Studies of Enzyme-mediated Reactions. Part 12.[1] Stereochemical Course of the Decarboxylation of (2*S*)-Tyrosine to Tyramine by Microbial, Mammalian, and Plant Systems

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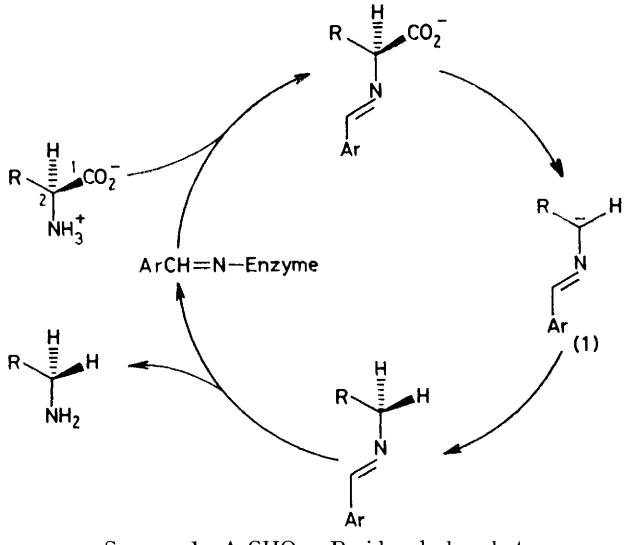
Tyrosine is proved to be decarboxylated with retention of configuration by three aromatic amino-acid decarboxylases of bacterial, mammalian, and plant origin by experiments based on the synthesis of (α*S*)-[α-^{3}H]tyrosine and the (α*R*)- and (α*S*)-isomers of [α-$^3H_1$]tyramine. Incubation of the labelled tyramines with the diamine oxidase from pea seedlings and the monoamine oxidase from rat liver shows that the former enzyme removes H_{Si} and the latter H_{Re}. These amine oxidases are used to assay the chirality of the [α-$^3H_1$]tyramines produced by incubation of suitably labelled tyrosines in tritiated water or in unlabelled water with the partially purified decarboxylases from *Streptococcus faecalis* and hog kidney. The plant enzyme was studied by administration of (α*S*)-[α-^{3}H,U-^{14}C]-tyrosine to intact *Papaver somniferum* plants followed by isolation of the resultant papaverine, which retained no tritium; morphine was also isolated to act as an internal standard.

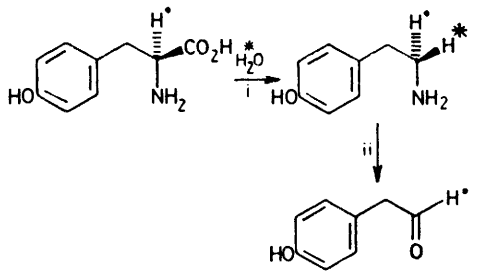
EARLIER work in this series has been concerned with a number of metabolic reactions in which one hydrogen is removed enzymically from a methylene group adjacent to nitrogen. This paper is the first of several in which reactions are studied where a methylene group is generated adjacent to nitrogen by enzymic decarboxylation of an α-amino-acid. The aim is to determine the stereochemistry of the replacement process (hydrogen for carboxy) for several examples in a variety of living systems to discover whether or not there is a unified pattern of behaviour.

Most amino-acid decarboxylases rely on pyridoxal phosphate as co-factor.[2,3] For a number of amino-acids it is known that the hydrogen at C-2 of the starting material is retained in the product,[4] as would be expected for a cyclic mechanism of the type proposed independently by Westheimer [4] and by Snell *et al.*[5] (Scheme 1). The key step from the stereochemical point of view is that in which a proton is added to the intermediate (1) produced by loss of carbon dioxide. In principle this could take place from above or below the illustrated planar system (the carbanion is delocalised over the π-system of the pyridoxal phosphate residue) and the outcome will be dictated by the nature of the surrounding catalytic groups.[6]



SCHEME 1 ArCHO ≡ Pyridoxal phosphate

Our initial work was concerned with the stereochemistry of decarboxylation of tyrosine and progresses



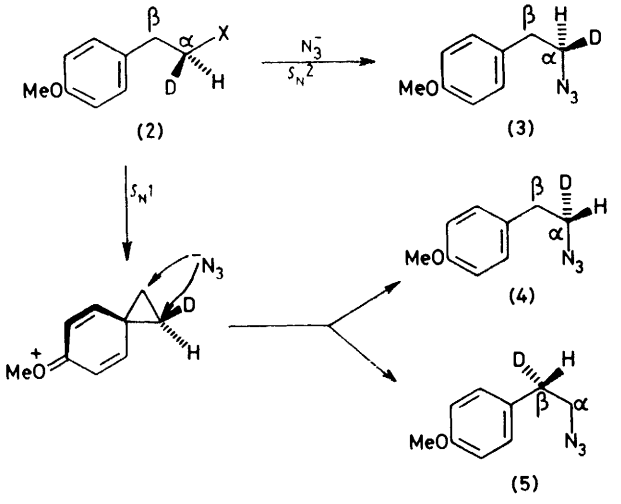
SCHEME 2 *Reagents:* i, tyrosine decarboxylase; ii, rat liver amine oxidase

from the pioneering investigation by Belleau and Burba [7,8] who incubated the enzyme from *Streptococcus faecalis* (E.C. 4.1.1.25) with [α-^{2}H]tyrosine in H_2O and with unlabelled tyrosine in D_2O to obtain two complementary labelled samples of [α-$^2H_1$]tyramine. The stereochemistry of labelling at C_α of the products was then determined by incubating the labelled tyramines with an amine oxidase from rat liver and comparing the relative rates of oxidation with those obtained in parallel control experiments carried out on samples of chirally-labelled tyramine produced synthetically (Scheme 2). The results were consistent with retention of configuration in the decarboxylation step. However, the methods available at the time of these important experiments could not reveal the degree of stereospecificity.

We have now re-investigated this problem using the more penetrating analytical techniques which have been developed recently for determining the stereochemistry of labelling at methylene groups adjacent to nitrogen.[9,10] The work has also been extended to cover tyrosine decarboxylases present in mammalian and plant systems as well as the microbiological source.

It seemed desirable in the light of our earlier ex-

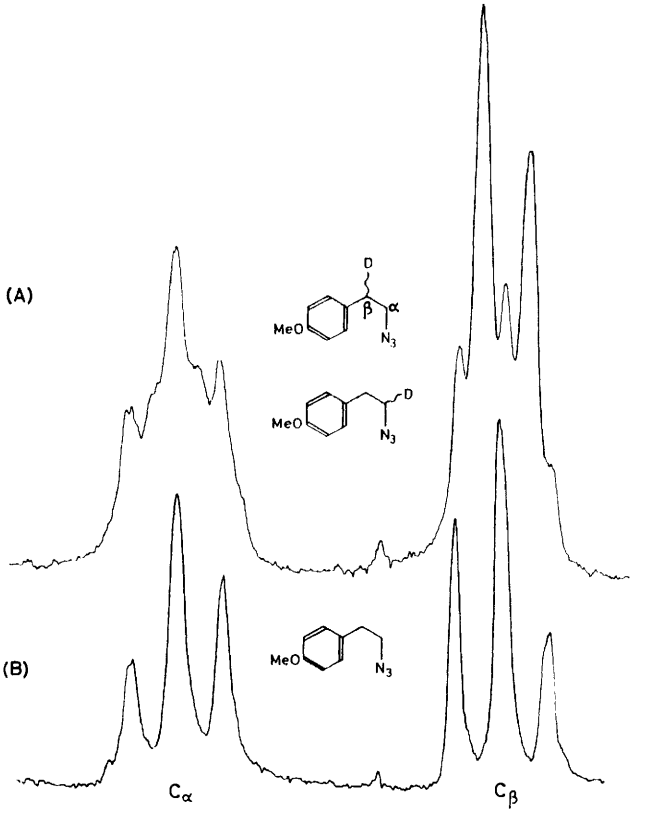
perience [11] to re-investigate the methods used for the synthesis of tyramines chirally labelled at C_α. Clearly, the validity of the approach hinges on the reliability of the chemical synthesis. The key step for the earlier



route [7,8] was the nucleophilic displacement of a leaving group by azide at a chiral centre β to an aryl substituent in the synthetic intermediate (2). The product, assuming a straightforward S_N2 mechanism, would be (3). However, there is a risk that the aryl ring will participate in the process leading to a two-stage reaction in which equal amounts of (4) and (5) will be formed instead of (3). A small percentage of such aryl participation would do no more than lower the configurational purity of the resultant tyramine at the crucial C_α position. Serious aryl participation would, however, cause obvious problems. This possibility was checked by synthesising the racemic tosylate corresponding to the (α*S*)-form (2; X = OTs) and converting it into the azide under conditions designed to suppress the S_N1 reaction. The ^1H n.m.r. spectrum of the product (Figure) showed the presence of deuterium at C_β: thus, the signal for that position consisted of a triplet superimposed on the expected doublet whilst the signal for C_α showed a corresponding doublet superimposed on the normal triplet. In contrast, the starting tosylate [as (2; X = OTs)] showed no evidence in its ^1H n.m.r. spectrum of scrambling of the label, so even under conditions designed to promote an S_N2 reaction, the alternative pathway has competed to an appreciable extent (roughly 30% of the product was calculated to be produced by aryl participation). Assuming a similar balance between the two competing pathways in Belleau and Burba's synthesis, it eventuates that their conclusion that *S. faecalis* tyrosine decarboxylase operates largely with retention of configuration is unaffected. The present work on this enzyme thus aimed at confirmation of this by a different approach and, further, at a determination of the degree of stereospecificity of the enzymic action.

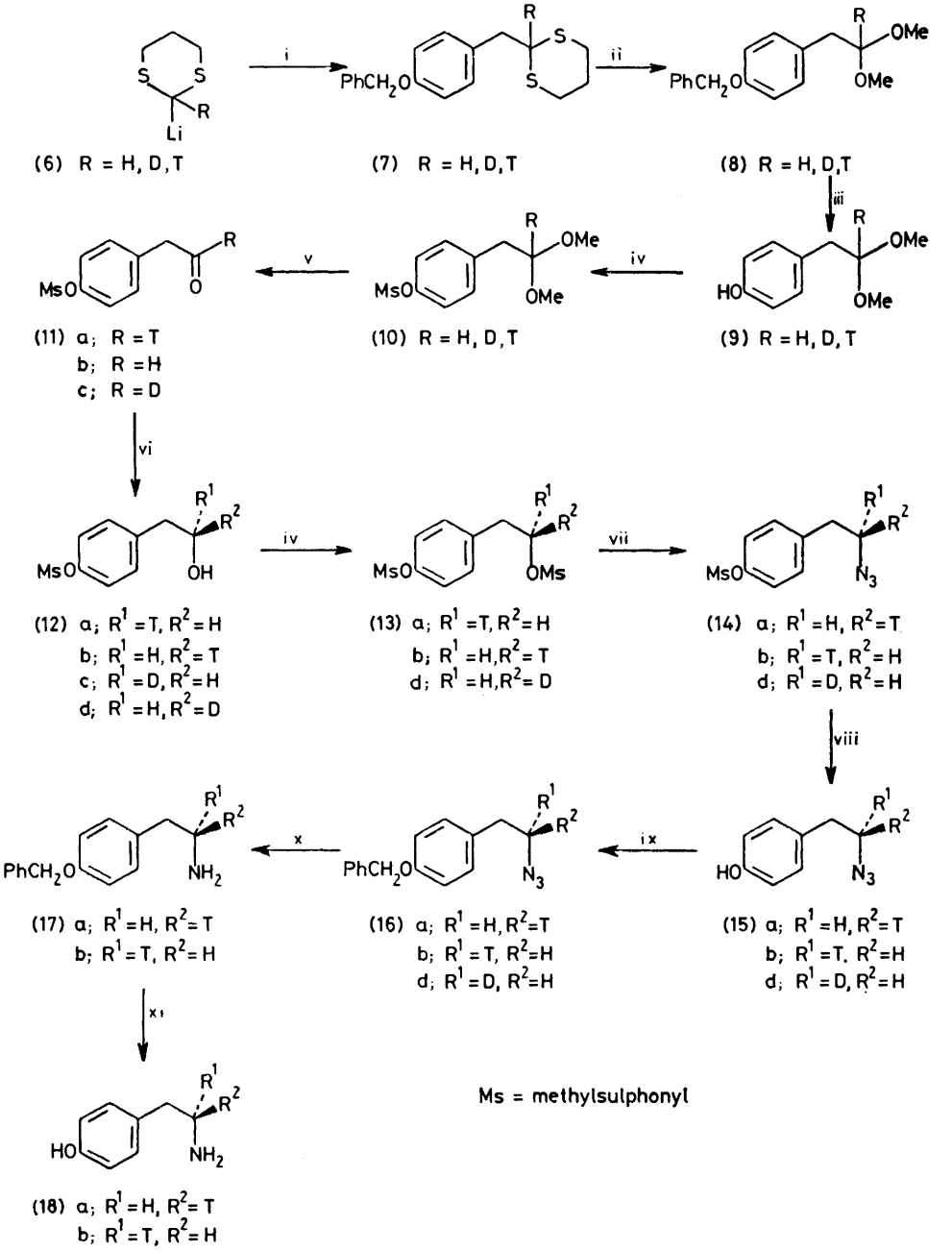
It had been shown earlier that the problem of aryl participation can be overcome by using an electron-withdrawing sulphonate group in place of an electron-donating ether group to protect the phenolic hydroxy-group in the key displacement step.[11] This tactic also proved successful in the present synthesis of chirally-labelled tyramines (Scheme 3). In essence, steps (i)—(v) provide a convenient synthesis of a substituted phenylacetaldehyde which is readily adaptable to produce compounds labelled with a hydrogen isotope in the aldehydic group. This intermediate is then reduced stereospecifically by transfer of hydrogen from ethanol (or another alcohol) catalysed by NAD^+ in the presence of liver alcohol dehydrogenase.[12] By appropriate choice of labelled aldehyde (11) and alcohol, all four chiral alcohols of general structure (12) can be prepared. Steps (vi)—(x) are designed to replace the hydroxy-group by an amino-group stereospecifically with the protecting group being finally removed to yield tyramine.

The use of different protecting groups at the various stages of this synthesis deserves comment. A benzyl protecting group is used in step (i) because a methanesulphonyl group would be unstable to strong base. The benzyl group must be replaced by a methanesulphonyl group prior to the displacement stage (step vii). Fortunately the sulphonyl-protected aldehydes (11) are acceptable substrates for liver alcohol dehydrogenase and so the replacement can be carried out conveniently at an early stage. With the formation of the azide in step (viii), the chiral centre is configurationally stable and so it is now safe to return to an *O*-benzyl protecting group for the hydride reduction step.



^1H N.m.r. signals from the side-chain of illustrated azides in C_6D_6 (A) deuteriated series, (B) unlabelled series

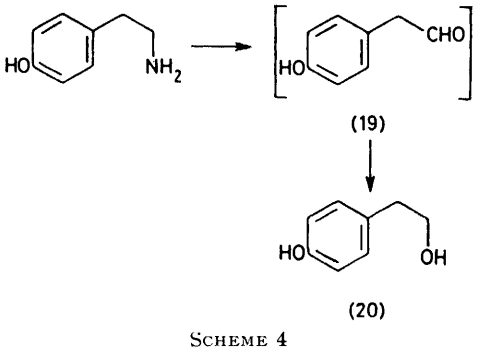
The reliability of this synthetic approach was checked as before by experiments with materials labelled with deuterium. This was achieved by reducing the unlabelled aldehyde (11) by transfer of deuterium from [1-^{2}H]cyclopentanol mediated by a catalytic quantity of NAD^+ in the presence of liver alcohol dehydrogenase. The chirally-labelled product (12d) was converted into the corresponding azide (14d), the ^{1}H n.m.r. spectrum of which was consistent with complete retention of the label at C_α. Thus, a small triplet was superimposed on the doublet for the signal from C_β but there was no matching triplet signal from C_α. The triplet was therefore attributed to the presence of unlabelled molecules (*ca.* 10% of the total) produced by the enzyme-mediated reduction. The high percentage of unlabelled molecules was unexpected because the labelled cyclopentanol used as reducing agent was >99% deuteriated at the carbinol carbon. Clearly an alternative source of reducing power is competing in the enzymic reduction step. We suspect either a small amount of contamination by ethanol in the commercial enzyme used or possibly the aldehyde itself is being partly oxidised to the acid in the



SCHEME 3 *Reagents:* i, *p*-Benzyloxybenzyl chloride; ii, HgO–$HgCl_2$, MeOH–MeCN; iii, Raney nickel; iv, $MeSO_2Cl$, Et_3N; v, H_3O^+, THF; vi, liver alcohol dehydrogenase, NAD^+, and ethanol or labelled cyclopentanol; vii, NaN_3; viii, H_2O–NaOH; ix, $PhCH_2Cl$, K_2CO_3 in DMF; x, $LiAlH_4$; xi, Pd–C, H_2

type of dismutation process described previously.[1] Whatever the explanation for the source of protium, the main point to be settled at this stage was quite clear: use of the methanesulphonyl protecting group on the phenolic hydroxy-group suppresses aryl participation, and so a reliable method was available for synthesising tyramines stereospecifically labelled at C-1.

Tritium is a more convenient tracer than deuterium for the planned incubation studies. The two [α-$^3H_1$]-tyramines (18a and b) were therefore prepared according to the route in Scheme 3. The preparation of the tritio-aldehyde (11a) followed that of the deuterio-analogue from the suitably tritium-labelled dithian (6). The reduction step (vi) was carried out by hydrogen transfer from ethanol. The product was then converted in subsequent steps into (1*R*)-[α-$^3H_1$]tyramine (18a). The enantiomeric (α*S*)-tyramine (18b) was prepared by reducing the unlabelled aldehyde (11b) by tritium transfer from [1-^{3}H]cyclopentanol to give the alcohol (12b). Subsequent steps to (18b) are strictly complementary to those



SCHEME 4

leading to (18a) and so the final products should have the same degree of configurational purity. Indeed the subsequent incubation studies showed that each of the tyramines was essentially free of its enantiomer.

The foregoing (α*R*)- and (α*S*)-[α-$^3H_1$]tyramines (18a) and (18b) were then used as standards to examine by radiochemical methods the stereospecificity of tyrosine decarboxylases. The most convenient assay of configuration involves incubation of the primary amine with the diamine oxidase (DAO) from pea seedlings (E.C. 1.4.3.6). This was carried out by the now standard method (Scheme 4) in which the initially produced aldehyde (19) is reduced as it is formed to tyrosol (20) [2-(4-hydroxyphenyl)ethanol] by added liver alcohol dehydrogenase, NAD^+, and a large excess of ethanol as hydride source.[9,10] With DAO, the turnover of tyramine is sufficiently high for the product to be isolated without dilution and so the measure of isotopic retention could be determined simply by comparing the specific activity of the alcohol (20) with that of the starting amine. Both the amine and the alcohol were converted into radiochemically pure crystalline dibenzoate derivatives prior to counting. The results in Table 1 show that DAO abstracts specifically the (*Si*)-hydrogen from the α-methylene group of tyramine as it does in every primary amine tested so far.[9,10,13] The values also

TABLE 1

Incubation of [α-$^3H_1$]tyramines with DAO and MAO

Expt. no.	Enzyme	Substrate	Specific activity: Substrate [a]	Specific activity: Tyrosol [a]	% Retention of ^{3}H in tyrosol
1	DAO	(α*S*)-Isomer (18b)	1.92	0.01	0.6
2	DAO	(α*S*)-Isomer (18b)	1.84	<0.01	<0.5
3	MAO	(α*S*)-Isomer (18b)	5.8	5.3	91
4	MAO	(α*S*)-Isomer (18b)	5.8	5.1	88
5	DAO	(α*R*)-Isomer (18a)	1.65	1.58	95
6	DAO	(α*R*)-Isomer (18a)	1.66	1.55	93
7	MAO	(α*R*)-Isomer (18a)	5.1	<0.01	<0.02
8	MAO	(α*R*)-Isomer (18a)	5.1	<0.01	<0.02

[a] Radio-assay as dibenzoyl derivative; μCi mmol^{-1}.

confirm that the modified synthesis described above produces amines of high configurational purity. Interestingly, however, there appears to be a significant loss of label from the (α*R*)-[α-$^3H_1$]tyramine (18a) (Experiments 5 and 6). That this was not due to a lack of configurational purity in the amine was established by the complementary incubation experiments (Experiments 7 and 8) described below using a monoamine oxidase (MAO) isolated from rat liver.

Trial experiments showed that with MAO (E.C. 1.4.3.4) the turnover of tyramine was not sufficiently high to allow the product alcohol to be isolated directly. It was therefore extracted after dilution with inactive material and the tritium retention was measured against an internal ^{14}C-standard which had been added at the outset. The results from Experiments 3, 4, 7, and 8 in Table 1 demonstrate (*a*) that MAO specifically removes the (*Re*)-hydrogen from C_α of tyramine, in agreement with earlier results,[7,8] and (*b*) that the synthetic amines are essentially pure enantiomers. Again however the (α*R*)-amine (18a), which should have led to complete retention of tritium, showed instead a small but significant loss and similar examples appear in later Tables.

We have not yet been able to account for this loss (but *cf.* ref. 13). The unchanged tyramine after treatment with MAO (Experiment 4, Table 1) showed no appreciable change in the $^3H : ^{14}C$ ratio on re-isolation. When the aqueous medium from the incubation was recovered by distillation *in vacuo* it was found to contain *ca.* 30% of the tritium originally present in tyramine, a much higher amount than could be accounted for on the basis of the tyrosol produced. Apparently the MAO preparation used contained other enzymes which metabolise tyramine in some way to release tritium.

Fortunately none of these side reactions affect any of the conclusions reached above. We could thus move ahead with confidence to use the DAO from pea seedlings and MAO from rat liver as enzymes of rigorously-established stereospecificity to assay the tyramine samples produced by decarboxylation of tyrosine. Two tyrosine decarboxylases were studied.

Our first candidate was that examined earlier,[2,8] *viz.* the enzyme from *S. faecalis*. This is available commercially as an acetone powder which could be used without further purification. A typical incubation

TABLE 2

Decarboxylation of labelled tyrosines and configurational assay of labelled tyramines

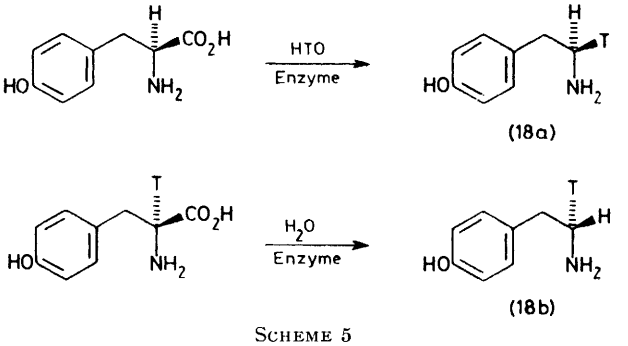
Expt. no.	Substrate for enzyme	Source of enzyme and medium	Tyramine [a] ^3H : ^{14}C ratio	Tyrosol [a] ^3H : ^{14}C ratio (% ^3H-retention)
9	(αS)-[α-^{3}H,β-^{14}C]Tyrosine	*S. faecalis*, H_2O	10.3	0.07 [b] (0.7)
10	(αS)-[α-^{3}H,β-^{14}C]Tyrosine	*S. faecalis*, H_2O	8.2	0.03 [b] (0.4)
11	(αS)-[β-^{14}C]Tyrosine	*S. faecalis*, HTO	1.19	1.15 [b] (97)
12	(αS)-[β-^{14}C]Tyrosine	*S. faecalis*, HTO	21.4	20.4 [b] (95)
13	(αS)-[α-^{3}H,β-^{14}C]Tyrosine	Kidney, H_2O	7.7	0.04 [b] (0.5)
14	(αS)-[α-^{3}H,β-^{14}C]Tyrosine	Kidney, H_2O	9.1	7.0 [c] (77)

[a] Radio-assay as dibenzoyl derivative. [b] Stereochemical assay by pea seedling DAO. [c] Stereochemical assay by rat liver MAO.

contained tyrosine and the enzyme in 0.2M-acetate buffer (pH 5.5) at 30 °C. After 3 h, the tyramine was isolated by ion exchange and was purified as its hydrochloride salt. A portion was then converted into the dibenzoate for radiochemical assay, the rest being subjected to the stereochemical assay using pea seedling DAO. For convenience, the tritium was measured with respect to an internal ^{14}C-standard. Two complementary sets of incubation conditions were used which should lead to tyramines having opposite chirality at C_α. In one, (αS)-[β-^{14}C]tyrosine was incubated in tritiated water; in the other (αS)-[α-^{3}H,β-^{14}C]tyrosine was incubated in unlabelled water.

The [α-^{3}H]tyrosine required for these experiments was prepared by exchange on the related azlactone derived from *ON*-diacetyltyrosine (see Experimental section). After mixing with a suitable quantity of commercially available [β-^{14}C]tyrosine, the amino-acid was resolved prior to incubation with the decarboxylase. The results in Table 2 (Experiments 9—12) show that the decarboxylation is stereospecific, within experimental error. They also confirm the earlier conclusion [7,8] that it is the (*Re*)-hydrogen of the product tyramine which is added from the medium corresponding to *retention of configuration* (Scheme 5).

It has been shown [4] that certain amino-acid decarboxylases will bring about exchange with the medium of one of the two enantiotopic hydrogens on the carbon adjacent to the nitrogen of the amine which is their normal product. We have studied the stereochemistry of this exchange for tyramine using the enzyme from *S. faecalis*. The labelled tyramine produced by incubation in tritiated water was subjected to stereochemical assay with both pea seedling DAO and MAO; in the latter case, an internal reference of ^{14}C was used. The results in Table 3 confirm the conclusion of Mandeles *et al.*[4] that the exchange is stereospecific and further they show that the hydrogen which undergoes exchange is H_{Re}, the same as that introduced in the normal decarboxylation reaction. It seems probable therefore that this exchange takes place at the active site for decarboxylation by a partial reversal of the final step of the decarboxylation process.



SCHEME 5

We next wished to compare the results for the foregoing bacterial enzyme with a tyrosine decarboxylase of mammalian origin. An acetone powder derived from hog kidneys was partially purified by extraction, centrifugation, dialysis, and osmotic concentration. It was then incubated with (αS)-[α-^{3}H,β-^{14}C]tyrosine in unlabelled water (in this case the turnover was too low for the complementary experiment in tritiated water to give an experimentally useful incorporation of tritium). The resultant tyramine was assayed by both pea seedling DAO and rat liver MAO. The results in Table 2 (Experiments 13 and 14) establish that (αS)-[α-$^3H_1$]-

TABLE 3

Stereochemical assay of [α-$^3H_1$]tyramine produced by exchange with HTO in the presence of the decarboxylase from *S. faecalis*

Expt. no.	Tyramine: μCi mmol^{-1} (^{3}H)	Tyramine: ^3H : ^{14}C ratio	Assay	Tyrosol: μCi mol^{-1} (% ^{3}H retention)	Tyrosol: ^3H : ^{14}C ratio
15	27.4		DAO	25.2 (92)	
16	57.0		DAO	54.0 (95)	
17		3.0	MAO		0.01 (0.3)

TABLE 4

Administration of (αS)-[α-^{3}H,U-^{14}C]tyrosine to *P. somniferum* plants

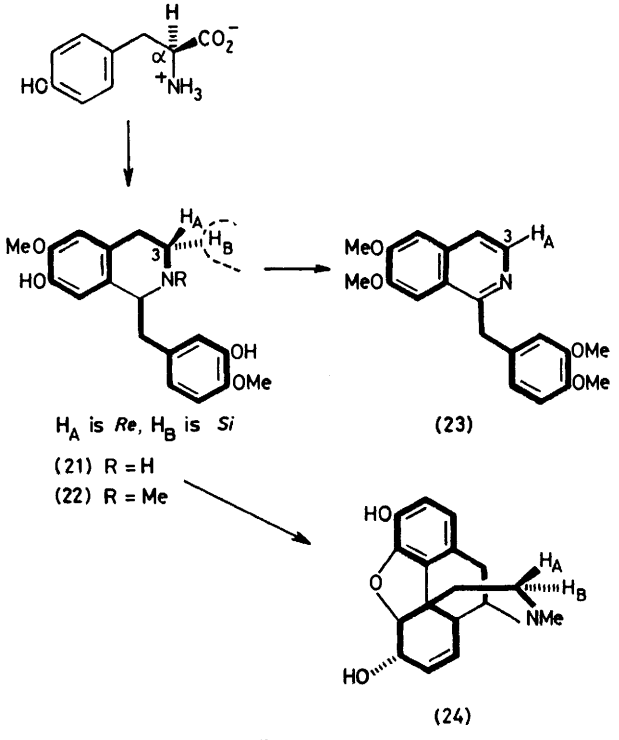
		Papaverine (23)			Morphine (24)		
Expt. no.	Tyrosine ^{3}H : ^{14}C ratio	% Incorporation (^{14}C)	^{3}H : ^{14}C ratio	Retention ^{3}H (%)	% Incorporation (^{14}C)	^{3}H : ^{14}C ratio	Retention ^{3}H (%)
18	10.1	0.26	0	0			
19	5.8	0.22	0	0	*b*	3.0	46 [a]

[a] Corrected for the loss of 1/9 of the labelled carbons from both C_6–C_2 units. [b] Incorporation into morphine is normally higher than into papaverine; here accidental loss of most of the morphine fraction reduced the value to 0.02%.

tyramine (18b) was produced and therefore that decarboxylation again takes place with retention of configuration (see Scheme 5).

Finally, a higher plant, the opium poppy (*Papaver somniferum*), was used for the third study. The L-aromatic amino-acid decarboxylase has not been isolated in this case but it was possible to determine the stereochemistry of its action by means of incorporation experiments carried out on whole plants. Thus, ^{14}C-labelled tyrosine has been shown to be incorporated specifically into both C_6–C_2 units of the benzylisoquinoline alkaloids papaverine [14] (23) and morphine [15,16] (24) and both units are labelled to the same extent. Nor-reticuline (21) is an established intermediate for the biosynthesis of both papaverine [17,18] and morphine [19] and its conversion into papaverine involves stereospecific loss of the (*Si*)-hydrogen (H_B) at C-3 of the heterocyclic ring.[20] Therefore the stereochemistry of the decarboxylation step,* which occurs early in the biosynthetic pathway, can be discovered by determining whether the α-hydrogen of the amino-acid is retained at C-3 in the phenethylamine residue of papaverine (it is necessarily lost from the second C_6–C_2 unit which is incorporated *via* an intermediate phenylpyruvate derivative [22,23]). This was achieved by incorporation studies with (αS)-[α-^{3}H,U-^{14}C]tyrosine. If the decarboxylation step proceeds with inversion of configuration, the ^{3}H : ^{14}C ratio in the papaverine should be somewhat over half (see note *a*, Table 4) that in the amino-acid administered to the plant; decarboxylation with retention should lead to complete loss of tritium.

There are hidden dangers in the use of intact plants because the administered tyrosine and subsequent biosynthetic intermediates will be exposed to many enzymes other than those involved in the steps considered above. Of particular concern is the possibility that the amino-acid might be stripped of its hydrogen label at C_α prior to the decarboxylation step by the reversible deamination–amination process which connects amino-acids and the corresponding pyruvic acids. Fortunately it was possible to detect whether there had been such a spurious loss of tritium by isolating the morphine (24), which is formed in the same plants, along with papaverine (23), from the same precursor (21) *via* reticuline (22) [24] but without loss of hydrogen from C_β. The corrected isotopic ratio in the morphine should therefore be half that in the amino-acid and so it provides a convenient basis for comparison with the ratio of papaverine. The results in Table 4 show that the hydrogen at C_α of tyrosine is not retained in the phenethylamine unit of papaverine but that it is largely retained in the equivalent unit of morphine. It can be concluded, therefore, that the aromatic amino-acid decarboxylase in *P. somniferum* brings about decarboxylation with retention of configuration.



SCHEME 6

The work described in this paper thus demonstrates a stereochemical uniformity of action for three tyrosine decarboxylases from bacterial, mammalian, and plant sources. There is retention of configuration in each case and this is also the outcome for the two histidine decarboxylase enzymes covered in the following paper.[13]

EXPERIMENTAL

General directions are given in ref. 25.

2-(4-Methoxybenzyl)-1,3-dithian.—n-Butyl-lithium (31.6 ml; 1.84mM in hexane) was added dropwise to a stirred solution of 1,3-dithian (7.15 g) in tetrahydrofuran (190 ml) at −78 °C. The solution was allowed to warm to −23 °C

* Many earlier studies [15,21,22] on alkaloid biosynthesis have shown that tyrosine, tyramine, 3,4-dihydroxyphenylalanine (DOPA), and 3,4-dihydroxyphenethylamine form an interrelated network of metabolites. It is therefore conceivable that at least some of the decarboxylation process mediated by the aromatic amino-acid decarboxylase in the opium poppy occurs on DOPA.

and stirred for a further 1.5 h. 4-Methoxybenzyl chloride (9.32 g) was added and the solution allowed to stand at 0 °C for 14 h, then poured into ice–water (100 g) and extracted with chloroform (3 × 200 ml). The product from evaporation of the chloroform was purified by chromatography on silica gel with dichloromethane as eluant. The *dithian* crystallised on evaporation of the dichloromethane from ethyl acetate (9.1 g), m.p. 71—72° (Found: C, 60.1; H, 6.7; S, 27.0. $C_{12}H_{16}OS$ requires C, 60.0; H, 6.7; S, 26.7%); δ 7.14 (2 H, d, ArH, *J* 8 Hz), 6.95 (2 H, d, ArH, *J* 8 Hz), 4.2 (1 H, t, *J* 7 Hz, S–CH–S), 3.76 (3 H, s, MeO), 2.95 (2 H, d, *J* 7 Hz, $ArCH_2$), 2.82 (4 H, m, 2 × SCH_2), and 2.0 (2 H, m, C–CH_2–C); $\nu_{max.}$ 1 620, 1 590, and 1 520 cm^{-1}; *m/e* 240 (M^+, 5%), 121 (8), 119 (100), and 143 (5).

2-(4-*Methoxyphenyl*)-1-O-*tosylethane* [*as* (2; X = OTs)].—Ceric ammonium nitrate (13.8 g) was added to a stirred solution of the above dithian (1.5 g) in methanol–acetonitrile (75 ml; 3 : 1 v/v) at room temperature.[26] After 3 min, the colour of the ceric ion had been discharged, and the solution was poured into water (100 ml) and extracted with diethyl ether (3 × 200 ml). Evaporation of the ether gave a yellow oil (1.18 g); δ 7.17 (2 H, d, *J* 9 Hz, ArH), 6.8 (2 H, d, *J* 9 Hz, ArH), 4.48 [1 H, t, *J* 6 Hz, $CH(OMe)_2$], 3.75 (3 H, s, MeO), 3.38 [6 H, s, $CH(OMe)_2$], and 2.98 (2 H, s, $ArCH_2$).

2N-Aqueous hydrochloric acid (25 ml) was added to a stirred solution of this acetal (1.18 g) in tetrahydrofuran (25 ml) at room temperature. After 1 h, the solution was extracted with chloroform (3 × 150 ml). The aldehyde was recovered after evaporation as a yellow oil (925 mg); δ 9.7 (1 H, t, *J* 2.5 Hz, CHO), 7.13 (2 H, d, *J* 9 Hz, ArH), 6.87 (2 H, d, *J* 9 Hz, ArH), 3.77 (3 H, s, MeO), and 3.57 (2 H, d, *J* 2.5 Hz, $ArCH_2$); $\nu_{max.}$ 1 730, 1 640, 1 590, and 1 515 cm^{-1}.

Solid sodium borohydride (900 mg) was added slowly to a stirred solution of all this aldehyde in methanol (100 ml) at room temperature. After 10 min, the solution was acidified (hydrochloric acid) and evaporated to a white slurry, which was partitioned between diethyl ether (150 ml) and water (20 ml). Evaporation of the organic phase gave a yellow oil (930 mg); δ 7.07 (2 H, d, *J* 9 Hz, ArH), 6.76 (2 H, d, *J* 9 Hz, Ar*H*), 3.69 (3 H, s, MeO), 3.69 (2 H, t, *J* 6 Hz, CH_2OH), 2.73 (2 H, t, *J* 6 Hz, $ArCH_2$), and 2.73 (1 H, s, O*H*).

Tosyl chloride (2.4 g) was added in two batches over 5 min to a stirred solution of all this alcohol in pyridine (10 ml) at 0—10 °C. After 4 h at 0 °C, the solution was partitioned between diethyl ether (50 ml) and 3N-aqueous sulphuric acid (40 ml). The aqueous phase was extracted with diethyl ether (3 × 50 ml) and the combined organic phases yielded a yellow solid which was chromatographed on silica gel with dichloromethane as eluant. Evaporation of the eluate gave the crystalline tosyl derivative, (850 mg), m.p. 57.5—58.5° (from benzene–hexane) (lit.,[27] 58.6—59.6°) (Found: C, 62.8; H, 6.1; S, 10.5. Calc. for $C_{16}H_{18}O_4S$: C, 62.7; H, 5.9; S, 10.5%); δ 7.24 (2 H, d, *J* 8 Hz, MeAr*H*), 7.26 (2 H, d, *J* 8 Hz, MeAr*H*), 7.0 (2 H, d, *J* 9 Hz, MeOAr*H*), 6.75 (2 H, d, *J* 9 Hz, MeOAr*H*), 4.17 (2 H, t, *J* 7 Hz, CH_2OTs), 3.74 (3 H, s, MeO), 2.88 (2 H, t, *J* 7 Hz, $ArCH_2$), and 2.4 (3 H, s, *Me*Ar); $\nu_{max.}$ 1 620, 1 605, 1 590, and 1 520 cm^{-1}; *m/e* 326 (M^+, 5%), 134 (100), 121 (80), and 91 (30).

1-*Azido*-2-(4-*methoxyphenyl*)*ethane* [*as* (3)].—The above tosylate (300 mg) was added to a stirred solution of sodium azide (128 mg) in 7 : 3 methanol–water (1.45 ml) at 70 °C.[28] After 24 h, the cooled mixture was extracted with ether (3 × 50 ml) and the residue from the organic phase purified by preparative t.l.c. with benzene as eluant. A sample of the oil recovered from the plate was distilled for analysis, b.p. 95—98° at 0.7 mmHg (Found: C, 60.8; H, 6.4; N, 24.1%; M^+, 177.098 6. $C_9H_{11}ON_3$ requires C, 61.0; H, 6.25; N, 23.7%; *M*, 177.090 1). The remaining data were for the crude azide (130 mg); δ (C_6D_6) 6.81 (2 H, d, *J* 10 Hz, ArH), 6.65 (2 H, d, *J* 10 Hz, ArH), 3.21 (3 H, s, MeO), 2.92 (2 H, t, *J* 7.5 Hz, CH_2N_3), and 2.42 (2 H, t, *J* 7.5 Hz, $ArCH_2$); $\nu_{max.}$ 2 110, 1 615, 1 585, and 1 510 cm^{-1}; *m/e* 177 (M^+, 30%), 122 (20), 121 (100), and 91 (15).

[2-$^2H_1$]-2-(*Methoxybenzyl*)-1,3-*dithian*.—n-Butyl-lithium (6 ml; 1.84M in hexane) was added to a stirred solution of 2-(methoxybenzyl)-1,3-dithian (2.0 g) in tetrahydrofuran (30 ml) at −78 °C. After 1.5 h at −23 °C, deuterium oxide (1 ml; 95 atom % ^2H) was added and the mixture kept for 14 h at 0 °C.[29-31] The solution was poured into ice–water (10 g) and the mixture extracted with chloroform (3 × 50 ml). The crystalline residue from the chloroform (1.76 g) had m.p. 71—72°; δ 7.17 (2 H, d, *J* 8 Hz, ArH), 6.84 (2 H, d, *J* 8 Hz, ArH), 3.76 (3 H, s, MeO), 3.22 (0.25 H, t, *J* 6 Hz, S–CH–S), 2.8 (2 H, s, $ArCH_2$), 2.85 (4 H, m, 2 × SCH_2), and 2.0 (2 H, m, C–CH_2–C); $\nu_{max.}$ 1 620, 1 590, and 1 520 cm^{-1}; *m/e* 241 [M^+ (D), 20%], 240 [M^+ (H), 5], 120 (100), and 119 (30) (Found: M^+, 241.070 0. $C_{12}H_{15}DOS_2$ requires *M*, 241.070 4).

(1RS)-[1-$^2H_1$]-2-(4-*Methoxyphenyl*)-1-O-*tosylethane* [*as* (2)].—The tosylate was prepared from the above deuterio-1,3-dithian as for the unlabelled series (0.79 g), m.p. 57—58.5°; δ 7.64 (2 H, d, *J* 8 Hz, MeAr*H*), 7.26 (2 H, d, *J* 8 Hz, MeAr*H*), 7.0 (2 H, d, *J* 9 Hz, MeOAr*H*), 6.75 (2 H, d, *J* 9 Hz, MeOAr*H*), 4.16 (1.25 H, t, *J* 7 Hz, *CH*DOTs), 3.74 (3 H, s, MeO), 2.87 (2 H, d, *J* 7 Hz, CH_2), and 2.4 (3 H, s, CH_3Ar); $\nu_{max.}$ 1 620, 1 605, 1 590, and 1 520 cm^{-1}; *m/e* 327 [M^+ (D), 5%], 326 [M^+ (H), 1.5], 135 (80), 134 (50), 121 (100), and 91 (50) (Found: M^+, 307.099 6. $C_{16}H_{17}DO_4S$ requires *M*, 307.098 8).

(1RS)-[1-$^2H_1$]-1-*Azido*-2-(4-*methoxyphenyl*)*ethane* *and* (2RS)-[2-$^2H_1$]-1-*Azido*-2-(4-*methoxyphenyl*)*ethane* (4 + 5).—The mixture of deuterio-azides was prepared from the deuterio-tosylate as for the unlabelled series (344 mg), b.p. 92° at 0.6 mmHg; δ (C_6D_6) 6.84 (2 H, d, *J* 8 Hz, ArH), 6.78 (2 H, d, *J* 8 Hz, Ar*H*), 3.34 (3 H, s, MeO), 2.96 (1.37 H, m, *J* 7 Hz, $CHDN_3$), and 2.45 (1.88 H, m, partially deuteriated C-2 methylene); $\nu_{max.}$ 2 100, 1 615, 1 585, and 1 515 cm^{-1}; *m/e* 178 [M^+ (D), 30%], 177 [M^+ (H), 10], 122 (30), 121 (100), 92 (7), 91 (7), and 78 (30) (Found: M^+, 178.096 1. $C_9H_{12}DN_3O$ requires *M*, 178.096 4).

4-*Benzyloxybenzyl Chloride*.—A solution of 4-benzyloxybenzyl alcohol (3 g) [32] and redistilled thionyl chloride (9 ml) in diethyl ether (90 ml) was heated at reflux for 2 h. The solution was evaporated and the residue (3.1 g) crystallised from benzene–hexane, m.p. 77—79° (Found: C, 72.4; H, 5.6; Cl, 15.2. $C_{14}H_{13}ClO$ requires C, 72.4; H, 5.7; Cl, 15.1%); δ 7.32 (5 H, s, ArH), 7.24 (2 H, d, *J* 8 Hz, C_6H_4), 6.89 (2 H, d, *J* 8 Hz, C_6H_4), 4.98 (2 H, s, $ArCH_2$), and 4.67 (2 H, s, $ArCH_2$); $\nu_{max.}$ 1 615, 1 590, and 1 515 cm^{-1}; *m/e* 234 [M^+ (^{37}Cl), 30%], 232 [M^+ (^{35}Cl), 100], 197 (35), 91 (80), and 65 (40).

2-(4-*Benzyloxybenzyl*)-1,3-*dithian* (7).—n-Butyl-lithium (8.7 ml; 1.84M-solution in hexane) was added to a stirred solution of 1,3-dithian (1.92 g) in tetrahydrofuran (40 ml) at −78 °C. After stirring for 1½ h at −23 °C, a solution of

the foregoing benzyl chloride (4.11 g) in tetrahydrofuran (20 ml) was added.[29-31] The mixture was kept at 0 °C for a further 14 h, and then poured into water (50 ml) and extracted with chloroform (3 × 60 ml). The residue from the organic phase was chromatographed on silica gel using benzene as eluant. The recovered *dithian* crystallised from ethyl acetate, m.p. 94—96° (4.2 g) (Found: C, 68.1; H, 6.4; S, 19.9. $C_{18}H_{20}OS_2$ requires C, 68.3; H, 6.4; S, 20.25%); δ 7.38 (5 H, s, ArH), 7.16 (2 H, d, *J* 8 Hz, C_6H_4), 6.91 (2 H, d, *J* 8 Hz, C_6H_4), 5.02 (2 H, s, $ArCH_2O$), 4.20 (1 H, t, *J* 8 Hz, C*H*), 2.96 (2 H, d, *J* 8 Hz, $ArCH_2$), 2.82 (4 H, m, 2 × SCH_2), and 2.68 (2 H, m, $C{-}CH_2{-}C$); $\nu_{max.}$ 1 605, 1 585, and 1 505 cm^{-1}; *m/e* 316 (M^+, 70%), 154 (30), 121 (70), 120 (70), 119 (100), 91 (100), and 65 (50).

2-(4-*Benzyloxyphenyl*)-1,1-*dimethoxyethane* (8).—Mercuric oxide (6.79 g) and mercuric chloride (8.45 g) were added to a stirred solution of the foregoing dithian (4.69 g) in methanol (410 ml) and acetonitrile (175 ml), which was heated at reflux for 6 h. After cooling, the mixture was filtered through Celite, evaporated to dryness, and the residue partitioned between water (20 ml) and chloroform (100 ml). The chloroform was washed with water (20 ml) and saturated brine (20 ml) and dried and evaporated (<30 °C) to give the *acetal* (8) as a clear gum (3.6 g); δ 7.32 (5 H, s, ArH), 7.12 (2 H, d, *J* 9 Hz, C_6H_4), 6.85 (2 H, d, *J* 9 Hz, C_6H_4), 4.96 (2 H, s, $ArCH_2$), 4.36 [1 H, t, *J* 6 Hz, $CH(OMe)_2$], 3.18 (6 H, s, OMe), and 2.82 (2 H, d, *J* 6 Hz, $ArCH_2$); *m/e* 272 (M^+, 15%) and 91 (100) (Found: M^+, 272.140 8. $C_{17}H_{20}O_3$ requires *M*, 272.141 2).

2-(4-*Hydroxyphenyl*)-1,1-*dimethoxyethane* (9).—Raney nickel [33] (prepared from 6 g alloy) was added to a solution of the foregoing acetal (0.579 g) in ethanol (20 ml) and the solution was heated at reflux for 15—20 min. The cooled solution was filtered through Celite and evaporated (<30 °C). The residue in methanol (10 ml) was shaken with 10% palladium–charcoal (20 mg) and hydrogen until uptake was complete. The catalyst was filtered off and the solution evaporated (<30 °C) to give the *phenol* (402 mg); δ 7.04 (2 H, s, *J* 8 Hz, ArH), 6.70 (2 H, s, *J* 8 Hz, ArH), 6.36br (1 H, s, OH), 4.52 [1 H, t, *J* 6 Hz, $CH(OMe)_2$], 3.32 (6 H, s, OMe), and 2.63 (2 H, d, *J* 6 Hz, $ArCH_2$); *m/e* 182 (M^+, 10%), 181 (58), 151 (70), 119 (50), and 79 (100) (Found: M^+, 182.094 9. $C_{10}H_{14}O_3$ requires *M*, 182.094 2).

1,1-*Dimethoxy*-2-(4-*methylsulphonyloxyphenyl*)*ethane* (10).—Methanesulphonyl chloride (1.18 ml) was added over 10 min to a stirred solution of the foregoing phenol (2.0 g) in dichloromethane (55 ml) containing triethylamine (2.3 ml) at 0—10 °C. After a further 10 min, the reaction was worked up in the usual way to give the *methanesulphonyl derivative* as a yellow oil (2.5 g), m.p. 40—43° (from benzene–hexane) (Found: C, 50.45; H, 6.15; S, 12.2. $C_{11}H_{16}O_5S$ requires C, 50.75; H, 6.20; S, 12.3%); δ 1.28 (2 H, d, *J* 10 Hz, ArH), 1.16 (2 H, d, *J* 10 Hz, ArH), 4.70 [1 H, t, *J* 6 Hz, $CH(OMe)_2$], 3.31 (6 H, s, MeO), 3.08 (3 H, s, MeS), and 2.90 (2 H, d, *J* 6 Hz, $ArCH_2$); *m/e* 260 (M^+, 6%), 259 (5), 230 (100), 149 (100), 121 (80), and 107 (80).

2-(4-*Methylsulphonyl*)*ethanol* [*as* (12)].—2N-Aqueous hydrochloric acid (50 ml) was added to a stirred solution of the foregoing acetal (1.34 g) in tetrahydrofuran (50 ml) at 18 °C. After 40 min, the solution was extracted with chloroform (3 × 50 ml) and the chloroform extracts were washed with water (30 ml), saturated sodium hydrogencarbonate (30 ml), and saturated brine (30 ml), and then dried over anhydrous potassium carbonate. Evaporation of the chloroform gave 4-methylsulphonyloxyphenylacetaldehyde as a clear oil (1.12 g); δ 9.72 (1 H, t, *J* 3 Hz, CHO), 7.27 (4 H, s, ArH), 3.71 (2 H, d, *J* 3 Hz, $ArCH_2CHO$), and 3.12 (3 H, s, MeS).

Sodium borohydride (0.1 g) was added slowly to a stirred solution of the foregoing product (1.92 g) in methanol (60 ml) at room temperature. After 10 min, acetone (5 ml) was added to the solution, which was acidified after a further 3 min with dilute aqueous sulphuric acid. Extraction with ether gave the *alcohol* as a clear oil which crystallised from benzene–hexane (1.83 g), m.p. 46—48°; δ 7.19 (4 H, s, ArH), 3.80 (2 H, t, *J* 5 Hz, $ArCH_2$), 3.08 (3 H, s, MeS), 2.82 (2 H, t, *J* 5 Hz, CH_2OH), and 1.91 (1 H, s, O*H*); *m/e* 216 (M^+, 100%), 202 (40), 186 (40), 185 (40), 107 (40), 97 (40), and 95 (50) (Found: M^+, 216.045 4. $C_9H_{12}O_4S$ requires *M*, 216.045 6).

1-*Methylsulphonyloxy*-2-(4-*methylsulphonyloxyphenyl*)-*ethane* [*as* (13)].—Methanesulphonyl chloride (0.43 ml) was added over 10 min to a stirred solution of the foregoing alcohol (0.85 g) in dichloromethane (19 ml) containing triethylamine (0.79 ml) at 0—10 °C. After a further 10 min the solution was worked up by extraction as usual to give the *bismethanesulphonyl derivative* as a clear oil (1.03 g); δ 7.21 (4 H, s, ArH), 4.37 (2 H, t, *J* 6 Hz, $ArCH_2$), 3.07 (3 H, s, $MeSO_3Ar$), 3.02 (2 H, t, *J* 6 Hz, CH_2O), and 2.84 (3 H, s, $MeSO_3CH_2$); *m/e* 295 (M^+, 5%), 230 (60), 229 (80), 198 (80), 149 (60), 137 (100), 136 (80), and 108 (60) (Found: M^+, 294.023. $C_{10}H_{14}O_6S_2$ requires *M*, 294.023).

1-*Azido*-2-(4-*methylsulphonyloxyphenyl*)*ethane* [*as* (14)].—A stirred mixture of the above product (1.03 g), sodium azide (1.37 g), acetone (14 ml), and water (14 ml) was heated at reflux overnight, then cooled and extracted with diethyl ether (3 × 20 ml). The combined organic phases were washed with water (20 ml) and saturated brine (20 ml) and dried over anhydrous potassium carbonate. Evaporation gave the *azide* as an oil (730 mg); δ 7.26 (4 H, s, ArH), 3.54 (2 H, t, *J* 6 Hz, $ArCH_2$), 3.12 (3 H, s, MeS), and 2.92 (2 H, t, $ArCH_2$); *m/e* 243 (M^+, 60%), 187 (40), 186 (70), 185 (100), 134 (20), and 107 (80) (Found: M^+, 241.051 7. $C_9H_{11}N_3O_3S$ requires *M*, 241.052 0).

1-*Azido*-2-(4-*hydroxyphenyl*)*ethane* [*as* (15)].—Sodium hydroxide pellets (700 mg) were added to a stirred solution of the foregoing azide (540 mg) in 1 : 1 v/v methanol–water (60 ml) at 70 °C. After 1 h, the solution was poured onto ice and washed with dichloromethane (10 ml). The aqueous solution was acidified with 3N-hydrochloric acid and extracted with diethyl ether (3 × 20 ml). The extracts were washed with water (10 ml), saturated sodium hydrogencarbonate solution (10 ml), and brine (10 ml), and dried and evaporated to give the *phenol* as a clear oil (310 mg); δ 7.02 (2 H, d, *J* 8 Hz, ArH), 6.72 (2 H, d, *J* 8 Hz, ArH), 3.80br (1 H, OH), 3.40 (2 H, t, *J* 6 Hz, $ArCH_2$), and 2.76 (2 H, t, *J* 6 Hz, CH_2N_3); $\nu_{max.}$ 3 300, 2 100, and 1 610 cm^{-1}; *m/e* 163 (M^+, 80%) and 107 (100) (Found: M^+, 163.074 6. $C_8H_9N_3O$ requires *M*, 163.074 5).

1-*Azido*-2-(4-*benzyloxyphenyl*)*ethane* [*as* (16)].—A stirred solution of the foregoing phenol (626 mg), benzyl chloride (0.8 ml), and anhydrous potassium carbonate (800 mg) in methanol (58 ml) was heated at reflux overnight. After dilution with an excess of water, it was extracted with diethyl ether (3 × 20 ml) to give the *azide* (828 mg), m.p. 43—45° (from benzene–hexane) (Found: C, 71.2; H, 6.1; N, 16.8. $C_{15}H_{15}N_3O$ requires C, 71.1; H, 6.0; N, 16.6%); δ 7.35 (5 H, s, C_6H_5), 7.10 (2 H, d, *J* 8 Hz, C_6H_4), 6.89 (2 H, d, *J* 8 Hz, C_6H_4), 5.01 (2 H, s, $ArCH_2O$), 3.44 (2 H, t, *J* 6 Hz, $ArCH_2CH_2$), and 2.81 (2 H, t, *J* 6 Hz, CH_2N_3);

$\nu_{max.}$ 2 190, 1 605, and 1 580 cm^{-1}; *m/e* 235 (M^+, 5%), 91 (100), and 65 (20).

2-(4-*Benzyloxyphenyl*)*ethylamine Hydrochloride* [*as* (17)].—A solution of the foregoing azide (828 mg) in diethyl ether (95 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (860 mg) in diethyl ether (95 ml) and the mixture was heated at reflux for 1 h and then cooled. Water (0.86 ml) and 10% aqueous sodium hydroxide (1.29 ml) was added with stirring and the filtered ethereal solution was washed with water (20 ml), saturated sodium hydrogencarbonate (20 ml), and saturated brine (20 ml), and dried with anhydrous potassium carbonate. The residue from evaporation in the minimum volume of hot methanol was acidified with ethereal hydrogen chloride to give the amine hydrochloride (499 mg), m.p. 204—206° (from methanol–diethyl ether) (lit.,[34] 196—206°); δ (CF_3CO_2D) 7.35 (5 H, s, C_6H_5), 7.23 (2 H, d, *J* 10 Hz, C_6H_4), 7.05 (2 H, d, *J* 10 Hz, C_6H_4), 5.17 (2 H, s, $ArCH_2O$), 3.50 (2 H, m, $CH_2\overset{+}{N}H_3$), and 3.07 (2 H, t, *J* 6 Hz, $ArCH_2$); *m/e* 227 (M^+, 5%), 198 (30), 107 (25), 91 (100), and 65 (10).

Tyramine Hydrochloride [*as* (18)].—10% Palladium–charcoal (50 mg) was added to a degassed solution of the above benzyloxy-derivative (499 mg) in methanol (100 ml) and the mixture was shaken under hydrogen until uptake ceased. The filtered solution was evaporated and a filtered solution of the residue in hot ethanol was acidified with ethereal hydrogen chloride to give tyramine hydrochloride (237 mg), m.p. 263—265° (from ethanol–ether) (lit.,[35] 269°); δ (CF_3CO_2D) 7.23 (2 H, d, *J* 8 Hz, ArH), 6.98 (2 H, d, *J* 8 Hz, ArH), 6.80br (3 H, $\overset{+}{N}H_3$), 3.56 (2 H, q, *J* 6 Hz, CH_2N), and 3.12 (2 H, t, *J* 6 Hz, $ArCH_2$); *m/e* 137 (M^+ — HCl, 10%), 108 (100), and 107 (50).

[2-^{2}H]-1,3-*Dithian* [*as* (6)].—n-Butyl-lithium (8.2 ml; 1.84M in hexane) was added to a degassed solution of 1,3-dithian (2.0 g) in tetrahydrofuran (50 ml) under nitrogen at −70 °C. After 1.5 h at −23 °C, deuterium oxide was added and the mixture was warmed to 20 °C before being worked up as previously described for 2-(4-benzyloxybenzyl)-1,3-dithian. This entire sequence was then repeated to give the dideuteriodithian with only 4% protium at C-2 (1.4 g), m.p. 49—51°(from methanol); δ 3.76 (0.08 H, s, S–CHD–S), 2.09 (4 H, m, 2 × CH_2S), and 2.08 (2 H, m, C–CH_2–C); *m/e* 122 (M^+, 100%) (Found: M^+, 122.018 9. $C_4H_6D_2S_2$ requires *M*, 122.019 2).

2-(4-*Benzyloxybenzyl*)-[2-^{2}H]-1,3-*dithian* [*as* (7)].—This was prepared from the foregoing product as for the unlabelled material described earlier; n.m.r. showed 95% deuteriation, confirmed by mass spectrometry (Found: M^+, 317.099 8. $C_{18}H_{19}DOS_2$ requires *M*, 317.101 7).

Methylsulphonyloxyphenyl[formyl-^{2}H]*acetaldehyde* (11c).—This was prepared from the foregoing labelled product as for the unlabelled series. Deuteriation was shown to be 95% by n.m.r. and mass spectrometry (Found: M^+, 215.036 6. $C_9H_9DO_4S$ requires *M*, 215.036 2).

(1S)-2-(4-*Methylsulphonyloxyphenyl*)[1-$^2H_1$]*ethanol* (12c).—A solution of the above deuterio-aldehyde (105 mg) in ethanol (11 ml) was added to 0.01M-phosphate buffer (pH 7; 100 ml) at 36 °C followed by a suspension of LADH in ethanol (0.5 ml; 100 mg per 10 ml) and NAD^+ (25 mg) in 0.01M-phosphate buffer (pH 7; 10 ml). After being kept at 36 °C for 18 h, the mixture was saturated with sodium chloride and extracted with ethyl acetate (4 × 40 ml). The combined organic phases were washed with saturated sodium hydrogencarbonate solution (30 ml) and saturated brine (30 ml) and dried over anhydrous potassium carbonate. The residue from evaporation was purified by preparative t.l.c. on silica with diethyl ether to give material (82 mg) identical, apart from the isotope, with authentic unlabelled material.

(1R)-2-(4-*Methylsulphonyloxyphenyl*)[1-$^2H_2$]*ethanol* (12d).—A sample of 4-methylsulphonyloxyphenylacetaldehyde was obtained by hydrolysis of the dimethyl acetal as described earlier for the preparation of the unlabelled alcohol. A solution of liver alcohol dehydrogenase (LADH) (100 mg, ethanol free) and NAD^+ (50 mg) in phosphate buffer (0.01M; pH 7; 5 ml) was added to a suspension of the aldehyde (184 mg) in dioxan (10 ml), [1-^{2}H]cyclopentanol (1.7 ml) and 0.01M-phosphate buffer (pH 7; 200 ml). After incubation at 37 °C overnight, the mixture was saturated with sodium chloride and stirred with ethyl acetate (40 ml). After filtration through Celite, the phases were separated and the aqueous phase extracted with more ethyl acetate (3 × 40 ml). The work-up then followed that in the preceding preparation to give the (1*R*)-[1-$^2H_1$]-alcohol (91 mg); δ 7.19 (4 H, s, ArH), 3.80 (1.14 H, t, *J* 5 Hz, *CH*DOH), 3.08 (3 H, s, MeS), 2.82 (2 H, d, *J* 5 Hz, $ArCH_2$), and 1.91br (1 H, s, O*H*); *m/e* 217 (M^+, 50%), 186 (40), 185 (95), 121 (20), 108 (30), and 107 (100) (Found: M^+, 217.051 8. $C_9H_{11}DO_4S$ requires *M*, 217.051 9).

(1S)-1-*Azido*-2-(4-*benzyloxybenzyl*)[1-$^2H_1$]*ethane* (13d).—The azide, prepared from the foregoing (1*R*)-deuterioethanol by the route previously described for unlabelled material, showed δ 7.35 (5 H, s, C_6H_5), 7.10 (2 H, d, *J* 8 Hz, C_6H_4), 6.89 (2 H, d, *J* 8 Hz, ArH), 5.01 (2 H, s, $ArCH_2O$), 3.44 (2 H, t + d, *J* 6 Hz, $ArCH_2$), 3.20 (1.13 H, t, *J* 6 Hz, $CHDN_3$); *m/e* 254 (M^+, 10%), 135 (20), 134 (20), 91 (100), and 65 (50) (Found: M^+, 254.126 8. $C_{15}H_{14}NOD$ requires *M*, 254.127 7).

[1-$^3H_1$]*Cyclopentanol*[25] [*as* (6)].—Sodium borohydride (10 mg) was added to a stirred solution of freshly redistilled cyclopentanone (761 mg) in 0.01N-aqueous sodium hydroxide (10 ml). After 1 h, potassium borotritiide (11.25 mg, 0.208mM, 12.5 mCi) was added to 1 ml of the above solution and the reaction mixture stirred for 18 h at 20 °C. Further sodium borohydride (10 mg) was added and after another 1.5 h, the solution was acidified, then adjusted to pH 10, and the volume made up to 10 ml (0.096 mM ml^{-1}; 1.248 mCi ml^{-1}).

(1R)-2-(4-*Methylsulphonyloxyphenyl*)[1-$^3H_1$]*ethanol* (12b).—A solution of 4-methylsulphonyloxyphenylacetaldehyde (297 mg) in dioxan (33 ml) was added to a stirred solution of LADH (40 mg), NAD^+ (40 mg), and the above cyclopentanol solution (2.08 ml; 2.6 mCi) in 0.01M-phosphate buffer (pH 7; 300 ml). The reaction was followed by taking 2 μl samples of the incubation mixture, overspotting with unlabelled alcohol on a t.l.c. plate, and developing in ether. The spot containing alcohol was eluted and counted and the mixture was worked up as earlier when the activity of the alcohol was constant. The product (130 mg) was diluted with unlabelled alcohol (524 mg).

(αS)-[α-$^3H_1$]*Tyramine Hydrochloride* (18b).—The salt was prepared from the above (1*R*)-tritio-alcohol as previously described for unlabelled tyramine hydrochloride (72 mg, 28.2 μCi $mmol^{-1}$).

[2-$^3H_1$]-1,3-*Dithian* [*as* (6)].—n-Butyl-lithium (0.345 ml; 1.84M in hexane) was added slowly to a stirred solution of 1,3-dithian (67 mg) in tetrahydrofuran (0.15 ml) at −78 °C and then kept at −23 °C for 1.5 h. A solution of tritiated trifluoroacetic acid (0.556mM) in tetrahydrofuran (0.5 ml)

was then added and the solution was kept at 0 °C overnight; it was worked up as described previously for [2-$^2H_1$]-1,3-dithian. The product was purified by preparative t.l.c. on silica with toluene and crystallised from methanol (28 mg, 41%), m.p. 50—52°.

2-(4-*Benzyloxybenzyl*)-[2-$^3H_1$]-1,3-*dithian* [*as* (7)].—This was prepared from [2-$^3H_1$]-1,3-dithian (709 mg) and benzyloxybenzyl chloride (1.37 g) by the method previously described for unlabelled material (810 mg), m.p. 91—93° (from ethyl acetate), 120 μCi mmol^{-1}, identified by comparison with authentic material.

(1S)-2-(*Methylsulphonyloxybenzyl*)-[1-$^3H_1$]*ethanol* (12a).—The tritio-alcohol was prepared from the foregoing tritio-1,3-dithian by the route described for the deuterio-alcohol. The product (220 mg) was diluted with unlabelled alcohol (741 mg).

(αR)-[α-$^3H_1$]*Tyramine Hydrochloride* (18a).—The diluted alcohol from above was converted into the amine by the route described earlier (237 mg; 20.8 μCi mmol^{-1}).

(αRS)[α-^{3}H]*Tyrosine* (cf. *ref.* 36).—*ON*-Diacetyltyrosine (150 mg) was suspended in acetic anhydride (0.45 ml). Tritiated water (0.06 ml; 200 mCi) was distilled under vacuum into the reaction mixture. After being heated at reflux in a sealed tube for 3 h, the solution was evaporated and the residue was redissolved in water (1 ml) and evaporated again. This was repeated several times and the final residue in the minimum water was adjusted to pH 6 and the (α*RS*)-[α-^{3}H]tyrosine crystallised at 0 °C (82 mg), 12.7—0.2 mCi mmol^{-1}.

(αS)-[α-^{3}H,U-^{14}C]*Tyrosine and* (2S)-[α-^{3}H,β-^{14}C]-*Tyrosine*.—The foregoing (α*RS*)-[α-^{3}H]tyrosine was mixed in suitable amount with the appropriate (α*RS*)-[^{14}C]tyrosine from a commercial source and recrystallised. The racemate was then resolved by standard preparation of the *N*-chloroacetyl derivative followed by treatment with carboxypeptidase. This involved suspending the chloro-amide (199 mg) in water (7 ml) and adjusting the pH to 7.2 with ammonia. Carboxypeptidase (0.05 ml of suspension containing 16.5 mg protein ml^{-1} at 60 units per mg) was added and the mixture was incubated for 48 h at 37 °C. After adjustment to pH 5, the solution was left at 0 °C; the precipitated labelled (α*S*)-tyrosine was collected, dissolved in 1N-hydrochloric acid, and treated with charcoal. The filtered solution was again adjusted to pH 5 to give pure (α*S*)-tyrosine in labelled form (55 mg).

ON-*Dibenzoyltyrosol*.—Sodium hydroxide (400 mg) was added to stirred solution of 2-(4-methylsulphonyloxyphenyl)-ethanol (160 mg) in 1 : 1 methanol–water (12 ml) at 70 °C. After 1 h, the solution was poured onto ice, washed with chloroform (5 ml), and acidified with hydrochloric acid. The aqueous phase was extracted with diethyl ether (3 × 10 ml) and the extracts were washed with water (10 ml), saturated sodium hydrogencarbonate (10 ml), and brine and dried. The residue from evaporation was purified by preparative t.l.c. on silica (1 : 1 ethyl acetate–toluene as eluant) to give tyrosol, R_F 0.54, m.p. 89—91° (lit.,[37] 89—91°) (Found: M^+, 138.067 9. Calc. for $C_8H_{10}O_2$: *M*, 138.068 0). This product was stirred at 0 °C with benzoyl chloride (0.4 ml) and pyridine (10 ml) for 18 h. The residue from evaporation was purified by preparative t.l.c. on silica with benzene to give *ON*-dibenzoyltyrosol (R_F 0.35) (48 mg), m.p. 110—112° (from 95% ethanol) (lit.,[37] 110—112°) (Found: C, 76.0; H, 5.4. Calc. for $C_{22}H_{18}O_4$: C, 76.3; H, 5.2%); δ 8.11—7.50 (10 H, m, C_6H_5), 7.36 (2 H, d, *J* 9 Hz, C_6H_4), 7.15 (2 H, d, *J* 9 Hz, C_6H_4), 4.56 (2 H, t, *J* 7 Hz, ArCH_2), and 3.11 (2 H, t, *J* 7 Hz, CH_2O); *m/e* 224 (M^+ − 122, 40%) and 107 (100).

Incubation of Tyramine with Pea Seedling Diamine Oxidase.—A solution of tyramine hydrochloride (19 mg), pea seedling diamine oxidase extract[9] (0.38 ml), liver alcohol dehydrogenase (0.2 ml; suspension in ethanol; 100 mg in 10 ml), NAD^+ (5 mg), ethanol (0.79 ml), and catalase (2 mg) in phosphate buffer (0.1M, pH 8, 16 ml) was incubated at 37 °C for 32 h. The mixture was then acidified, saturated with sodium chloride, and stirred with diethyl ether. After filtration through Celite, the solution was continuously extracted with diethyl ether overnight. The combined ether extracts were dried and evaporated to give tyrosol (7 mg) which was converted into its *OO*-dibenzoyl derivative (12 mg), m.p. 110—112°, identical with material prepared as above.

Incubation of Tyramine with Rat Liver Monoamine Oxidase.—A solution of doubly-labelled tyramine (3 mg), amine oxidase extract (1 ml),[10] liver alcohol dehydrogenase, LADH (0.1 ml ethanol suspension as above), NAD^+ (10 mg), ethanol (0.1 ml), and catalase (0.1 mg) in phosphate buffer (0.1M; pH 7) was incubated at 32 °C for 24 h. More LADH (0.1 ml) and NAD^+ (5 mg) were then added and the mixture was kept for a further 24 h at 32 °C, and then acidified with concentrated hydrochloric acid, saturated with sodium chloride, and stirred with diethyl ether. The protein precipitate was filtered off through Celite and the aqueous phase was extracted with diethyl ether (3 × 20 ml). The combined ethereal extracts were dried and evaporated to give tyrosol (4 mg) which was converted as above into its dibenzoyl derivative. This was diluted with unlabelled dibenzoyltyrosol (7 mg) before rigorous purification as above for radio-assay, m.p. 109—110°.

Decarboxylation of Tyrosine by the Enzyme from S. faecalis.—(a) (αS)-[α-^{3}H,carboxy-^{14}C]*Tyrosine in* H_2O. (α*S*)-[α-^{3}H,*carboxy*-^{14}C]Tyrosine (2.85 mg) and acetone powder of *S. faecalis* (10 mg) (Sigma) were suspended in 0.1M-acetate buffer (3 ml; pH 5.5) at 30 °C. After 3 h, the mixture was boiled, filtered, and evaporated. The residue was twice redissolved in water (5 ml) and evaporated to dryness. It was then dissolved in water (10 ml) and applied to a column of Amberlite IRC-50 resin (2 g, dry sodium salt), purified according to the method of Hirs *et al.*,[38] and prepared by soaking in water, washing in 3N-hydrochloric acid, and then with distilled water until the washings were neutral. The column was washed with water (20 ml) and then with 4N-aqueous ammonia (20 ml) to give an ammoniacal solution containing the labelled tyramine. This was diluted with unlabelled tyramine hydrochloride (51 mg). The residue on evaporation was dissolved in absolute alcohol and the solution filtered through Celite. The hydrochloride (43 mg) (70—87% radiochemical yield) was precipitated as earlier. Part of the [α-^{3}H,β-^{14}C]tyramine hydrochloride was converted into *ON*-dibenzoyltyramine for counting and the rest was incubated with pea seedling diamine oxidase to establish the stereochemistry at C-1 as previously described.

(b) (αR)-[α-^{3}H,β-^{14}C]*Tyrosine in tritiated water*. (α*S*)-[β-^{14}C]Tyrosine (2 mg) and acetone powder of *S. faecalis* (15 mg) were suspended in 0.2M-acetate buffer (1.5 ml; pH 5.5, containing 300 mCi tritiated water). The incubation was carried out and worked up as described under (a) except that unlabelled tyramine (110 mg) was used in the dilution prior to isolation of the labelled amine (85 mg)

(62% radiochemical yield). Derivatisation and stereochemical assay were as described for (a).

Incubation of Tyramine with the Decarboxylase from S. faecalis.—[β-^{14}C]Tyramine hydrochloride (4 mg) was dissolved in 0.2M-acetate buffer (1.5 ml; pH 5.5; containing 300 mCi tritiated water) and mixed with acetone powder of *S. faecalis* (10 mg). The incubation was carried out and worked up as previously described, after dilution at the end with unlabelled tyramine hydrochloride (51.3 mg). The recovered tyramine (44 mg) was in part derivatised and the rest subjected to the pea seedling diamine oxidase as above. For stereochemical assay by the rat liver monoamine oxidase, the same procedure was adopted, except that less unlabelled tyramine (10 mg) was used to dilute the doubly-labelled tyramine.

Concentration of Mammalian Aromatic L-*Amino-acid Decarboxylase.*—All operations were carried out at 0—5 °C. Hog kidney acetone powder (1.2 g) was stirred in 0.075M-phosphate buffer (pH 8) for 1 h, then centrifuged (0.5 h, 11 000 r.p.m., 2°) and the supernatant was transferred to dialysis tubing. This enzyme preparation was concentrated by osmosis against sucrose, which was regularly changed. When the volume of the extract had been reduced to *ca.* 8 ml, the slack in the dialysis tube was taken up and the solution was dialysed for 2 h against 0.075M-phosphate buffer (pH 8). The resulting purified extract (9.2 ml) was used immediately as below.

Decarboxylation of Tyrosine with the Mammalian Enzyme.—(α*S*)-[α-^{3}H,β-^{14}C]Tyrosine (2 mg) was incubated with the foregoing mammalian decarboxylase extract (5 ml) for 48 h at 36 °C. The incubation was worked up after dilution with unlabelled tyramine hydrochloride as described previously (radiochemical yield 68—74%).

Tracer Experiments on Papaver somniferum *Plants.*—Methods for cultivation of the plants, administration of precursors, and purification of the alkaloids have been described previously.[39] Here, (α*S*)-[α-^{3}H,U-^{14}C]tyrosine (23.1 mg, 42.2 μCi ^{14}C, ^{3}H : ^{14}C ratio 10.1) in water (25 ml) was injected into the capsules of 16 plants, the heads and 3—4 in of stem below being harvested 5 days later for work-up. Unlabelled papaverine and morphine (50 mg each) were added as carriers.

The crude non-phenolic alkaloids (180 mg) were fractionated by preparative t.l.c. on silica using 2 developments with methanol–benzene to give papaverine, R_F 0.6 (64 mg), which was purified to constant specific activity and ^{3}H : ^{14}C ratio by multiple recrystallisation of its picrate from ethanol. The free base was recovered by percolating a solution of the picrate in chloroform through grade III alumina (2.5 g) and was recrystallised from aqueous methanol to give pure papaverine (38 mg), m.p. 146—148°.

Morphine was isolated and purified as previously.[39]

Related Intermediates.—Several useful materials prepared in exploratory studies are described below.

4-*Methylsulphonyloxybenzaldehyde.*—Methanesulphonyl chloride (7.2 ml) was added over 10 min to a stirred 0.2M-solution of 4-hydroxybenzaldehyde (10 g) in methylene chloride (400 ml) at 0—10 °C containing triethylamine (17.1 ml). After a further 10 min, the solution was worked up as usual.[40] The *aldehyde* (14.7 g, 90%), m.p. 62—63 °C, crystallised from benzene–hexane (Found: C, 48.0; H, 4.1. C_8H_8OS requires C, 48.0; H, 4.0%); δ 9.99 (1 H, s, CHO), 7.95 (2 H, d, *J* 11 Hz, ArH), 7.43 (2 H, d, *J* 11 Hz, ArH), and 3.18 (3 H, s, Me); *m/e* 200 (M^+, 80%), 135 (80), and 121 (100).

Methylsulphonyloxy-(4-*methylsulphonyloxyphenyl*)*methane.*—Sodium borohydride (34 mg) was added to a stirred solution of the above aldehyde (402 mg) in methanol–isopropyl alcohol (1 : 4 v/v) (100 ml) at room temperature. After 5 min, acetone (5 ml) was added and after a further 1 min, the solution was passed down a column of Amberlite IRC-120 (H^+) resin, previously washed with 2N-hydrochloric acid and isopropyl alcohol. The column was further eluted with isopropyl alcohol (2 × 20 ml) and the combined eluates were evaporated to a slurry, which was partitioned between water (20 ml) and diethyl ether (80 ml). The residue from the organic phase crystallised from benzene–hexane (366 mg, 90%), m.p. 42—44°.

Methanesulphonyl chloride (5.95 ml, 1.1 equiv.) was added over 10 min to a stirred 0.2M-solution of the above alcohol (13 g) in dichloromethane (324 ml) containing triethylamine (14.5 ml, 1.5 equiv.) at 0—10 °C. After a further 10 min at 0—10 °C the mixture was worked up as usual,[40] to give the *sulphonate ester* (14.6 g, 81%), m.p. 95—97° (Found: C, 38.5; H, 4.5. $C_9H_{12}O_6S_2$ requires C, 38.55; H, 4.3%); δ 7.36 (2 H, d, *J* 9 Hz, ArH), 7.21 (2 H, d, *J* 9 Hz, ArH), 5.12 (2 H, s, ArCH_2), 3.04 (3 H, s, MeS), and 2.86 (3 H, s, MeS); *m/e* 280 (M^+, 30%), 201 (80), 185 (65), 123 (30), 122 (40), 121 (20), and 107 (100).

4-*Methylsulphonyloxybenzyl Iodide.*—Tetra-n-butylammonium iodide (0.06 g) was added to a stirred mixture of the above bis-ester (0.2 g), dichloromethane (1.4 ml), and potassium iodide (0.383 g) in water (2 ml) at 20 °C. After 2.5 h, the product was extracted with dichloromethane (3 × 20 ml) and the combined organic phases were washed with water (10 ml), saturated sodium thiosulphate solution (10 ml), and water and dried. The residue after evaporation was purified by preparative t.l.c. on silica with dichloromethane as eluant and then crystallised from benzene–hexane to afford the *iodide* (177 mg, 80%), m.p. 98—99° (Found: C, 31.1; H, 3.0; S, 10.0; I, 40.6. $C_8H_9IO_3S$ requires C, 30.8; H, 2.9; S, 10.3; I, 40.7%); δ 7.42 (2 H, d, *J* 9 Hz, ArH), 7.17 (2 H, d, *J* 9 Hz, ArH), 4.41 (2 H, s, CH_2), and 3.1 (3 H, s, MeS); *m/e* 312 (M^+, 1%), 185 (10), 184 (100), 108 (10), 107 (100), 106 (25), and 91 (25).

4-*Methylsulphonyloxybenzyl Cyanide.*—Tetra-n-butylammonium iodide (0.6 g) was added to a stirred mixture of the above bismethanesulphonyl ester (12.2 g), dichloromethane (120 ml), and a solution of sodium cyanide (9.8 g) in water (120 ml) at 20 °C. After 4 h the *cyanide* was recovered by extraction into dichloromethane and crystallised from benzene–hexane (7.6 g, 82%), m.p. 72—73° (Found: C, 51.2; H, 4.3; N, 6.6. $C_9H_9NO_3S$ requires C, 51.3; H, 4.3; N, 6.6%); δ 7.35 (2 H, d, *J* 10 Hz, ArH), 7.23 (2 H, d, *J* 10 Hz, ArH), 3.69 (2 H, s, CH_2), and 3.08 (3 H, s, Me); $\nu_{max.}$ 2 260 cm^{-1}; *m/e* 211 (M^+, 15%), 133 (100), 132 (20), and 103 (20).

4,4,6-*Trimethyl-*2-(4-*methylsulphonyloxybenzyl*)-3,4,5,6-*tetrahydro-*2H-1,3-*oxazine.*[41]—2-Methyl-2,4-pentanediol (3.34 ml) was added to a stirred solution of the above nitrile (3.0 g) in concentrated sulphuric acid (5 ml) at −15 °C. After 2 h at 0 °C, the mixture was poured onto ice (30 gm) and washed with chloroform (3 × 10 ml). The aqueous phase was neutralised and extracted with diethyl ether (3 × 20 ml), and the extracts were dried over anhydrous potassium carbonate and evaporated to give the 5,6-dihydro-2*H*-1,3-oxazine (2.14 g, 46%); δ 7.37 (2 H, d, *J* 9 Hz, ArH), 7.20 (2 H, d, *J* 9 Hz, ArH), 4.20 (1 H, m, OC*H*Me), 3.47 (2 H, s, ArCH_2), 3.13 (3 H, s, MeS), 1.66 (2 H, m, ring H, C-5), 1.30 (3 H, d, *J* 4 Hz, MeCH, C-6), and

1.12 (6 H, s, Me_2C, C-4); *m/e* 311 (M^+, 100%), 310 (30), 184 (90), 107 (40), and 90 (40) (Found: M^+, 311.118 9. $C_{15}H_{21}NO_4S$ requires M, 311.119 0).

An alkaline aqueous solution of sodium borohydride (0.72 g) was added dropwise to a stirred solution of the dihydro-oxazine (2.14 g) in ethanol–tetrahydrofuran (1 : 1 v/v) (120 ml) at −35 to −45 °C. 2N-Hydrochloric acid was added as necessary to maintain pH 6—8 and after 1 h the solution was poured into water (100 ml) and the pH adjusted to 10. The aqueous phase was extracted with diethyl ether (3 × 60 ml) and the combined organic phases were washed with saturated brine (30 ml) and dried over anhydrous potassium carbonate. Evaporation gave the *perhydro-oxazine* (1.41 g, 66%), m.p. 113—114° (from benzene–hexane) (Found: C, 57.6; H, 7.5; N, 4.3. $C_{15}H_{23}NO_4S$ requires C, 57.5; H, 7.4; N, 4.5%); δ 7.33 (2 H, d, *J* 9 Hz, ArH), 7.14 (2 H, d, *J* 9 Hz, ArH), 4.43 (1 H, t, *J* 5 Hz, CH_2CH), 3.73 [1 H, m, J_1 6 Hz, J_2 3 Hz, CH(Me)], 3.09 (3 H, s, MeS), 2.84 (2 H, d, *J* 5 Hz, $ArCH_2$), 1.42 (2 H, dd, J_1 12 Hz, J_2 3 Hz, CH_2CHMe), 1.10 (3 H, s, Me), 1.08 (3 H, s, Me), and 0.95 (3 H, d, *J* 6 Hz, MeCH); *m/e* 313 (M^+, 10%), 214 (30), 185 (30), 129 (40), 128 (100), and 106 (50).

4-(*Phenylsulphonyloxy*)*benzaldehyde*.—Benzenesulphonyl chloride (2.5 ml) was added dropwise over 10 min to a stirred solution of 4-hydroxybenzaldehyde (2 g) in dichloromethane (90 ml) and triethylamine (3.5 ml) at 0—10 °C. After a further 15 min, the mixture was worked up as usual to give the *aldehyde* (3.85 g, 91%), m.p. 79.5—81.5° (from benzene–hexane) (Found: C, 59.3; H, 3.9; S, 12.2. $C_{13}H_{10}O_4S$ requires C, 59.5; H, 3.8; S, 12.2%); δ 9.96 (1 H, s, CHO) and 8.5 (9 H, m, ArH); $\nu_{max.}$ 1 705 cm^{-1}; *m/e* 262 (M^+, 26%), 141 (60), and 77 (100).

4-(*Phenylsulphonyloxy*)*benzyl Benzenesulphonate*.—Sodium borohydride (40 mg) was added to stirred solution of the above aldehyde (500 mg) in methanol (25 ml) at 20 °C. After 3 h, the mixture was acidified with 2N-sulphuric acid and evaporated to a slurry, which was extracted with ether to give the benzyl alcohol (460 mg). This, in dichloromethane (9 ml), was stirred and treated at 0—10 °C with triethylamine (0.38 ml) and benzenesulphonyl chloride (0.25 ml) added dropwise over 10 min. After 2 h, the organic solution was worked up as earlier and the product fractionated on silica with dichloromethane. The *diester* crystallised from ethyl acetate (0.3 g, 43%), m.p. 89—91° (Found: C, 56.4; H, 4.0; S, 15.9. $C_{19}H_{16}S_2O$ requires C, 56.25; H, 4.1; S, 16.0%); δ 8.5 (10 H, m, ArH), 5.0 (2 H, s, $ArCH_2$); *m/e* 404 (M^+, 10%), 282 (30), 263 (35), 247 (50), 141 (100), 125 (50), and 107 (40).

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