Studies of Enzyme-mediated Reactions. Part III.[1,2] Stereoselective Labelling at C-2 of Tyramine: Stereochemistry of Hydroxylation at Saturated Carbon

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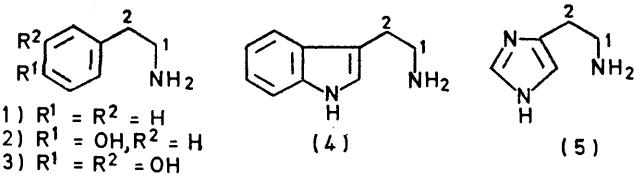
Synthetic routes to tyramine have been developed which allow stereoselective labelling with isotopic hydrogen at C-2 in configurations established by chemical correlation with [2-$^2H_1$]succinic acid of known absolute configuration. The various labelled tyramines have been converted into samples of *O*-methylnorbelladine [as (33)] which are used to prove that the hydroxylation step at saturated carbon in haemanthamine biosynthesis occurs (*a*) stereospecifically with removal of the *pro-R* hydrogen atom and (*b*) with retention of configuration.

MANY enzymic conversions in animals, plants and micro-organisms involve removal of a hydrogen atom from C-1 or C-2 (or both) of phenethylamine (1), tyramine (2), or dopamine (3) or their derivatives. The same holds true for tryptamine (4) and histamine (5). These five amines are formed in nature by decarboxylation of the corresponding α-amino-acids, a reaction which creates the

[1] Part II, P. G. Strange, J. Staunton, H. R. Wiltshire, A. R. Battersby, K. R. Hanson, and E. A. Havir, *J.C.S. Perkin I*, 1972, 2364.

[2] Preliminary account, A. R. Battersby, J. E. Kelsey, and J. Staunton, *Chem. Comm.*, 1971, 183.

C-1 methylene group. All these conversions fall into one stereochemical class *viz.* the generation or transformation of prochiral centres.[3,4] Knowledge of the stereochemistry of these processes is essential for a full understanding of the mechanism of the various enzymic

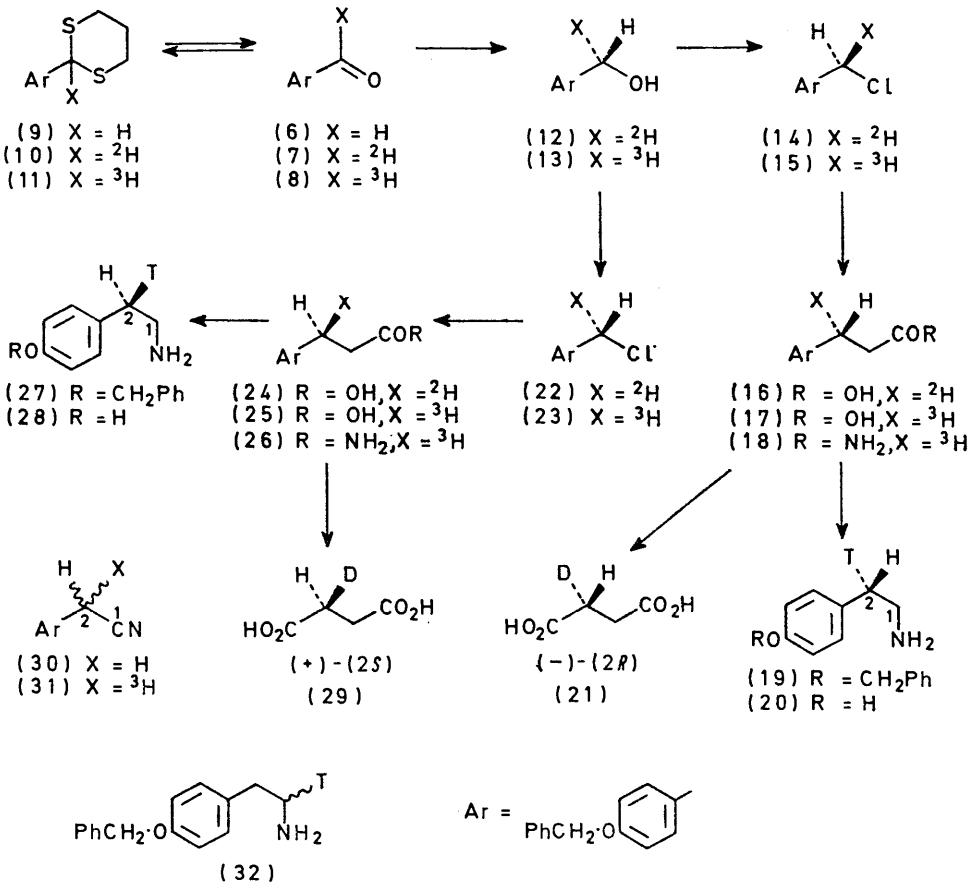


(1) $R^1 = R^2 = H$
(2) $R^1 = OH, R^2 = H$
(3) $R^1 = R^2 = OH$

(4) (5)

reactions. Accordingly, we are developing routes for synthesis of the amines (1)—(5) labelled with deuterium and/or tritium at C–1 and C–2; this paper describes the preparation and use of 2-labelled tyramines. The final aim in all cases is *stereospecific labelling* (approaching 100% configurational purity). But for many purposes, amines which are labelled *stereoselectively* (70—90% configurational purity) can provide rigorous stereochemical information about the enzymic step of interest.[5]

Synthetic Work.—Chirality was generated almost at the outset of the sequence by harnessing the stereospecific action of liver alcohol dehydrogenase[6] (LAD). Thus, 4-benzyloxybenzaldehyde (6) was converted into the dithian[7] (9) and the derived anion was quenched with deuterium oxide to yield the labelled derivative (10). The regenerated [*formyl*-^{2}H]aldehyde (7) was reduced with LAD and the coenzyme NADH, the latter being continuously regenerated in a 'coupled system'[8] by having an excess of ethanol in the medium. It was expected[6] that (*S*)-4-benzyloxy[*methylene*-$^2H_1$]benzyl alcohol (12) would be formed by addition of 'hydride' to the *re*-face of the aldehyde (7); supporting evidence will be given later.



(9) X = H
(10) X = ^{2}H
(11) X = ^{3}H

(6) X = H
(7) X = ^{2}H
(8) X = ^{3}H

(12) X = ^{2}H
(13) X = ^{3}H

(14) X = ^{2}H
(15) X = ^{3}H

(27) R = CH_2Ph
(28) R = H

(24) R = OH, X = ^{2}H
(25) R = OH, X = ^{3}H
(26) R = NH_2, X = ^{3}H

(22) X = ^{2}H
(23) X = ^{3}H

(16) R = OH, X = ^{2}H
(17) R = OH, X = ^{3}H
(18) R = NH_2, X = ^{3}H

(30) X = H
(31) X = ^{3}H

(+)-(2*S*)
(29)

(−)-(2*R*)
(21)

(19) R = CH_2Ph
(20) R = H

(32)

Treatment of the alcohol (12) with triphenylphosphine and carbon tetrachloride[9] gave the inverted chloride (14), which by a standard malonate synthesis afforded the deuteriated propionic acid (16). The *O*-benzyl group was hydrogenolysed and the phenol was oxidised by ozone and performic acid to yield monodeuteriosuccinic acid. This contained (by mass spectrometry)

[3] K. R. Hanson, *J. Amer. Chem. Soc.*, 1966, **88**, 2731.
[4] Reviewed by D. Arigoni and E. L. Eliel, *Topics Stereochem.*, 1969, **4**, 127.
[5] A. R. Battersby, *Accounts Chem. Res.*, 1972, **5**, 148.
[6] Reviewed by R. Bentley, 'Molecular Asymmetry in Biology,' Academic Press, New York, 1970, vol. II, p. 1.
[7] D. Seebach, B. W. Erickson, and G. Singh, *J. Org. Chem.*, 1966, **31**, 4303.
[8] *E.g.* H. R. Levy, F. A. Loewus, and B. Vennesland, *J. Amer. Chem. Soc.*, 1957, **79**, 2949; I. A. Rose, *ibid.*, 1958, **80**, 5835.
[9] R. G. Weiss and E. I. Snyder, *Chem. Comm.*, 1968, 1350, 1358; H. R. Hudson, *Synthesis*, 1969, 112.

87 ± 2% of $^2H_1$-species in agreement with the deuterium content found for the acid (16) of 89 ± 2% $^2H_1$-species. Professor J. W. Cornforth and Dr. G. Ryback kindly provided facilities for o.r.d. measurements[10] on the succinic acid which showed it to contain 72 ± 4% of the (−)-(*R*)-enantiomer (21). This finding established the configuration (16) for the major component in the phenylpropionic acid and supported the configurations illustrated for the substances earlier in the sequence.

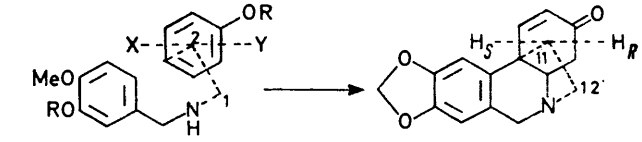
With a practical route for stereoselective labelling available, the sequence was repeated, with tritium in place of deuterium, using strictly equivalent conditions over the series (9) → (11) → (8) → (13) → (15) → (17). Hypochlorite and alkali converted the amide (18) into *O*-benzyl[2-$^3H_1$]tyramine containing 72 ± 4% of the (2*S*)-enantiomer (19); the corresponding tyramine hydrochloride [as (20)] is readily obtained from it by mild hydrogenolysis.

The ideal synthesis of the enantiomeric [2-^{3}H]tyramine (28) would first involve transfer of tritium from a tritiated alcohol to NAD^+, with subsequent transfer from [^{3}H]NADH to the unlabelled aromatic aldehyde to yield the enantiomer of alcohol (13). This approach was used successfully to prepare (*R*)-4-benzyloxy[*methylene*-$^2H_1$]benzyl alcohol [enantiomer of (12)]. However, when the same scheme without refinement was applied in the tritium series it proved to be highly inefficient because of a large isotope effect which presumably operates at both C–H bond-breaking steps. A full study of the factors involved has recently allowed this problem to be overcome.[11] The present work took a different tack.

The alcohol (12) was converted by thionyl chloride, largely with retention of configuration, into the halide (22) which as earlier yielded the acid (24). Ozonolysis gave monodeuteriosuccinic acid (85 ± 2% $^2H_1$) containing 68 ± 4% of the (+)-(*S*)-isomer (29) (by o.r.d.), thereby rigorously establishing the absolute configuration of the acid (24), this being the major component. Exact repetition of the reaction sequence in the tritium series (13) → (23) → (25) → (26) gave *O*-benzyl[2-$^3H_1$]-tyramine (27) containing 68 ± 4% of the illustrated (2*R*)-isomer.

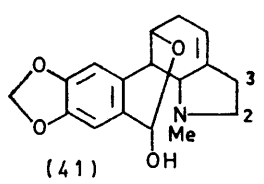
Finally, (2*RS*)-*O*-benzyl[2-$^3H_2$]tyramine [(19) + (27)] was prepared by equilibrating the C-2 hydrogen atoms of the nitrile (30) with tritiated methanol using magnesium methoxide to give (31); hydride reduction of the nitrile then gave the C-2 randomly labelled amine.

Enzymic Work.—Mixed function oxidases are able to hydroxylate 'unactivated' saturated carbon atoms (≥CH ⟶ ≥C–OH), with oxygen generally being the oxidising species.[12] There is obvious importance (and practical potential) in understanding this process and, as a contribution to this end, the stereochemistry of hydroxylation is being studied in a range of systems. The availability of the labelled amines (19), (27), and [(19) + (27)] allowed such a determination to be made in a higher plant for the case of haemanthamine (40). This base was chosen because its absolute configuration is firmly established[13] and much is known about its biosynthesis.[14] The pathway leads from phenylalanine and tyrosine to *O*-methylnorbelladine (33) and then by stages to oxocrinine (39), with hydroxylation occurring beyond this point.[15] The stereochemical problem can thus be solved by synthesising three samples of *O*-methylnorbelladine (33) from the labelled tyramine derivatives (19), (27) and [(19) + (27)] already prepared; this was carried out by the known route.[14,16] The products were mixed with *O*-methyl[1-^{14}C]norbelladine prepared similarly from *O*-benzyl[1-^{14}C]tyramine.

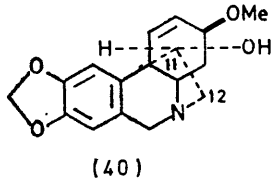


(33) R = H, X = H_S, Y = H_R
(34) R = H, X = ^{3}H, Y = H
(35) R = H, X = H, Y = ^{3}H
(36) R = CH_2Ph, X = ^{3}H, Y = H
(37) R = CH_2Ph, X = H, Y = ^{3}H
(38) R = CH_2Ph, X = Y = H

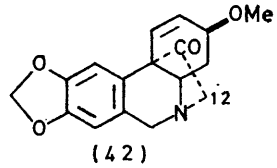
(39)



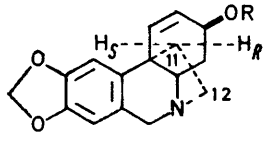
(41)



(40)



(42)



(43) R = H
(44) R = Me

Solutions of the hydrochlorides of (2*S*)-, (2*R*)-, and (2*RS*)-*O*-methyl[2-^{3}H,1-^{14}C]norbelladine [(34), (35), and [(34) + (35)] were injected in separate experiments into the flower stems of King Alfred daffodils. After 2 weeks, the plants were extracted to give haemanthamine (40) and oduline[17] (41). Carbon atoms 2 and 3 of oduline correspond biosynthetically[16] to positions 1 and 2 of

[10] (*a*) J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popják, G. Ryback, and G. J. Schroepfer, jun., *Proc. Roy. Soc. B.*, 1966, **163**, 436; (*b*) J. Rétey, J. Seibl, D. Arigoni, J. W. Cornforth, G. Ryback, W. P. Zeylemaker, and C. Veeger, *Eur. J. Biochem.*, 1970, **14**, 232.

[11] A. R. Battersby, J. Staunton, and H. R. Wiltshire, in preparation.

[12] O. Hayaishi, *Ann. Rev. Biochem.*, 1969, **38**, 21; G. A. Hamilton, *Adv. Enzymol.*, 1969, **32**, 55.

[13] J. Clardy, F. M. Hauser, D. Dahm, R. A. Jacobson, and W. C. Wildman, *J. Amer. Chem. Soc.*, 1970, **92**, 6337.

[14] W. C. Wildman, H. M. Fales, and A. R. Battersby, *J. Amer. Chem. Soc.*, 1962, **84**, 681; D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J. Chem. Soc.*, 1963, 4545.

[15] A. R. Battersby, C. Fuganti, and J. Staunton, unpublished work.

[16] A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J. Chem. Soc.*, 1964, 1595.

[17] W. Döpke and M. Biernet, *Pharmazie*, 1966, **21**, 323.

O-methylnorbelladine (33) and since these methylenes remain untouched, oduline serves as an internal standard. Table 1 shows that no significant loss of tritium occurred in experiments 1—3 as the various labelled *O*-methylnorbelladines were incorporated into oduline (100, 93, and 105 ± 4% retention, respectively). The haemanthamine isolated from the (2*RS*)-sample (experiment 1) retained 50 ± 2% of the original tritium, in agreement[4,5] with a stereospecific hydroxylation process.

In contrast, the haemanthamine from the (2*S*)-sample, (experiment 2) retained 66 ± 4% of the tritium whereas that from the (2*R*)-sample (experiment 3) retained only 31 ± 2%. Jones oxidation of the haemanthamine from experiment 2 or oxidation under Sarett's conditions gave oxohaemanthamine (42), which retained its carbon-14 but carried no tritium. It will be shown later that tritium at C-12 of haemanthamine is appreciably retained during the preparation and isolation of oxohaemanthamine (40), *i.e.* incomplete exchange *via* enolisation. It follows that the tritium in haemanthamine from experiment 2 is as expected entirely at C-11.

The foregoing results show that hydroxylation has occurred by direct attack at C-11 of (39), or a later intermediate (43) or (44), with stereospecific removal of the *pro-R* hydrogen atom. Comparison of structures (33), (39), and (40) shows that this corresponds to retention of configuration as for examples in other areas.[19] Our findings are in agreement with a mechanism in which the equivalent of an oxygen atom is inserted into the C–H bond, the so-called ' oxene ' or ' oxenoid ' mechanism.[12,20]

Kirby and Michael,[21] using stereoselectively labelled tyrosines, have independently shown that the hydroxylation step in haemanthamine biosynthesis occurs with retention of configuration.

EXPERIMENTAL

For general directions, see ref. 1.

2-(4-*Benzyloxyphenyl*)-1,3-[2-^{2}H]*dithian* (10) *and* -[2-^{3}H]-*dithian* (11).—Anhydrous dimethylformamide (100 ml) was treated with deuterium oxide (0·1 ml) and then redried, finally by distillation from sodium hydride directly into the protected reaction flask. This contained 2-(4-benzyloxyphenyl)-1,3-dithian[1] (3·02 g) which had been dried over phosphoric oxide; the mixture at 60° under dry nitrogen was treated with sodium hydride (1·5 g of 50% suspension in oil). After 4·5 h, deuterium oxide (1 ml) was added; the mixture was stirred at 20° for 16 h, then diluted with water (200 ml), adjusted to pH 2 with 2N-hydrochloric acid, and extracted with methylene chloride. The extracted material crystallised from chloroform to give the [2-^{2}H]dithian (3·0 g), m.p. 144—145° (lit.[1] for ^{1}H analogue, 144—145°), 96% $^2H_1$ species by mass spectrometry.

In a similar way, the unlabelled dithian (2·4 g) was treated with sodium hydride (0·39 g) and finally with tritiated water (0·06 ml; 300 mCi) to yield the [2-^{3}H]dithian (2·35 g), m.p. 144—145° (33 mCi).

4-*Benzyloxy*[formyl-^{2}H]*benzaldehyde* (7) *and* 4-*Benzyloxy*-[formyl-^{3}H]*benzaldehyde* (8).—The foregoing [2-^{2}H]dithian (3 g), mercuric oxide (2·44 g), and mercuric chloride (6·09 g) were stirred in 9 : 1 methanol–water (500 ml) and heated under reflux for 1 day. The filtered solution was evaporated (to *ca.* 50 ml), diluted with water (500 ml), and extracted with methylene chloride. The organic solution was washed with aqueous ammonium acetate (10%; 200 ml) and water (200 ml), then dried and evaporated. Crystallisation of the residue from light petroleum (b.p. 60—80°) gave the [*formyl*-^{2}H]benzaldehyde (2·0 g), m.p. 69—70°, 94% $^2H_1$ species by mass spectrometry.

Similar steps in the ^{3}H-series converted the [2-^{3}H]dithian

TABLE 1

Incorporation of labelled *O*-methylnorbelladine into haemanthamine and oduline

Expt. no.	Labelling	Structure precursor	Ratio ^{3}H : ^{14}C			Incorporation (%)	
			Precursor	Haemanthamine (40)	Oduline (41)	(40)	(41)
1	2*RS*-[2-$^3H_2$, 1-^{14}C]	(34) + (35)	9·17 ± 0·15	4·63 ± 0·1	9·19 ± 0·15	0·01	0·02
2	2*S*-[2-$^3H_1$, 1-^{14}C]	(34)	3·40 ± 0·08	2·24 ± 0·08	3·16 ± 0·08	0·006	0·016
3	2*R*-[2-$^3H_1$, 1-^{14}C]	(35)	7·50 ± 0·15	2·34 ± 0·08	7·87 ± 0·15	0·085	0·10
4	1*RS*-[1-$^3H_2$, 1-^{14}C]	as (38)	4·60 ± 0·1	4·27 ± 0·1	4·26 ± 0·1	0·6	0·26

Since the recorded configurations for the *O*-methylnorbelladines (34) and (35) refer to the major enantiomer present (*ca.* 70% in each case; see before), the foregoing interlocking results establish a high degree of stereospecificity (at least) in the hydroxylation step with removal of the *pro-R* hydrogen atom. This could occur by direct oxidation or by introduction of unsaturation between C-11 and C-12 (then epoxidation *etc.*). Accordingly, the (1*RS*)-amine (32) was prepared by reduction of the nitrile (30) with borotritiide in the presence of cobalt chloride.[18] It was used as before to synthesise (1*RS*)-*O*-methyl[1-$^3H_2$,1-^{14}C]norbelladine [as (33), random ^{3}H at C-1] for experiment 4. This showed no significant difference between the ^{3}H : ^{14}C ratios for the derived haemanthamine (40) and oduline (41); clearly, the hydroxylation process does not involve C-12. The haemanthamine was oxidised with Jones reagent to yield oxohaemanthamine (42) which retained 26% of the original tritium and this was completely eliminated by treatment with mild base.

[18] *Cf.* T. Satoh, S. Suzuki, Y. Suzuki, Y. Miyaji, and Z. Imai, *Tetrahedron Letters*, 1969, 4555.

[19] *E.g.* S. Bergstrom, S. Linstedt, G. Samuelson, E. J. Corey, and G. A. Gregoriou, *J. Amer. Chem. Soc.*, 1958, **80**, 2337; G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reincke, *ibid.*, 1972, **94**, 672; Y. Fujita, A. Gottlieb, B. Peterkofsky, S. Undenfriend, and B. Witkop, *ibid.*, 1964, **86**, 4709; C. J. Sih, Y. Y. Tsong, and B. Stein, *ibid.*, 1968, **90**, 5300; D. F. Jones, *J. Chem. Soc.* (*C*), 1968, 2827; E. Heinz, A. P. Tullock, and J. F. T. Spencer, *J. Biol. Chem.*, 1969, **244**, 882.

[20] G. A. Hamilton, *J. Amer. Chem. Soc.*, 1964, **86**, 3391.

[21] G. W. Kirby and J. Michael, *J.C.S. Perkin I*, 1973, 115.

(2·35 g) into the [*formyl*-^{3}H]benzaldehyde, which was chromatographed in benzene on silica gel and crystallised above; yield 1·56 g (30 mCi), m.p. 69—70°.

(S)-4-*Benzyloxy*[methylene-$^2H_1$]*benzyl Alcohol* (12) *and the* ^{3}H-*Analogue* (13).—A solution of nicotinamide adenine dinucleotide (Boehringer; 180 mg) in ethanol (5 ml) was added to pH 7 aqueous 0·01M-phosphate buffer (1·8 l) at 30°, followed by a suspension of liver alcohol dehydrogenase (Boehringer; 3 ml containing 30 mg enzyme). 4-Benzyloxy[*formyl*-^{2}H]benzaldehyde (0·6 g) in ethanol (45 ml) and dioxan (30 ml) was then added dropwise over 3 h. After a further 12 h, extraction with ether gave a product which was fractionated on silica gel in chloroform. This yielded starting material (80 mg) and the (*S*)-[methylene-$^2H_1$]*benzyl alcohol* (520 mg), m.p. 89—90° identified by direct comparison with authentic [^{1}H]alcohol.

The (*S*)-[methylene-$^3H_1$]*benzyl alcohol* (648 mg, 16 mCi) was prepared similarly from the corresponding aldehyde (1·21 g).

(3R)-3-(4-*Benzyloxyphenyl*)[3-$^2H_1$]*propionic Acid* (16), *the* ^{3}H-*Analogue* (17), *and its Amide* (18).—Tetrahydrofuran (20 ml), freshly distilled from lithium aluminium hydride, triphenylphosphine (2·26 g), and carbon tetrachloride (2·4 g) were mixed and a solution of the foregoing *S*-[$^2H_1$]benzyl alcohol (1·62 g) in anhydrous tetrahydrofuran (40 ml) was added. After the solution had been heated under reflux for 5 h, it was removed, with washings, from the triphenylphosphine oxide and added to a solution of diethyl sodiomalonate in tetrahydrofuran [22] [prepared by treating sodium hydride (365 mg; 50% suspension) in tetrahydrofuran (20 ml) with diethyl malonate (1·34 g)]. The mixture was heated under reflux in nitrogen for 3 h, diluted with water, acidified, and extracted with chloroform. Chromatography of the extract in benzene on silica gel gave a mixture of diethyl malonate and a product; the mixture was heated under reflux for 16 h with potassium hydroxide (1·4 g) in 1 : 1 water–ethanol (50 ml). After dilution with water (600 ml), the acidic product was isolated in methylene chloride and recovered therefrom as an oil (1·04 g). This was heated at 140° for 5 h under nitrogen and crystallised from benzene–light petroleum (b.p. 60—80°) to give (3R)-3-(4-*benzyloxyphenyl*)[3-$^2H_1$]*propionic acid* (0·8 g), m.p. 122—124° (lit.,[23] m.p. 123—124° for ^{1}H-material), not depressed by admixture with authentic material prepared by hydrogenation of 4-benzyloxycinnamic acid; *m/e* 257 (M^+), 89 ± 2% $^2H_1$ species.

The same procedures applied to (*S*)-4-benzyloxy[*methylene*-$^3H_1$]benzyl alcohol (560 mg) afforded (3R)-3-(4-*benzyloxyphenyl*)[3-$^3H_1$]*propionic acid* (260 mg), m.p. 123—124°.

A solution of this ^{3}H-product (256 mg) in anhydrous benzene (15 ml) was treated with oxalyl chloride (165 mg) and dimethylformamide (1 drop), and after 1 h was evaporated. The residue in diethyl ether (30 ml) was treated with an excess of dry ammonia and the solvent was evaporated off. A solution of the residue in ethyl acetate (50 ml) was washed with water, saturated aqueous sodium hydrogen carbonate, and finally water. The material from the ethyl acetate crystallised from chloroform to give (3R)-3-(4-*benzyloxyphenyl*)[3-$^3H_1$]*propionamide* (220 mg), m.p. 162—163° (Found for unlabelled material: C, 75·0, H, 6·6, N, 5·6. $C_{16}H_{17}NO_2$ requires C, 75·3; H, 6·7; N, 5·5%); $\nu_{max.}$ (Nujol) 3400, 3220, 1645, and 1618 cm^{-1}.

(3S)-3-(4-*Benzyloxyphenyl*)[3-$^2H_1$]*propionic Acid* (24), *the* ^{3}H-*Analogue* (25), *and its Amide* (26).—A solution of (*S*)-4-benzyloxy[*methylene*-$^2H_1$]benzyl alcohol (828 mg) in anhydrous diethyl ether (50 ml) was treated with thionyl chloride (920 mg), freshly purified from triphenyl phosphite.[24] After being stirred at 20° for 3 h, the solution was evaporated and the residue in tetrahydrofuran (10 ml) was added to diethyl sodiomalonate (3·8 mmol) in tetrahydrofuran. The subsequent reactions and work-up were as for the (3*R*)-isomer to yield (3S)-3-(4-*benzyloxyphenyl*) [3-$^2H_1$]-*propionic acid* (520 mg), m.p. 121—124°.

(3S)-3-(4-*Benzyloxyphenyl*) [3-$^3H_1$]*propionic acid* (525 mg; 10 mCi) was prepared similarly from (*S*)-4-benzyloxy-[methylene-$^3H_1$]benzyl alcohol (648 mg, 15 mCi).

(3S)-3-(4-*Benzyloxyphenyl*)[3-$^3H_1$]*propionamide* (522 mg), m.p. 160—161° was prepared as for the (3*R*)-isomer from the foregoing ^{3}H-acid (525 mg).

(3R)- *and* (3S)-3-(4-*Hydroxyphenyl*)[3-$^2H_1$]*propionic Acids*.—The (3*R*)-*O*-benzyl ether (16) (0·8 g) in 3 : 1 methanol–ethyl acetate (40 ml) was shaken with hydrogen and 10% palladised charcoal (125 mg) at 20° for 3 h. The filtered solution was evaporated and the residue crystallised from ether to give (3R)-3-(4-*hydroxyphenyl*)[3-$^2H_1$]*propionic acid* (0·5 g), m.p. 129—130° (lit.,[25] m.p. 129—130°). The (3S)-*enantiomer* was prepared in the same way.

Ozonolysis to yield Succinic Acids.—(3*R*)-3-(4-Hydroxyphenyl)[3-$^2H_1$]propionic acid (0·5 g) in methanol (100 ml) was treated at 20° with a stream of ozonised oxygen for 12 h and then evaporated. A solution of the residue in 98% formic acid (11 ml) and 30% hydrogen peroxide (6 ml) was heated at 100° for 15 h and then stirred with 10% palladised charcoal (30 mg) until gas evolution ceased. The filtered solution was evaporated and the chloroform-insoluble part of the residue was crystallised from water and then several times from ethyl acetate to give (2*R*)-[2-$^2H_1$]succinic acid (55 mg), m.p. 186—188°. The acid was converted into succinic anhydride and this was examined mass spectrometrically by Cornforth and Ryhage's methods [26] with an A.E.I. MS12 instrument at 70 eV (direct inlet; water cooled source at 40°). At least four spectra were run for each sample and the average intensities of the ions at *m/e* 56 and 57 were used in the calculations: found, 87 ± 2% $^2H_1$ species. O.r.d. measurements were made on the acid (6·03 mg) in water (248·4 mg) as shown in the *R* columns of

TABLE 2

Wavelength (nm)	Standard [10b] [α]^0	Found (*R*) [α]^0	Found (*S*) [α]^0	% of standard *R*	*S*
323	2·50	−1·05		42	
313	3·13	−1·21		39	
303	3·71	−1·37	+1·00	37	27
294	4·22	−1·55	+1·18	37	28
286	5·32	−1·92	+1·58	36	30
278	6·38	−2·42	+1·98	38	31
270	7·89	−2·89	+2·56	37	31
263	10·3	−3·92	+3·26	38	32
256	12·9	−5·10	+4·20	40	33
250	17·4	−7·01	+6·00	40	34
244	24·4	−9·93		41	
238	34·7	−14·3		41	

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Table 2. The average observed value for the *R*-acid over the range 313—244 nm is 38% of the standard corresponding (by allowance for 87% $^2H_1$ species) to 44% of the standard. Thus the deuterio-acid is composed of 72% (−)-(*R*)-succinic acid and 28% (+)-(*S*)-succinic acid.

Similar degradation of (3*S*)-3-(4-hydroxyphenyl)[3-$^2H_1$]-propionic acid (270 mg) gave (2*S*)-[2-$^2H_1$]succinic acid containing 85 ± 2% $^2H_1$ species. This acid (7·28 mg) in water (252 mg) gave the optical values as shown in the *S* columns of Table 2. The average observed value for the *S*-acid over the range 303—250 nm is 31% of the standard, corresponding (by allowance for 85% $^2H_1$ species) to 36% of the standard. Thus the deuterio-sample is composed of 68% (+)-(*S*)-succinic acid and 32% of (−)-(*R*)-succinic acid.

(*R*)-4-*Benzyloxy*[methylene-$^2H_1$]*benzyl Alcohol* [*Enantiomer of* (12)] *and its* ^{3}H-*Analogue* [*Enantiomer of* (13)].—3,3-Dimethylacrylic acid (8·5 g) in anhydrous ether (100 ml) was added to lithium aluminium deuteride (2·8 g) in ether (250 ml) over 1 h and the mixture was then heated under reflux for 2 h and kept at 20° for 16 h. Water (2·8 ml) was added, slowly followed by aqueous 15% potassium hydroxide (2·8 ml) and then water (2·8 ml). The solid was washed with ether and the combined organic solutions were washed with near-saturated aqueous potassium carbonate. The residue from the ether was distilled (B.P. 140—142°) to give 3,3-dimethyl[1-$^2H_2$]allyl alcohol (4 g). A solution of this product (1·55 g) and 4-benzyloxy-benzaldehyde (0·4 g) in dioxan (24 ml) was added in portions (1 ml) over 4 h to a solution at 30° containing the foregoing deuterio-allyl alcohol (1·55 g), 0·1M-K_2HPO_4 (45 ml), 0·1M-KH_2PO_4 (35 ml), water (720 ml), nicotinamide adenine dinucleotide (120 mg), ethanol-free liver alcohol dehydrogenase (70 mg; Sigma), and albumin (160 mg). The mixture was kept at 30° for 15 h and then worked up as for the *S*-enantiomer to give starting aldehyde (10 mg) and (R)-4-*benzyloxy*-[methylene-$^2H_1$]*benzyl alcohol* (380 mg), identified by comparison with the earlier *S*-product; 98 ± 2% $^2H_1$ species by mass spectrometry.

The tritium experiment was run in a similar way using the following quantities: 4-benzyloxybenzaldehyde (150 mg), dioxan (8 ml), and [1-$^3H_2$]isobutyl alcohol (total 3 g, 3 mCi used in two portions as above), nicotinamide adenine dinucleotide (30 mg), ethanol-free liver alcohol dehydrogenase (30 mg), albumin (40 mg), and phosphate buffer (225 ml). The [1-$^3H_2$]isobutyl alcohol was prepared by reducing isobutyraldehyde with borotritiide. Isolation of the [*methylene*-$^3H_1$]benzyl alcohol (60 mg) as before gave a product carrying 0·06 mCi total ^{3}H activity.

(2S)-4-*Benzyloxy*-N-(3-*benzyloxy*-4-*methoxybenzyl*)[2-$^3H_1$]-*phenethylamine* (36) *and the* (2R)-*Analogue* (37).—A solution of (3*R*)-3-(4-benzyloxyphenyl)[3-$^3H_1$]propionamide (220 mg) in methanol (30 ml) was treated with aqueous sodium hypochlorite (3 ml; 9% available chlorine) at 90° for 40 min. After addition of water (50 ml), the solution was evaporated (to 10 ml) and extracted with methylene chloride to afford the crude urethane. This was heated under reflux for 24 h with aqueous 4N-potassium hydroxide (15 ml) and methanol (15 ml); the solution was then diluted with water (50 ml), acidified to pH 2, and extracted with chloroform. Basification of the aqueous layer and extraction with chloroform yielded (2S)-O-*benzyl*[2-$^3H_1$]*tyramine* (105 mg), identified by comparison with radioinactive material. It yielded a hydrochloride m.p. 210—213° (lit.,[16] 210—215°).

[27] M. Tomita, K. Nakaguchi, and S. Takagi, *J. Pharm. Soc. Japan*, 1951, **71**, 1045.

The labelled tyramine was converted into the (2S)-[2-$^3H_1$]*phenethylamine* (36) essentially as earlier [16] and it was isolated as the *hydrochloride* (163 mg), m.p. 124—127°, 1 mCi (lit.,[16] m.p. 126—128°).

By the same sequence, (3*S*)-3-(4-benzyloxyphenyl)[3-$^3H_1$]-propionamide (522 mg) yielded (2R)-O-*benzyl*[2-$^3H_1$]*tyramine* (115 mg) and the (2R)-[2-$^3H_1$]*phenethylamine* (37) as its *hydrochloride* (224 mg), m.p. 126—128°, 2·3 mCi.

4-*Benzyloxyphenyl*[2-$^3H_2$]*acetonitrile* (31).—The corresponding radioinactive acetonitrile [14,27] (0·33 g) in tetrahydrofuran (5 ml) was added to fresh magnesium methoxide [28] (0·35 g) and the protected mixture was treated with tritiated water (0·1 ml; 200 mCi). After being heated under reflux for 24 h, the mixture was diluted with aqueous N-acetic acid (150 ml) and extracted with chloroform to yield the [2-$^3H_2$]*acetonitrile* (0·3 g) after recrystallisation from light petroleum (b.p. 40—60°); m.p. 68—69°, 36 mCi.

(2RS)-4-*Benzyloxy*-N-(3-*benzyloxy*-4-*methoxybenzyl*)[2-$^3H_2$]*phenethylamine* [(36) *and* (37].—A solution of the foregoing nitrile (0·3 g) in tetrahydrofuran (10 ml) was added under nitrogen to a solution (5 ml) of aluminium hydride [prepared by adding 100% sulphuric acid (522 mg) at 0° to a solution of lithium aluminium hydride (0·53 g) in tetrahydrofuran (5 ml)]. The solution was kept at 20° for 12 h and then worked up as for dimethyl[1-$^2H_2$]allyl alcohol; the material extracted into ether was separated as usual into a neutral fraction [unchanged nitrile (110 mg)] and a basic fraction {(2*RS*)-*O*-benzyl[2-$^3H_2$]tyramine (151 mg)}. The latter was converted as earlier into the (2RS)-[2-$^3H_2$]-*phenethylamine* [(36) + (37)], isolated as the *hydrochloride* (240 mg), m.p. 126—128°, 11·6 mCi.

(1RS)-4-*Benzyloxy*-N-(3-*benzyloxy*-4-*methoxybenzyl*)-[1-$^3H_2$]*phenethylamine* (38; random ^{3}H at C-1).—4-Benzyloxyacetonitrile (75 mg) was added at 20° to a stirred solution of cobalt chloride hexahydrate (160 mg) under nitrogen, followed by sodium borohydride (12·8 mg) in portions over 15 min. After 1 h, sodium borotritiide (8 mg; 100 mCi) was added, followed 1 h later by sodium borohydride (108 mg), and the mixture was kept for 1 h. 3N-Hydrochloric acid (0·67 ml) was added, and after the solution had been stirred for a further 16 h it was diluted with water and extracted with ether. Basification of the aqueous phase and extraction with ether gave an organic solution which was treated with hydrogen chloride to yield (1RS)-O-*benzyl*[1-$^3H_2$]*tyramine hydrochloride* (50 mg), m.p. 203—206° without recrystallisation. This as before yielded the (1RS)-[1-$^3H_2$]*phenethylamine* (38; random ^{3}H at C-1) as its *hydrochloride* (83 mg), m.p. 124—127°, 12 mCi.

4-*Benzyloxy*-N-(3-*benzyloxy*-4-*methoxybenzyl*)[1-^{14}C]-*phenethylamine* [*as* (38)].—4-Benzyloxyphenyl[1-^{14}C]acetonitrile [14] (220 mg; 0·8 mCi) was reduced as for the preparation [(36) + (37] to give the corresponding tyramine (152 mg), which as before gave the [1-^{14}C]phenethylamine hydrochloride (315 mg), m.p. 126—128°, 0·5 mCi.

^{3}H,^{14}C-*Labelled* O-*Methylnorbelladines*.—The foregoing four samples of ^{3}H-labelled dibenzyl ether hydrochlorides were mixed in ethanol solution with appropriate amounts of the ^{14}C-labelled hydrochloride to produce the ^{3}H : ^{14}C ratios shown in Table 1. These various solutions were shaken at 20° and 1 atm with hydrogen and 10% palladised charcoal (5—10 mg) until uptake ceased. The resultant *O*-methylnorbelladine hydrochlorides were recrystallised

[28] D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, 'Purification of Laboratory Chemicals,' Pergamon, Oxford, 1966, p. 157.

from ethanol–ethyl acetate; m.p. 208—211° (lit.,[16] m.p. 209—211°). Small portions of each of these four products were diluted with radioinactive *O*-methylnorbelladine hydrochloride and the salts were then recrystallised to constant ^3H : ^{14}C ratio to give the accurate values quoted in Table 1.

Administration of Labelled Precursors and Work-up of Plants.—Aqueous solutions of the labelled hydrochlorides were injected into the flower stems of King Alfred daffodils (12—30 plants used in different experiments) and the plants were worked up as previously [16] after 2 weeks. The total alkaloids (from the chloroform extraction) were fractionated on Spence alumina, first in benzene and then with benzene–ethyl acetate mixtures containing progressively more of the ester. Finally, pure ethyl acetate was used and the separation was controlled by t.l.c. (1 : 1 benzene–ethanol). The haemanthamine crystallised from acetone (typical yield 0·32 g), m.p. and mixed m.p. with authentic material 201—203°; i.r. that of haemanthamine (Found: C, 67·6; H, 6·1. Calc. for $C_{17}H_{19}NO_4$: C, 67·8; H, 6·4%).

The oduline from acetone (typical yield 165 mg) had m.p. 171—173°, $[\alpha]_D^{25}$ + 232° (*c* 0·45 in $CHCl_3$) {lit.,[17,29] m.p. 168°, $[\alpha]_D^{27}$ + 239° (*c* 0·35 in $CHCl_3$)}, $\nu_{max.}$ 3100, 2800, 1620, 1500, 1250, and 1155 cm^{-1} as reported,[17,29] τ 3·10—3·13 (2H, s, ArH), 4·00 (2H, s, O·CH_2·O), 4·05 (1H, s, O·CH·OH), 4·50br (1H, s, >C=CH), and 7·88 (3H, s, NMe) (Found: C, 67·5; H, 6·1; N, 4·5%; M^+, 301·1313. $C_{17}H_{19}NO_4$ requires C, 67·7; H, 6·3; N, 4·6%; *M*, 301·1314).

Oxohaemanthamine (42).—Haemanthamine (80 mg) from experiment 2 in ' stabilised ' acetone (20 ml) was treated at 20° for 25 min with Jones reagent [0·5 ml made by dissolving chromium trioxide (13·35 g) and conc. sulphuric acid (11·5 ml) in water (36·45 ml) to give 50 ml of solution]. Aqueous 2N-ammonia (1 ml) was then added and chloroform extraction gave a mixture (65 mg) which was fractionated by p.l.c. on silica (1 : 1 ethanol–benzene) to yield oxohaemanthamine (25 mg), m.p. 162—164° (from ether) (lit.,[30] 164°), identical (mixed m.p. and i.r.) with an authentic sample; found ^3H : ^{14}C ratio <0·02.

Similar oxidation of haemanthamine (50 mg) from experiment 4 gave oxohaemanthamine (15 mg), m.p. 161—162° found ^3H : ^{14}C ratio 1·12 corresponding to 26% retention of ^3H. Part (9 mg) of this sample was dissolved in 1 : 1 : 1 dioxan–triethylamine–water (total 0·6 ml) and kept at 20° for 3 weeks. The recovered base was recrystallised from ether; m.p. 161—163° (7 mg); found ^3H : ^{14}C ratio <0·02.

A solution of haemanthamine (85 mg) from experiment 2 in pyridine (2 ml) was added to a solution of chromium trioxide (0·19 g) in water (0·1 ml) and pyridine (3 ml). After 24 h at 20°, the solution was diluted with water (50 ml) and extracted with chloroform; the extracted base was purified as before to give oxohaemanthamine (35 mg), m.p. and mixed m.p. 162—163°; found ^3H : ^{14}C ratio <0·02.

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