Studies of Enzyme-mediated Reactions. Part VII.[1,2] Stereospecific Syntheses of Tritium-labelled (2*R*)- and (2*S*)-Dopamines: Stereochemical Course of Hydroxylation of Dopamine by Dopamine β-Hydroxylase (E.C. 1.14.17.1)

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An efficient synthesis has been developed for the preparation in high configurational purity of dopamines (3,4-dihydroxyphenethylamines) stereospecifically labelled at C-2 with isotopes of hydrogen. By incubating (2*R*)-, and (2*S*)-[2-$^2H_1$]dopamines with dopamine β-hydroxylase (E.C. 1.14.17.1) it has been shown that the *pro-R*-hydrogen atom is lost in the formation of noradrenaline; thus the hydroxylation of the benzylic methylene group occurs with retention of configuration.

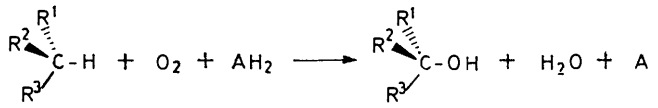
Of the wide range of natural oxidative processes, none is more intriguing from the mechanistic viewpoint than that in which a saturated carbon atom (C–H) is hydroxylated (to C–OH). This process, catalysed by a family of enzymes known as the mixed function oxidases,[3] is remarkable in that it requires not only an oxidising agent (molecular oxygen) but also a reducing agent (typically NADPH). Its general form is shown in

[1] Part VI, A. R. Battersby, J. Staunton, and M. C. Summers, preceding paper.

[2] Preliminary report, A. R. Battersby, P. W. Sheldrake, J. Staunton, and D. C. Williams, *J.C.S. Chem. Comm.*, 1974, 566.

[3] S. Kaufmann in 'Oxygenases,' ed. O. Hayaishi, Academic Press, New York, 1962, p. 158.

Scheme 1: one of the atoms supplied by a particular oxygen molecule is incorporated into the new hydroxy-group; the other is converted into water at the expense



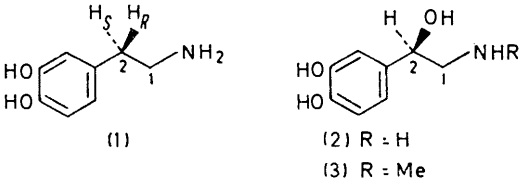
SCHEME 1

of two hydrogen atoms derived from the reducing agent AH_2. Investigations of the stereochemistry of such transformations, mainly with steroids and fatty acids, have revealed another intriguing feature which surely has mechanistic significance: with one exception,[4] all cases studied so far have been found to proceed with retention of configuration as indicated, *i.e.* the new hydroxy-group takes up the same configuration as the hydrogen atom it replaces.

In principle, the mechanism of the process could involve initial activation of the substrate to form a reactive species such as a radical or carbanion centre at the relevant carbon atom; this could then react with oxygen (or a suitable derivative generated on the enzyme) leading ultimately to the hydroxylated product. Alternatively an 'oxenoid' mechanism[5] may be considered which in its simplest form can be envisaged as direct insertion of a single oxygen atom (oxene), produced on the enzyme from molecular oxygen, into an unactivated C–H bond of the substrate. Such a mechanism would be related to analogous carbene insertions and would have the advantage that, unlike a radical or carbanion mechanism, it would necessarily proceed with retention of configuration. Moreover, an oxenoid mechanism would account for the ability of mixed function oxidases to hydroxylate carbon atoms remote from functional groups capable of stabilising a radical or carbanion intermediate.

Nevertheless, even if oxygen insertion is the normal mechanism, the possibility remains that when hydroxylation takes place at a carbon atom where stabilisation is available an alternative process may be employed. Perhaps significantly, the one exception[4] to the so far normal pattern of retention of configuration involved hydroxylation at an allylic centre. With this consideration in mind, we are studying hydroxylations at benzylic carbon atoms and have reported[6] that one such reaction, in the biosynthesis of narcotine, occurs with retention of configuration. This paper reports work on a further case, the hydroxylation of dopamine (1) to form (−)-noradrenaline (2), the biological precursor of adrenaline (3). These three catecholamines (1)—(3) are all important in the chemistry of the central nervous system.[7]

The key hydroxylation reaction is catalysed by a mixed function oxidase, dopamine β-hydroxylase (E.C. 1.14.17.1), which employs ascorbic acid as hydrogen donor. The properties of the enzyme have been studied extensively.[8] Early work established that the oxygen atom introduced at C-2 is derived from molecular



(1) (2) R = H (3) R = Me

oxygen[9] rather than water, and that only one hydrogen atom is removed from C-2 in the oxidation.[10] The reaction thus shows several features characteristic of hydroxylations mediated by other mixed function oxidases.

For our stereochemical studies it was necessary to prepare dopamines stereospecifically labelled at C-2 with isotopes of hydrogen. The route employed is illustrated in Scheme 2 by the synthesis initially of (2*R*)-[2-$^3H_1$]-dopamine (14). The choice of protecting groups used in this synthesis deserves comment because Suckling,[11] in this laboratory, had previously used essentially the same sequence of reactions illustrated [(4)→(10c)] to prepare the acid (16). He used *O*-benzyl throughout to protect the phenolic hydroxy-group; chemical yields were good and the product (16) had the expected configuration but the configurational purity was very low. It seemed clear that an S_N1 process was competing seriously with the desired S_N2 displacement step [analogous to (6b)→(8)] and we reasoned that the powerfully electron-withdrawing methylsulphonyl group should suppress this unwanted interference. In practice this ploy was highly successful as will be apparent later.

The starting material, *O*-methylsulphonyl[*formyl*-^{3}H]-vanillin (4), was prepared from unlabelled material (5) by reduction with potassium borotritiide followed by reoxidation with chromium trioxide–pyridine complex;[12] the ^{3}H isotope effect operated here to our advantage. This aldehyde was then converted into the (*S*)-alcohol (6a) by stereospecific hydrogen transfer from ethanol in a reaction mediated by liver alcohol dehydrogenase in the presence of a catalytic quantity of the coenzyme NAD^+.[13] Treatment of the alcohol (6a)

[4] 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; for collected refs. on examples showing retention of configuration, see *J.C.S. Perkin I*, 1973, 1609.

[5] G. A. Hamilton, *Adv. Enzymol. Related Areas Mol. Biol.*, 1969, **32**, 55.

[6] A. R. Battersby, J. Staunton, H. R. Wiltshire, B. J. Bircher, and C. Fuganti, *J.C.S. Perkin I*, 1975, 1162.

[7] C. R. Creveling and J. W. Daly in 'Analysis of Biogenic Amines and their Related Enzymes,' ed. D. Gluck, Wiley–Interscience, New York, 1971.

[8] *E.g.* S. Friedman and S. Kaufman, *J. Biol. Chem.*, 1965, **240**, 4763; A. Foldes, P. L. Jeffrey, B. N. Preston, and L. Anstu, *J. Neurochem.*, 1973, **20**, 1431.

[9] S. Kaufman, W. F. Bridges, F. Eisenburg, and S. Friedman, *Biochem. Biophys. Res. Comm.*, 1962, **9**, 497.

[10] S. Senoh, B. Witkop, C. R. Creveling, and S. Udenfriend, *J. Amer. Chem. Soc.*, 1959, **81**, 1768.

[11] K. E. Suckling, Ph.D. Thesis, Cambridge, 1971; A. R. Battersby, J. Staunton, K. E. Suckling, and R. N. Woodhouse, in preparation.

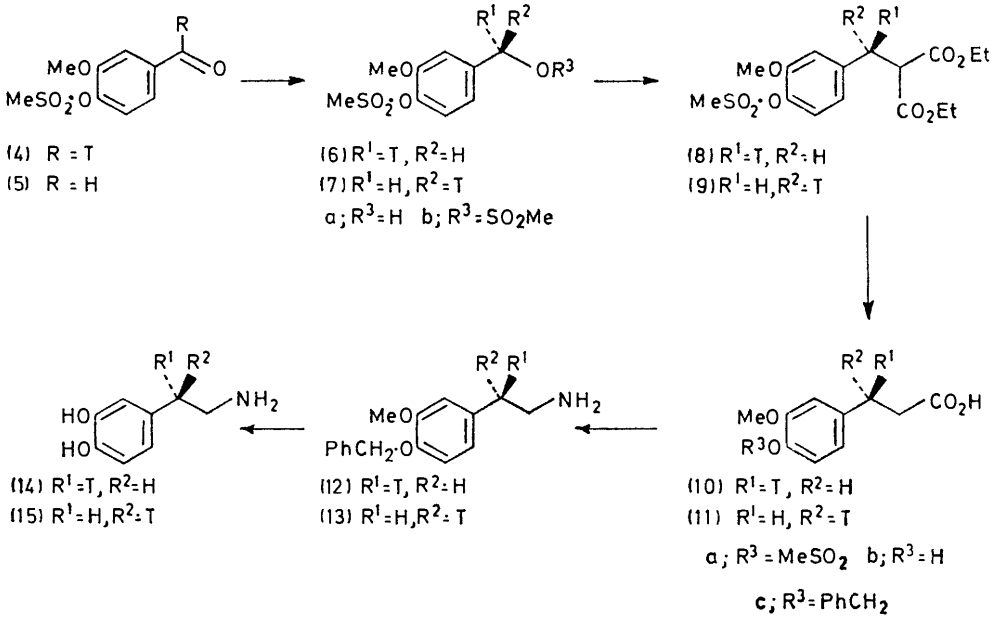
[12] R. Ratcliffe and R. Rodehorst, *J. Org. Chem.*, 1970, **35**, 4000.

[13] *E.g.* F. A. Loewus, F. H. Westheimer, and B. Vennesland, *J. Amer. Chem. Soc.*, 1953, **75**, 5018; see also V. Prelog, 3rd International Symposium on Chemistry of Natural Products, Special Lectures, 1964, p. 119.

with methanesulphonyl chloride and triethylamine [14] in dichloromethane gave the diester (6b), in which one methylsulphonyl group serves to activate and the other to deactivate the molecule. This product was converted into the ester (8) by treatment with the anion of diethyl malonate under conditions designed to promote an S_N2 displacement. The derived phenylpropionic acid (10a) was converted by base into the phenol (10b), in which the chiral centre was now secure; reprotection by *O*-benzylation was therefore appropriate. A Curtius reaction on this *O*-benzyl ether (10c) led first to the isocyanate,[15] which without purification was converted into the corresponding urethane and by hydrolysis into the phenethylamine (12). Ether cleavage with hydrobromic acid [16] then yielded (2*R*)-[2-$^3H_1$]dopamine (14), isolated as its hydrochloride.

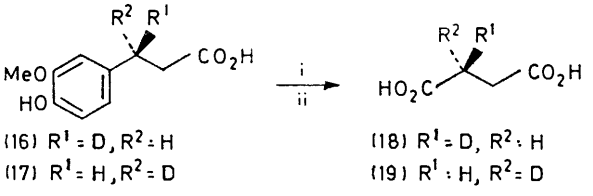
The preparation of (2*S*)-[2-$^3H_1$]dopamine (15) involved reduction of the unlabelled aldehyde (5) by stereospecific transfer of tritium from [1-^{3}H]cyclohexanol [17] catalysed by liver alcohol dehydrogenase and its coenzyme NAD^+ to give the (*R*)-alcohol (7a). This was subsequently elaborated to the labelled (2*S*)-dopamine (15) by a sequence of reactions exactly parallel to those used for the (2*R*)-isomer, *i.e.* (7b)→(9)→(11a)→(11c)→(13)→(15). An important consequence of this approach is that the two enantiomers of [2-$^3H_1$]dopamine have an essentially identical degree of configurational purity and so should give complementary results in the subsequent enzymic experiments. (2*RS*)-[2-$^3H_1$]Dopamine was prepared by base-catalysed exchange of the methylene group of 4-benzyloxy-3-methoxyphenylacetonitrile against tritiated methanol followed by reduction of the nitrile with diborane and finally cleavage of the protecting groups as before.

It was now necessary to determine the configurational purity of the products from the syntheses described above and, to this end, the deuteriated analogue (16) of the ^{3}H-labelled sample (10b) was prepared. The route started from the aldehyde (4; D in place of T), which was prepared from *O*-benzyl[*formyl*-^{2}H]vanillin [11]



SCHEME 2

by cleavage of the *O*-benzyl group and reaction with methanesulphonyl chloride. Apart from this inconsequential variation, the synthesis ran exactly parallel to the tritium series so that the configurational purity of the deuteriated acid (16) matches that of the tritiated acid (10b). Ozonolysis of the acid (16) gave, as was hoped, (2*S*)-[2-$^2H_1$]succinic acid (18) (Scheme 3).



SCHEME 3 i, O_3; ii, HCO_3H

Importantly, the o.r.d. curve [18] (see Table 2 in Experimental section) showed that this succinic acid was, within experimental error, configurationally pure. This result was confirmed by a complementary synthesis of

[14] R. K. Crossland and K. L. Servis, *J. Org. Chem.*, 1970, **35**, 3195.
[15] *Cf.* J. Weinstock, *J. Org. Chem.*, 1961, **26**, 3511.
[16] P. A. Wehrli, F. Pigøtt, and V. Chu, *Canad. J. Chem.*, 1972, **50**, 3075.
[17] A. R. Battersby, J. Staunton, and H. R. Wiltshire, *J.C.S. Perkin I*, 1975, 1156.
[18] J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, G. Ryback, and G. J. Schroepfer, jun., *Proc. Roy. Soc.* 1966, *B*, **168**, 436; D. Arigoni, J. W. Cornforth, G. Ryback, W. P. Zeylmaker, and C. Veeger, *European J. Biochem.*, 1970, **14**, 232.

the enantiomeric acid (17); this exactly followed the earlier work which had yielded the ^{3}H-labelled acid (11b). Degradation of the acid (17) as before gave enantiomerically pure (2*R*)-[2-$^2H_1$]succinic acid (19) (see Table 2).

The foregoing studies rigorously define the chiral sense and configurational purity of the deuteriated arylpropionic acids (16) and (17), and this information holds good for the tritiated dopamines (14) and (15), since the steps (10)→(14) and (11)→(15) in the syntheses do not affect the chiral centre.

The three samples [(2*R*), (2*S*), and (2*RS*)] of tritiated dopamine [(14), (15), and (14 and 15), respectively] were each mixed with a suitable quantity of [1-^{14}C]-dopamine to provide an internal standard and were then incubated separately with an homogenate of bovine adrenal medulla containing ascorbic acid (a cofactor), fumaric acid (an activator), and promiazid (an inhibitor of monoamine oxidase).[19] Prior to work-up of each incubation mixture, radio-inactive dopamine (1) and noradrenaline (2) were added and the amines were acylated *in situ* by addition of isobutyric anhydride. *NOO*-Tri-isobutyrylnoradrenaline was isolated by preparative t.l.c., and then was subjected to further purification after addition of radio-inactive *NOO*-tri-isobutyryldopamine until the specific activity and ^{3}H : ^{14}C ratio of the noradrenaline derivative remained

TABLE 1

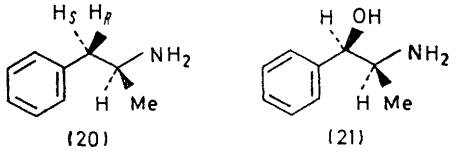
Incubation of labelled dopamines with dopamine β-hydroxylase

Dopamine		Noradrenaline		
Configuration	^{3}H/^{14}C [a]	Conversion (%)	^{3}H/^{14}C [b]	% ^{3}H retention [b]
(2*R*)-[2-$^3H_1$, 1-^{14}C] (14)	14.2 ± 0.6	0.8	0.07 ± 0.02	0.5
(2*S*)-[2-$^3H_1$, 1-^{14}C] (15)	13.9 ± 0.3	0.6	13.5 ± 0.3	97 ± 3
(2*RS*)-[2-$^3H_1$, 1-^{14}C] (14 + 15)	16.9 ± 0.6	0.6	8.3 ± 0.2	49 ± 2

[a] Mean value and range from assays of ^{3}H : ^{14}C ratio on the hydrochloride and on the *NOO*-tri-isobutyryl derivative. [b] Radio-assay on the *NOO*-tri-isobutyryl derivative.

constant. The observed conversion of dopamine into noradrenaline was in line with that normally found for the homogenate system, *i.e.* around 1%. The results for ^{3}H retention in Table 1 establish that the hydroxylation is stereospecific and that it is the *pro-R*-hydrogen atom which is eliminated from C-2 of dopamine. Earlier work [20] had shown that noradrenaline has the illustrated (2*R*)-configuration (2) and it therefore follows that the hydroxylation reaction (1)→(2) takes place with retention of configuration.

This conclusion is in accord with the results of Taylor,[21] who demonstrated that conversion of the unnatural substrate (1*S*)-amphetamine (20) by dopamine β-hydroxylase into (1*S*,2*R*)-ephedrine (21) involves loss



of the *pro-R*-hydrogen atom from C-2. More recently Bachan and his co-workers [22] have shown independently, using deuteriated phenethylamine, that hydroxylation of this second unnatural substrate again takes place with retention.

This consistent stereochemistry of action now established for dopamine β-hydroxylase in attack at saturated benzylic carbon is in agreement with what is known for the few other similar enzymic reactions so far studied; *i.e.* benzylic hydroxylation of ethylbenzene,[23] of *p*-hydroxyphenylacetonitrile,[24] and in the biosynthesis of narcotine [6] all occur with retention of configuration.

EXPERIMENTAL

For general directions see refs. 1 and 25.

3-Methoxy-4-methylsulphonyloxybenzaldehyde (5).—A solution of 4-hydroxy-3-methoxybenzaldehyde (20 g) in anhydrous pyridine (80 ml) was treated at 0 °C with methanesulphonyl chloride (16 ml) and kept at 4 °C for 16 h. The mixture was poured into an excess of 2N-sulphuric acid at 0 °C, and after 45 min the product was collected, washed with water, dried, and recrystallised from 95% ethanol; yield 24 g; m.p. 93—94° (lit.,[26] 93—94°) (Found: C, 47.0; H, 4.2; N, 13.9. $C_9H_{10}O_5S$ requires C, 47.0; H, 4.3; N, 13.9%); $\lambda_{max.}$ 255 and 307 nm (ε 9 100 and 3 300); $\nu_{max.}$ 1 711 cm^{-1}; τ 0.04 (1 H, s, CHO), 2.50 (3 H, m, ArH), 6.08 (3 H, s, OMe), and 6.90 (3 H, s, $MeSO_2$); *m/e* 230 (M^+, 99%), 151 (100), and 95 (100).

3-Methoxy-4-methylsulphonyloxy[formyl-^{3}H]*benzaldehyde* (4).—The foregoing aldehyde (464 mg) in AnalaR methanol (40 ml) was treated with potassium borohydride (4.5 mg), then with potassium borotritiide (11.0 mg; 100 mCi), and finally with potassium borohydride (15 mg). The solution was acidified with 2N-hydrochloric acid, the methanol was evaporated off, saturated brine (20 ml) was added, and the product was extracted into chloroform. Removal of the solvent left an oil (475 mg). A solution of this product in methylene chloride was added to chromium trioxide–pyridine complex [prepared by treating chromium trioxide (1.2 g) with a stirred solution of anhydrous pyridine (1.94 ml) in dry dichloromethane (30 ml) for 15 min]. After being stirred for 15 min, the solution was decanted and the residue extracted with dichloromethane (5 × 10 ml). The combined organic layers were washed with 2N-potassium hydroxide (3 × 15 ml), 2N-hydrochloric acid (2 × 15 ml), saturated sodium hydrogen carbonate (15 ml), and brine (15 ml). The product was purified by p.l.c. on silica with

[19] J. H. Phillips, *Biochem. J.*, 1973, **136**, 579.
[20] P. Pratesi, A. La Manna, A. Campiglio, and V. Ghislandi, *J. Chem. Soc.*, 1958, 2069; 1959, 4062.
[21] K. B. Taylor, *J. Biol. Chem.*, 1974, **249**, 454.
[22] L. Bachan, C. B. Storm, J. W. Wheeler, and S. Kaufman, *J. Amer. Chem. Soc.*, 1974, **96**, 6799.
[23] R. E. McMahon, H. R. Sullivan, J. C. Craig, and W. E. Peveira, jun., *Arch. Biochem. Biophys.*, 1969, **132**, 575.
[24] M. A. Rosen, K. J. F. Farnden, E. E. Conn, and K. R. Hanson, in preparation.
[25] 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.
[26] T. Kametani, O. Umezawa, K. Sekine, T. Oda, M. Ishiguro, and D. Mizuno, *Yakugaku Zasshi*, 1964, **84**, 237 (*Chem. Abs.*, 1964, **61**, 600f).

ether to give the labelled aldehyde (407 mg; 63 mCi), which was identified by comparison with unlabelled material.

(S)-3-*Methoxy*-4-*methylsulphonyloxy*[methylene-$^3H_1$]*benzyl Alcohol* (6a).—To a mixture of the foregoing tritiated aldehyde (464 mg) in ethanol (25 ml) was added 0.002M-phosphate buffer (pH 8; 475 ml), followed by NAD^+ (150 mg; Boehringer), and a suspension of horse liver alcohol dehydrogenase (2 ml; 10 mg per ml; Boehringer). The solution was incubated at 37 °C. After 1 h, more enzyme suspension (0.8 ml) was added and incubation was continued for a further 1.5 h. The solution was then saturated with sodium chloride and extracted with chloroform (5 × 100 ml) to give an oil which was purified by p.l.c. on silica with ether to yield the alcohol (406 mg, 87%), m.p. 65—66°; $\lambda_{max.}$ 274 and 281 nm; $\nu_{max.}$ 3 560 cm^{-1}; τ [$(CD_3)_2SO$] 2.6—3.2 (3 H, m, ArH), 4.72 (1 H, s, OH), 5.48 (2 H, s, CH_2), 6.16 (3 H, s, MeO), and 6.76 (3 H, s, $MeSO_2$); *m/e* 232 (M^+, 49%), 153 (100), 121 (22).

3-*Methoxy*-4-*methylsulphonyloxy*[methylene-$^3H_1$]*benzyl Methanesulphonate* (6b).—The foregoing alcohol (406 mg) in dry dichloromethane was treated at 0 °C with triethylamine (0.46 ml) and methanesulphonyl chloride (0.17 ml). After being stirred for 40 min, the solution was washed with water, 2N-hydrochloric acid, saturated sodium hydrogen carbonate, and brine. The product was recrystallised from dichloromethane–ether to give the *methanesulphonate* (435 mg, 80%), m.p. 114—115° (Found: C, 38.8; H, 4.35; S, 20.5. $C_{10}H_{14}O_7S_2$ requires C, 38.7; H, 4.5; S, 20.65%); $\lambda_{max.}$ 279 nm; $\nu_{max.}$ 1 600, 1 510, 1 170, and 1 150 cm^{-1}; τ 3.06—2.50 (3 H, m, ArH), 4.79 (2 H, s, CH_2), 6.10 (3 H, s, MeO), and 6.82 and 7.04 (each 3 H, s, $MeSO_2$); *m/e* 310 (M^+, 17%), 231 (44), 215 (12), 153 (22), 152 (22), 137 (18), and 96 (100).

(S)-*Diethyl* (3-*Methoxy*-4-*methylsulphonyloxy*[α-$^3H_1$]-*benzyl*)*malonate* (8).—Diethyl malonate (0.55 ml) was added to sodium hydride (56.5 mg; 60% dispersion in oil) in dry tetrahydrofuran under nitrogen at 0 °C. When the stirred solution became clear, the above methanesulphonate (434 mg) in dry tetrahydrofuran (40 ml) was added over 1 h, and the mixture was stirred at 20 °C for 24 h. The solution was acidified with 2N-hydrochloric acid, the tetrahydrofuran was evaporated off, and the residue was partitioned between 2N-hydrochloric acid (15 ml) and chloroform (20 ml). After re-extraction of the aqueous layer with chloroform, the chloroform solution was evaporated and the oil was purified by p.l.c. on silica with ether to give the diester (44 mg, 83%), m.p. 75—79°; $\lambda_{max.}$ 275 and 281 nm; $\nu_{max.}$ 1 720 cm^{-1}; τ 2.7—3.3 (3 H, m, ArH), 5.84 (4 H, q, *J* 7 Hz, OCH_2), 6.16 (3 H, s, MeO), 6.37 (1 H, t, *J* 8 Hz, CH), 6.80 (2 H, d, *J* 8 Hz, ArCH_2), 6.88 (3 H, s, $MeSO_2$), and 8.79 (6 H, t, *J* 7 Hz, Me); *m/e* 374 (M^+, 32%), 329 (10), 295 (67), and 246 (100).

(3S)-3-(3-*Methoxy*-4-*methylsulphonyloxyphenyl*)[3-$^3H_1$]-*propionic Acid* (10a).—The foregoing ester (439 mg) was heated under reflux in 5N-hydrochloric acid (20 ml) for 22 h. Extraction of the cold solution with ethyl acetate (4 × 10 ml), washing of the extract with brine, and evaporation gave the *acid* (301 mg, 93%), m.p. 116—118° (Found: C, 47.9; H, 5.1; S, 11.7. $C_{11}H_{14}O_6S$ requires C, 48.2; H, 5.1; S, 11.7%); $\lambda_{max.}$ 275 and 281 nm; $\nu_{max.}$ 3 300—2 500 and 1 700 cm^{-1}; τ 1.2br (1 H, s, CO_2H), 3.3—2.7 (3 H, m, ArH), 6.18 (3 H, s, MeO), and 6.89 (3 H, s, $MeSO_2$); *m/e* 274 (M^+, 52%), 232 (52), 195 (76), 151 (100), and 149 (43).

(3S)-3-(4-*Hydroxy*-3-*methoxyphenyl*)[3-$^3H_1$]*propionic Acid* (10b).—The foregoing acid (242 mg) in aqueous 2N-potassium hydroxide (23 ml) was kept at 20 °C for 24 h. The solution was acidified and extracted with ethyl acetate (5 × 10 ml) to give the phenolic acid, which was recrystallised from ether–light petroleum (b.p. 60—80°), m.p. 88—90° (80% yield) (lit.,[27] m.p. 89—90°).

(3S)-3-(4-*Benzyloxy*-3-*methoxyphenyl*)[3-$^3H_1$]*propionic Acid* (10c).—The phenolic acid (252 mg) in dry acetone (20 ml) was heated under reflux for 7 h with benzyl chloride (0.45 ml), tetramethylammonium iodide (0.5 g), and anhydrous potassium carbonate (2 g). The residue from evaporation of the acetone was dissolved in an excess of sodium hydroxide and heated under reflux for 6 h. After the cold solution had been extracted with ether, it was acidified and extracted again with ether (5 × 20 ml) to give the *O*-benzyl acid, m.p. 98—100° (from di-isopropyl ether) (279 mg, 76%); it was identified by comparison with authentic material,[11] m.p. 99°.

Methyl (2R)-N-[2-(4-*Benzyloxy*-3-*methoxyphenyl*)[2-$^3H_1$]-*ethyl*]*carbamate*.—The foregoing acid (286 mg) was treated in AnalaR acetone (15 ml) at 0 °C with triethylamine (0.15 ml) and ethyl chloroformate (0.105 ml). After 0.5 h, saturated aqueous sodium azide (85 mg) was added and the mixture was stirred for 2 h more. The acetone was evaporated off, brine was added and the azide was extracted into ether, recovered ($\nu_{max.}$ 2 145 and 1 718 cm^{-1}), and dissolved in anhydrous toluene (20 ml). After heating on the steam-bath for 30 min, the toluene was evaporated off and the resultant isocyanate ($\nu_{max.}$ 2 260 cm^{-1}) was treated with sodium methoxide (1.1 equiv.) in methanol. The solution was acidified after 1 h, then evaporated; the residue was mixed with water and the crude product was extracted with chloroform. The *carbamate* (236 mg, 75%) (from cyclohexane) had m.p. 72—75° (Found: C, 68.7; H, 6.55; N, 4.4. $C_{18}H_{21}NO_4$ requires C, 68.55; H, 6.7; N, 4.45%); τ 2.60 (5 H, m, ArH), 3.20 (3 H, m, ArH), 4.80 (2 H, s, CH_2O), 6.11 (3 H, s, MeO), 6.31 (3 H, s, CO_2Me), 6.60 (2 H, t, *J* 6 Hz, CH_2N), and 7.30 (2 H, t, *J* 6 Hz, CH_2); *m/e* 315 (M^+, 61%), 239 (4), 137 (100), and 91 (100).

(2R)-2-(4-*Benzyloxy*-3-*methoxyphenyl*)[2-$^3H_1$]*ethylamine Hydrochloride* (12).—The foregoing carbamate (248 mg) in AnalaR methanol (15 ml) and water (15 ml) was treated with potassium hydroxide (7 g). After the mixture had been heated under reflux for 52 h, the methanol was evaporated off, water (90 ml) was added, and the suspension was adjusted to pH 1 with conc. hydrochloric acid. Extraction with chloroform (3 × 20 ml) removed neutral and acidic materials and the aqueous layer was basified (pH 9) with solid potassium hydroxide. It was then extracted with dichloromethane (4 × 20 ml); the extracts were evaporated and the residue was treated in ether with an excess of dry hydrogen chloride. Evaporation of the ether left a solid which was recrystallised from methanol–ether; yield 180 mg, m.p. 167—169°, τ 2.7 (5 H, m, ArH), 3.25 (3 H, m, ArH), 4.91 (2 H, s, $PhCH_2$), 6.10 (3 H, s, OMe), and 7.3br (4 H, $CH_2{\cdot}CH_2$); *m/e* 257 (M^+, 22%), 228 (85), 137 (56), and 91 (100).

(2R)-2-(3,4-*Dihydroxyphenyl*)[2-$^3H_1$]*ethylamine Hydrochloride* (14).—The foregoing amine hydrochloride (50 mg) was heated under reflux for 6 h in constant boiling AnalaR hydrobromic acid [10 ml; redistilled from tin(II) chloride] under nitrogen. The acid was evaporated off to yield dopamine hydrobromide, which was converted into the hydrochloride by recrystallisation from concentrated

[27] F. Tieman and N. Nagai, *Ber.*, 1878, **11**, 646.

hydrochloric acid. Occasionally the product was slightly coloured and impurities were removed by p.l.c. on silica (elution with 85 : 15 : 1 acetone–water–hydrochloric acid). Dopamine hydrochloride was recovered from the plate with methanol acidified with 2N-hydrochloric acid and was recrystallised as before then finally from methanol–ether to give white crystals, m.p. 238—243°, identified by comparison with an authentic sample.

(R)-3-*Methoxy*-4-*methylsulphonyloxy*[methylene-$^2H_1$]-*benzyl Alcohol* (7a).—Cyclohexanone (6.12 mg) in aqueous 0.01N-potassium hydroxide (1 ml) was treated with potassium borohydride solution (0.1 ml; 0.276 mg ml^{-1}) and then after 1 h, with potassium borotritiide (100 mCi; 7.6 Ci $mmol^{-1}$). The solution was kept overnight at 20 °C, then an excess of potassium borohydride was added, and after 1 h the solution was acidified, rebasified with ammonium hydroxide, and made up to 5 ml with 0.002M-phosphate buffer (pH 8.7). This solution was mixed with 0.002M-potassium phosphate buffer (pH 8; 240 ml), NAD^+ (95 mg), albumin (40 mg), and a solution of 3-methoxy-4-methylsulphonyloxybenzaldehyde (235 mg) in dioxan (12 ml). Horse liver alcohol dehydrogenase (110 mg; ethanol-free) was added and the mixture was incubated at 37 °C for 1¼ h. More enzyme (150 mg) was added in 3 portions over the next 3 h, and potassium borohydride was then added to complete the reduction. The product (7a) was isolated, purified, and identified as for its enantiomer to give the (*R*)-benzyl alcohol (233 mg, 52%; 39 mCi).

(2S)-2-(3,4-*Dihydroxyphenyl*)[2-$^3H_1$]*ethylamine Hydrochloride* (15).—The foregoing product was diluted with the radio-inactive alcohol (110 mg), and the whole was carried through exactly the same synthetic series described above for the (2*R*)-isomer.

2-(3,4-*Dihydroxyphenyl*)[1-^{14}C]*ethylamine Hydrochloride*.—4-Benzyloxy-3-methoxyphenylaceto[^{14}C]nitrile [28] (99 mg; *ca.* 2 mCi) in anhydrous tetrahydrofuran was stirred under nitrogen with sodium borohydride (240 mg), and boron trifluoride–ether complex (1.35 ml; redistilled from calcium hydride) was injected. The mixture was heated under reflux for 5 h, then water and 2N-hydrochloric acid were added, and the tetrahydrofuran was evaporated off. After the solution had been extracted with dichloromethane, it was basified and re-extracted to give basic material, which was converted into the hydrochloride (94 mg) in ether with ethereal hydrogen chloride. The two ether protecting groups were cleaved as above in similar yield to give the [^{14}C]dopamine hydrochloride.

(2RS)-2-(3,4-*Dihydroxyphenyl*)[2-$^3H_1$]*ethylamine Hydrochloride* (14 *and* 15).—Magnesium (60 mg) was dissolved in anhydrous methanol (10 ml) and the residue from evaporation was treated with a solution of 4-benzyloxy-3-methoxyphenylacetonitrile (150 mg) in dry tetrahydrofuran (5 ml), followed by tritiated water (50 mg) in tetrahydrofuran (5 ml). After the mixture had been heated under reflux for 25 h, it was diluted with water and extracted with chloroform (4 × 15 ml) to give the labelled nitrile (143 mg; 46 mCi $mmol^{-1}$). This was then converted into (2*RS*)-labelled dopamine as in the previous experiment.

(3S)-3-(4-*Hydroxy*-3-*methoxyphenyl*)[3-$^2H_1$]*propionic Acid* (16).—A solution of 4-benzyloxy-3-methoxy[*formyl*-^{2}H]-benzaldehyde [11] (2.29 g; 98% $^2H_1$) in tetrahydrofuran was heated under reflux for 1.5 h with 5N-hydrochloric acid (100 ml). Extraction with ethyl acetate (4 × 25 ml) gave the phenolic aldehyde, which was re-extracted from the organic solution into 2N-sodium hydroxide (4 × 200 ml). After acidification of the aqueous solutions and extraction with ethyl acetate, the phenolic aldehyde was chromatographed on silica gel with chloroform. The pure product was converted into its *O*-methylsulphonyl derivative as earlier (63% overall; 98% $^2H_1$), m.p. 88—90°.

This product was then treated exactly as for the preparation of the ^{3}H-labelled material in order to obtain the (3*S*)-labelled propionic acid (16).

(R)-3-*Methoxy*-4-*methylsulphonyloxy*[methylene-$^2H_1$]-*benzyl Alcohol* [*as* (7a), D *in place of* T].—A solution of cyclohexanone (4 g) in water (100 ml) containing 2N-sodium hydroxide (6.5 ml) was treated with $NaBD_4$ (488 mg) and stirred for 3 h. After addition of a slight excess of glacial acetic acid, the solution was saturated with sodium chloride and extracted with ether (4 × 10 ml). The extracts were washed with saturated aqueous sodium hydrogen carbonate and brine, then dried and evaporated. Distillation of the residue gave [1-^{2}H]cyclohexanol (2.78 g); 91% $^2H_1$.

A solution of the aldehyde (5) (1.41 g) and [1-^{2}H]cyclohexanol (2.41 ml) in dioxan (65 ml) was diluted with pH 8 0.002M-phosphate buffer (1 330 ml). After addition of NAD^+ (300 mg), alcohol dehydrogenase (35 mg; Sigma; ethanol-free) was added and the mixture was incubated at 37 °C for 1.5 h, three further additions of alcohol dehydrogenase (25 mg) being made at 1.5 h intervals. After 6 h, the solution was saturated with sodium chloride and extracted with chloroform (4 × 250 ml) to give an oil which was purified by p.l.c. in ether. The resultant alcohol (1.09 g) had a deuterium content of 0.90 atom mol^{-1}.

(3R)-3-(4-*Hydroxy*-3-*methoxyphenyl*)[3-$^2H_1$]*propionic Acid*.—The foregoing alcohol was carried through exactly the same sequence of reactions used to prepare the ^{3}H analogue, to give the (3*R*)-labelled propionic acid (17).

Ozonolysis of (3S)- *and* (3R)-3-(4-*Hydroxy*-3-*methoxyphenyl*)[3-$^2H_1$]*Propionic Acids* [(16) *and* (17)].—Ozonised oxygen (*ca.* 5%; 20 l h^{-1}) was bubbled through a solution of the (3*S*)-acid (200 mg) in AnalaR methanol (80 ml) for 18 h. The solution was purged with nitrogen and evaporated, and the residue was heated under reflux with formic acid (12 ml) and AnalaR hydrogen peroxide (100 mol; 6 ml) for 3 h. The residue from evaporation was extracted with hot ethyl acetate, which after filtration and concentration yielded crystalline succinic acid. This was purified by recrystallisation from ethyl acetate, sublimation at 135° and 0.1 mmHg and further recrystallisation to yield pure (2*S*)-[2-$^2H_1$]succinic acid (37%), m.p. 184—186°. The (3*R*)-propionic acid was degraded in the same way.

Succinic anhydride was prepared from the labelled succinic acids on a micro-scale by using acetyl chloride, and was assayed for deuterium content by mass spectrometry.[29] In addition, Professor J. W. Cornforth and Dr. G. Ryback kindly converted portions of the two succinic acids with ethereal diazomethane into the dimethyl esters, and determined the deuterium content with their mass spectrometer. The combined results (average of 6—8 determinations in each case) were: (2*S*)-acid 0.97 (on anhydride), 0.98 (on dimethyl ester) atoms D per mole; (2*R*)-acid 0.89 (on anhydride), 0.93 (on dimethyl ester) atoms D per mole. We regard these values as safely accurate to ±3%.

[28] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.

[29] G. Popjak, D. S. Goodman, J. W. Cornforth, R. H. Cornforth, and R. Ryhage, *J. Biol. Chem.*, 1961, **261**, 1934.

O.r.d. measurements on the (2*S*)-isomer (8.07 mg) in water (257 mg) and the (2*R*)-isomer (10.295 mg) in water (284 mg) are given in Table 2. The enantiomeric purities are reported after correction for the ^2H content; essentially both samples were enantiomerically pure.

TABLE 2

O.r.d. measurements on the succinic acids

λ/nm	Standard [18] (2*S*)-isomer [α] (°)	(2*S*)-Isomer [α] (°)	(2*R*)-Isomer [α] (°)
244	+26.2	+25.8	−24.4
250	+18.2	+17.9	−17.3
256	+13.0	+12.9	−12.6
263	+10.1	+10.0	−9.6
270	+7.9	+7.9	−7.5
278	+6.3	+6.1	−6.0
286	+5.1	+4.9	−4.9
294	+4.1	+4.0	−4.0
303	+3.4	+3.3	−3.3
313	+2.7	+2.8	−2.7
323	+2.3	+2.2	−2.3
333	+1.9	+1.9	−2.0
Enantiomeric purity		100 ± 5%	104 ± 5%

Enzymic Conversion of (2R)-, (2S)-, *and* (2RS)-[$^3H_1$]-*Dopamines* [(14), (15), *and* (14 *and* 15)] *into Noradrenaline* (2).—Ten adrenal glands, obtained from freshly slaughtered animals and immediately cooled to 0 °C until use 1—2 h later, were defatted and the medullae were dissected. These were homogenised at 'high speed' for 20 s in a Waring blender with pH 6.5 0.1M-phosphate buffer (100 ml) at 4 °C and the mixture was stored at 4 °C. To this suspension (*ca.* 40 ml) were added fumaric acid (42 mmol), ascorbic acid (0.42 mmol), proniazid phosphate (0.84 mmol), Triton X-100 (0.084% w/v), *p*-hydroxymercuribenzoate (33 μmol), and ethanol (0.75%) in amounts to give the concentrations indicated in parentheses. After addition of catalase (830 units), the appropriate sample of [2-$^3H_1$,1-^{14}C]-dopamine was added (*ca.* 1 mg) and the mixture was incubated at 37 °C for 0.5 h. Radio-inactive noradrenaline hydrogen tartrate (37 mg) and dopamine hydrochloride (25 mg) were then added, the mixture was centrifuged, and the pellet was washed and discarded. Sufficient sodium carbonate was added to the combined solutions to give a 1M-solution, which was immediately shaken with an excess of isobutyric anhydride. The two isobutyryl derivatives (see below) were extracted with chloroform and separated by p.l.c. on silica with ether. After the tri-isobutyryl-noradrenaline had been diluted with more radio-inactive tri-isobutyryldopamine, it was rechromatographed and this procedure was repeated until the noradrenaline derivative showed constant specific activity and ^3H : ^{14}C ratio. The results are collected in Table 1.

NOO-*Tri-isobutyryldopamine.*—This *derivative* was prepared by standard acylation of the phenolic base as in the foregoing experiment; m.p. 110—111° (from ethyl acetate–hexane) (Found: C, 66.2; H, 8.2; N, 3.9. $C_{20}H_{29}NO_5$ requires C, 66.1; H, 8.05; N, 3.85%); $\nu_{max.}$ 3 320, 1 756, 1 643, and 1 542 cm^{-1}; τ 3.0 (3 H, m, ArH), 4.38br (1 H, NH), 6.53 (2 H, m, CH_2N), 7.21 (4 H, m, $ArCH_2$ and 2 CH), 7.71 (1 H, septet, *J* 6 Hz, CH), and 8.73 and 8.91 (12 H and 6 H, d, *J* 6 Hz, Me); *m/e* 363 (M^+, 5%), 293 (5), 276 (6), 237 (9), 223 (5), 206 (50), and 136 (100).

NOO-*Tri-isobutyrylnoradrenaline.*—This *derivative* was prepared similarly; m.p. 105—107° (from ethyl acetate–hexane) (Found: C, 63.1; H, 7.6; N, 3.7. $C_{20}H_{29}NO_6$ requires C, 63.3; H, 7.7; N, 3.7%); $\nu_{max.}$ 3 350, 3 290, 1 765, 1 660, and 1 622 cm^{-1}; τ 2.87 (3 H, m, ArH), 3.86br (1 H, NH), 5.26 (1 H, m, ArCH), 6.11 (1 H, s, OH), 6.40 and 6.80 (1 H, each, m, CHN), 7.23 and 7.66 (2 H and 1 H, septets, *J* 6 Hz, CH), and 8.75 and 8.90 (12 H and 6 H, d, *J* 6 Hz, Me); *m/e* 379 (M^+, 5%), 362 (1), 292 (10), 221 (16), 152 (13), 139 (16), and 101 (100).

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