolution. Control experiments had been carried out in advance with [α-^{3}H]tyrosine. No significant (>3%) loss of tritium was observed but epimerisation involving cleavage of a C–T bond (control experiment) may well have taken place more slowly than that involving cleavage of a C–H bond (preparative experiment) owing to a kinetic isotope effect. An independent test of the configurational purity of (β*R*)-DL-[β-^{3}H]- and (β*S*)-DL-[β-^{3}H]-tyrosine was made in collaboration with Professors Floss and Zenk.[29] The labelled tyrosines were treated with the enzyme tyrosine ammonia-lyase to yield 4-hydroxy-*trans*-cinnamic acid. Elimination ammonia took place in *anti* fashion with removal of the *pro-S* hydrogen atom from the L-form of the amino-acid.[30] For example, the isomer (11) gave 4-hydroxycinnamic acid with high retention of tritium. Again, the tritium retention values for the two labelled forms of tyrosine showed that each was *ca.* 85% stereochemically pure. It was clear therefore that both the hydroxylase of daffodils and tyrosine ammonia-lyase were highly stereospecific in their removal of β-hydrogen atoms. N. Johns and Dr. S. Narayanaswami have obtained similar results, in these laboratories, with [β-^{3}H]phenylalanine. Asymmetrically labelled samples were prepared by hydrogenation of the cinnamic acid (2; R^1 = H, R^2 = Ph, T in place of D) followed by resolution of the resulting racemate *via* hydrolysis (HBr), *N*-chloroacetylation, and enzymic hydrolysis (carboxypeptidase). The separate forms were α-epimerised and assayed with phenylalanine ammonia-lyase from potatoes, an enzyme known [6,31,32] to catalyse stereospecific *anti*-elimination of ammonia. The assays showed, once more, typical configurational purities of 85%. Presumably, highly stereospecific labelling could be achieved if the racemates [(3) + (4)] were resolved, before removal of protecting groups, by chemical means involving, for example, crystallisation of diastereoisomeric salts derived from brucine or α-phenylethylamine. However, enzymic resolution is convenient for small-scale work and was adequate for the present investigations.

The oxazolinone route to stereospecifically labelled amino-acids has been developed independently by Hanson, Battersby, and their co-workers.[6,30,32] Ife and Haslam [31] have devised an alternative procedure for phenylalanine using asymmetrically labelled benzyl alcohol.

EXPERIMENTAL

Activities (^{3}H and ^{14}C) were measured with a Beckman CPM-100 liquid scintillation spectrometer calibrated with [1,2-$^{3}H_2$]- and [1-^{14}C]-hexadecane (Radiochemical Centre, Amersham). N.m.r. spectra were measured at 60 MHz.

α-(4-*Methoxyphenyl*)-α-*morpholino*[α-^{2}H]*acetonitrile*.— 4-Methoxybenzaldehyde was converted,[7] with morpholine perchlorate and sodium cyanide in morpholine, into the corresponding morpholinoacetonitrile derivative, m.p. 81—82° [ether–light petroleum (b.p. 60—80°)] (90% yield). This derivative was treated, in dry dimethylformamide under nitrogen, with sodium hydride (2 mol. equiv.) for 2 h at 50 °C to generate the α-carbanion. The solution was cooled then quenched with deuterium oxide (6 mol. equiv.) followed by an excess of thionyl chloride. α-(4-Methoxyphenyl)-α-morpholino[α-^{2}H]acetonitrile (81%) was isolated in the usual way.[7] The n.m.r. spectrum ($CDCl_3$) showed no detectable α-H signal at τ 5·2.

(Z)-4-(4-*Methoxy*[α-^{2}H]*benzylidene*)-2-*methyloxazolin*-5-*one* (1; R^1 = MeO, R^2 = Me).—The foregoing morpholinonitrile was hydrolysed with 2N-hydrochloric acid under reflux for 1 h to yield 4-methoxy[α-^{2}H]benzaldehyde (97%

[26] A. R. Battersby, H. M. Fales, and W. C. Wildman, *J. Amer. Chem. Soc.*, 1961, **83**, 4098.
[27] *E.g.* S. Bergstrom, S. Linstedt, G. Samuelson, E. J. Corey, and G. A. Gregoriou, *J. Amer. Chem. Soc.*, 1958, **80**, 2337; L. J. Morris, *Biochem. J.*, 1970, **118**, 681; see also citations in ref. 24.
[28] W. C. Wildman and N. E. Heimer, *J. Amer. Chem. Soc.*, 1967, **89**, 5265; I. T. Bruce and G. W. Kirby, *Chem. Comm.*, 1968, 207.
[29] B. E. Ellis, M. H. Zenk, G. W. Kirby, J. Michael, and H. G. Floss, unpublished work.
[30] *Cf.* P. G. Strange, J. Staunton, H. R. Wiltshire, A. R. Battersby, K. R. Hanson, and E. A. Havir, *J.C.S. Perkin I*, 1972, in the press.
[31] R. Ife and E. Haslam, *J. Chem. Soc.* (*C*), 1971, 2818.
[32] R. H. Wightman, J. Staunton, A. R. Battersby, and K. R. Hanson, *J.C.S. Perkin I*, 1972, 2355.

$^2H_1$ by n.m.r.). This aldehyde (6·8 g) was heated with *N*-acetylglycine (5·85 g) and anhydrous potassium carbonate (6·9 g) in acetic anhydride (20 ml) at 80 °C for 1 h. The crystals which separated overnight from the mixture at 5 °C were collected, washed with aqueous acetic acid, and recrystallised from ether–light petroleum (b.p. 60—80°) to yield the oxazolinone (1·7 g), m.p. 111—114° (lit.,[8] 114° for non-deuteriated material), (*ca.* 93% $^2H_1$ by n.m.r.).

Hydrogenation of (Z)-α-*Acetamido*-4-*methoxy*[β-^{2}H]*cinnamic Acid* (2; R^1 = MeO, R^2 = Me).—The foregoing oxazolinone (200 mg) was heated at 100 °C in water (6 ml) containing sodium hydroxide (100 mg) until a clear solution was obtained (*ca.* 0·5 h). Acidification gave the acid (2; R^1 = MeO, R^2 = Me) (220 mg), m.p. 225—232° (lit.,[8] 216° for non-deuteriated material) (aqueous ethanol). This acid (164 mg) in ethanol (20 ml) containing 10% palladium–carbon (22 mg) was hydrogenated at ambient temperature and pressure for 1 h. The mixture was filtered through Celite and the filtrate evaporated to yield the racemic *N*-acetyl-4-methoxy[β-^{2}H]phenylalanine [(3) + (4); R^1 = MeO, R^2 = Me] (158 mg), m.p. 149—150° (lit.,[33] 150° for non-deuteriated material) (acetone–benzene).

(Z)-4-([α-^{2}H]*Benzylidene*)-2-*phenyloxazolin*-5-*one* (1; R^1 = H, R^2 = Ph).—[α-^{2}H]Benzaldehyde [7] (0·9 g), *N*-benzoylglycine (1·6 g), and potassium carbonate (0·6 g) in acetic anhydride (5 ml) were heated on a steam-bath for 2—3 min to induce an exothermic reaction then set aside for 30 min. The crystalline product was collected and washed with water. The deuteriated oxazolinone (1·6 g) had m.p. 164—166° (lit.,[34] 167—168° for non-deuteriated material) (ether).

Hydrogenation of (Z)-α-*Benzoylamino*[β-^{2}H]*cinnamic Acid*.—The foregoing oxazolinone (1; R^1 = H, R^2 = Ph) was hydrolysed with aqueous methanolic alkali [as for (1; R^1 = MeO, R^2 = Me)] to give the corresponding (*Z*)-cinnamic acid (95%), which was hydrogenated in either of two ways to give the racemate [(3) + (4); R^1 = H, R^2 = Ph]: (i) with 10% palladium–carbon in ethanol {as for [(3) + (4); R^1 = MeO, R^2 = Me]}; (ii) with $(Ph_3P)_3RhCl$ (10% by weight) in benzene–ethanol (1 : 1) at ambient temperature and pressure for 48 h.

Hydrogenation of (E)-α-*Benzoylamino*[β-^{2}H]*cinnamic Acid* (with S. Narayanaswami).—(*Z*)-4-([α-^{2}H]Benzylidene)-2-phenyloxazoline-5-one (1; R^1 = H, R^2 = Ph) was suspended in 48% hydrobromic acid and the mixture was saturated with dry hydrogen bromide at 0 °C then kept overnight at 0 °C. The *E*-isomer was collected, washed thoroughly with water, dried *in vacuo* (KOH), and crystallised from chloroform–light petroleum (b.p. 40—60 °C); m.p. 149° (lit.,[12] 146—148° for non-deuteriated material). Hydrolysis gave the corresponding (*E*)-cinnamic acid which was hydrogenated (Pd–C) in the usual way with the results described earlier in the text.

N-*Benzoyl*-O-*methyl*[β-^{2}H]*tyrosine* [(3) + (4); R^1 = MeO, R^2 = Ph].—This racemate was prepared from 4-methoxy-[α-^{2}H]benzaldehyde *via* the (*Z*)-oxazolinone (1; R^1 = MeO, R^2 = Ph) and the (*Z*)-cinnamic acid (2; R^1 = MeO, R^2 = Ph) as described for the phenylalanine derivative [(3) + (4); R^1 = H, R^2 = Ph] (57% overall yield).

[β-^{2}H]*Tyrosine* [(3) + (4); R^1 = OH, R^2CO = H].—The racemate [(3) + (4); R^1 = MeO, R^2 = Ph] (2·5 g) was heated under reflux in 48% hydrobromic acid (40 ml) for 1 h. After *ca.* 5 min a clear solution was obtained and after *ca.* 15 min benzoic acid began to crystallise in the condenser. The solution was cooled and benzoic acid (0·8 g) filtered off. The filtrate was evaporated to dryness and the residue treated with water and re-evaporated. This operation was repeated once. A solution of the crystalline product in dilute aqueous sodium hydroxide was acidified with acetic acid. [β-^{2}H]Tyrosine (1·58 g) crystallised out after 1 h at 0 °C.

Degradation of [β-^{2}H]*Tyrosine to* [β-^{2}H]*Aspartic Acid*.—The labelled tyrosine [(3) + (4); R^1 = OH, R^2CO = H] (500 mg) in water (20 ml) and formic acid (10 ml) was ozonised at 0 °C until the u.v. absorption at 275 nm had disappeared (4—6 h). The mixture was treated with 30% hydrogen peroxide (5 ml) in formic acid (5 ml) at room temperature for 24 h then at 100 °C for 1 h. The solution was evaporated to dryness, the residue dissolved in water, and this solution evaporated. The residue was washed twice with warm ethanol and crystallised from water (*ca.* 1 ml) to yield [β-^{2}H]aspartic acid [racemate of (10)] (58 mg).

(βR)-DL-[β-^{3}H]- *and* (βS)-DL-[β-^{3}H]-*Tyrosine*.—The racemic mixture of (β*R*)-L-[β-^{3}H]- and (β*S*)-D-[β-^{3}H]-tyrosine was prepared exactly as described for the deuterio-analogue. The racemate was acylated [19] in aqueous sodium hydroxide at 0 °C by addition of chloroacetic anhydride in portions with alternating addition of sodium hydroxide to maintain an alkaline pH. The resulting *N*-chloroacetyl[β-^{3}H]tyrosine was incubated at pH 7·5 with carboxypeptidase A (*ex* bovine pancreas; Koch–Light Laboratories Ltd.). (β*R*)-L-[β-^{3}H]-Tyrosine (99%) crystallised slowly from the incubation mixture. (β*S*)-D-*N*-Chloroacetyl[β-^{3}H]tyrosine was isolated from the remaining solution and hydrolysed with 6N-hydrochloric acid under reflux for 1 h to give (β*S*)-D-[β-^{3}H]tyrosine (90%). The enantiomers were recrystallised several times by dissolution in the minimum amount of aqueous sodium hydroxide and adjustment of the pH to *ca.* 6 with acetic acid. Both forms were α-epimerised by heating in sealed tubes in 10N-hydrochloric acid at 180 °C for 3 days then crystallised (typically 75% recovery).

(βR)-DL-[β-^{3}H]- *and* (βS)-DL-[β-^{3}H]-*Phenylalanine* (with N. Johns and S. Narayanaswami).—These labelled derivatives were prepared from [α-^{3}H]benzaldehyde *via* the oxazolinone (1; R^1 = H, R^2 = Ph, T in place of D) by strict analogy with the method for tyrosine. Each form was mixed with DL-[*carboxy*-^{14}C]phenylalanine and the doubly labelled specimens were assayed [6,31,32] with the ammonia-lyase from potatoes. The resulting cinnamic acids were diluted with inactive material, crystallised, and counted for ^{3}H and ^{14}C. Typical assays gave a configurational purity of 85% for the β-^{3}H.

Feeding Experiments in Daffodils.—Doubly labelled samples of tyrosine were prepared by mixing (β*R*)-DL-[β-^{3}H]- and (β*S*)-DL[β-^{3}H]-tyrosine each with DL-[α-^{14}C]-tyrosine. Aqueous solutions (*ca.* 0·1 mCi ^{3}H, 0·02 mCi ^{14}C) at *ca.* pH 5 were injected into the hollow flower stalks of 'Texas' daffodils. Plants were harvested after 1 week. Alkaloids were isolated and purified by reported methods.[23,35] The activities of norpluvine, narwedine, and haemanthamine are in the Table.

Conversion of Haemanthamine into Epihaemanthamine.—Labelled haemanthamine, derived from either form of tyrosine, was oxidised (CrO_3–pyridine) to haemanthaminone.[25] This did not crystallise and was therefore reduced with sodium borohydride in methanol at 0 °C. The

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crystalline product was a mixture (t.l.c.). Repeated crystallisation from ethyl acetate gave pure epihaemanthamine, m.p. 212—216° (lit.,[25] 216—217°). This material had the same ^{14}C molar activity as the haemanthamine but contained no detectable ^{3}H.

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