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THE BIOSYNTHESIS OF *SCELETIUM* ALKALOIDS IN *SCELETIUM SUBVELUTINUM* L. BOLUS.[1]

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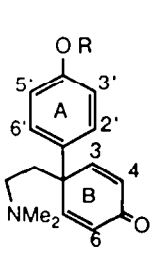
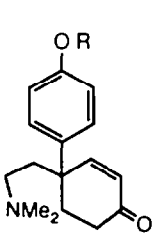
Abstract : Six *Sceletium* (Mesembrine) alkaloids (1)-(6) are identified, together with *N,N*-dimethyltyramine (10), as constituents of *Sceletium subvelutinum*. The alkaloids (1)-(6) incorporate label from [7-^{14}C]tyramine [as (8)] and 3-([3,5-$^{3}H_2$]-4-hydroxyphenyl)propionic acid (13) as expected; notably 3-([3,5-$^{3}H_2$]-4-hydroxyphenyl)propanal (15) is a more efficient alkaloid precursor than is the acid (13) and the aldehyde is deduced to be a key intermediate in the biosynthesis of *Sceletium* alkaloids. The *N*-methylamine (21) is an important late intermediate in the biosynthesis of *Sceletium* alkaloids, or is closely related to that intermediate. The amine (20) is less efficiently incorporated than (22)=(21) is.

Plants of the *Sceletium* genus elaborate a small, unique group of alkaloids exemplified by joubertiamine (4) and mesembrine (7).[2] (A change in the naming of the members of this genus from *Mesembryanthemum* to *Sceletium* alters[2] the name given to this family of alkaloids from "Mesembrine" to "*Sceletium*"). In important pioneering work it has been shown that the biosynthetic origins of these alkaloids lie separately in the α-amino acids tyrosine[4] and phenylalanine.[4,5] The former amino acid is utilized in biosynthesis *via* tyramine (8) and *N*-methyltyramine (9) to give ring B and attached C_2-*N* fragment, see (1).[2-4] Phenylalanine (11) is used by way of 3-(4-hydroxyphenyl)propionic acid (12) and closely related compounds[6] and incorporation of these compounds is into ring A, see (1), with complete loss of the side chain originally present in phenylalanine (11). Late stages of biosynthesis involve formation of the heterocyclic ring seen in, *e.g.* (7) and, as a major pathway, the introduction of the second aromatic oxygen substituent which is found in, *e.g.*, (7) occurs after formation of this heterocyclic ring rather than at an earlier stage.[6] Intermediates more complex than (12) and (9) have not until now been identified in spite of attempts to do so.[2] Although there is an apparent structural relationship with some Amaryllidaceae alkaloids, precursors



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



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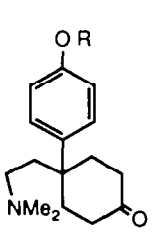
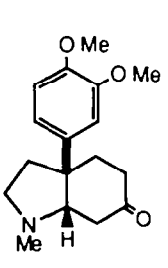
(4) R=H



(5) R=Me
(6) R=H



(7)

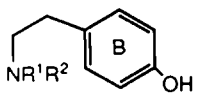
of type Ar-C_2-N-C_1-Ar, which are implicated in the biosynthesis of these alkaloids, have been clearly shown not to be involved in the biosynthesis of *Sceletium* alkaloids.[5] We report here results[1] which show that the biosynthesis of *Sceletium* alkaloids proceeds from (12) by way of the aldehyde (14) and results which identify the amine (21) as a key, late biosynthetic intermediate or as a compound that is closely related to that intermediate. Our experiments were carried out using *Sceletium subvelutinum* L. Bolus which were grown from seed in a greenhouse in Leeds.

The alkaloids produced by *S. Subvelutinum* were isolated and extensively purified to give in order of increasing polarity : (1), (3), (5), (2), (4), and (6). The structures of the alkaloids, joubertiamine (4) and (1)-(3), (5) and (6) were deduced from the 400 MHz ^{1}H n.m.r. and characteristic mass spectra of the compounds (*cf.* refs. 7 and 8). The alkaloids (3) and (5) had previously been isolated from this plant[7] and (3) was found to have the normal (relevant) stereochemistry associated with *Sceletium* alkaloids, *e.g.* (7). It is reasonable to assume that the other alkaloids in *S. subvelutinum* are of the same absolute stereochemistry but we have not proved this to be so. *N,N*-Dimethyltyramine (hordenine) (10) was also found by us to be a constituent of *S. subvelutinum* as it is in *S. joubertii* along with (2), (4), and (6).[8]

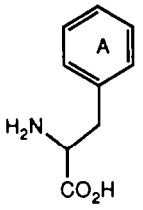
It has been established for numerous alkaloids of richly varied structure and embodying one or two aromatic rings that a pivotal biosynthetic step involves the condensation of an amine with an aldehyde.[9,10] Given that 3-(4-hydroxyphenyl)propionic acid (12) is a precursor for *Sceletium* alkaloids, it then follows that the corresponding aldehyde (14) might also be a precursor. Condensation of (14) with the established precursor *N*-methyltyramine (9) would give (25). Following reduction would yield (21) as an important late intermediate. Biosynthesis would then follow reasonably as shown in the Scheme (solid arrows).

To test these ideas labelled samples of 3-(4-hydroxyphenyl)propionic acid (12), 3-(4-hydroxyphenyl)propanal (14), and the amines (20) and (22) were prepared. Tritium labelling was introduced in all cases by exchange *ortho* to the phenolic group in commercially available 3-(4-hydroxyphenyl)propionic acid (12) following an exellent general method.[11] The labelling sites were established by first carrying out the preparation of ^{2}H-labelled material.

Esterification of 3-([3,5-$^3H_2$]-4-hydroxyphenyl)propionic acid (13) followed by benzylation and reduction gave the alcohol (17) which was oxidized, using $AgNO_3$-celite,[12] to the aldehyde (18); reductive debenzylation gave 3-([3,5-$^3H_2$]-4-hydroxyphenyl)propanal (15). Preparation of the aldehyde (14) by oxidation of (16) with pyridinium chlorochromate[13] gave the required product in very low yield and, by



(8) $R^1=R^2=H$
(9) $R^1=Me$, $R^2=H$
(10) $R^1=R^2=Me$



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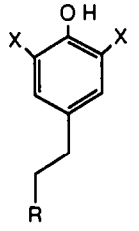
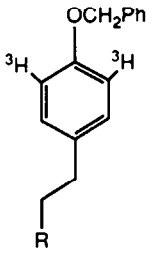
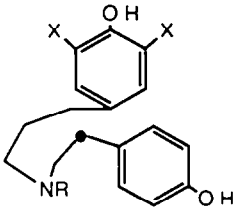


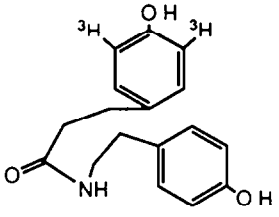
(12) $R=CO_2H$, $X={}^1H$
(13) $R=CO_2H$, $X={}^3H$
(14) R=CHO, $X={}^1H$
(15) R=CHO, $X={}^3H$
(16) $R=CH_2OH$, $X={}^1H$



(17) $R=CH_2OH$
(18) R=CHO



(19) R=H, $X={}^1H$, $\bullet={}^{12}C$
(20) R=H, $X={}^3H$, $\bullet={}^{12}C$
(21) R=Me, $X={}^1H$, $\bullet={}^{12}C$
(22) R=Me, $X={}^3H$, $\bullet={}^{12}C$
(23) R=Me, $X={}^3H$, $\bullet={}^{14}C$



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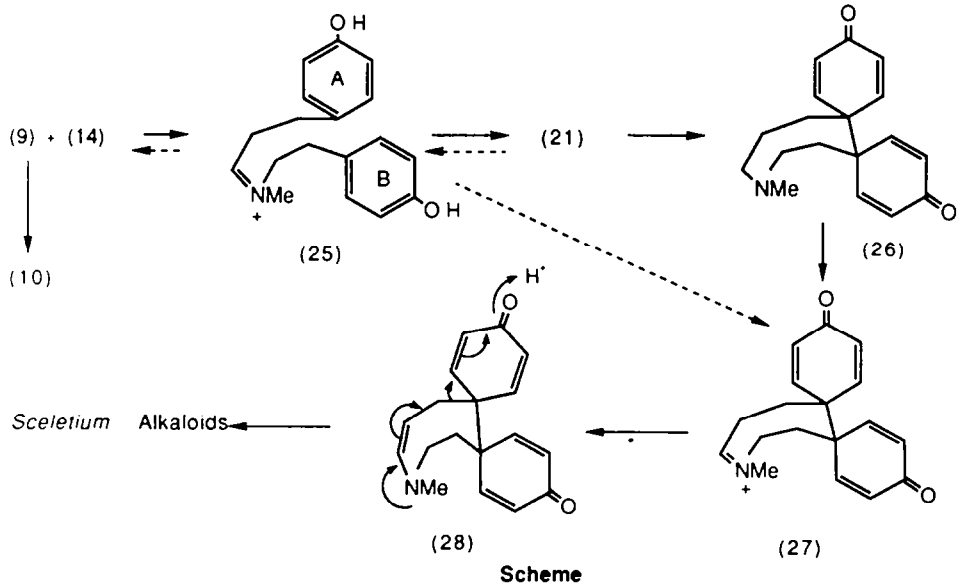


Scheme

Table 1. **Incorporation of Precursors into *S. subvelutinum* Alkaloids** (Summer 1988)

With the exception of entries in the last row, all entires are % specific incorporations. The values given in brackets are the weights (mg) of alkaloid isolated.

	Aldehyde[a] (15)	Acid[b] (13)	[7-^{14}C]Tyramine[b] [(as 8)]	Amine[c] (22)	Amine[d] (20)
(1)	0.17 (1.3)	- (0)	-	1.12 (2.2)	0.02 (2.1)
(2)	0.76 (2.1)	0.01 (2.1)	0.01	0.08 (2.2)	0.035 (1.3)
(3)	0.055 (6.8)	0.003 (5.1)	0.003	0.02 (6.5)	0.02 (5.5)
(4)	0.62 (3.2)	0.004 (3.5)	0.01	0.1 (1.7)	0 04 (1.4)
(5)	0.08 (2.1)	- (0)	-	- (0)	0.025 (3.3)
(6)	0.07 (4.8)	0.005 (4.1)	0.02	0.02 (2.9)	0.025 (0.7)
(10)	$<10^{-3}$ (6.2)	$<10^{-3}$ (3.2)	0.01	$<10^{-3}$ (5.4)	$<10^{-3}$ (2.2)
% total incorp.	0.07	0.006	0.012	0.2	0.017

a : 31.6 mg, 52.2μCi; aqueous solution of bisulphite complex.
b : These two precursors were fed as a mixture in aqueous solution; (13) : 8mg, 232μCi; tyramine: 9mg,50μCi.
c : 16 mg, 30μCi; 80% EtOH:H_2O.
d : 20mg, 39μCi; 80% EtOH:H_2O.

Table 2. **Incorporation of Precursors into *S. subvelutinum* Alkaloids (Summer 1989)**

The values given in brackets are the weights (mg) of the alkaloids isolated.

	Aldehyde (15) (11 mg, 37 μCi)[a] % Specific incorp.	Amine (23) (26.5mg, 21μCi, ^{3}H:^{14}C=1.94)[b] % specific incorp.	^{3}H:^{14}C
(1)	0.1 (0.75)	0.036 (2.7)	2.06
(2)	0.03 (2.7)	0.19 (3.3)	2.35
(3)	0.005 (21.1)	0.013 (23.4)	1.97
(4)	0.004 (4.2)	0.04 (4.7)	1.76
(5)	0.004 (4.5)	0.064 (2.5)	1.68
(6)	0.016 (4)	0.23 (1.3)	1.4
(10)	0 (8.2)	0.004 (9.35)	- 0.3
% total incorp.	0.017	0.07	

a : fed as an 80% EtOH:H_2O solution;
b : fed as a 55% EtOH:H_2O solution.

Swern oxidation[14], no aldehyde product was obtained.

Condensation of 3-([3,5-$^3H_2$]-4-hydroxyphenyl)propionic acid (13) with tyramine (8) through the use of DCC afforded the amide (24). Reduction of this amide with borane-thf gave *N*-[2-(4-hydroxyphenyl)ethyl]-*N*-3-[([3,5-$^3H_2$]-4-hydroxyphenyl)propyl]amine (20). Treatment of this amine with formaldehyde followed by sodium borohydride (*cf.* ref. 15) gave the corresponding *N*-methyl compound (22). Doubly labelled *N*-methyl compound (23) was prepared by mixing tritiated material with material prepared from [7-^{14}C]tyramine [as (8)].

Precursors were administered to *S. subvelutinum* plants on three occasions in successive summers. On the first occasion, [7-^{14}C]tyramine was fed to confirm the presence of alkaloids and active biosynthesis. On the second occasion 3-([3,5-$^3H_2$]-4-hydroxyphenyl)propanal (15) was fed as its water-soluble bisulphite addition compound (*cf.* ref. 16) in parallel with a mixture of 3-([3,5-$^3H_2$]-4-hydroxyphenyl)propionic acid (13) and [7-^{14}C]tyramine [as (8)]. Incorporation of radioactivity into the alkaloids (1)-(6) was as shown in Table 1. It is clear that the aldehyde (15) is a better precursor for the alkaloids than are the acid (13) and [7-^{14}C]tyramine. The small amounts of alkaloid available precluded degradation to establish the specificity of labelling and, in addition, the inevitable loss of the side-chain during the course of biosynthesis prevented us from labelling the aldehyde function itself with tritium in (14) to prove that (14) is implicated in biosynthesis without oxidation to the acid (12). (*cf.* refs 9a and 16) but in any case the significantly higher incorporation of (15) compared to (13) argues against this. Additional strengthening of the results was obtained by examining separately the incorporations into each of the six alkaloids which are produced (Table 1). Further, the *N,N*-dimethyltyramine (10) which was isolated in these experiments provides an independent check on the random incorporation of tritium through C_1-metabolism and through *de novo* synthesis of tyrosine. In all the experiments including those with (20) and (22) the incorporation of tritium label was reassuringly, negligible [Table 1, see also results for the aldehyde (15) in Table 2]. The results obtained for the aldehyde(15) (Tables 1 and 2) point strongly to the aldehyde (14) being a biosynthetic intermediate which lies on the pathway after the acid (12). Thus the *Sceletium* alkaloids belong to that group of diverse alkaloids which have in common the condensation of an amine with an aldehyde as a key step of biosynthesis. In the case of the *Sceletium* alkaloids such a condensation leads reasonably through (25) to (21) as biosynthetic intermediates.

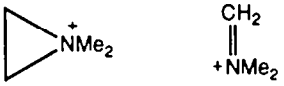
We have examined samples of (22) and (20) as precursors and have found that (22) is an efficient precursor (Table 1). The compound (20) was utilized much less well which is consistent with the conclusion[3] that *N*-methyltyramine (9) is a biosynthetic intermediate after tyramine.

In the third set of experiments (Table 2) we sought to examine whether or not (21) is an intact alkaloids precursor through the use of the doubly labelled *N*-[2-(4-hydroxyphenyl)[2-^{14}C]ethyl-*N*-methyl-*N*-[3-([3,5-$^{3}H_2$]-4-hydroxyphenyl)propyl]amine (23). The aldehyde (15) was fed chiefly to act on this occasion as a reference precursor. The results, which are again strengthened by being obtained separately for the six alkaloids, indicate that (21) can act as an intact precursor for *Sceletium* alkaloids [maintenance of ^{3}H : ^{14}C ratio for (1), (3) and (4) ± 10%]. It is notable that the most abundant alkaloid on this occasion, *i.e.* (3), gave the best isotope ratio. The total incorporation is once again higher than for the aldehyde (15) but is lower than in the previous year (0.07% vs. 0.2%). Also, the ^{3}H : ^{14}C ratios for dehydrojoubertiamine (2) and dihydrojoubertiamine (6) differ substantially from the ratio in the precursor. The (6) could not be freed completely from contaminating *N,N*-dimethyltyramine which had incorporated ^{14}C-label through degradation of the precursor. This can account then for the substantially lowered ratio obtained for dihydrojoubertiamine (6). But the ratio for (2) in particular is adrift of the expected value. This may be simply because at the level of incorporation observed there is sufficient degradation and random reincorporation of label to affect the results. There are, however, alternative explanations, which are suggested by the observation that the *N,N*-dimethyltyramine (10) incorporates essentially only ^{14}C-label.

If the conversion of (25) into (21) is reversible then it is possible for the labelled (21), which was fed, to be converted back into (14) and *N*-methyltyramine (9). Because of the labelling used in the precursor the *N*-methyltyramine (9) will contain ^{14}C label (and no ^{3}H label). Radioactive (10) would be formed by methylation of (9). New synthesis of (25) and (21) would be expected to result in some loss of the integrity of the ^{3}H :^{14}C ratio in the precursor fed.

The reasonable pathway outlined in the Scheme requires the reduction of the immonium salt (25) to give (21) and later oxidation of (26) to give the immonium salt (27). It is entirely possible that (25) normally and directly affords (27) by phenol-oxidative coupling. In this case (21) is a shunt metabolite which may be converted into (25) for further transformation into *Sceletium* alkaloids. Such a shunt metabolite might well serve as an alkaloid precursor with impaired efficiency. The arguments are summarized by addition of the broken arrows to the Scheme. These and other questions about the biosynthesis of this unique group of alkaloids is the subject of further investigation.

Our results and those of Jeffs *et al.* are accommodated by the routes shown in the Scheme. The fragmentation of the bis-dienone has chemical precedent. Previously compounds of type (21) were found not to act as alkaloid precursors and this was attributed to poor solubility[2,17]; we used aqueous alcohol satisfactorily as solvent.



(A) (B)

EXPERIMENTAL

General

Melting points were determined on a Kofler hot stage and are uncorrected. Ultra-Violet spectra were recorded on a Pye Unicam Pu 8800 Uv/Vis spectrophotometer and refer to solutions in ethanol. Infra-red spectra were recorded on a Perkin-Elmer 1420 Ratio Recording spectrophotometer. ^{1}H n.m.r. spectra were obtained on a Perkin-Elmer R32 or a Jeol FX90Q spectrometer at 90MHz or a Bruker WH-400 specrtrometer at 400MHz. Unless otherwise stated, all n.m.r. spectra were obtained in deuterochloroform solution with tetramethylsilane (TMS) as internal standard. Low-resolution mass spectra were determined on a Kratos MS25 mass spectrometer. Accurate mass measurements were obtained on a AEI MS950 mass spectrometer.

Column chromatography was carried out using Kieselgel G type 60 (Merck 7731) silica gel. Thin layer chromatography was carried out using Kieselgel G type 60 (Merck 7731) and GH_{254} (Merck 7730).

Unless otherwise stated, all organic extracts of aqueous solutions were dried with anhydrous magnesium sulphate.

Radioactive compounds were purchased from Amersham International plc. Radioactivity was measured with a Canberra Packard scintillation spectrometer model 300C. Samples were dissolved in methanol (1 ml) and the liquid scintillant (N.E. 21; 15 ml) was then added. A standard solution of methanol (1ml) and scintillant (15ml) was counted at the beginning of each count to determine the background radiation. The efficiency of counting was determined by adding a known weight of ^{3}H or ^{14}C labelled, standard hexadecane to the sample and recounting. Radioactive incorporation and specific incorporations were calculated as follows : Incorporation = 100 x total activity of alkaloid/total activity of compound fed. Specific incorporation = 100 x molar activity of alkaloid/molar activity of compound fed.

Feeding experiments were carried out in a greenhouse in the summer. The precursor solution was administered to the plant by capillary tubes the points of which were pressed into the stems and leaves. The

plants were left for two weeks, harvested, washed, and worked up for alkaloids. The precursor solutions were as follows : the acid (13) was dissolved in a basic aqueous solution (dilute NaOH), and the pH was adjusted to 7-8 with dilute mineral acid. The amines (20), (22), and (23) were dissolved in aqueous ethanol. For the preparation of the bisulphite addition compound of (15) the aldehyde was dissolved in a minimum volume of ether (approx. 3 ml). Then an excess of a saturated aqueous solution (approx. 1 ml) of sodium bisulphite was added. The ether was removed *in vacuo* and the solid which formed was dissolved in distilled water. The pH was adjusted to 6-7.

***Isolation and purification of alkaloids from* S. subvelutinum**

Whole plant material was macerated with ethanol (500 ml) in a Waring Blender. The mixture was filtered and the residue was re-extracted overnight with ethanol using a Soxhlet extractor. The extract was combined with the filtrate above and the solution was evaporated *in vacuo*. The residue was taken up in chloroform (300 ml) and washed with a saturated aq. solution of sodium carbonate (3 x 100 ml), then extracted with 1M HCl (3 x 150 ml). The combined acid extracts were reduced in volume to give a solution with a filterable precipitate. The filtrate was basified with sodium carbonate. This solution was extracted with chloroform (5 x 100 ml), and the combined extracts were dried. The solvent was removed *in vacuo* to give crude alkaloid (0.047% of the weight of wet plant material).

The crude alkaloid mixture was purified by column chromatography eluting with $CHCl_3$:MeOH:conc.NH_3 (80 ml:20 ml: 20 drops), followed by h.p.l.c. (Varian 5000). Either a silica column (Tecnicol spherisorb 5 silica column eluting with 1:1 MeOH, $CHCl_3$ +conc.NH_3) and/or a reverse phase column (PLRP-S 100A 10 micron 300 x 7.5 mm eluting with 1:1 CH_3CN, H_2O + conc.NH_3) was used to give in order of increasing polarity : (1), (3), (5), (2), (4), (10), (6).

O-*Methyldehydrojoubertiamine (1)* : λmax. 271, 228, 199 nm; νmax. ($CHCl_3$) 1650, 1500 cm^{-1}; δ 7.23(2H, d, J=9Hz, H-2′, and H-6′), 6.88(2H, d, J=9Hz, H-3′ and H-5′), 6.89(2H, d, J=10Hz, H-3 and H-7), 6.33(2H, d, J=10Hz, H-4 and H-6), 3.80(3H, s, OMe); 2.30-2.25(2H,m,2xH-1), 2.23(6H, s, NMe_2), 2.23-2.18(2H, m, 2xH-2); m/z 271.15696 (M^+, $C_{17}H_{21}NO_2$ requires 271.15722; 5.4%), 72 (A,14.5%),58 (B, 100%).

O-*Methyljoubertiamine (3)* : λmax. 272, 221 nm; νmax. ($CHCl_3$) 1670, 1505 cm^{-1}; δ 7.20(2H, d, J=9Hz, H-2′ and H-6′), 3.88(2H, d, J=9Hz, H-3′ and H-5′), 7.12(1H, d, J=10Hz, H-3), 6.15(1H, d, J=10Hz, H-4), 3.81(3H, s, OMe), 2.39-2.30(2H, m, 2xH-1), 2.27-1.95(6H, m, 2xH-2, 2xH-6 and 2xH7), 2.17(6H, s, NMe_2); m/z 273.17261 M^+, $C_{17}H_{23}NO_2$ requires 273.17287; 2.2%), 72 (A, 3.2%), 58 (B, 100%).

O-*Methyldihydrojoubertiamine (5)*: vmax. ($CHCl_3$) 1705 cm^{-1}; δ 7.28(2H, d, J=9Hz, H-2′ and H-6′) 6.95(2H, d, J=9Hz, H-3′, and H-5′), 3.83(3H, s, OMe), 2.71-2.66(2H, m, 2xH-1), 2.65(6H, s, NMe_2), 2.55-2.47(2H, m, 2xH-2), 2.41-2.23(4H, m, 2xH-4, and 2xH-6), 2.05-1.91 (4H, m, 2xH-3, and 2xH-7); m/z 275.1881 (M^+, $C_{17}H_{25}NO_2$ requires 275.18852; 1.9%), 72 (A, 2.2%). 58 (B, 100%).

***Dehydrojoubertiamine (2)*:** λmax. 271,228,199 nm; δ (CD_3OD) 7.19(2H, d, J=9Hz, H-2′ and H-6′), 6.78(2H, d, J=9Hz, H-3′ and H-5′), 7.09(2H, d, J=10Hz, H-3 and H-7), 6.33(2H, d, J=10Hz, H-4 and H-6); 2.67-2.61(2H, m, 2xH-1); 2.56(6H, s, NMe_2), 2.44-2.38(2H, m, 2xH-2); m/z 257.14146 (M^+, $C_{16}H_{19}NO_2$ requires 257.14157; 4.4%), 72 (A, 10.6%), 58 (B, 100%).

***Joubertiamine (4)*:** λmax. 273, 221 nm; δ (CD_3OD) 7.26(1H, d, J=10Hz, H-3); 7.18(2H, d, J=9Hz, H-2′ and H-6′); 6.78(2H, d, J = 9Hz, H-3′ and H-5′); 6.10(1H, d, J=10Hz, H-4); 2.45-1.96(8H, m, 2xH-1, 2xH-2, 2xH-6 and 2xH-7); 2.27(6H, s, NMe_2); m/z 259.15664 (M^+, $C_{16}H_{21}NO_2$ requires 259.15220; 5%), 72 (A, 3.2%), 58 (B, 100%).

N,N-*Dimethyltyramine (10)*: δ (CD_3OD) 7.01(2H, d, J=9Hz), 6.69(2H, d, J=9Hz), 2.71-2.65(2H, m), 2.53-2.47 (2H, m), 2.29 (6H, s); m/z 165.11556 (M^+, $C_{10}H_{15}NO$ requires 165.115358) 58. Authentic material was prepared by a published procedure.[18] The material prepred according to instructions is obtained as the hydrochloride after sublimation in the presence of NH_4Cl (N-Me : δ = 2.9 ppm). The free base was sublimed at 85° (bath temp.) and 0.01 mm and had m.p. 115-117°.

***Dihydrojoubertiamine (6)*:** δ (CD_3OD) 7.28(2H, d, J-9Hz, H-2′ and H-6′), 6.81(2H, d, J=9Hz, H-3′ and H-5′), 2.57-2.49(2H, m, 2xH-1), 2.32-2.56(4H, m, 2xH-4 and 2xH-6), 2.11(6H, s, NMe_2), 2.09-2.03(2H, m, 2xH-2), 1.95-1.86(4H, m, 2xH-3, and 2xH-7); m/z 261.17221 (M^+, $C_{16}H_{23}NO_2$ requires 261.172869; 2%), 72 (3%), 58 (100%).

3-([3,5-$^2H_2$]-4-Hydroxyphenyl)propionic acid [as (12)]

A mixture of 3-(4-hydroxyphenyl)propionic acid (19mg, 0.114 mmol), deuterium oxide (1 ml), dry dimethylformamaide (1 ml), and triethylamine (68mg, 0.97 mmol) was heated at 100°C in a sealed tube *in vacuo* for six days. The mixture was taken to dryness *in vacuo*. The residue was dissolved in methanol (5ml) and the methanol was removed *in vacuo*, this procedure being repeated twice. The residue was purified by colum chromatography eluting with $CHCl_3$:CH_3COCH_3:MeOH (60ml:20ml:20ml) to give the dideuterio-acid (16mg, 0.095mmol, 83%); m/z 168 (M^+); the signals for the 3 and 5 protons were absent in the ^1H n.m.r. spectrum and the intensities of the other signals were unaffected.

3-([3,5-$^3H_2$]-4-Hydroxyphenyl)propionic acid (13)

A mixture of 3-(4-hydroxyphenyl)propionic acid (420mg, 2.53mmol), tritiated water (approx. 1ml), dry dimethylformamide (2ml), and triethylamine (3ml) was heated at 100°C in a sealed tube *in vacuo* for five days. Then the triethylamine, water, and DMF were transferred to another tube using a vacuum line. The crude product was dissolved in methanol (20ml) and the methanol was removed *in vacuo*, this procedure being repeated three times. The residue was purified by column chromatography eluting with $CHCl_3$:MeOH (84:16) to give the tritiated acid (13) (318mg, 1.915mmol, 9.327mCi, 75.7%).

Methyl 3-(4-hydroxyphenyl)propionate

3-(4-hydroxyphenyl)propionic acid (180mg, 1.08mmol) was dissolved in dry methanol (10ml) and hydrogen chloride was passed through the solution for approx. 2 min. The mixture was refluxed overnight. The solvent was removed *in vacuo*. The residue was dissolved in chloroform (20ml) and washed with a saturated aq. solution of sodium bicarbonate (10ml). The organic layer was dried and evaporated *in vacuo*. The oily residue was trituriated with light petroleum (40-60°C). The crystalline *methyl 3-(4-hydroxyphenyl)propionate* was collected and washed with light petroleum (40-60°C) (2x20ml), then dried *in vacuo* (181mg, 92.7%), m.p. 38-40°C (lit.,[19] 39-40.5°C). δ 7.05("H, d, J=9Hz), 6.73(2H, d, J=9Hz), 3.65(3H, s), 3.00-2.47(4H, m).

Methyl 3-(4-benzyloxyphenyl)propionate

A mixture of methyl 3-(4-hydroxylphenyl)propionate (50mg, 0.28mmol), potassium iodide (65mg), anhydrous potassium carbonate, benzyl chloride (0.05ml), and acetone (2ml) was refluxed overnight, cooled, and filtered. The filtrate was evaporated *in vacuo*. The residue was recrystallised from petroleum (60-80°C) to give the ester (46mg, 61.5%), m.p. 78°C (lit.,[20] 78-79°C). δ 7.50-7.25(5H, m), 7.11(2H, d, J=9Hz); 6.88(2H, d, J=9Hz), 5.02(2H, s), 3.63(3H, s), 3.00-2.47(4H, m).

3-(4-Benzyloxyphenyl)propan-1-ol [as (17)]

To $LiAlH_4$ (100mg, 2.7mmol) in dry ether (10ml) was added dropwise methyl 3-(4-benzyloxyphenyl)propionate (460mg, 1.78mmol) in dry ether (10ml). The mixture was stirred at room temperature for 2.5h under nitrogen. The excess reagent was destroyed by careful addition of ethyl acetate. The reaction mixture was washed with a saturated aq. solution of sodium potassium tartrate (40ml). The organic layer was separated and the aqeuous layer was re-extracted with ether (2x30ml). The extracts were combined with the above organic layer and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was recrystallised from petroleum (60-80°C) to give the alcohol [as (17)] (314mg, 76.6%), m.p. 62-64°C (lit.,[20] 64-65°C). Found : C, 79.25; H, 7.6, Calc. for $C_{16}H_{18}O_2$: C, 79.3; H, 7.43%. δ 7.50-7.20(5H, m), 7.10(2H, d, J=9Hz), 6.88(2H, d, J=9Hz), 5.02(2H, s), 3.66(2H, t, J=6Hz), 2.65(2H, t, J=6Hz), 2.03-1.67(2H, m).

3-(4-Benzyloxyphenyl)propanal [as (18)].

3-(4-Benzyloxyphenyl)propan-1-ol (100mg, 0.434mmol) was dissolved in dry benzene (25ml). Ag_2CO_3-celite[12] (3g, 5.09 mmol, of Ag_2CO_3) was then added. The mixture was refluxed for 1 h. Then more reagent (1g) was added and the reflux was continued for a further 1h. The solution was filtered through celite. The filtrate was evaporated *in vacuo*. The residue was purified by column chromatography eluting with chloroform to give the aldehyde [as (18)] (45mg, 0.197mmmol, 45.3%). δ 9.77(1H, t, J=1.5Hz), 7.50-7.23(5H, m), 7.10(2H, d, J=9Hz), 6.87(2H, d, J=9Hz), 5.00(2H,s); 3.01-2.55(4H,m).

3-(4-Hydroxyphenyl)propanal (14).

A solution of 3-(4-benzyloxyphenyl)propan-1-ol (30mg, 0.13mmol) in dry methanol (5ml) plus Pd/C (10%; 30mg) was hydrogenated at room temeprature and pressure until one equivalent of hydrogen had been taken up. The mixture was filtered through celite. The solvent was removed *in vacuo* to give the phenolic aldehyde (14) (single component on t.l.c.; 19mg, 100%). δ 9.78(1H, t, J=1.5Hz), 7.05(2H, d, J=9Hz), 6.73(2H, d, J=9Hz), 5.07(1H, broad s), 3.03-2.60(4H, m); m/z, 150(M^+, 37%); 107(100%).

3-([3,5-$^3H_2$]-4-Hydroxyphenyl)propanal (15)

The tritiated aldehyde (15) (11mg, 37.3μCi) was prepared from 3-([3,5-$^3H_2$]-4-hydroxyphenyl)propionic acid (above) in the same way as described for the unlabelled compound. The product (15) was purified by column chromatography eluting with chloroform.

N-*[2-(4-hydroxyphenyl)ethyl]-3-(4-hydroxyphenyl)propionamide [as (24)]*

Tyramine (276mg, 2mmol), and 3-(4-hydroxyphenyl)propionic acid (332mg, 2mmol) were dissolved in a mixture of $CHCl_3$ (20ml) and methanol (1ml). Dicyclohexylcarbodiimide (775mg, 3.73mmol) was then added. The mixture was stirred at room temperature for 6h. The mixture was evaporated *in vacuo*. The solid residue was suspended in THF (25ml). The suspended solid was removed by filtration and the solvent was removed from the filtrate *in vacuo*. The residue was purified by column chromatography eluting with $CHCl_3$ and increasing the polarity to 8% MeOH in $CHCl_3$ to give the required amide [as (24)] which was recrystallised from ethyl acetate (274mg, 48%), m.p. 175°. Found: C,71.65; H,6.4; N,4.7. $C_{17}H_{19}NO_3$ requires C,71.57; H,6.66; N,4.91%. δ (DMSO) 9.26(2H, s,2xOH), 7.92(1H,t,J=5Hz, NH), 7.07(2H,d,J=8Hz), 7.03(2H,d,J=8Hz), 6.77(4H,d,J=8Hz), 3.4-3.1(2H,m), 2.9-2.5(4H,m), 2.5-2.2(2H,m); m/z 285.13686 (M^+, $C_{17}H_{19}NO_3$ requires 285.13649; 5%), 165(15%), 149(3%), 120(100%), 107(81%), 77(13%), 43(4%).

N-*[2-(4-Hydroxyphenyl)ethyl]*-N-*[3-(4-hydroxyphenyl)propyl]amine* (19)

To a stirred solution of the amide [as (24)] (53mg, 0.18mmol) in THF (2ml) was added diborane/THF in THF (2ml, 1.6mmol). The mixture was refluxed overnight under nitrogen. Methanol was then added to

destroy the excess reagent. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in methanol (5ml) which was then acidified with HCl gas. This solution was refluxed for 2h. The solvent was removed *in vacuo*. The residue was dissolved in methanol (10ml), and the methanol was evaporated; this procedure was repeated twice. The residue was dissolved in water (5ml) and the solution was basified with conc.NH_3. The resulting solution was extracted with ether (3x20ml). The combined ether extracts were dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by column chromatography eluting with ether:methanol (60ml:40ml) to give the amine (19) (39mg, 77.7%). δ (CD_3COCD_3) (400MHz), 7.04(2H, d, J=9Hz), 6.99(2H, d, J=9Hz), 6.75-6.66(4H,m), 2.8(2H, broad s, 2xOH), 2.77-2.50(8H, m), 1.73-1.65(2H, m); m/z 271.15715 (M^+, $C_{17}H_{21}NO_2$ requires 271.15722; 1%); 164(47%); 121(12%); 107(33%); 44(100%).

N-[2-(4-Hydroxyphenyl)ethyl]-N-[3-(4-hydroxyphenyl)propyl]-N-methylamine (21).

The amine (19) (50mg, 0.18mmol) was dissolved in methanol (1ml). Formaldehyde (0.25ml, 37.4%) was then added. The mixture was refluxed for 45min. Sodium borohydride was added carefully after cooling the mixture. The mixture was stirred at room temperature for 1h. Two drops of 2M HCl was added to destroy any excess of $NaBH_4$. The mixture was basified with conc.NH_3 (pH 8-9) then partitioned between water (2ml) and ether (15ml). The aqueous layer was re-extracted with ether (2x15ml). The extracts were combined with the above oroganic layer and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by column chromatography eluting with $CHCl_3$:MeOH:conc.NH_3 (84ml:16ml:16 drops) to give the amine (21) (41mg, 77.5%). δ 7.12(4H, d, J=8Hz), 6.82(4H, d, J=8Hz), 2.90-2.50(8H, m), 2.44(3H, s), 2.12-1.70(2H, m); m/z 285.17188 (M^+, $C_{18}H_{23}NO_2$ requires 285.17287; 0.1%); 178(22%); 107(13%), 58(100%).

N[2-(4-Hydroxyphenyl)ethyl]-N-[3-([3,5-$^3H_2$]-4-hydroxyphenyl)propyl]amine (20), N-[2-(4-hydroxyphenyl)ethyl]-N[3-([3,5-$^3H_2$]-4-hydroxyphenyl)propyl]-N-methylamine (22) and N-[2-([2-^{14}C]-4-hydroxyphenyl)ethyl]-N-[3-([3,5-$^3H_2$]-4-hydroxyphenyl)propyl]-N-methylamine (23).

These labelled compounds were prepared as described for the unlabelled materials above. The tritiated amines (20) (173mg, 0.34 mCi) and (22) (55mg, 0.1mCi) were prepared from the tritiated acid (13). [7-^{14}C]Tyramine was used to prepare ^{14}C-labelled [as (21)] (47mg, 25μCi) which was mixed with tritiated amine prior to feeding (^{3}H:^{14}C, 1.94).

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