Biosynthesis. Part 24.[1] Speculative Incorporation Experiments with 1-Benzylisoquinolines and a Logical Approach *via* C_6–C_2 and C_6–C_3 Precursors to the Biosynthesis of Hasubanonine and Protostephanine

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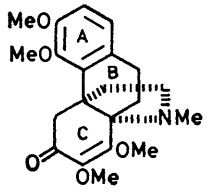
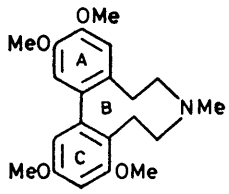
Many possible 1-benzyltetrahydroisoquinolines have been examined as possible advanced precursors of the alkaloids hasubanonine (1) and protostephanine (2) in *Stephania japonica* plants, but none was incorporated significantly. Administration of various precursor molecules having only one aromatic ring, such as tyrosine, has demonstrated that both alkaloids are derived from two different C_6–C_2 biogenetic units. The subsequent failure of further 1-benzyltetrahydroisoquinolines and bisphenethylamines to be incorporated suggested the intermediacy of either (*a*) modified 1-benzylisoquinolines or (*b*) trioxygenated C_6–C_2 building blocks. Precursors designed to examine the first possibility, such as 1-benzyl-3,4-dihydroisoquinolines or 1-benzyl-1-carboxytetrahydroisoquinolines, were not incorporated into (1) and (2) whereas two 3′,4′,5′-trioxygenated 2-phenylethylamines were incorporated. These findings allow further delineation of the requirements for later precursors of the alkaloids (1) and (2).

THE two alkaloids hasubanonine (**1**) and protostephanine (**2**), both produced by *Stephania japonica* Miers, are of considerable biosynthetic interest because of their unusual structural features (see earlier papers in this series [1]). We therefore undertook incorporation experiments with labelled precursors to provide insight into the way the plants construct these interesting substances.

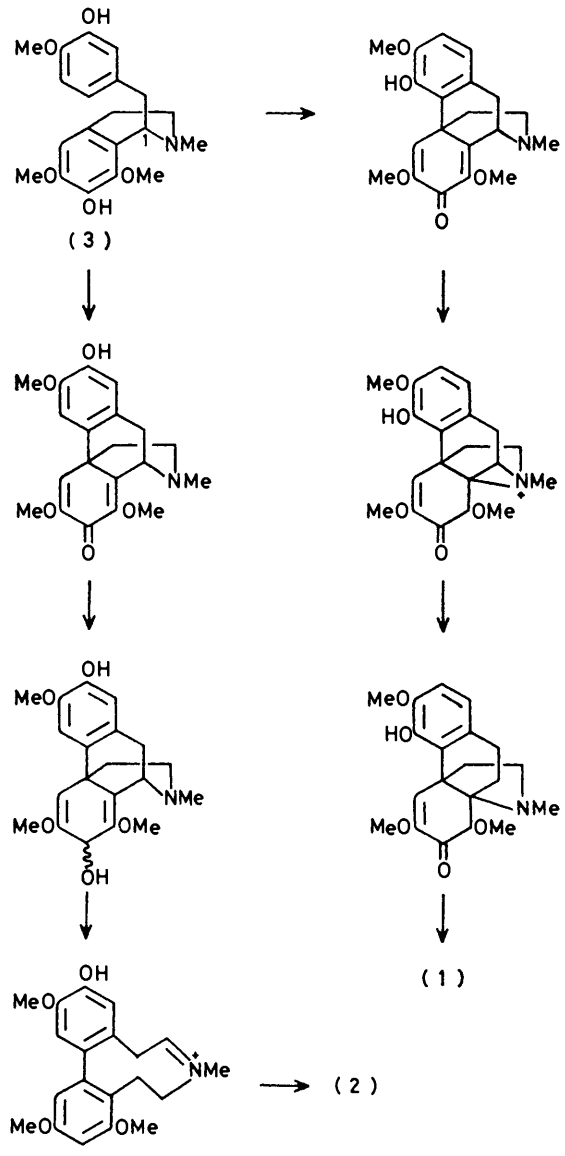


(1)



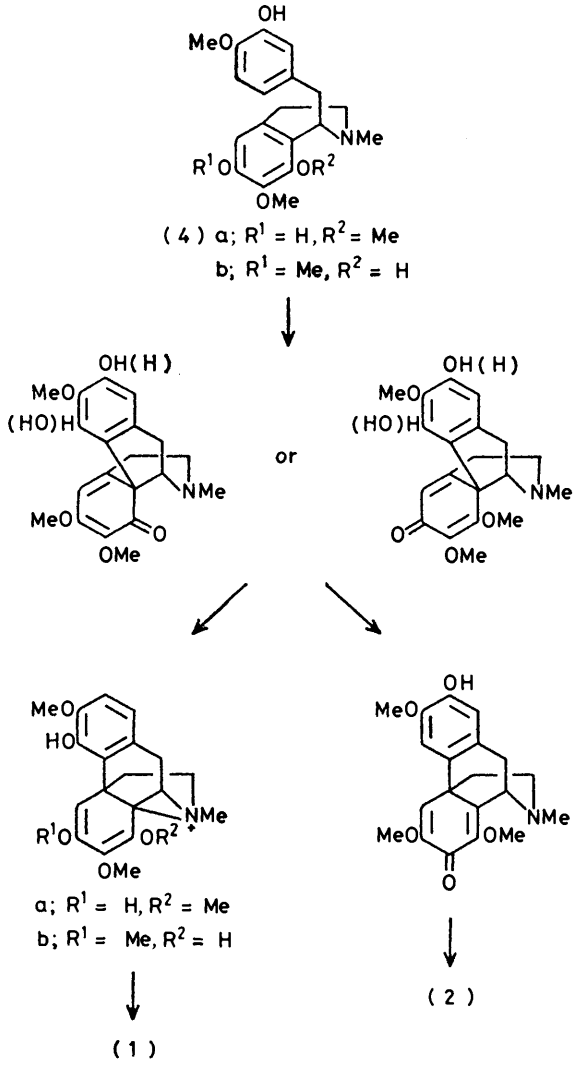
(2)

Brief consideration of the speculative proposals for the biosynthesis of structures (1) and (2) that had been made at the outset of our work, and some later modifications, is important as they guided our choice of labelled substances for the initial feeding experiments. One of the earliest speculations is that of Barton for the biosynthesis of protostephanine,[2] and later extended to account also for hasubanonine formation [3]; this is shown in Scheme 1. Phenol coupling of a dihydroxy-1-benzyltetrahydroisoquinoline (**3**), presumably derived from two molecules of tyrosine, to give a morphinandienone structure, followed by reduction to a dienol and rearrangement–fragmentation (in a manner closely related to that observed in the morphine alkaloids [4]) could produce protostephanine (2). An alternative mode of phenol coupling followed by internal Michael-type addition in the resultant dienone could lead to hasubanonine (1) *via* reduction of the aziridinium intermediate and further elaboration. Another proposal (Scheme 2), using closely related isoquinoline precursors, is that of Battersby, put forward originally to account for hasubanonine biosynthesis [5] and extended later to protostephanine.[6] A phenolic oxygen in the trioxygen-



SCHEME 1

ated isoquinoline nucleus of (4) now directs phenol coupling to give, depending on the orientation of the other phenolic ring, four alternative substitution patterns in the resultant dienones. N-Assisted rearrangement and aziridinium reduction leads to the hasubanan



SCHEME 2

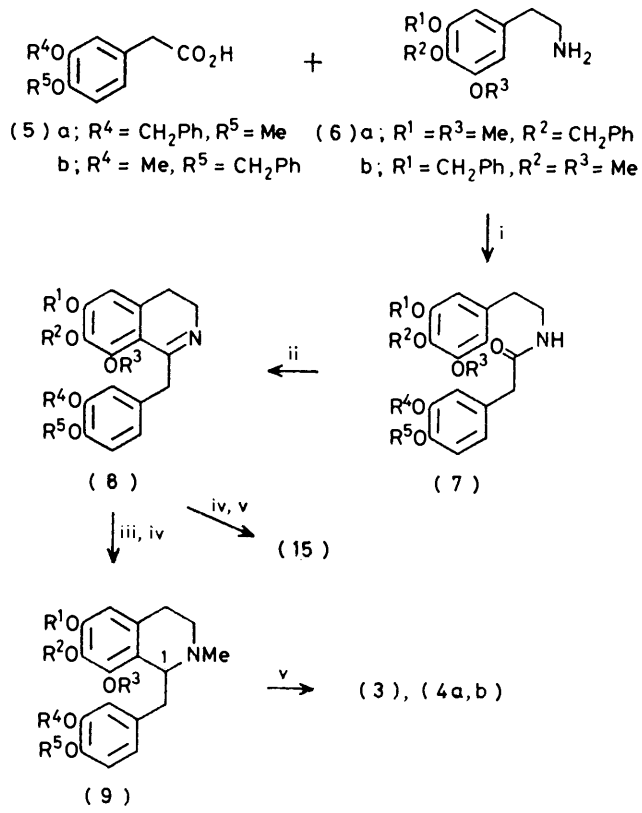
system from one pair whereas rearrangement–methylation of the alternative pair could produce the same dienone invoked in Scheme 1 *en route* to protostephanine.

These represent only some of the many possible routes that can be envisaged to structures (1) and (2).[7]

RESULTS AND DISCUSSION

The suggested intermediacy of 1-benzyltetrahydroisoquinolines on the pathways to the alkaloids (1) and (2) pre-supposes the construction of such a molecule from C_6–C_2 or C_6–C_3 precursors that can be derived *in vivo* from tyrosine or its relatives. Thus as a first step, (2*RS*)-[2-^{14}C]tyrosine was administered to whole plants of *S. japonica*. Examination of the protostephanine (2) and hasubanonine (1) isolated after a 7—10-day growing period showed that radioactivity had been incorporated into both alkaloids (see Table 1). Although the nature and specificity of the incorporations were not examined at this stage, these findings supported our general ideas about the origin of the carbon skeletons of the two alkaloids.* In addition, all our subsequent precursor feedings were checked by running a parallel feeding of (2*RS*)-[2-^{14}C]tyrosine as a control to test for the active synthesis of the alkaloids (1) and (2) at the time of the experiment.

The three pentaoxygenated diphenolic 1-benzyltetrahydroisoquinoline precursors (3), (4a), and (4b) suggested by Schemes 1 and 2 were then synthesised, using the general route outlined in Scheme 3. Condens-



(7)–(9) a; $R^1 = R^3 = R^5 = Me$, $R^2 = R^4 = CH_2Ph$
b; $R^1 = R^4 = CH_2Ph$, $R^2 = R^3 = R^5 = Me$
c; $R^1 = R^2 = R^5 = Me$, $R^3 = R^4 = CH_2Ph$
d; $R^1 = R^3 = R^4 = Me$, $R^2 = R^5 = CH_2Ph$

SCHEME 3 *Reagents:* i, (6) + acid chloride of (5), CH_2Cl_2–aq. NaOH; ii, $POCl_3$; iii, MeI; iv, $NaBH_4$; v, H_2–Pd

ation of appropriately substituted phenylacetyl chlorides, prepared from the acids (5), with 2-phenylethylamines (6) gave the amides (7) which were converted into 3,4-dihydroisoquinolines (8) by Bischler–Napieralski cyclis-

* The incorporation of tyrosine into protostephanine and hasubanonine with *S. japonica* plants has also been achieved by Professors Sir Derek Barton and G. W. Kirby and their colleagues; their work was not continued and we thank them warmly for exchange of information.

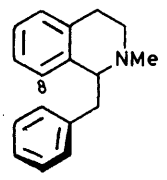
TABLE 1

Tracer experiments on *S. japonica* plants

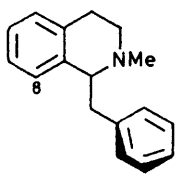
Expt. no.	Precursor	% Incorpn. into (1)	% Incorpn. into (2)	% of radioactivity in ring-c ethanamine chain: Base (1)	Base (2)
1	(2*RS*)-[2-^{14}C]Tyrosine (19)	0.64–3.1	0.028–0.18	53	57
2	(2*RS*)-[2-^{14}C]Dopa (20)	0.02	0.008	98	92
3	[2-^{14}C]Tyramine (21)	1.2	0.005	99	93
4	[2-^{14}C]Dopamine (22)	0.17	0.005	99	94
5	[1-^{14}C]Amine (26a)	<0.002	<0.0018		
6	[1-^{14}C]Amine (26b)	<0.002	<0.0018		
7	[1-^{14}C]Amine (26c)	1.1	0.011	99	95
8	[1-^{14}C]Amine (26d)	1.0	0.017	99	93

ation using phosphorus oxychloride. *N*-Methylation, followed by hydride reduction and unmasking of the phenolic groups by hydrogenolysis of the benzyl protecting groups, led to the desired 1-benzyltetrahydroisoquinolines (3), (4a), and (4b).

In the cyclisation of (7b), a mixture of 6- and 8-benzyloxydihydroisoquinolines was formed which was separated at the methiodide stage, before reduction to the tetrahydroisoquinolines. The separated isomers (9b) and (9c) were identified by n.m.r. spectroscopy in $CDCl_3$ solution.[8] *N*-Methyltetrahydroisoquinolines adopt conformation (10) for the 1-benzyl residue when



(10)

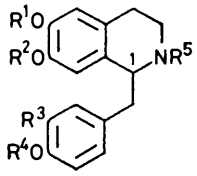


(11)

C-8 is unsubstituted with the result that (*a*) the *N*-methyl resonance shows the expected chemical shift of *ca.* δ 2.5 and (*b*) the C-8 proton is shifted upfield as it lies over the aromatic ring. When C-8 carries a substituent larger than hydrogen, the alternative conformation (11) is favoured, and the *N*-methyl signal is now observed at higher field. In general, the larger the group at C-8, the higher field is the *N*-methyl resonance observed.* In this way, isomer (9b), *N*-methyl resonance at δ 2.26, was distinguished from the 8-benzyloxy-isomer (9c), *N*-methyl at δ 2.11.

The C_6–C_2 building blocks (5) and (6) for Scheme 3 were both prepared from the corresponding benzaldehydes. Sodium borohydride reduction of these aldehydes, chlorination of the resultant alcohols, exchange with cyanide ion, and basic hydrolysis of the phenylacetonitriles so formed, gave the phenylacetic acids (5). The phenylethylamines (6) were obtained by condensation of an appropriate benzaldehyde with nitromethane followed by hydride reduction of the resultant nitrostyrene. Use of potassium [^{14}C]cyanide in the former sequence permitted the synthesis of the phenol (3) with a carbon-14 label at C-1. The isoquinolines (4a and b) were labelled in the aromatic rings by acid-catalysed exchange with tritiated water.[10]

An obvious modification to the proposals of Scheme 1 is that the fifth oxygen is inserted at a late stage and we therefore also synthesised reticuline (12), a known precursor to the morphine group of alkaloids,[11] and norlaudanosoline (13), the tetrahydroxybenzylisoquinoline



(12) $R^1 = R^4 = R^5 = Me$, $R^2 = H$, $R^3 = OH$
(13) $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OH$
(14) $R^1 = H$, $R^2 = R^4 = R^5 = Me$, $R^3 = OH$
(17) $R^1 = Me$, $R^2 = R^3 = R^4 = R^5 = H$
(18) $R^1 = R^5 = Me$, $R^2 = R^3 = R^4 = H$
(23) $R^1 = R^2 = R^3 = R^4 = R^5 = H$

that is known to act as a precursor of reticuline and other methoxylated benzylisoquinolines.[12] Protosinomenine (14), the *N*-demethyl derivative of which is an established precursor to the erythrinan skeleton [13] in the Leguminoseae, was also included since it corresponds to a precursor from Scheme 2 lacking one methoxy-group. All three compounds were ^{3}H-labelled in the aryl rings.

These six compounds [(3), (4a and b), and (12)—(14)] were fed to *S. japonica* plants, but examination of the hasubanonine (1) and protostephanine (2) isolated from these feedings indicated that essentially no incorporation had taken place with any of the isoquinolines.†,‡

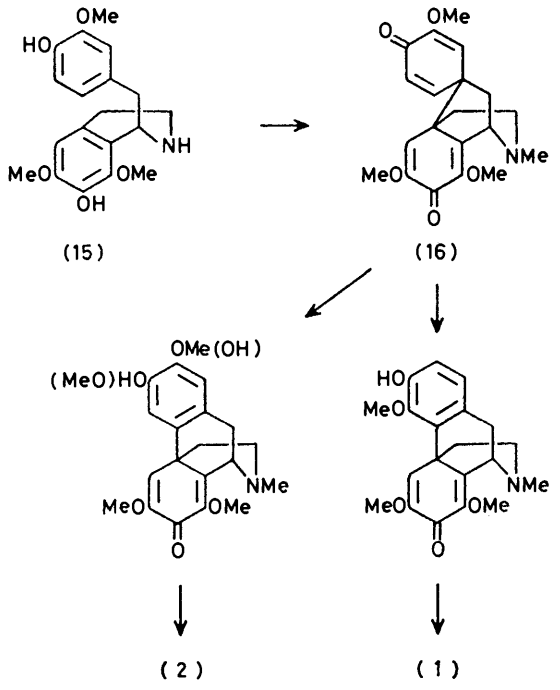
At this stage a further biosynthetic proposal was made, both in our group [6,14] and independently,[15] that the 1-benzyl residue of the 1-benzyltetrahydroisoquinoline precursor should carry 4-hydroxy-3-methoxy-substitution rather than the 3-hydroxy-4-methoxy-pattern suggested thus far. This proposal, which involves a bis-

* Brossi and Teitel [9a] record δ 2.20 for the NCH_3 resonance in 8-benzyloxy-6,7-dimethoxy-1-(4-methoxybenzyl)-2-methyl-1,2,3,-4-tetrahydroisoquinoline whereas Kubota *et al.*[9b] report δ 2.33 or the 6,7,8-trimethoxy-analogue.

† Incorporations ranged from ≪1% to 3% of the incorporation found for (2*RS*)-[2-^{14}C]tyrosine in a parallel feeding, almost all being below 1% of the value for tyrosine.

‡ Professors Sir Derek Barton and G. W. Kirby and their co-workers have also found no incorporation into (1) and (2) in *S. japonica* of the isoquinoline (3), its *N*-demethyl derivative, and reticuline (12). In a parallel feeding,[3] tyrosine was incorporated (1.7%) into hasubanonine (1) but not into protostephanine (2); however, the latter alkaloid was not produced in significant amounts on that occasion.

dienone (16), is outlined in Scheme 4. The isoquinoline (15) was synthesised from (7d) by the general route of Scheme 3 and with [*aryl*-^{3}H]-labelling was fed to *S. japonica* plants. Two further speculative feedings, of the widely occurring alkaloid coclaurine (17) and its *N*-methyl derivative (18), again [*aryl*-^{3}H]-labelled, were made to test the possibility that the precursors used so far had been over oxygenated. These three feedings also gave no incorporation into the isolated hasubanonine (1) and protostephanine (2).

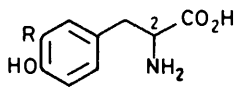


SCHEME 4

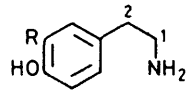
At this stage it was clear that what had seemed to be shrewd speculations did not in this case (unlike many others) allow identification of an advanced precursor in the 1-benzylisoquinoline series. So a logical approach was adopted. The plan was to discover the origin of the complete carbon skeletons of hasubanonine (1) and protostephanine (2) by incorporation experiments with the appropriate C_6–C_2 and C_6–C_3 building blocks. Also, all labelling was to be with ^{14}C in the skeletons of the precursors to allow unambiguous degradation by methods already described.[1] Such degradations are generally more difficult for ^{3}H-labelling and other problems, such as the NIH shift,[16] can cause complications. Finally, with the consistent lack of incorporation of label in the work so far, we were concerned about possible loss of ^{3}H-label by exchange from polyoxygenated intermediates.

The units chosen for this study were tyrosine (19) and 3,4-dihydroxyphenylalanine (dopa) (20), both labelled with ^{14}C at C-2, and the amines tyramine (21) and dopamine (22), ^{14}C-labelled at the C-2 benzylic carbon atom. These were administered separately to *S. japonica* plants in the usual way. The hasubanonine (1) and protostephanine (2) were isolated * and all four precursors were found to have been incorporated into both alkaloids (see Table 1).

The degradative schemes described in the preceding paper [1] allow separation of the two C_2-units in each of (1) and (2). In the case of hasubanonine (1), the ethanamine bridge is removed to leave a phenanthrene containing only the ring-A C_2-residue whereas for (2) the C_2-unit attached to ring A is isolated ultimately as a derivative of acetic acid. Application of these methods to the alkaloids isolated from the foregoing feedings showed (Tables 1 and 2) that tyrosine (19) was incorporated into both C_2-units of each molecule, with 53% [for (1)] or 57% [for (2)] of the activity residing in the ring-C ethanamine side-chain. In contrast, the other



(19) R = H
(20) R = OH



(21) R = H
(22) R = OH

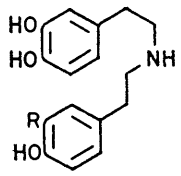
incorporated molecules (20), (21), and (22) labelled *only* the ring-C ethanamine side-chain. It is reasonable to conclude therefore (*a*) that both alkaloids are built from two different C_6–C_2 units derivable from tyrosine [this eliminates any possibility that the vicinally trioxygenated ring C of (1) or the *meta*-dioxygenated ring C of (2) might be derived from a polyacetate source and confirms the origin of both aromatic rings in the shikimate pathway], (*b*) that one unit is a phenethylamine that can be formed from both tyramine and dopamine and that it generates ring C with its attached ethanamine side-chain for both alkaloids, and (*c*) that dopa (20) affords only this same phenylethylamine unit. Apparently dopamine may be formed from tyrosine *via* either dopa or tyramine, a situation that is not unique and has been found, for example, in simple isoquinoline alkaloids.[17] The type of construction suggested for (1) and (2) from a C_6C_2N portion and a C_6C_2 unit, both available from tyrosine, is well known among systems derived from 1-benzylisoquinolines,[18] including the morphine group.[18a,19]

The nature of the C_6–C_2 unit providing ring A of the alkaloids (1) and (2) was still in doubt. Other work in cacti on simple 1-alkylisoquinolines,[20] and also with the morphine alkaloids,[21] has shown that the C-1 position of natural isoquinolines can arise from the C-2 carbon of α-keto-acids, whereas in certain circumstances an aldehyde may supply this carbon atom.[22] Accordingly, (3,4-dihydroxyphenyl)[2-^{14}C]pyruvic acid was synthesised from *N*-benzoyl[2-^{14}C]glycine by the azlactone method (see Experimental section). It was fed to *S. japonica* plants but no incorporation into protostephanine (2) was observed. Hasubanonine (1) was labelled but degradation as above showed the radioactivity to be

* The protostephanine was isolated from these feedings by dilution with radioinactive protostephanine as carrier because the amount produced by the plants in these, and subsequent feedings, was small.

almost completely in the ring-C ethanamine side-chain, and not in the C_6–C_2 unit attached to ring A. Incorporation of the keto-acid into the C_6C_2N part of the alkaloid (1) can be accounted for by a facile enzymic amination of the pyruvic acid to provide dopa (20) which is then incorporated as earlier. Thus, if a keto-acid is involved in biosynthesis of ring A of these alkaloids, it must carry no more than one oxygen substituent in the aromatic ring, at the 4-position.

The foregoing experiments were in keeping with our initial view that the alkaloids (1) and (2) are probably derived from 1-benzyltetrahydroisoquinolines. Tests were therefore made of the possibility that the advanced precursors used earlier were either wrongly, or too highly, *O*-methylated and/or too highly oxygenated. The isoquinoline (23), which has the minimum oxygenation indicated by the studies with simple precursors, and (13), together with their *N*-methyl derivatives, were synthesised analogously to Scheme 3 in a [1-^{14}C]-labelled form. The NH compounds were obtained by direct reduction of the 3,4-dihydroisoquinoline intermediates [*cf.* (8)]. Diborane reduction of the intermediate *N*-

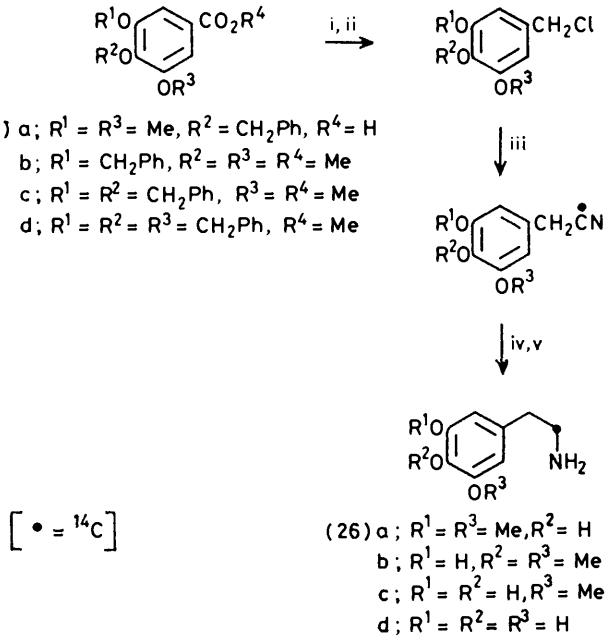


(24) a; R = H
b; R = OH

phenethyl-2-phenylacetamides [*cf.* (7)] gave the bis-phenethylamines (24a and b), similarly labelled. Such bisphenethylamines feature in an early proposal [23] for the biosynthesis of hasubanonine (1) and protostephanine (2). All six compounds were tested in *S. japonica* plants but incorporation of radioactivity into alkaloids (1) and (2) was insignificant in all cases.

A reasonable conclusion from these results is either (*a*) that oxidation to generate a trioxygenated aromatic ring must occur early on the biosynthetic pathway, before the C_6C_2 and C_6C_2N fragments are joined, or (*b*) that if 1-benzyltetrahydroisoquinolines are involved as precursors, hydroxylation occurs on some intermediate between isoquinoline ring-closure and formation of a tetrahydroisoquinoline. Discrimination between these possibilities was made by synthesis of two further sets of putative precursors.

The first were the four ^{14}C-labelled 3,4,5-trioxygenated 2-phenethylamines (26a—d). A 3,4,5-substitution pattern was preferred to the possible 2,3,4-oxygenation because of the presence of a 3,4,5-trisubstituted C_6C_2N moiety in hasubanonine (1), *i.e.* ring C and the ethanamine bridge. The synthesis of these amines was achieved from the appropriately substituted benzoic acid derivatives (25) (Scheme 5), all ultimately available from gallic acid. Benzyl ethers were used to protect phenolic functions during this sequence, which consisted of reduction of the methyl benzoates (25b—d) and the benzoic acid (25a) to the corresponding benzyl alcohols, chlorination, and exchange of the halogen for a cyano-group with potassium [^{14}C]cyanide. Reduction of the phenylacetonitriles so formed and removal of the protecting groups by hydrogenolysis completed the synthesis of the radio-labelled amines.



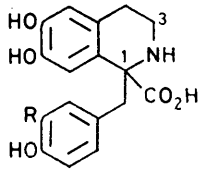
(25) a; $R^1 = R^3 = Me$, $R^2 = CH_2Ph$, $R^4 = H$
b; $R^1 = CH_2Ph$, $R^2 = R^3 = R^4 = Me$
c; $R^1 = R^2 = CH_2Ph$, $R^3 = R^4 = Me$
d; $R^1 = R^2 = R^3 = CH_2Ph$, $R^4 = Me$

[• = ^{14}C]

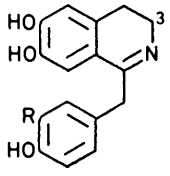
(26) a; $R^1 = R^3 = Me$, $R^2 = H$
b; $R^1 = H$, $R^2 = R^3 = Me$
c; $R^1 = R^2 = H$, $R^3 = Me$
d; $R^1 = R^2 = R^3 = H$

SCHEME 5 *Reagents:* i, $LiAlH_4$; ii, $SOCl_2$; iii, $K^{14}CN$; iv, $NaBH_4$–BF_3; v, H_2–Pd

The second set (*cf.* refs. 20 and 21) consisted of the 1-benzyl-1-carboxyisoquinolines (27a and b) and the 1-benzyl-3,4-dihydroisoquinolines (28a and b). [1-^{14}C]-Dopamine (22) and its dibenzyl ether were both prepared from 3,4-bisbenzyloxybenzaldehyde by reduction to the benzyl alcohol and then by steps related to Scheme 5. The 1-carboxy[3-^{14}C]isoquinolines were obtained by



(27) a; R = H
b; R = OH



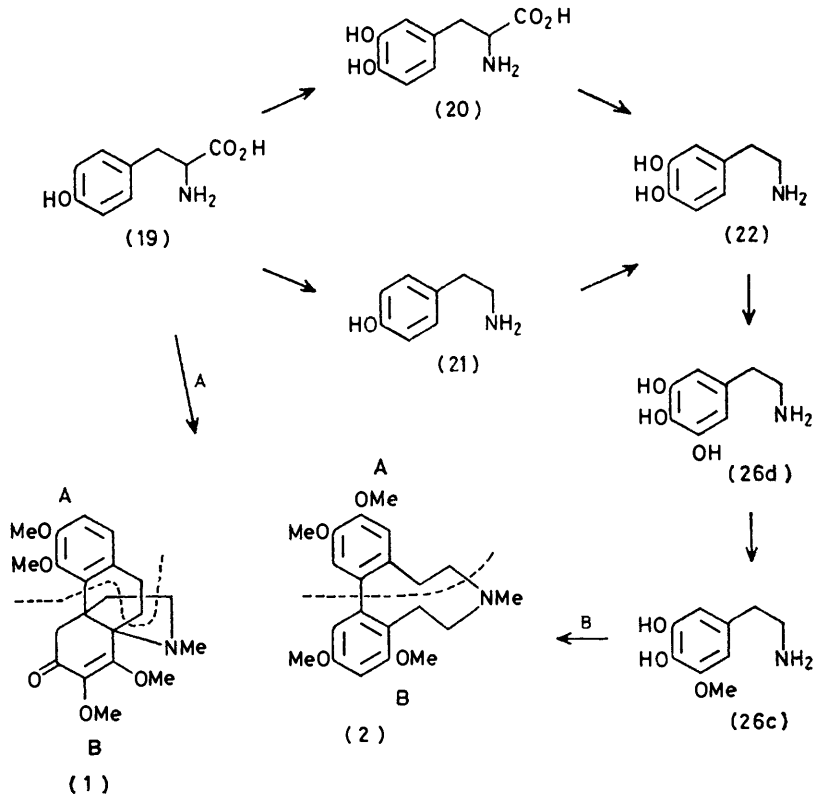
(28) a; R = H
b; R = OH

Pictet–Spengler condensation [24] at pH 5 of the labelled dopamine with either 4-hydroxy- or 3,4-dihydroxy-phenylpyruvic acid (both prepared by the hydantoin method [25]). Yields in this condensation were low (25—40%), probably due to decomposition of the starting materials under the reaction conditions. Coupling of dibenzyl[1-^{14}C]dopamine with an appropriately substituted and protected phenylacetyl chloride and Bischler–Napieralski cyclisation (*cf.* Scheme 3) led to

protected 3,4-dihydroisoquinolines which could be debenzylated in acid to give (28a and b) directly, both ^{14}C-labelled at C-3.

The four phenethylamines (26a—d) and the four isoquinolines (27a and b) and (28a and b) were fed to *S. japonica* plants and the alkaloids (1) and (2) isolated. The isoquinolines were not incorporated but two of the amines, the trihydroxy-compound (26d) and the dihydroxy-amine (26c), gave good incorporations into both (1) and (2). Degradation (see Table 2) demonstrated that, as is the case with tyramine and dopamine, the trioxygenated C_6C_2N unit had been used specifically to form ring c and its ethanamine side-chain. These findings show that the biosynthesis of (1) and (2) in *S. japonica* involves the first alternative above, *viz.* formation of a trioxygenated ring early on the pathway, and that this ring becomes mono-*O*-methylated. Rejection by the plant of the amines (26a and b) indicates in addition that a further *O*-methylation is not the next step, in contrast with the situation in cacti.[26] This conclusion is consistent with our earlier negative results using postulated advanced precursors of the penta-oxygenated 1-benzylisoquinoline type; in all these cases, the trioxygenated ring was di-*O*-methylated.

Knowledge of the biosynthetic pathways to hasubanonine (1) and protostephanine (2) from the present work is summarised in Scheme 6. By combining building block (26c) with a C_6C_2 residue of the type (29) or (30), a set of isoquinolines (and bisphenethylamines) can be designed for testing in the plants. This work, which demonstrated the involvement of 1-benzyltetrahydroisoquinolines on the biosynthetic pathway to these unusual *Stephania* alkaloids, is described in the following paper.[27]

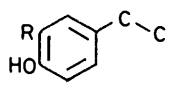


SCHEME 6

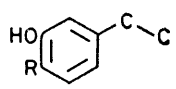
TABLE 2

Degradation of labelled protostephanine (2)

Expt. no. (Table 1)	Relative molar activities: Protostephanine dihydro-α-methine	Relative molar activities: *p*-Bromophenacyl acetate (see ref. 1)
1	100	53
2	100	8
3	100	7
4	100	6
7	100	5
8	100	7



(29) R = H, OMe



(30) R = OH, OMe

EXPERIMENTAL

General directions are as detailed in Part 23 [1] with the following additions. In addition to the instruments listed, some i.r. spectra were recorded on Unicam S.P. 200 and Perkin-Elmer 125 spectrophotometers, and some n.m.r. spectra on Varian A60 or Perkin-Elmer R.12 spectrometers; n.m.r. data measured at 60 MHz are indicated in the text.

Radioactive samples were counted using Packard liquid-scintillation counters, models 3003, 3325, 3375, and 3385. Toluene solutions of scintillators were used and efficiency was determined by addition of [^{14}C]hexadecane as internal standard. Synthesis of labelled compounds is described only where the procedure used differed from the unlabelled series. Radiochemical purity of synthetic materials was established using t.l.c. and a thin layer scanner, Panax RTLS-1A, and wherever possible by crystallisation to constant activity.

Plant Cultivation and Administration of Precursors.—Whole plants of *S. japonica* Miers were grown at 32 °C and high humidity. The precursors (usually 0—40 mg, activity 50—350 μCi) were fed in aqueous solution (1—2 ml) where possible, or in 9 : 1 (v/v) water–DMSO for less soluble compounds; amines were administered as their hydrochlorides. They were introduced into healthy young plants, having at least four stems, using drawn out capillary tubes pierced directly into the plant stems. Approximately ten tubes per plant were used and four whole plants per feeding. Rapid uptake of the solutions was achieved by careful choice of the stems, the best being young stems with above average amounts of foliage. Following uptake, a further 1—2 ml of water was administered to the plant *via* the same tubes to rinse any residual material into the plant. The complete feeding apparatus was rinsed with methanol and this solution assayed for radioactivity; typically 1—2% of the activity remained in the apparatus.

The plants were grown for a further 10 days, harvested whole, but free of soil, and then stored at −25 °C until required.

Isolation of the Alkaloids.—The four plants, including roots, were cut into small pieces and macerated in a blender with methanol (1—2 l). The macerate was poured into a large glass column and eluted with a solution of tartaric acid in methanol (2% w/v; 3—4 l), followed by methanol (2—4 l). The total eluate was evaporated to near-dryness on a rotary evaporator using a continuous-feed system. The total residues were partitioned between water (400 ml) and ether (500 ml), the aqueous layer (pH 2) was further extracted with ether (typically 3 × 500 ml) and then basified by careful addition of solid sodium hydrogen carbonate, followed by aqueous sodium hydroxide, to pH 9. Extraction of this basic solution with dichloromethane (3 × 500 ml) and evaporation of the organic layers gave the total alkaloid fraction as a gum (2—4 g). This gum, in dichloromethane (200 ml), was washed with aqueous sodium hydroxide (5% w/v; 4 × 150 ml) to remove phenolic bases and the aqueous layers were back-extracted with dichloromethane (1 × 200 ml). The combined organic solutions were washed with water (2 × 200 ml), dried, and evaporated to afford the non-phenolic alkaloids as a gum (2—4 g).

For some feedings it was necessary to add radioinactive synthetic protostephanine both to the initial eluate after masceration and/or to the crude non-phenolic fraction as the natural production of this alkaloid was low. This has proved unnecessary in later work.[27]

This non-phenolic mixture was further separated into hasubanonine- and protostephanine-containing fractions by chromatography (Merck Kieselgel 60; 70—320 mesh), eluting with (i) benzene, then (ii) benzene–methanol (9 : 1 v/v) followed by (iii) benzene–ethyl acetate–diethylamine (7 : 2 : 1 v/v/v). A typical result was as follows: non-phenolic alkaloids 3.17 g, adsorbent 80 g, eluants (i) 50 ml, (ii) 500 ml, (iii) 700 ml. Fractions collected (a) 550 ml, 5 mg; (b) 180 ml, 2.65 g; (c) 50 ml, 33 mg; (d) 300 ml, 202 mg; (e) 150 ml, 135 mg. Fraction (b) was found to contain hasubanonine whereas fractions (c) and (d) contained protostephanine. The alkaloids were detected by t.l.c. on silica plates in two different solvent systems, benzene–methanol (9 : 1 v/v), and benzene–diethylamine (96 : 4 v/v).

Hasubanonine (1).—The hasubanonine-containing fraction, (b) above, was separated by preparative t.l.c. on silica plates developed in ethyl acetate. The main band (R_F 0.4) was removed and the alkaloid extracted with ethyl acetate–methanol (9 : 1 v/v) to afford hasubanonine as a yellow syrup (0.7—1.3 g). To this in ethanol (5 ml) was added dilute nitric acid (5M; 0.3 ml) at 0 °C. The nitrate (0.15—0.9 g) was collected and recrystallised from methanol as needles, m.p. 218—220 °C (lit.,[28] 222 °C). Further purification if necessary could be achieved by recovering the free base from the nitrate, subjecting it to further preparative t.l.c. on silica, developing with benzene–diethylamine (95 : 5 v/v), and re-isolating the hasubanonine as the nitrate. For incorporation studies, the hasubanonine nitrate was recrystallised to constant activity. All samples were dried at 0.1 mmHg for 24 h prior to counting; the nitrate was counted directly by dissolving the sample in methanol–water (1 : 2 v/v; 0.3 ml) before addition of scintillator solution. Incorporations were calculated as usual[29] and are based on the weight of hasubanonine nitrate first obtained.

Protostephanine (2).—The protostephanine-containing fractions, (c) and (d) above, were purified by preparative t.l.c. on silica plates developed twice with benzene–diethylamine (96 : 4 v/v). The protostephanine band was removed and extracted with benzene–ethyl acetate–diethylamine (7 : 2 : 1 v/v/v). The gum obtained on evaporation (50—100 mg) was percolated in chloroform solution down a column of alumina (1 g : 10 mg alkaloid). Evaporation of the eluate gave protostephanine, which, in hot methanol, was treated with methanolic picric acid (1 mol equiv.) to give the picrate, m.p. 190—192 °C (from methanol) (lit.,[23] 209 °C). Samples were dried at 0.1 mmHg and 65 °C for 16 h over P_2O_5 (Found: C, 55.0; H, 5.3; N, 9.4. $C_{27}H_{30}N_4O_{11}$ requires C, 55.3; H, 5.2; N, 9.55%). For incorporation studies, the picrate was recrystallised to constant activity and counted as the free base by percolating a weighed sample of the picrate through alumina (*ca.* 25 mm in a Pasteur pipette) with chloroform (total 5 ml) into a counting vial. The chloroform was then removed under a stream of nitrogen before addition of scintillator solution. Incorporations were calculated as for hasubanonine, and based on the weight of material obtained after preparative t.l.c. and alumina filtration.

4-Benzyloxy-3,5-dimethoxybenzaldehyde.—4-Hydroxy-3,5-dimethoxybenzaldehyde (9.6 g) in the minimum of anhydrous methanol was mixed with potassium hydroxide (3.25 g), also in the minimum of dry methanol. Benzyl chloride (dried and freshly distilled; 7.35 g) was added and the mixture heated at reflux with the exclusion of moisture until benzylation was complete (t.l.c.). The cooled mixture was diluted with water, made basic (pH 10—11), and extracted with chloroform. The chloroform extracts were washed with dilute sodium hydroxide solution, and then water, dried, and evaporated to yield 4-benzyloxy-3,5-dimethoxybenzaldehyde (12.7 g; 88%), m.p. 60 °C (lit.,[30] 63 °C), $\nu_{max.}$ 1 690 cm^{-1}; $\lambda_{max.}$ 217 and 290 nm; δ (60 MHz)

3.86 (6 H, s, 2 × OCH_3), 5.12 (2 H, s, benzyl-CH_2), and 7.12 (2 H, s, ArH); *m/e* 272 (M^+).

3-*Benzyloxy*-4,5-*dimethoxybenzaldehyde*.—Methyl 3-benzyloxy-4,5-dihydroxybenzoate [31] (6.5 g) in acetone (22 ml) was treated with dimethyl sulphate (12 ml) and potassium carbonate (16 g). The mixture was heated at reflux for 3 h, filtered, and the solid dissolved in water (50 ml). The aqueous layer was extracted with chloroform (3 × 50 ml) and the chloroform solutions, combined with the acetone filtrate, were evaporated to dryness. Sodium hydroxide solution (2M; 75 ml) was added and after 2 h at 20 °C the mixture was extracted with chloroform (3 × 50 ml) to give methyl 3-benzyloxy-4,5-dimethoxybenzoate, m.p. 73—75 °C from ethanol (lit.,[32] 74 °C), $\nu_{max.}$ 1 710, 1 590, and 1 500 cm^{-1}; $\lambda_{max.}$ 222 and 263 nm; δ 3.87 and 3.90 (9 H, 2s, 3 × OCH_3), 5.13 (2 H, s, CH_2O), and 7.25—7.5 (7 H, m, ArH); *m/e* 302 (M^+).

This ester (20 g) in absolute ethanol (25 ml) was heated under reflux with hydrazine dihydrate (15 ml) for 12 h to give the hydrazide (19.5 g), m.p. 136 °C (lit.,[33] 137 °C). This (16.4 g) in anhydrous pyridine (75 ml) was stirred at 0 °C during dropwise addition of benzenesulphonyl chloride (12 ml), then for 15 min at 0 °C and for 2 h at 20 °C. Hydrochloric acid (2M) was added and the solid was collected and washed with cold methanol to afford the benzenesulphonyl hydrazide, m.p. 213—214 °C (from methanol) (lit.,[33] 215 °C).

The sulphonyl hydrazide (17 g) was suspended in ethylene glycol (175 ml) and anhydrous sodium carbonate was carefully added. The solution was heated for a further 3 min and then boiling water (100 ml) was added. The solution was cooled, poured into more water (200 ml) and extracted with ether (3 × 200 ml and 1 × 250 ml). The washed ethereal extracts were evaporated to yield crude 3-benzyloxy-4,5-dimethoxybenzaldehyde (9.4 g). Recrystallisation from ether gave pure material (7.1 g), m.p. 64.5 °C (lit.,[34] 67—68 °C), $\nu_{max.}$ 1 684 cm^{-1}; $\lambda_{max.}$ 235 and 292 nm; δ(60 MHz) 3.88 and 3.92 (each 3 H, s, OCH_3), 5.14 (2 H, s, benzyl-CH_2), 7.25 (7 H, m, ArH), and 9.68 (1 H, s, CH=O).

4-*Benzyloxy*-3,5-*dimethoxy*-β-*nitrostyrene*.—The corresponding benzaldehyde (see earlier) (14 g) in absolute ethanol (100 ml) was treated with freshly distilled nitromethane (5.75 ml) and cooled to 0 °C. Potassium hydroxide (7.65 g) in ice-cold ethanol (80 ml) was added over 10 min to the mixture, maintained at 0 °C. After a further 10 min, the mixture was poured into dilute hydrochloric acid (15% w/v; 130 ml) and the nitrostyrene was collected, washed with water, and dried (9.8 g, 61%), m.p. 132 °C (from methanol) (lit.,[30] 133 °C), $\nu_{max.}$ 1 635 and 1 585 cm^{-1}; $\lambda_{max.}$ 212 and 353 nm; δ(60 MHz) 3.82 (6 H, s, 2 × OCH_3), 5.18 (2 H, s, benzyl-CH_2), 6.72 (2 H, s, ArH), and 7.46 and 7.93 (each 1 H, d, *J* 13.5 Hz, CH=CH *trans*); *m/e* 315 (M^+).

3-Benzyloxy-4,5-dimethoxy-β-nitrostyrene was prepared as above from the corresponding benzaldehyde, and had m.p. 101—102 °C (from ethanol) (lit.,[35] 103—103.5 °C), $\nu_{max.}$ 1 635, 1 583, 1 523, and 1 333 cm^{-1}; δ(60 MHz) 3.87 and 3.92 (each 3 H, s, OCH_3), 5.12 (2 H, s, benzyl-CH_2), and 7.43 and 7.90 (each 1 H, d, *J* 14.2 Hz, CH=CH *trans*).

2-(4-*Benzyloxy*-3,5-*dimethoxyphenyl*)*ethylamine* (6a).—The appropriate β-nitrostyrene (see above) (5.5 g) in dry tetrahydrofuran (THF) (150 ml) was added dropwise over 3 h to a vigorously stirred suspension of lithium aluminium hydride (6.9 g) in THF (100 ml) heated under reflux. This heating was continued for a further 6 h and the mixture was then cooled. The excess of hydride was destroyed by cautious addition of a saturated aqueous solution of Rochelle salt. The mixture was filtered and the residue repeatedly extracted with boiling THF. The residue from evaporation of the combined THF solutions in dilute hydrochloric acid (100 ml) was washed with chloroform (3 × 100 ml), and the aqueous layer then basified with aqueous sodium hydroxide. Extraction with chloroform (4 × 100 ml) (extracts dried over anhydrous K_2CO_3) and evaporation gave the amine (3.3 g, 66%), $\nu_{max.}$ 3 550 and 1 595 cm^{-1}; δ(CCl_4; 60 MHz) 3.78 (6 H, s, 2 × OCH_3), 4.89 (2 H, s, *O*-benzyl-CH_2), and 6.36 (2 H, s, ArH). Treatment of this base in methanol with methanolic oxalic acid afforded the oxalate salt (3.96 g), m.p. 184 °C (decomp.) (from methanol) (lit.,[3] 195—197 °C).

2-(3-Benzyloxy-4,5-dimethoxyphenyl)ethylamine (6b) was prepared in similar fashion from the corresponding β-nitrostyrene and was isolated as the oxalate, m.p. 186 °C (from ethanol–water) (lit.,[35] 187—187.5 °C), $\nu_{max.}$ (KBr) 3 255, 1 722, 1 613, and 1 585 cm^{-1}; δ(TFA; 60 MHz) 3.98 and 4.00 (each 3 H, s, OCH_3), 5.22 (2 H, s, *O*-benzyl-CH_2), and 6.69—7.40 (7 H, m, ArH).

3-Benzyloxy-4-methoxyphenylacetic acid (5a), m.p. 125—126 °C (lit.,[36] 127—128 °C) was prepared from *O*-benzylisovanillin *via* the benzyl alcohol, chloride, and phenylacetonitrile.[36]

3-*Benzyloxy*-4-*methoxy*[1-^{14}C]*phenylacetonitrile*.—Rigorously purified 3-benzyloxy-4-methoxybenzyl chloride (200 mg) in dry dimethylformamide (DMF) (2.5 ml) was stirred at 20 °C for 2 h with radioinactive potassium cyanide (10 mg). Potassium [^{14}C]cyanide (1.43 mg; 1 mCi) was then added and the mixture stirred for 18 h before addition of further inactive KCN (48 mg) and a final stirring period of 36 h. Water (2.5 ml) was added and the mixture extracted with benzene–ether (1 : 1 v/v) (5 × 4 ml). The organic extracts were combined, washed with water (4 × 5 ml), dried, and evaporated to yield the nitrile (187 mg, 97%; 0.76 mCi), identical with a radioactive sample.[36]

4-*Benzyloxy*-3-*methoxyphenylacetic Acid* (5b).—The acid (5b), m.p. 116—117 °C (lit.,[37] 116 °C), was prepared from 4-benzyloxy-3-methoxybenzaldehyde [38] by the same methods as for the 3-benzyloxy-4-methoxy-isomer above, with the following modification for preparation of the nitrile: the corresponding benzyl chloride (26 g) in DMF (250 ml) was stirred with sodium cyanide (8.1 g) with exclusion of moisture for 36 h. The mixture was then poured into saturated brine (1 l) and extracted with ethyl acetate (1 × 500 and 2 × 250 ml), and the combined organic layers were washed with water, dried, and evaporated to leave a solid which was recrystallised from benzene–light petroleum (b.p. 60—80 °C) to give the pure nitrile (23.1 g, 94%), m.p. 65—67 °C (lit.,[39] 69—70 °C) (from ethanol).

N-(4-*Benzyloxy*-3,5-*dimethoxyphenethyl*)-2-(3-*benzyloxy*-4-*methoxyphenyl*)*acetamide* (7a).—3-Benzyloxy-4-methoxyphenylacetic acid (5a) (2.12 g) in dry benzene (50 ml) was stirred at 20 °C for 1 h with oxalyl chloride (2 ml) and DMF (2 drops). The solution was evaporated and the residue repeatedly taken up in benzene and re-evaporated to remove the excess of oxalyl chloride. The phenylacetyl chloride remaining [$\nu_{max.}$ (film) 1 790, 1 610, and 1 595 cm^{-1}] in dry dichloromethane (60 ml) was added dropwise to a vigorously stirred two-phase system of water (30 ml) and dichloromethane (40 ml) containing sodium hydrogen carbonate (4.55 g) and the oxalate salt of 4-benzyloxy-3,5-dimethoxyphenethylamine (6a) (4.1 g). After being stirred for 1½ h, the phases were separated, the organic layer being washed

with water, dilute acid, and water again, dried, and evaporated to leave the amide (3.8 g, 90%), m.p. 124 °C (from ethyl acetate) (lit.,[3] 124—126 °C), $\nu_{max.}$ 3 450, 1 665, and 1 600 cm^{-1}; δ(60 MHz) 2.64 (2 H, t, *J* 7 Hz, $ArCH_2CH_2$), 3.42 (2 H, s, $ArCH_2CO$), 3.75 (6 H, s, 2 × OCH_3), 3.86 (3 H, s, OCH_3), 4.98 and 5.12 (each 2 H, s, *O*-benzyl-CH_2), 5.45br (1 H, s, NH), 6.3 (2 H, s, ArH), and 6.74—6.76 (3 H, m, ArH).

N-(3-*Benzyloxy*-4,5-*dimethoxyphenethyl*)-2-(3-*benzyloxy*-4-*methoxyphenyl*)*acetamide* (7b) prepared similarly from (5a) and (6b) had m.p. 127—128 °C (from ethyl acetate) (Found: C, 73.0; H, 6.2; N, 2.7. $C_{33}H_{35}NO_6$ requires C, 73.2; H, 6.5; N, 2.6%); $\nu_{max.}$ 1 660 cm^{-1}; δ(60 MHz) 3.38 (2 H, s, $ArCH_2CO$), 3.77, 3.80, and 3.82 (each 3 H, s, OCH_3), 5.03 and 5.08 (each 2 H, s, *O*-benzyl-CH_2), and 5.47br (1 H, s, NH); *m/e* 541 (M^+).

N-(4-*Benzyloxy*-3,5-*dimethoxyphenethyl*)-2-(4-*benzyloxy*-3-*methoxyphenyl*)*acetamide* (7d) was likewise prepared from (5b) and (6a), and had m.p. 121 °C (Found: C, 73.1; H, 6.75; N, 2.8%; M^+, 541.245. $C_{33}H_{35}NO_6$ requires C, 73.15; H, 6.5; N, 2.6%; *M*, 541.245); $\nu_{max.}$ 3 440, 1 660, and 1 595 cm^{-1}; δ(60 MHz) 2.68 (2 H, t, *J* 7 Hz, $ArCH_2CH_2$), 3.41 (2 H, t, *J* 7 Hz, $ArCH_2CH_2$), 3.44 (2 H, s, $ArCH_2CO$), 3.73 (6 H, s, 2 × OCH_3), 3.83 (3 H, s, OCH_3), 4.95 and 5.11 (each 2 H, s, *O*-benzyl-CH_2), and 6.30 (2 H, s, ArH).

7-*Benzyloxy*-1-(3-*benzyloxy*-4-*methoxybenzyl*)-6,8-*dimethoxy*-2-*methyl*-1,2,3,4-*tetrahydroisoquinoline* (9a).—The amide (7a) (4.4 g) in dry toluene (100 ml) was heated at reflux for 45 min under nitrogen with freshly distilled phosphorus oxychloride (12.5 ml). The mixture was then repeatedly evaporated to dryness from toluene solution to remove the excess of phosphorus oxychloride to leave 7-benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6,8-dimethoxy-3,4-dihydroisoquinoline hydrochloride (8a) which was used without further purification, $\nu_{max.}$ 2 650br, 1 640, and 1 595 cm^{-1}.

This hydrochloride was converted into the free base by partitioning between ethyl acetate (50 ml) and sodium hydrogen carbonate solution (10% w/v; 25 ml) under nitrogen. The ethyl acetate layer was washed with water, dried, and evaporated. Redistilled methyl iodide (29 ml) was added to the residue and the solution under nitrogen was kept at 20 °C for 18 h. Evaporation gave the 2-methyl-3,4-dihydroisoquinolinium iodide (5.1 g, 93%), $\nu_{max.}$ 1 620 and 1 595 cm^{-1}. A solution of this methiodide in ethanol (800 ml) was stirred at 0 °C during addition of sodium borohydride (0.5 g) in portions over 30 min, and then for a further 1 h. The mixture was acidified, evaporated to low volume, diluted with water, and basified with sodium hydrogen carbonate. The basic aqueous phase was extracted with chloroform (3 × 100 ml) and the chloroform layers washed with water, dried (anhydrous potassium carbonate), and evaporated to yield the tetrahydroisoquinoline (9a) as a gum (4.1 g) that was converted in methanol into the picrate salt (5.2 g, 90%), m.p. 143.5—144.5 °C (from methanol) (Found for the free base: M^+, 539.269. $C_{34}H_{37}NO_5$ requires *M*, 539.267), $\nu_{max.}$ 1 605 and 1 595 cm^{-1}; δ(60 MHz) 2.30 (3 H, s, NCH_3), 3.80, 3.87, and 3.90 (each 3 H, s, OCH_3), 5.02 and 5.13 (each 2 H, s, *O*-benzyl-CH_2), 6.40 (1 H, s, ArH), and 6.80 (3 H, s, ArH).

6-*Benzyloxy*-1-(3-*benzyloxy*-4-*methoxybenzyl*)-7,8-*dimethoxy*-2-*methyl*-1,2,3,4-*tetrahydroisoquinoline* (9b) *and the* 8-*Benzyloxy*-6,7-*dimethoxy-isomer* (9c).—The corresponding amide (7b) was cyclised and *N*-methylated as described above, to afford a mixture of the 6-benzyloxy- and 8-benzyloxy-2-methyl-3,4-dihydroisoquinolinium iodides as a gum that solidified on trituration with ether. This solid in methanol was diluted with ether until the solution became cloudy. The 6-benzyloxy-isomer crystallised at 0 °C, m.p. 147—148 °C, $\nu_{max.}$ (KBr) 1 618 and 1 519 cm^{-1}; δ(TFA) 3.61 (3 H, s, $\overset{+}{N}CH_3$), 3.87, 3.95, and 3.97 (each 3 H, s, OCH_3), 4.41 (2 H, s, $ArCH_2C{=}N$), 4.59 and 5.26 (each 2 H, s, *O*-benzyl-CH_2), and *ca.* 3.0 (m, ArH). Evaporation of the mother-liquors yielded the amorphous 8-benzyloxy-isomer. The crystalline methiodide, without further purification, was reduced as above to afford the 6-*benzyloxy*-1,2,3,4-*tetrahydroisoquinoline* (9b), isolated as the *picrate*, m.p. 85.5—86.5 °C (Found: C, 62.5; H, 5.2; N, 7.05. $C_{40}H_{40}O_{12}N_4$ requires C, 62.5; H, 5.2; N, 7.15%). The free amine had δ 2.26 (3 H, s, NCH_3), 3.81, 3.84, and 3.87 (each 3 H, s, OCH_3), 5.02 and 5.06 (each 2 H, s, *O*-benzyl-CH_2), 6.39 (1 H, ArH), 6.75 (2 H, m, ArH), and 7.35 (5 H, m, ArH); *m/e* 539 (M^+). The 8-benzyloxy-isomer (9c) was prepared similarly, δ 2.11 (3 H, s, NCH_3).

7-*Benzyloxy*-1-(4-*benzyloxy*-3-*methoxybenzyl*)-6,8-*dimethoxy*-1,2,3,4-*tetrahydroisoquinoline* (9d).—This was prepared by cyclisation of the amide (7d) as above to give the 3,4-dihydroisoquinoline (8d) as its hydrochloride, $\nu_{max.}$ 1 640 and 1 595 cm^{-1}, which was reduced directly by sodium borohydride in methanol. Evaporation of the methanol, partition of the residue between water and chloroform, followed by washing (water), drying, and evaporation of the chloroform extract, afforded the 1,2,3,4-*tetrahydroisoquinoline* (9d) (Found: M^+, 525.243. $C_{33}H_{35}NO_5$ requires *M*, 525.252), $\nu_{max.}$ 1 605 and 1 590 cm^{-1}; δ(CCl_4; 60 MHz) 3.77, 3.82, and 3.96 (each 3 H, s, OCH_3), 4.98 and 5.01 (each 2 H, s, *O*-benzyl-CH_2), 6.34 (1 H, s, ArH), and 6.75 (3 H, m, ArH).

Preparation of Phenolic Isoquinolines for Incorporation Experiments.—The dibenzyl ether (9a) was recovered from its picrate (384 mg) by passage through a column of alumina (neutral grade 3) (8 g) in chloroform. The eluate was evaporated and the residue in ethanol (10 ml), water (2 ml), and concentrated hydrochloric acid (0.38 ml) was stirred under hydrogen (1 atm.) with 10% palladium–charcoal catalyst (140 mg) until uptake was complete (3 h). The suspension was filtered through Celite and the filtrate evaporated to leave the diphenolic tetrahydroisoquinoline (3) as its hydrochloride (210 mg; quantitative), $\nu_{max.}$ 3 550, 2 550br, 1 615, and 595 cm^{-1}; δ(TFA; 60 MHz) 3.06 (3 H, s, NCH_3), 3.97, 4.05, and 4.14 (each 3 H, s, OCH_3), 6.74 (1 H, s, ArH), and 7.02 (3 H, m, ArH). The [1-^{14}C]-labelled material was prepared similarly starting from 3-benzyloxy-4-methoxy[1-^{14}C]phenylacetic acid (see earlier). The *O*-benzyl groups were removed in a similar way from (9b) to give (4a) as the hydrochloride, $\nu_{max.}$ 3 545 cm^{-1}; *m/e* 359 (M^+ − HCl), from (9c) to yield (4b), and from the foregoing di-*O*-benzyl ether of (15) to form the hydrochloride of the diphenol (15), $\nu_{max.}$ 3 540, 1 615, and 1 590 cm^{-1}; δ(TFA; 60 MHz), 3.68, 3.98, and 4.07 (each 3 H, s, OCH_3), 6.70 (1 H, s, ArH), and 6.95 (3 H, m, ArH).

Norlaudanosoline (13),[36] reticuline (12),[11,40] protosinomenine (14),[41] coclaurine (17),[42] and *N*-methylcoclaurine (18) [42] were prepared by the reported methods.

Acid-catalysed Tritiation of Precursors.[10]—Typically, the phenolic benzylisoquinoline as its hydrochloride (15—20 mg) was heated at 100 °C with tritiated water (40—100 mCi), acidified to a concentration of 2—3M with hydrochloric acid or thionyl chloride for 7 days in an evacuated

sealed tube. The tritiated water was then removed *in vacuo* and the residue repeatedly evaporated to dryness from methanol to remove labile tritium. The purity of the products was checked by comparison (t.l.c.) with authentic radioinactive samples.

3-(3,4-*Dihydroxyphenyl*)[2-^{14}C]*pyruvic Acid*.—Radioinactive glycine (35.55 mg) and sodium hydroxide (100 mg) were added to [2-^{14}C]glycine (500 μCi; 56 mCi mmol^{-1}) in water (1.5 ml) followed by benzoyl chloride (200 mg). The mixture was shaken for 1 h, kept for 18 h, then acidified with concentrated hydrochloric acid. The precipitate was collected and heated at 100 °C and 0.1 mmHg to remove benzoic acid, and the residue (90.5 mg; 0.4 mCi) was [2-^{14}C]hippuric acid (t.l.c.), identical to inactive material. This acid was treated [43] with 3,4-dihydroxybenzaldehyde (74.39 mg) and lead acetate (90 mg) in acetic anhydride (0.5 ml) and THF (2 ml). The mixture was heated under reflux for 3½ h, then the excess of acetic anhydride was decomposed with water (50 ml). Extraction with dichloromethane (3 × 30 ml) afforded the azlactone. Dilution of the mother-liquors with inactive azlactone (27 mg) allowed the isolation of a second crop to give a total recovery of pure 4-(3,4-diacetoxybenzylidene)-2-phenyl[4-^{14}C]-Δ^2-oxazolin-5-one (138.1 mg; 0.296 mCi), m.p. 135—136 °C (from ethanol) (lit.,[44] 137—138 °C). This azlactone in water (9 ml) and concentrated hydrochloric acid (3 ml) were sealed in a tube at 0.1 mmHg under nitrogen, and heated at 115 °C for 24 h. After cooling, the contents were diluted with water (10 ml), washed with benzene (3 × 15 ml), and then extracted with ethyl acetate (5 × 15 ml). The product from the ethyl acetate was diluted with inactive 3,4-dihydroxyphenylpyruvic acid (20 mg) and purified by preparative t.l.c. using ethanol–dichloromethane–acetic acid (5 : 5 : 1 v/v/v). The appropriate band was eluted with ethyl acetate–methanol (9 : 1 v/v) and the product was recrystallised twice from ether–light petroleum (b.p. 40—60 °C) to afford the pyruvic acid (48 mg; 0.11 mCi), m.p. 179—183 °C, or 188—189 °C (capillary) (lit.,[45] 179—182 °C and 188—189 °C), identical with radioinactive material prepared separately.[46]

3,4-*Bisbenzyloxy*[1-^{14}C]*phenylacetonitrile*.—3,4-Bisbenzyloxybenzyl alcohol (67 mg) was converted into the benzyl chloride,[47] and this in DMF (2 ml) was stirred at 20 °C with potassium cyanide (3 mg) for 24 h before addition of potassium [^{14}C]cyanide (1.26 mg; 1 mCi). After a further 48 h stirring, inactive potassium cyanide (28 mg) was added and the mixture was stirred for a final 60 h. It was then poured into saturated brine (25 ml) and extracted with ether (2 × 25 ml), and the combined organic layers were washed with water (2 × 50 ml) and evaporated to yield the [^{14}C]nitrile (64 mg, 93%; 0.79 mCi), identical (t.l.c.) with an inactive sample having m.p. 50—54 °C (lit.,[48] 53—54 °C) (from ethyl acetate–benzene); $\nu_{max.}$ 2 245, 1 590, and 1 510 cm^{-1}; $\lambda_{max.}$ 229 and 281 nm; δ 3.58 (2 H, s, ArCH_2CN), 5.13 (4 H, s, 2 × *O*-benzyl-CH$_2$), 6.7—7.0 (3 H, m, ArH), and 7.2—7.5 (10 H, m, ArH); *m/e* 329 (M^+).

4-Benzyloxy[1-^{14}C]phenylacetonitrile was prepared by the same method from the corresponding benzyl alcohol; it was identified by comparison with an inactive sample of m.p. 63.5—66.5 °C from chloroform–light petroleum (b.p. 60—80 °C) (lit.,[49] 68—69 °C); $\nu_{max.}$ 2 250, 1 610, 1 582, and 1 510 cm^{-1}; $\lambda_{max.}$ 236, 277, and 284 nm; δ 3.70 (2 H, s, ArCH_2CN), 5.10 (2 H, s, *O*-benzyl-CH$_2$), 7.00 and 7.28 (each 2 H, d, *J* 8.5 Hz, ArH), and 7.3—7.5 (5 H, m, ArH); *m/e* 223 (M^+).

3,4-Dibenzyloxyphenylacetic acid was prepared by heating the corresponding nitrile (500 mg) in ethylene glycol (20 ml) and water (5 ml) containing potassium hydroxide (1 g) under reflux for 18 h (*cf.* ref. 36). Work-up gave the acid (400 mg, 76%), m.p. 106.5—107.5 °C [from benzene–light petroleum (b.p. 60—80 °C)] (lit.,[50] 109 °C), $\nu_{max.}$ 3 300—2 800vbr, 1 708, 1 600, 1 582, and 1 506 cm^{-1}; $\lambda_{max.}$ 230 and 281 nm; δ 3.45 (2 H, s, ArCH_2CO$_2$H), 5.06 (4 H, s, 2 × *O*-benzyl-CH$_2$), 6.7—6.9 (3 H, m, ArH), 7.2—7.5 (10 H, m, ArH), and 9.86br (1 H, s, CO$_2$*H*); *m/e* 348 (M^+).

4-Benzyloxyphenylacetic acid was prepared as above, m.p. 119—122 °C (lit.,[48] 119.5—120.5 °C), $\nu_{max.}$ 2 500—3 300vbr, 1 712, 1 612, 1 584, and 1 511 cm^{-1}; $\lambda_{max.}$ 227, 277, and 283 nm; δ 3.56 (2 H, s, ArCH_2CO$_2$H), 5.04 (2 H, s, *O*-benzyl-CH$_2$), 6.91 and 7.18 (each 2 H, d, *J* 8.5 Hz ArH), and 7.3—7.5 (5 H, m, ArH); *m/e* 242 (M^+).

3,4-*Bisbenzyloxyphenethylamine*.—3,4-Bisbenzyloxy-β-nitrostyrene [47] (10 g) in dry THF (120 ml) was added over 2¼ h to a stirred suspension of lithium aluminium hydride (6 g) in THF (150 ml) at 20 °C. The mixture was heated under reflux for 1½ h, cooled, and the excess of hydride decomposed by successive addition of water (6 ml), sodium hydroxide solution (6 ml; 15% w/v), and water (18 ml). The suspension was filtered, the solids were washed with THF and then extracted with ether (100 ml) under reflux for 30 min, and the mixture was filtered. Evaporation of the combined filtrates left a gum which was treated, in ethyl acetate, with a saturated solution of hydrogen chloride in ether. The precipitate was recrystallised from ethyl acetate containing a small amount of ethanol to give the amine hydrochloride (7.13 g; 70%), m.p. 129—133 °C (lit.,[47] 131 °C), $\nu_{max.}$ 1 580br and 1 500br cm^{-1}; $\lambda_{max.}$ 228 and 280 nm; δ 2.9—3.3br (4 H, m, ArCH_2CH_2N), 4.93 and 5.00 (each 2 H, s, *O*-benzyl-CH$_2$), 6.7—6.9 (3 H, m, ArH), 7.1—7.5 (10 H, m, ArH), and 8.3br (3 H, s, $\overset{+}{N}H_3$); *m/e* 333 (M^+ − HCl).

N-(3,4-*Bisbenzyloxyphenethyl*)-3,4-*bisbenzyloxyphenylacetamide*. Prepared from the appropriate acid and amine hydrochloride, by the method detailed earlier for (7a), the amide was obtained as crystals, m.p. 121.5—125 °C (from 95% ethanol) (lit.,[47] 123 °C), $\nu_{max.}$ 3 400br, 1 655, and 1 503 cm^{-1}; $\lambda_{max.}$ 236, 279, and 284 nm; δ 2.55 (2 H, t, *J* 6 Hz, ArCH_2CH$_2$NH), 3.34 (2 H, q, *J* 6 Hz, ArCH$_2$CH_2NH), 3.36 (2 H, s, ArCH_2CO), 5.06 (8 H, s, 4 × *O*-benzyl-CH$_2$), 5.32br (1 H, s, NH), 6.4—6.9 (6 H, m, ArH), and 7.2—7.5 (20 H, m, ArH).

N-(3,4-Dibenzyloxyphenethyl)-4-benzyloxyphenylacetamide was similarly prepared, m.p. 125—126 °C (from 95% ethanol) (lit.,[51] 122—124 °C), $\nu_{max.}$ 3 410br, 1 653, and 1 510 cm^{-1}; $\lambda_{max.}$ 232, 278, and 283 nm; δ 2.64 (2 H, t, *J* 6 Hz, ArCH_2CH$_2$NH), 3.44 (4 H, m, ArCH$_2$CH_2NH and ArCH_2-CO), 5.02 (2 H, s, *O*-benzyl-CH$_2$), 5.12 (4 H, s, 2 × *O*-benzyl-CH$_2$), 5.48br (1 H, s, NH), 6.5—7.1 (7 H, m, ArH), and 7.2—7.6 (15 H, m, ArH); *m/e* 557 (M^+).

NN-*Bis*-(3,4-*dibenzyloxyphenethyl*)*amine*.—The corresponding amide above (80 mg) was dissolved in dry THF (2 ml) and diborane solution in THF [52] (1M; 5 ml) was added. The solution was heated at reflux for 30 min under nitrogen and cooled, and the excess of hydride was decomposed by addition of hydrochloric acid (6N; 2 ml). The solvent was evaporated off and the residue partitioned between saturated aqueous sodium hydrogen carbonate (25 ml) and ethyl acetate (3 × 25 ml). The organic layers

afforded a gum which was purified by preparative t.l.c. on silica using benzene–ethyl acetate–diethylamine (7 : 2 : 1 v/v/v), and was converted in ethyl acetate–ether into the *amine hydrochloride* (62 mg; 75%), m.p. 143—146 °C (Found: C, 74.5; H, 6.3; N, 2.3. $C_{44}H_{44}ClNO_4 \cdot 1.5H_2O$ requires C, 74.1; H, 6.6; N, 2.0%), $\nu_{max.}$ 2 760, 2 450, 1 602, 1 587, and 1 510 cm^{-1}; $\lambda_{max.}$ 229 and 281 nm; δ 3.14 (8 H, m, 2 × $ArCH_2CH_2N$), 5.03br (8 H, s, 4 × *O*-benzyl-CH_2), 6.6—6.9 (6 H, m, ArH), 7.1—7.5 (20 H, m, ArH), and 10.1—10.5vbr (2 H, $\overset{+}{N}H_2$); *m/e* 650, 649 (M^+ − HCl), 648, and 647.

N-(4-*Benzyloxyphenethyl*)-N-(3,4-*bisbenzyloxyphenethyl*)-*amine*.—This was prepared similarly from the appropriate amide and isolated as either its *hydrochloride*, m.p. 152—156 °C, or *oxalate*, m.p. 182—186 °C (from ethanol) [Found (for the oxalate): C, 73.8; H, 6.3; N, 2.3. $C_{39}H_{39}NO_7$ requires C, 73.9; H, 6.2; N, 2.2%], $\nu_{max.}$ 2 720, 2 400, 1 600, 1 580, and 1 505 cm^{-1}; $\lambda_{max.}$ 226, 278, and 283 nm; δ 3.16 (8 H, m, 2 × $ArCH_2CH_2N$), 4.90 (2 H, s, *O*-benzyl-CH_2), 5.00 (4 H, s, 2 × *O*-benzyl-CH_2), 6.6—7.6 (22 H, m, ArH), and 10.1—10.4vbr (2 H, $\overset{+}{N}H_2$); *m/e* 543 (M^+ − HCl).

Preparation of Phenolic NN-*Bisphenethylamines for Incorporation Experiments*.—The foregoing tetrabenzyloxyamine hydrochloride (40 mg) was hydrogenated as detailed earlier for the benzyloxyisoquinolines in ethanol (10 ml) and concentrated hydrochloric acid (5 drops) over 10% palladium–charcoal (30 mg) for 2 h. The residue obtained after evaporation of solvents was triturated with propan-2-ol to give *NN*-bis-(3,4-dihydroxyphenethyl)amine (24b) as its hydrochloride (20 mg; quantitative), m.p. >325 °C, sinters at 105 °C, $\nu_{max.}$ (Nujol) 3 260, 3 110, 1 650w, 1 605, and 1 525 cm^{-1}; $\lambda_{max.}$ 225 and 283 nm; δ([$^2H_6$]DMSO) 2.6—3.2 (8 H, m, 2 × $ArCH_2CH_2N$), 3.31 (2 H, s, $\overset{+}{N}H_2$), 6.3—6.75 (6 H, m, ArH), and 9.7—9.8 (4 H, m, 4 × OH).

Similarly, amorphous *N*-(4-hydroxyphenethyl)-*N*-(3,4-dihydroxyphenethyl)amine (24a) was isolated as the hydrochloride, δ([$^2H_6$]DMSO) 2.7—3.2 (8 H, m, 2 × $ArCH_2CH_2N$), 3.2—3.7 (2 H, m, $\overset{+}{N}H_2$), 6.4—7.1 (7 H, m, ArH), and 8.5—9.5br (3 H, m, 3 × OH).

6,7-*Bisbenzyloxy*-1-(3,4-*bisbenzyloxybenzyl*)-3,4-*dihydroisoquinoline*.—The corresponding tetrakisbenzyloxy-amide above (104 mg) in acetonitrile (6 ml; dried and redistilled) was warmed under nitrogen to 60 °C, when phosphorus oxychloride (0.25 ml; freshly distilled) was added. After heating under reflux for 25 min the solution was evaporated to dryness. The residue in ethanol (1 ml) and hydrochloric acid (1M; 6 drops) was mixed with water (2.5 ml) and the precipitate was recrystallised from ethanol–ether to yield the dihydroisoquinoline hydrochloride (91 mg, 85%), m.p. 181—185 °C (lit.,[47] 198 °C) (Found: C, 73.1; H, 5.9; N, 2.1. Calc. for $C_{44}H_{40}ClNO_4 \cdot 2H_2O$: C, 73.6; H, 6.2; N, 2.0%), $\nu_{max.}$ 2 500vbr, 1 648, 1 602, 1 560, and 1 510 cm^{-1}; $\lambda_{max.}$ 219, 248, 309, and 356 nm; δ 5.1br and 5.2br (8 H, each s, 4 × *O*-benzyl-CH_2), 6.6—6.9br (5 H, m, ArH), and 7.1—7.5 (20 H, m, ArH); *m/e* 645 (M^+ − HCl), 644.

6,7-Bisbenzyloxy-1-(4-benzyloxybenzyl)-3,4-dihydroisoquinoline was prepared similarly from the corresponding amide and was isolated as the hydrochloride, m.p. 157—160 °C (lit.,[51] 154—160 °C), $\nu_{max.}$ 2 570vbr, 1 648, 1 601, 1 559, and 1 510 cm^{-1}; $\lambda_{max.}$ 226, 248, 305, and 353 (in base 229, 276, and 310) nm; δ 2.85 (2 H, m, $ArCH_2CH_2N$), 3.8 (2 H, m, $ArCH_2CH_2N$), 4.43br (2 H, s, $ArCH_2C{=}N$), 4.94, 5.14, and 5.21 (each 2 H, s, *O*-benzyl-CH_2), 6.9, 7.1, and 7.2 (6 H, m, ArH), and 7.2—7.5 (15 H, m, ArH); *m/e* 539 (M^+ − HCl).

6,7-*Bisbenzyloxy*-1-(3,4-*bisbenzyloxybenzyl*)-1,2,3,4-*tetrahydroisoquinoline*.—The corresponding imine hydrochloride (105 mg) was stirred in methanol solution (10 ml) at 0 °C with sodium borohydride (35 mg), added in portions over 15 min. After further stirring for 30 min, the methanol was evaporated off and the residue was partitioned between saturated brine (25 ml) and ethyl acetate (3 × 25 ml). The product from the ethyl acetate (98 mg) was converted in ethyl acetate–ether into the *tetrahydroisoquinoline hydrochloride* (97 mg, 92%), m.p. 204—209 °C [from ethanol–ether (softening at 150 °C)] (Found: C, 73.8; H, 6.2; N, 1.9. $C_{44}H_{42}ClNO_4 \cdot 2H_2O$ requires C, 73.4; H, 6.4; N, 1.9%), $\nu_{max.}$ 2 770vbr, 1 605, 1 585, and 1 510 cm^{-1}; $\lambda_{max.}$ 219 and 288 nm; δ 2.3 (2 H, m, $ArCH_2CH_2N$), 2.7—3.5br (5 H, m), 4.74 and 4.98 (each 2 H, s, *O*-benzyl-CH_2), 5.03 (4 H, s, 2 × *O*-benzyl-CH_2), 6.23 and 6.5—6.9 (5 H, m, ArH), and 7.1—7.5 (20 H, m, ArH).

6,7-*Bisbenzyloxy*-1-(4-*benzyloxybenzyl*)-1,2,3,4-*tetrahydroisoquinoline*.—The corresponding 3,4-dihydroisoquinoline was reduced in the same way and the product isolated as the *hydrochloride*, m.p. 185—186 °C (from ethanol) (Found: C, 74.1; H, 6.2; N, 2.4. $C_{37}H_{36}ClNO_3 \cdot 1.25H_2O$ requires C, 74.2; H, 6.5; N, 2.3%), $\nu_{max.}$ 2 750vbr, 1 605, 1 580, and 1 508 cm^{-1}; $\lambda_{max.}$ 229 and 285 nm; δ 1.85br (2 H, s, $ArCH_2CH_2N$), 2.8—3.5 (5 H, m), 4.66, 4.87, and 4.98 (each 2 H, s, *O*-benzyl-CH_2), 6.09 and 6.55 (each 1 H, s, ArH), 6.74 and 7.02 (each 2 H, d, *J* 8 Hz, ArH), and 7.1—7.4 (15 H, m, ArH).

6,7-*Dihydroxy*-1-(3,4-*dihydroxybenzyl*)-1,2,3,4-*tetrahydroisoquinoline* (13).—Prepared by hydrogenolysis from the foregoing tetrabenzyl ether as earlier, followed by trituration with water, the tetrahydroxytetrahydroisoquinoline (norlaudanosoline) was obtained as its hydrochloride (81%), m.p. 265—275 °C (decomp.) (lit.,[36] 278—281 °C), $\nu_{max.}$ (Nujol) 3 200vbr, 1 600, and 1 520 cm^{-1}; $\lambda_{max.}$ 225 and 287 nm; δ([$^2H_6$]DMSO) 2.7—3.5br (8 H, m), 4.45 (1 H, m), 6.5—6.8 (5 H, m, ArH), and 8.7—9.2br (4 H, m, 4 × OH).

6,7-Dihydroxy-1-(4-hydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (23) was similarly prepared from the tribenzyl ether as the hydrochloride, m.p. 258—268 °C (decomp.), $\nu_{max.}$ (Nujol) 3 400br, 3 200br, 1 608, 1 587, and 1 505 cm^{-1}; $\lambda_{max.}$ 229 and 286 nm; δ([$^2H_6$]DMSO) 2.7—3.5br (8 H, m), 4.44 (1 H, m), 6.51 and 6.56 (each 1 H, s, ArH), 6.7 and 7.1 (each 2 H, d, *J* 8.5 Hz, ArH), 8.7—9.5br (3 H, m, 3 × OH).

6,7-*Dihydroxy*-1-(3,4-*dihydroxybenzyl*)-2-*methyl*-1,2,3,4-*tetrahydroisoquinoline*.—The foregoing tetrakisbenzyloxy-3,4-dihydroisoquinoline hydrochloride (109 mg) was converted into the free base as described earlier for (8a) and then treated in ethyl acetate solution with methyl iodide (5 ml) under nitrogen for 6 days in the dark. The residue, after removal of the ethyl acetate, was reduced in ethanol (15 ml) at 0 °C with sodium borohydride (100 mg). The usual work-up gave a gum which was purified by preparative t.l.c. in benzene–diethylamine (9.5 : 0.5 v/v) to afford 6,7-*bisbenzyloxy*-1-(3,4-*bisbenzyloxybenzyl*)-2-*methyl*-1,2,3,4-*tetrahydroisoquinoline*, characterised as the *picrolonate* (32 mg), m.p. 149—150 °C (from dichloromethane–methanol) (Found: C, 71.6; H, 5.7; N, 7.6. $C_{55}H_{51}N_5O_9$ requires C, 71.3; H, 5.6; N, 7.6%). The free base (47 mg), recovered from the picrolonate (71 mg) by passage over alumina (activity III; 10 g) in dichloromethane, was hydrogenated as described earlier to yield the tetrahydroxy-

compound (laudanosoline) (25 mg), identical with authentic material.

6,7-Dihydroxy-1-(4-hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline.—This was prepared as above from the corresponding tribenzyloxy-3,4-dihydroisoquinoline *via* *6,7-bisbenzyloxy-1-(4-benzyloxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline*, characterised as the *hydrochloride*, m.p. 172—174 °C (from methanol–ether) (Found: C, 77.0; H, 6.3; N, 2.3. $C_{38}H_{38}ClNO_3$ requires C, 77.1; H, 6.5; N, 2.4%), $\nu_{max.}$ 2 420br, 1 605, and 1 510 cm^{-1}; δ 3.80 (3 H, s, NCH_3), and 4.63, 4.98, and 5.09 (each 2 H, s, *O*-benzyl-CH_2). This was debenzylated as usual to yield the *trihydroxy-compound*, obtained as its *hydrochloride*, a non-crystalline solid from methanol–ether (Found: C, 63.7; H, 6.6; N, 4.1. $C_{17}H_{20}ClNO_3$ requires C, 63.5; H, 6.3; N, 4.4%), δ([$^2H_6$]DMSO) 3.80 (3 H, s, NCH_3), 6.60 (2 H, s, ArH), and 6.71 and 6.96 (each 2 H, d, *J* 8 Hz, ArH).

4-Benzyloxy-3,5-dimethoxybenzoic Acid (25a).—Syringic acid (5 g) in hot methanol (40 ml) was treated with sodium hydroxide (2.2 g) in methanol (50 ml) followed by benzyl chloride (7 ml). After the mixture had been heated under reflux for 4 h, sodium hydroxide (1.1 g) in water (25 ml) was added before a further 5 h at reflux. The methanol was removed by evaporation and the solution diluted with water (50 ml), washed with dichloromethane (2 × 100 ml), and acidified with concentrated hydrochloric acid. The precipitate was washed with water to give the acid (5.97 g, 82%), m.p. 153—158 °C (lit.,[53] 140—150 °C), $\nu_{max.}$ 2 500—3 300vbr, 1 687, 1 588, and 1 498 cm^{-1}; $\lambda_{max.}$ 216 and 258 nm; δ 3.82 (6 H, s, 2 × OCH_3), 5.17 (2 H, s, *O*-benzyl-CH_2), 7.2—7.5 (7 H, m, ArH), and 11.75br (1 H, CO_2H); *m/e* 288 (M^+).

Methyl 4,5-Bisbenzyloxy-3-methoxybenzoate (25c).—Methyl 3-methoxy-4,5-dihydroxybenzoate[31] (2.63 g) was dissolved in acetone (50 ml) containing potassium iodide (2 g), anhydrous potassium carbonate (10 g), and benzyl chloride (7 ml), and the suspension was heated at reflux with mechanical stirring for 3 h. The cooled suspension was filtered and the solids washed with acetone to give a combined filtrate which afforded a solid on evaporation. This material was washed with light petroleum (b.p. 40—60 °C), and then water, to leave the *bisbenzyloxy-ester* (4.11 g, 82%), m.p. 85—87 °C (from methanol) (Found: C, 72.9; H, 5.85. $C_{23}H_{22}O_5$ requires C, 73.0; H, 5.85%).

Methyl 3,4,5-Trisbenzyloxybenzoate (25d).—Methyl 3-benzyloxy-4,5-dihydroxybenzoate[31] (1.9 g) was benzylated as above in acetone (40 ml) with potassium iodide (1 g), anhydrous potassium carbonate (5 g), and benzyl chloride (3.5 ml) for 18 h, to yield after work-up the trisbenzyloxy-ester (2.78 g, 88%), m.p. 96—98 °C (lit.,[54] 100.5—101.5 °C), $\nu_{max.}$ 1 715, 1 592, and 1 503 cm^{-1}; $\lambda_{max.}$ 223 and 265 nm; δ 3.89 (3 H, s, OCH_3), 5.14 (6 H, s, 3 × *O*-benzyl-CH_2), and 7.2—7.5 (17 H, m, ArH); *m/e* 454 (M^+).

4-Benzyloxy-3,5-dimethoxybenzyl Alcohol.—The acid (25a) (5.0 g) in THF (85 ml) was added dropwise over 1 h to a stirred suspension of lithium aluminium hydride (0.8 g) in THF (50 ml). The mixture was heated under reflux for 2 h, and then cooled in an ice-bath during the successive addition of water (0.8 ml), sodium hydroxide solution (15% w/v; 0.8 ml), and water (2.4 ml). The suspension was filtered and the solids were washed with ether and then boiled with ether (100 ml) for 30 min and filtered again. The combined filtrates were evaporated to dryness to yield the alcohol as an oil (4.75 g; quantitative), $\nu_{max.}$ (film) 3 400br, 2 840, 1 595, and 1 505 cm^{-1}; $\lambda_{max.}$ 235, 258, 264, and 269 nm; δ 2.20 (1 H, s, OH), 3.81 (6 H, s, 2 × OCH_3), 4.59 (2 H, s, CH_2OH), 5.00 (2 H, s, *O*-benzyl-CH_2), 6.69 (2 H, s, ArH), and 7.2—7.6 (5 H, m, ArH); *m/e* 274 (M^+).

The following benzyl alcohols were prepared as intermediates by the same method from the corresponding methyl benzoates: 3-benzyloxy-4,5-dimethoxybenzyl alcohol, as a gum, $\nu_{max.}$ (film) 3 400br, 1 593, and 1 507 cm^{-1}; $\lambda_{max.}$ 235, 258, 264, and 269 nm; δ 2.02br (1 H, s, OH), 3.88 (6 H, s, 2 × OCH_3), 4.58 (2 H, s, CH_2OH), 5.13 (2 H, s, *O*-benzyl-CH_2), 6.63 (2 H, s, ArH), and 7.25—7.5 (5 H, m, ArH); *m/e* 274 (M^+); 4,5-bisbenzyloxy-3-methoxybenzyl alcohol, as a gum, $\nu_{max.}$ 3 580, 3 420br, 1 590, and 1 500 cm^{-1}; $\lambda_{max.}$ 233, 257, 264, and 268 nm; δ 2.10 (1 H, s, OH), 3.79 (3 H, s, OCH_3), 4.52 (2 H, s, CH_2OH), 5.02 and 5.05 (each 2 H, s, *O*-benzyl-CH_2), 6.57 and 6.60 (each 1 H, s, ArH), and 7.2—7.5 (10 H, m, ArH); *m/e* 350 (M^+); and 3,4,5-trisbenzyloxybenzyl alcohol, m.p. 105—107 °C (lit.,[50] 111 °C), $\nu_{max.}$ 3 600, 3 420br, 1 592, and 1 504 cm^{-1}; $\lambda_{max.}$ 227, 257, 264, and 268 nm; δ 1.87br (1 H, s, OH), 4.53 (2 H, s, CH_2OH), 5.08br (6 H, s, 3 × *O*-benzyl-CH_2), 6.65 (2 H, s, ArH), and 7.2—7.5 (15 H, m, ArH); *m/e* 426 (M^+).

These four benzyl alcohols were converted into the benzyl chlorides with thionyl chloride by the method outlined previously,[36] and then converted without further purification into the phenylacetonitriles by reaction with potassium cyanide in DMF. The [1-^{14}C]phenylacetonitriles were prepared from the chlorides by the method used earlier in this paper for 3,4-bisbenzyloxy[1-^{14}C]phenylacetonitrile. The four nitriles were used without further purification.

2-(4-Benzyloxy-3,5-dimethoxyphenyl)ethylamine.—The corresponding phenylacetonitrile (462 mg) was dissolved in THF (10 ml) containing sodium borohydride (1.124 g), and boron trifluoride–ether (5 ml; redistilled) was added dropwise *via* the reflux condenser. The suspension was heated under reflux for 5 h, cooled, and treated with hydrochloric acid (6M; 20 ml), to destroy the excess of hydride, followed by water (50 ml). The solution was basified (sodium hydroxide pellets) and extracted with dichloromethane (3 × 50 ml). Removal of the solvents left the amine (491 mg) which, as the hydrochloride, crystallised from ethanol–ether (337 mg, 64%), m.p. 156—159 °C (lit.,[55] 156—158 °C), $\nu_{max.}$ (Nujol) 1 603, 1 591, 1 525, and 1 502 cm^{-1}; $\lambda_{max.}$ 228, 258, 264, and 268 nm; δ([$^2H_4$]methanol) 2.93 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.20 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.85 (6 H, s, 2 × OCH_3), 4.94 (2 H, s, *O*-benzyl-CH_2), 6.62 (2 H, s, ArH), and 7.25—7.55 (5 H, m, ArH); *m/e* 287 (M^+ − HCl).

The following amines were prepared by the same method: 2-(3-benzyloxy-4,5-dimethoxyphenyl)ethylamine, as the hydrochloride, m.p. 150—153 °C (from methanol–ether) (lit.,[56] 150—152 °C), $\nu_{max.}$ (Nujol) 2 500—3 100vbr, 1 608, 1 594, 1 525, and 1 510 cm^{-1}; $\lambda_{max.}$ 231, 258, 264, and 268 nm; δ([$^2H_4$]methanol) 2.91 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.18 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.80 and 3.87 (each 3 H, s, OCH_3), 5.14 (2 H, s, *O*-benzyl-CH_2), 6.64 and 6.67 (each 1 H, s, ArH), and 7.25—7.55 (5 H, m, ArH); *m/e* 287 (M^+ − HCl); 2-(4,5-*bisbenzyloxy-3-methoxyphenyl)ethylamine*, as the *oxalate*, m.p. 122—124 °C (from ethanol–ether) (Found: C, 66.5; H, 6.05; N, 3.2. $C_{25}H_{27}NO_7$ requires C, 66.2; H, 6.0; N, 3.1%), $\nu_{max.}$ (Nujol) 3 300—2 600vbr, 1 720, 1 700, and 1 590 cm^{-1}; $\lambda_{max.}$ 232, 258, 264, and 268 nm; δ([$^2H_4$]methanol) 2.90 (2 H, m, $ArCH_2CH_2N$), 3.15 (2 H, m, $ArCH_2CH_2N$), 3.83 (3 H, s, OCH_3), 4.93 and 5.08 (each 2 H, s, *O*-benzyl-CH_2), 6.6 (2 H, m, ArH), and 7.15—7.5 (10 H, m, ArH); *m/e* 363 ($M^+ - C_2H_2O_4$); and 2-(3,4,5-*tris-*

benzyloxyphenyl)ethylamine, as the *hydrochloride*, m.p. 153—156 °C (from ethanol–ether) (Found: C, 71.6; H, 6.3; N, 2.95. $C_{29}H_{30}ClNO_3 \cdot 0.5H_2O$ requires C, 71.8; H, 6.4; N, 2.9%), $\nu_{max.}$ (Nujol) 3 100—2 500vbr, 1 595, 1 510, and 1 498 cm^{-1}; $\lambda_{max.}$ 222, 258, 264, and 268 nm; δ([$^2H_4$]-methanol) 2.88 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.14 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 4.96 (2 H, s, *O*-benzyl-CH_2), 5.12 (4 H, s, 2 × *O*-benzyl-CH_2), 6.69 (2 H, s, ArH), and 7.1—7.6 (15 H, m, ArH); *m/e* 440 (M^+ − Cl).

Debenzylation of the Phenethylamines.—The above benzyloxyamines were debenzylated under the conditions already outlined to yield the corresponding phenolic amines (26a—d) as follows: 2-(4-hydroxy-3,5-dimethoxyphenyl)ethylamine (26a) (94%), as its hydrochloride, m.p. 253—256 °C (lit.,[55] 250 °C), $\nu_{max.}$ 3 300vbr, 1 618, and 1 517 cm^{-1}; $\lambda_{max.}$ 210, 226sh, and 270 nm; δ([$^2H_4$]methanol) 2.91 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.21 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.87 (6 H, s, 2 × OCH_3), and 6.58 (2 H, s, ArH); *m/e* 197 (M^+ − HCl); 2-(3-hydroxy-4,5-dimethoxyphenyl)ethylamine (26b), as the hydrochloride, m.p. 176—179 °C (lit.,[56] 178—179 °C), $\nu_{max.}$ (Nujol) 3 400br, 3 200—2 600vbr, 1 598, and 1 512 cm^{-1}; $\lambda_{max.}$ 230 and 270 nm; δ([$^2H_4$]methanol) 2.87 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.18 (2 H, t, *J* 7 Hz, $ArCH_2CH_2N$), 3.78 and 3.86 (each 3 H, s, OCH_3), and 6.46 (2 H, s, ArH); *m/e* 197 (M^+ − HCl); 2-(4,5-dihydroxy-3-methoxyphenyl)-ethylamine (26c), as the hydrochloride, m.p. 197—200 °C (from ethanol–ether) (lit.,[57] 206—207 °C), $\nu_{max.}$ (Nujol) 3 470, 3 250—2 600vbr, 1 620, 1 610, 1 538, and 1 519 cm^{-1}; $\lambda_{max.}$ 230 and 271 nm; δ([$^2H_4$]methanol) 2.82 (2 H, t, *J* 6.5 Hz, $ArCH_2CH_2N$), 3.15 (2 H, t, *J* 6.5 Hz, $ArCH_2CH_2N$), 3.84 (3 H, s, OCH_3), and 6.42 (2 H, s, ArH); *m/e* 183 (M^+ − HCl); and 2-(3,4,5-trishydroxyphenyl)ethylamine (26d), as the hydrochloride m.p. 215—219 °C (from methanol–ethyl acetate) (lit.,[58] 212.5—213 °C), $\nu_{max.}$ (Nujol), 3 300—2 500vbr, 1 604, 1 545, and 1 490 cm^{-1}; $\lambda_{max.}$ 234 and 270 nm; δ([$^2H_4$]-methanol) 2.76 (2 H, t, *J* 6 Hz, $ArCH_2CH_2N$), 3.11 (2 H, t, *J* 6 Hz, $ArCH_2CH_2N$), and 6.30 (2 H, s, ArH); *m/e* 169 (M^+ − HCl).

2-(3,4-Bisbenzyloxyphenyl)[1-^{14}C]ethylamine (*OO*-Dibenzyl[1-^{14}C]dopamine) was prepared from 3,4-bisbenzyloxy-[1-^{14}C]phenylacetonitrile (see earlier) by reduction with sodium borohydride–boron trifluoride as above to give material identical with radioinactive samples prepared by the nitrostyrene route (see earlier). This was hydrogenated by the usual method to leave 2-(3,4-dihydroxyphenyl)[1-^{14}C]ethylamine hydrochloride ([1-^{14}C]dopamine), m.p. 238—240 °C (from methanol–ether) (lit.,[59] 238—239 °C).

6,7-Dihydroxy-1-(3,4-dihydroxybenzyl)-3,4-dihydroisoquinoline (28b).—The corresponding tetrabenzyl ether (described earlier) (46 mg) in ethanol (5 ml) and hydrochloric acid (5M; 5 ml) was boiled in a slow stream of nitrogen. The solution was maintained at 5 ml by addition of water and heating continued until the evolution of benzyl chloride ceased. The residue after evaporation was dried *in vacuo* over P_2O_5 and recrystallised from dilute hydrochloric acid to yield the *tetrahydroxy-3,4-dihydroisoquinoline* as its *hydrochloride* (28 mg), decomp. >200 °C (Found: C, 59.7; H, 5.0; N, 4.4. $C_{16}H_{16}ClNO_4$ requires C, 59.5; H, 5.2; N, 4.3%), $\nu_{max.}$ 3 300—2 500, 1 645, 1 610, 1 570, and 1 520 cm^{-1}; δ([$^2H_6$]DMSO) 2.90 (2 H, t, $ArCH_2CH_2N$), 3.75 (2 H, t, $ArCH_2CH_2N$), 4.19 (2 H, s, $ArCH_2C{=}N$), and 6.5—7.4 (5 H, m, ArH).

6,7-Dihydroxy-1-(4-hydroxybenzyl)-3,4-dihydroisoquinoline (28a) was prepared as above from its tribenzyl ether (described earlier) (62 mg) and isolated, after recrystallisation from dilute hydrochloric acid, as the *hydrochloride* (29 mg), m.p. 209—211 °C (Found: C, 62.7; H, 5.2; N, 4.4. $C_{16}H_{16}ClNO_3$ requires C, 62.9; H, 5.3; N, 4.6%), δ([$^2H_4$]-methanol) 3.00 (2 H, t, $ArCH_2CH_2N$), 3.85 (2 H, t, $ArCH_2CH_2N$), 4.30 (2 H, s, $ArCH_2C{=}N$), and 6.7—7.5 (6 H, m, ArH).

1-Carboxy-6,7-dihydroxy-1-(4-hydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (27a).—2-(3,4-Dihydroxyphenyl)-ethylamine hydrochloride (30 mg) and 4-hydroxyphenylpyruvic acid[25] (42 mg) were dissolved in dilute aqueous ammonia (water–ammonia solution (*d*, 0.88) 19 : 1, v/v; 0.4 ml) and the solution was adjusted to pH 5 with 1M-hydrochloric acid. Buffer solution (0.1M-sodium acetate–0.1M-acetic acid, 1 : 1 v/v; 0.35 ml) was added and the solution was kept at 20 °C for 5 days. The crystalline precipitate was collected and boiled with 1M-hydrochloric acid to give the 1-carboxyisoquinoline hydrochloride (19.5 mg, 35%), m.p. 240—245 °C (decomp.) [lit.,[60] 260 °C (decomp.)], $\nu_{max.}$ (Nujol) 3 440vbr 3 290vbr, 1 605, 1 590, 1 561, and 1 510 cm^{-1}; $\lambda_{max.}$ 211, 225, and 284 nm.

1-Carboxy-6,7-dihydroxy-1-(3,4-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (27b) was prepared as above from the phenethylamine and 3,4-dihydroxyphenylpyruvic acid,[46] and isolated as the hydrochloride (41%), m.p. 205—210 °C (decomp.) [lit.,[21] 287—295 °C (decomp.); 285—290 °C (decomp.)], $\nu_{max.}$ (Nujol) 3 505, 3 450—2 500vbr, 1 720, 1 625, 1 605, 1 560, and 1 525 cm^{-1}; $\lambda_{max.}$ 214, 230sh, and 285 nm; δ([$^2H_6$]DMSO) 2.7—3.7 (8 H, m), 6.5—6.9 (4 H, m), 7.22 (1 H, s), and 8.5—9.7br (5 H, m). Both amino-acids (27a and b) were hygroscopic, m.p.s were variable, and satisfactory combusion analyses could not be achieved.

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