Alkaloid Biosynthesis. Part XVIII.[1,2] Biosynthesis of Colchicine from the 1-Phenethylisoquinoline System

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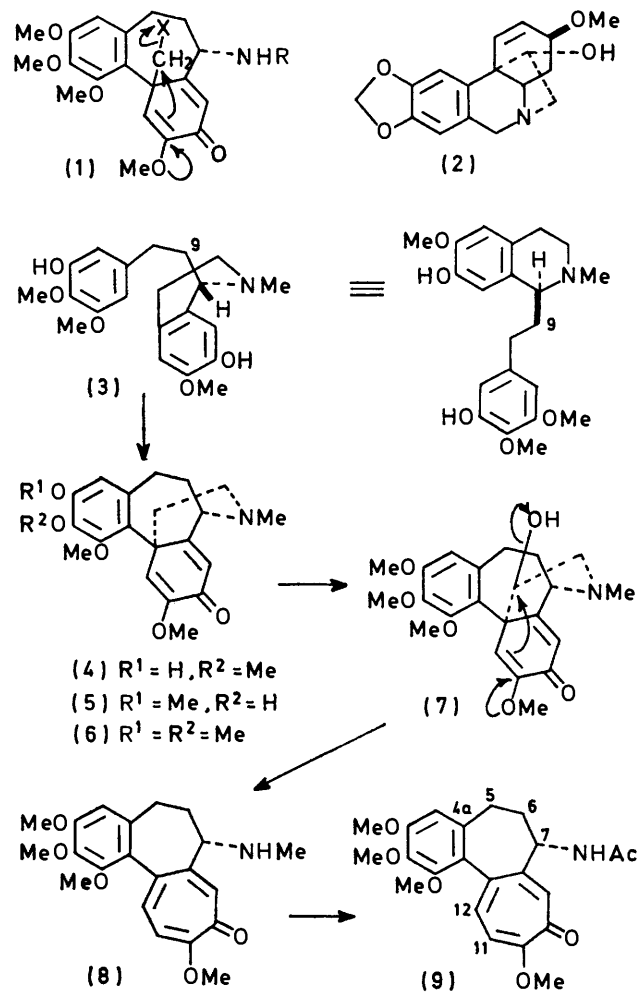
Extensive tracer experiments show that colchicine (9) is formed by a novel biosynthetic pathway from 1,2,3,4-tetrahydro-7-hydroxy-1-(3-hydroxy-4,5-dimethoxyphenethyl)-6-methoxy-2-methylisoquinoline (3) (autumnaline) with *O*-methylandrocymbine (6) as a key intermediate. The way in which autumnaline is constructed in the living system is also studied.

PART XVI [3] described tracer experiments on *Colchicum autumnale* plants which led to the suggestion that colchicine (9) and its tropolonoid relatives [4] are biosynthesised from a dienone of type (1). A process of ring expansion involving the departure of some good leaving group X with homoallylic assistance as illustrated accounted well for the observed labelling patterns. Crucial information became available at this stage when androcymbine, which occurs alongside colchicine in *Androcymbium melanthioides*, was proved [1] to have the novel structure and absolute stereochemistry shown (5). The close relationship between androcymbine (5) and the postulated precursor of colchicine (1) and also colchicine itself (9) pointed to a biosynthetic connection between compounds (5) and (9). On this basis, a pathway can be considered making use of a 1-phenethylisoquinoline [*e.g.* (3)] which by oxidative coupling [5] and subsequent *O*-methylation could generate *O*-methylandrocymbine (6). Hydroxylation of (6) to form structure (7), as occurs at a late stage in the biosynthesis of haemanthamine [6] (2), could provide a starting point [7] for ring expansion (see Scheme 1).

A decisive test of these ideas required labelled *O*-methylandrocymbine (6), which was prepared by methylating androcymbine (5) with tritiated diazomethane.[8] When this product was administered to *C. autumnale* plants in spring,† incorporation into colchicine occurred to the remarkably high extent of over 15% (Expt. 9, Table 1). No randomisation of the label occurred in the biological system; thus, the 3,4,5-trimethoxyphthalic acid isolated after oxidative degradation of the colchicine had essentially unchanged molar activity. These results supported the suspected biosynthetic relationship between colchicine and a 1-phenethylisoquinoline system, and they opened the way to a detailed study of this remarkable sequence of reactions.

Further progress depended upon proving that the diphenol (3), hereafter called autumnaline, also acts as a specific biological precursor of colchicine. The synthesis of compound (3) was designed to insert a ^{14}C label at



SCHEME 1

† Experiments were carried out in spring on *C. autumnale* and in autumn on *C. byzantinum*. Incorporations were invariably higher in springtime by a factor varying over the range 4—15 (Tables 1 and 2).

[1] Part XVII, A. R. Battersby, R. B. Herbert, L. Pijewska, F. Šantavý, and P. Sedmera, preceding paper.

[2] Preliminary report, A. R. Battersby, R. B. Herbert, E. McDonald, R. Ramage, and J. H. Clements, *Chem. Comm.*, 1966, 603.

[3] A. R. Battersby, T. A. Dobson, D. M. Foulkes, and R. B. Herbert *J.C.S. Perkin I*, 1972, 1730.

[4] Reviewed by W. C. Wildman in 'The Alkaloids,' ed. R. H. F. Manske, Academic Press, New York, 1960, vol. VI, p. 247.

[5] Reviewed by A. R. Battersby in 'Oxidative Coupling of Phenols,' eds. W. I. Taylor and A. R. Battersby, Dekker, New York, 1967, p. 119.

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

[7] A. R. Battersby, *Pure and Appl. Chem.*, 1967, **14**, 117.

[8] *Cf.* K. J. Van Der Merwe, P. S. Steyn, and S. H. Eggers, *Tetrahedron Letters*, 1964, 3923.

C-9, corresponding to C-6 of colchicine (9). An unambiguous degradation of compound (9) was available [9] for radiochemical assay of this centre.

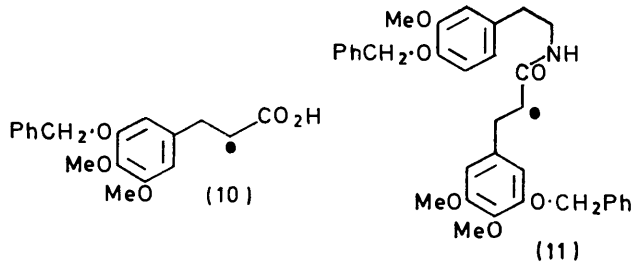
TABLE 1

Tracer experiments on *Colchicum autumnale* in spring

Expt.	Precursor	Incorporation (%) into colchicine
1	[2-^{14}C]Tyramine	0·4
2	[*Ar*-^{3}H]Dopamine	1·1
3	[8-^{3}H]Isoquinoline (19)	0·007
4	[9-^{14}C]Isoquinoline (20) *	1·4
5	[9-^{14}C]Isoquinoline (23) *	3·8
6	[9-^{14}C]Isoquinoline (24) *	0·52
7	[9-^{14}C]Autumnaline (3) *	9·6
8	*N*-Nor[*Ar*-^{3}H]autumnaline (22)	0·04
9	*O*-[^{3}H]Methylandrocymbine (6)	15·2

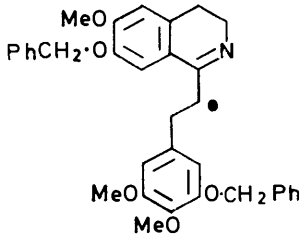
* The 9-position is the α-position of the phenethyl group.

[2-^{14}C]Malonic acid condensed with 3-benzyloxy-4,5-dimethoxybenzaldehyde to yield the corresponding cinnamic acid, which was converted by controlled hydrogenation into the acid (10). 4-Benzyloxy-3-methoxyphenethylamine reacted with the acid chloride from (10) and the resultant amide (11) was cyclised to form the 3,4-dihydroisoquinoline (12). *N*-Methylation and reduction with borohydride followed by hydrogenolysis of the *O*-benzyl groups then completed the

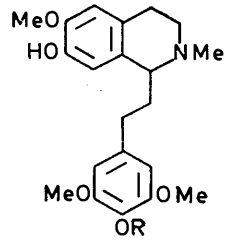


(10)

(11)



(12)



(13) R = Me
(14) R = H

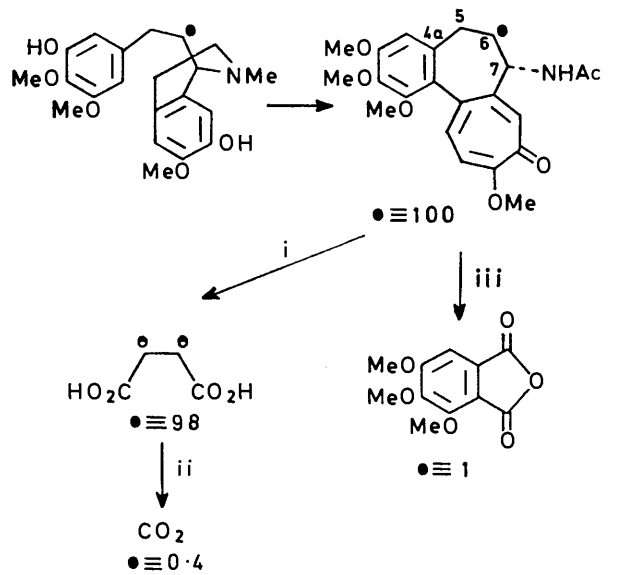
synthesis of (*RS*)-[9-^{14}C]autumnaline [as (3)]. This was fed as an aqueous solution of its hydrochloride to *C. byzantinum* plants, and satisfactory incorporations were achieved into both demecolcine (8) and colchicine (9) (Expt. 8, Table 2). A much higher incorporation was obtained when the same precursor [as (3)] was subsequently administered to *C. autumnale* plants in spring (Expt. 7, Table 1).

* The trioxygenated ring could in principle be rotated to allow *ortho–para* coupling. Tracer evidence on this point will be presented in a later paper.

[9] A. R. Battersby, R. Binks, J. J. Reynolds, and D. A. Yeowell, *J. Chem. Soc.*, 1964, 4257.

The labelled site in the isolated colchicine was established by the set of degradations shown in Scheme 2. Since the derived succinic acid (corresponding to carbon atoms 4a, 5, 6, and 7 of colchicine) carried essentially all the original activity yet yielded inactive carbon dioxide, it must have been labelled at one or both of the methylene groups (corresponding to carbon atoms 5 and 6 of colchicine). The isolation of radioinactive trimethoxyphthalic anhydride located the label unambiguously at C-6 of colchicine as expected.

The results outlined so far define part of the biological pathway to colchicine as (3) ⟶ (6) ⟶ (9). Colchicine thus falls into place as a considerably modified isoquinoline system rather than being, as it was, a puzzling inconsistency.



i, CrO_3-H_2SO_4; ii, HN_3-H_2SO_4; iii, $K_3Fe(CN)_6$-OH^-

SCHEME 2

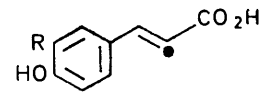
The oxidative coupling step (3) ⟶ (4) as written * produces a new carbon–carbon bond between the positions *para* to the phenolic hydroxy-groups. Direct tracer study of such carbon–carbon couplings has consistently shown that phenolic hydroxy-groups *ortho* or *para* to the new bond are essential.[5,10] On this basis, the phenols (13) [1] and (14) should not serve as precursors of colchicine despite their close resemblance to autumnaline (3). These two phenols were prepared by standard methods and were labelled with tritium in the aromatic rings by exchange with tritiated water.[11] When they were administered in separate experiments to *Colchicum* plants, only insignificant incorporations were observed relative to the high values found (Table 2) for autumnaline (3) and *O*-methylandrocymbine (6). Thus, compounds (13) and (14), fed in autumn to *C. byzantinum* gave maximum incorporations of 0·005 and 0·02%,

[10] D. H. R. Barton and T. Cohen, 'Festschr. A. Stoll,' Birkhauser, Basle, 1957, p. 117.

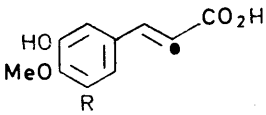
[11] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914; A. R. Battersby, T. H. Brown, and J. H. Clements, *ibid.*, p. 4550.

respectively, into demecolcine (8). By showing the ineffectiveness of close relatives, these results strengthen further the position of autumnaline as precursor of colchicine.

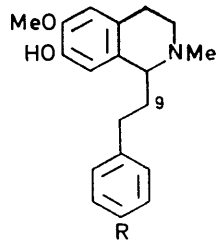
Attention was turned next to a study of the pathway by which autumnaline (3) is produced in *Colchicum* plants. Earlier work[9,12] had shown that colchicine (and therefore autumnaline too) is built from tyrosine and cinnamic acid, the latter being formed in turn from phenylalanine. The various experiments now summarised were designed to cast light on the subsequent steps. Tyramine and 3,4-dihydroxyphenethylamine



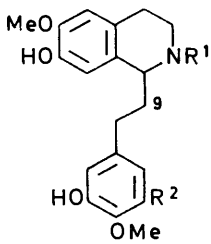
(15) R = H
(16) R = OMe



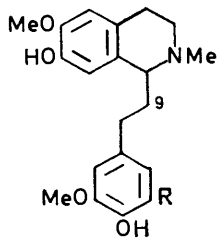
(17) R = H
(18) R = OMe



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



(21) R^1 = Me, R^2 = H
(22) R^1 = H, R^2 = OMe



(23) R = H
(24) R = OH

(dopamine) were both incorporated with satisfactory efficiency into colchicine (Expts. 1 and 2, Table 1). Support is thus given for decarboxylation of the related amino-acids before combination of the nitrogenous unit with that derived from cinnamic acid. None of the cinnamic acids (15)—(18), labelled as shown, acted as effective precursors (Expts. 1—4, Table 2) so presumably something other than hydroxylation occurs as a first step (reduction?). Further work is necessary on this aspect.

The sequence at the isoquinoline level was examined by preparing a set of 1-phenethylisoquinolines having various degrees of oxygenation and of *O*- and *N*-methylation. By comparing the levels of incorporation of these substances into colchicine and demecolcine, an indication of the biological order of events was gained. No stronger interpretation of our results is made because a number of factors can affect incorporation values. The base (19) is evidently not on the biosynthetic pathway (Expt. 3, Table 1) but introduction of a single hydroxy-group produces a reasonably efficient precursor (20) of

[12] E. Leete, *Tetrahedron Letters*, 1965, 333.

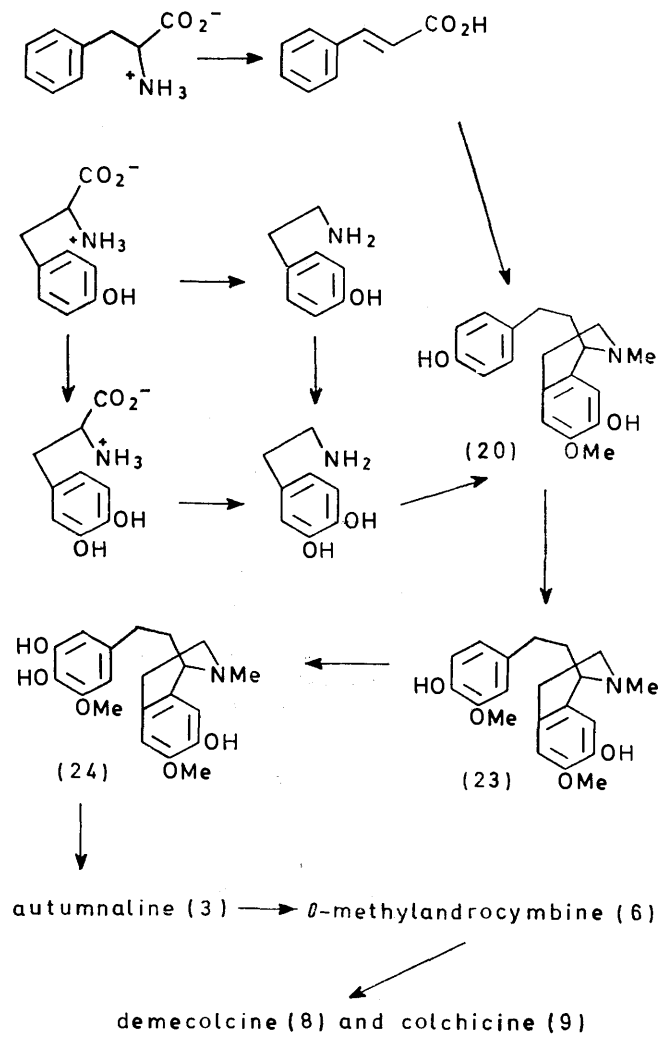
TABLE 2

Tracer experiments on *Colchicum byzantinum* in autumn

Expt.	Precursor	Incorporation (%) Demecolcine	Colchicine
1	*p*-Hydroxy[2-^{14}C]cinnamic acid (15)	*a*	<0·01
2	[2-^{14}C]Ferulic acid (16)	*a*	<0·01
3	[2-^{14}C]Isoferulic acid (17)	0·003	0·001
4	3-Hydroxy-4,5-dimethoxy-[2-^{14}C]cinnamic acid (18)	0·006	0·003
5	[9-^{14}C]Isoquinoline (20) [b]	0·30	0·012
6	[9-^{14}C]Isoquinoline (23) [b]	0·22	0·030
7	[9-^{14}C]Isoquinoline (21) [b]	0·06	0·012
8	[9-^{14}C]Autumnaline (3) [b]	1·22	0·26
9	[*Ar*-^{3}H]Isoquinoline (14)	0·02	0·002
10	*O*-Methyl[*Ar*-^{3}H]autumnaline (13)	0·005	*a*
11	*O*-[^{3}H]Methylandrocymbine (6)	*a*	0·65

[a] Not determined. [b] See footnote to Table 1.

the tropolone alkaloids (Expt. 4, Table 1). Expts. 4, 5, and 7 in Table 1 and 5, 6, 7, and 8 in Table 2 point to the sequence (20) ⟶ (23) ⟶ autumnaline (3). The triphenolic base (24) might be expected on chemical grounds to lie on the pathway between (23) and



SCHEME 3

autumnaline (3) so the observed incorporation (Expt. 6, Table 1) was surprising. This result may be due to the marked sensitivity of (24) to destruction by oxidation. Finally, Expt. 8 (Table 1), in which *N*-norautumnaline (22) was fed, interlocks with several of the foregoing ones in showing that *N*-methylation occurs early in the sequence.

The results reported here show that the tropolone alkaloids of *Colchicum* species are derived from the 1-phenethylisoquinoline system by way of the dienone *O*-methylandrocymbine (6). Further, these findings, when combined with results of earlier work, support the sequence shown in Scheme 3. Subsequent papers will describe research on the step by which autumnaline is converted into *O*-methylandrocymbine (6) and, particularly, on the mechanism of the remarkable ring-expansion step which generates the tropolone nucleus.

EXPERIMENTAL

For general directions concerning radioactive work see Part III; [13] the cultivation of *Colchicum* plants is described in Part VI.[9]

Administration of Labelled Precursors.—(a) *Spring feedings to* C. autumnale. Aqueous solutions of the precursors, generally as hydrochlorides of bases or sodium salts of acids, were injected into the hollow seed capsules. Substances which were difficult to dissolve were dissolved first in a few drops of dimethyl sulphoxide and this solution was then diluted with water.

(b) *Autumn feedings to* C. autumnale *and spring and autumn feedings to* C. byzantinum. The plants were set in pots so that at least the top third of each corm was exposed. A wet cotton wick was threaded with a curved needle through the flesh of the corm and the ends were dipped into the solution of precursor in a small glass tube. After most of the solution had been absorbed, distilled water was added to the tube and the process was repeated at least once. Finally, the tubes were checked for radioactive material and, if necessary, small corrections were made to the amount of activity fed. Some feedings to *C. byzantinum* were carried out as above but with the bare corm resting in a Petri dish.

Isolation of the Alkaloids.—A solution of the total crude alkaloid from four plants was obtained in chloroform as earlier.[9] This was passed through a column of Merck neutral alumina (activity I; 10 g) and the column was washed with chloroform. Evaporation of the total chloroformic eluate gave a gum (*ca.* 0·2 g) which was shaken with water (1 ml) and sufficient ethyl acetate to form an emulsion. A volume of light petroleum (b.p. 40—60°) equal to that of the ethyl acetate was then added, and the thoroughly emulsified mixture was adsorbed evenly on Celite (1 g). This was packed on to the top of a column prepared by mixing Celite (15 g) with water (15 ml) which had been equilibrated with an equal volume of 1 : 1 (v/v) ethyl acetate–light petroleum (b.p. 40—60°). The column was eluted with the organic layer from the foregoing equilibration, and material from an initial yellow band was discarded. The demecolcine which followed it was collected and crystallised from ethyl acetate; m.p. 184—185° (lit.,[14] 186°); yield *ca.* 30 mg from *C. autumnale* and 120 mg from *C. byzantinum*. Elution was continued with dry ethyl acetate to yield colchicine, m.p. 148—149° (from ethyl acetate) (lit.,[14] 148—150°); yield *ca.* 90 mg from *C. autumnale* and 110 mg from *C. byzantinum*.

Degradations of Labelled Colchicine.—These were carried out as earlier.[9] Colchicine from Expt. 8, Table 2 ($1·9 \times 10^4$ dis. per 100 s per mmol; 100%) yielded succinic acid ($1·86 \times 10^4$ dis. per 100 s per mmol; 98%) which by Schmidt degradation gave carbon dioxide (1×10^2 dis. per 100 s per mmol; 0·4%). Ferricyanide oxidation [15] of a further portion of this colchicine sample gave 3,4,5-trimethoxyphthalic anhydride (2×10^2 dis. per 100 s per mmol; 1%).

3,4-Diphenylmethylenedioxy-5-methoxybenzaldehyde.— The corresponding benzoic acid [16] (34·8 g) suspended in benzene (150 ml) was dried by azeotropic distillation of part of the solvent. Oxalyl chloride (10 ml) was added to the cooled solution, followed by dimethylformamide (0·2 ml). When effervescence had ceased, the solution was evaporated and the crude acid chloride was redissolved in dry xylene (150 ml). Dry hydrogen was passed into this solution under reflux in the presence of 10% palladium–barium sulphate (3 g) and 1% sulphur in quinoline (0·05 ml), and after 8 h filtration and evaporation gave the crude aldehyde. Chromatography gave a pure specimen, but the major by-product, 1,2-diphenylmethylenedioxy-3-methoxybenzene, m.p. 106°, was more easily removed after formation of the cinnamic acid. The *aldehyde* had m.p. 120—121° (from aqueous methanol) (Found: C, 76·0; H, 4·9. $C_{21}H_{16}O_4$ requires C, 75·9; H, 4·9%); $\nu_{max.}$ 1690 and 2800 cm^{-1}; $\lambda_{max.}$ 305 nm; τ 0·35 (1H, s, CHO) and 6·05 (3H, s, OMe).

Synthesis of [2-^{14}C]*Cinnamic Acids.*—A mixture of sodium [2-^{14}C]malonate (6·13 mg; 0·5 mCi) and radioinactive sodium malonate (142 mg) was dissolved in the minimum amount of water and treated with 2N-hydrochloric acid (1·05 ml). The solution was evaporated and the residue was dried at 20° for 16 h (P_2O_5) and then mixed with dry pyridine, the appropriate aromatic aldehyde (2 mmol) and piperidine (0·05 ml). After the solution had been heated at 100° for 2 h and at 120° for 1 h it was evaporated, and the residue was partitioned between 3 : 1 ether–chloroform and saturated aqueous sodium hydrogen carbonate. The aqueous layer was acidified and the cinnamic acid, extracted into ethyl acetate, was crystallised from the indicated solvent. The following were prepared in labelled form (87—98% yield) after characterisation had been completed on radioinactive material: 4-Hydroxy-3-methoxy[2-^{14}C]cinnamic acid (16), m.p. 170° (from ethyl acetate) (lit.,[17] 168—169°); 3-hydroxy-4-methoxy[2-^{14}C]-cinnamic acid (17), m.p. 231·5° (from methanol) (lit.,[18] 224—225°); *3-hydroxy-4,5-dimethoxy*[2-^{14}C]*cinnamic acid* (18), m.p. 144—145° (from aqueous ethanol) (Found: C, 58·5; H, 5·2. $C_{11}H_{12}O_5$ requires C, 58·9; H, 5·4%); *3-benzyloxy-4,5-dimethoxy*[2-^{14}C]*cinnamic acid*, m.p. 105—106° (from methanol) (Found: C, 68·7; H, 5·7. $C_{18}H_{18}O_5$ requires C, 68·8; H, 5·8%); $\nu_{max.}$ 1630 and 1690 cm^{-1}; $\lambda_{max.}$ 293 nm; τ −0·90 (1H, s, CO_2H), 2·30 and 3·70 (both d, 2H, *J* 16 Hz, *trans*-CH=CH), 4·87 (s, 2H, O·CH_2Ar), 6·09

[13] A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J. Chem. Soc.*, 1964, 1595.
[14] J. W. Cook and J. D. Loudon in 'The Alkaloids,' ed. R. H. F. Manske, Academic Press, New York, 1952, vol. II, p. 266.
[15] E. Leete and P. E. Nemeth, *J. Amer. Chem. Soc.*, 1960, **82**, 6055.
[16] L. Jurd, *J. Amer. Chem. Soc.*, 1959, **81**, 4608.
[17] F. Tiemann and N. Nagai, *Ber.*, 1878, 646.
[18] R. Robinson and S. Sugasawa, *J. Chem. Soc.*, 1931, 3163.

(3H, s, OMe), and 6·12 (3H, s, OMe); 3,4-*diphenylmethylenedioxy*-5-*methoxy*[2-^{14}C]*cinnamic acid*, m.p. 197—199° (from aqueous methanol) (Found: C, 73·9; H, 4·8. $C_{23}H_{18}O_5$ requires C, 73·8; H, 4·8%); and 3,4-*dibenzyloxy*-5-*methoxycinnamic acid*, m.p. 152—154° (Found: C, 73·3; H, 5·6. $C_{24}H_{22}O_5$ requires C, 73·8; H, 5·7%).

Preparation of 3-*Phenyl*[2-^{14}C]*propionic Acids*.—The foregoing cinnamic acids were hydrogenated as in the following example.

3-(3-*Benzyloxy*-4,5-*dimethoxyphenyl*)[2-^{14}C]*propionic acid* (10). A solution of the corresponding cinnamic acid (1·44 g) in ethyl acetate (15 ml) was shaken with hydrogen and Adams platinic oxide (15 mg) until 1 mol. equiv. of hydrogen had been absorbed. Evaporation of the filtered solution and crystallisation of the residue from ether–light petroleum (b.p. 40—60°) gave the *propionic acid* (1·42 g), m.p. 85—87° (Found: C, 68·0; H, 6·6. $C_{18}H_{20}O_5$ requires C, 68·3; H, 6·4%); $\nu_{max.}$ 1710 cm^{-1}. The labelled sample was prepared analogously on a smaller scale.

3-(4-*Benzyloxyphenyl*)[2-^{14}C]*propionic acid* had m.p. 123—125° (from aqueous ethanol) (Found: C, 74·8; H, 6·2. $C_{16}H_{16}O_3$ requires C, 75·0; H, 6·3%). 3-(3,4-*Diphenylmethylenedioxy*-5-*methoxyphenyl*)[2-^{14}C]*propionic acid* had m.p. 169—170° (from methanol) (Found: C, 73·2; H, 5·5. $C_{23}H_{20}O_5$ requires C, 73·4; H, 5·4%). 3-(4-Hydroxy-3-methoxyphenyl)[2-^{14}C]propionic acid had m.p. 90—91° [from ether–light petroleum (b.p. 60—80°)] (lit.,[17] 89—90°).

Preparation of 3-*Phenyl*[2-^{14}C]*propionamides*.—The following standard method was used in each case.

N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-(3-*benzyloxy*-4,5-*dimethoxyphenyl*)[2-^{14}C]*propionamide* (11). A solution of the appropriate 3-phenylpropionic acid (139 mg) in benzene (10 ml) was dried by azeotropic distillation of part of the solvent, and the cooled solution was treated with oxalyl chloride (130 mg) and dimethylformamide (0·02 ml). When effervescence had ceased, the crude acid chloride was freed from solvent, redissolved in dry dichloromethane (5 ml), and added dropwise to a vigorously stirred emulsion of 4-benzyloxy-3-methoxyphenethylamine (126 mg) in dichloromethane (5 ml) and saturated aqueous sodium hydrogen carbonate (1 ml). After 1 h the organic layer was washed with dilute acid and water, and then evaporated. Crystallisation of the residue from ethyl acetate–light petroleum (b.p. 60—80°) gave the *amide* (167 mg, 68%), m.p. 105—107° (Found: C, 73·8; H, 6·8; N, 2·5. $C_{34}H_{37}NO_6$ requires C, 73·4; H, 6·7; N, 2·5%); $\nu_{max.}$ 1660 cm^{-1}. For this and the following amides, the [^{14}C]-samples were prepared similarly on a small scale.

N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-(4-*benzyloxy*-3-*methoxyphenyl*)[2-^{14}C]*propionamide* had m.p. 129—131° [from ethyl acetate–light petroleum (b.p. 60—80°)] (Found: C, 75·2; H, 6·7; N, 2·7. $C_{33}H_{35}NO_5$ requires C, 75·4; H, 6·7; N, 2·7%). N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-(3-*benzyloxy*-4-*methoxyphenyl*)[2-^{14}C]*propionamide* had m.p. 141—142° (from ethyl acetate) (Found: C, 75·5; H, 6·7; N, 2·7. $C_{33}H_{35}NO_5$ requires C, 75·4; H, 6·7; N, 2·7%). N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-(4-*benzyloxyphenyl*)-[2-^{14}C]*propionamide* had m.p. 149—150° (from ethyl acetate–ether) (Found: C, 77·5; H, 6·7; N, 2·8. $C_{32}H_{33}NO_4$ requires C, 77·5; H, 6·7; N, 2·8%). N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-(3,4-*dihydroxy*-5-*methoxyphenyl*)[2-^{14}C]-*propionamide* had m.p. 160—162° (from methanol) (Found: C, 68·6; H, 6·5. $C_{26}H_{29}NO_6$,0·25H_2O requires C, 68·5; H, 6·5%) (this compound was prepared by treatment of the corresponding diphenylmethylenedioxy-derivative with hot aqueous acetic acid [16]). N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-(3,4-*dibenzyloxy*-5-*methoxyphenyl*)[2-^{14}C]*propionamide* had m.p. 107·5—108·5° (from ethyl acetate–ether) (Found: C, 75·8; H, 6·6. $C_{40}H_{41}NO_6$ requires C, 76·1; H, 6·5) (prepared by standard *O*-benzylation of the foregoing diphenolic amide). N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-*phenylpropionamide* had m.p. 112° [from ethyl acetate–light petroleum (b.p. 60—80°)] (Found: C, 76·9; H, 7·0; N, 3·6. $C_{25}H_{27}NO_3$ requires C, 77·1; H, 7·0; N, 3·6%). N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-(4-*benzyloxy*-3,5-*dimethoxyphenyl*)*propionamide* had m.p. 108—109° [from ethyl acetate–light petroleum (b.p. 60—80°)] (Found: C, 73·2; H, 6·8; N, 2·6. $C_{34}H_{37}NO_6$ requires C, 73·5; H, 6·7; N, 2·5%).

Preparation of 3,4-*Dihydro*-1-[α-^{14}C]*phenethylisoquinolines*.—The following case illustrates the standard procedure.

7-*Benzyloxy*-1-(3-*benzyloxy*-4,5-*dimethoxy*[α-^{14}C]*phenethyl*)-3,4-*dihydro*-6-*methoxy*-2-*methylisoquinolinium iodide*. A solution of the corresponding amide (10·2 g) in toluene (250 ml) was dried by azeotropic distillation of part of the solvent, heated at 100° for 2 h with phosphoryl chloride (7 ml), and then evaporated. The residue was dissolved in ethyl acetate (100 ml) and mixed with 2N-sodium hydroxide (100 ml) (vigorous agitation with nitrogen). After 15 min, the organic phase was washed with water and dried, and the volume was adjusted to 150 ml before addition of methyl iodide (15 ml) at 0°. The crystalline *isoquinolinium iodide* was collected after 16 h; yield 9·9 g, m.p. 158—160° (from acetone–ethyl acetate) (Found: C, 60·4; H, 5·7. $C_{35}H_{38}INO_5,H_2O$ requires C, 60·2; H, 5·8%); $\nu_{max.}$ 1625 cm^{-1}; $\lambda_{max.}$ 246 (log ε 4·22), 307 (3·91), and 352 nm (3·93); τ 3·16, 3·57, and 3·62 (each 1H, s, ArH), 4·88 and 4·94 (both 2H, s, O·CH_2Ph), 6·06 (3H, s, OMe), 6·22 (6H, s, 2 × OMe), and 6·40 (3H, s, $\overset{+}{N}$Me). The labelled sample was prepared in a similar way on a small scale.

7-*Benzyloxy*-1-(4-*benzyloxy*-3-*methoxy*[α-^{14}C]*phenethyl*)-3,4-*dihydro*-6-*methoxy*-2-*methylisoquinolinium iodide* had m.p. 132—136° (from dichloromethane–ethyl acetate) (Found: C, 62·7; H, 5·6. $C_{34}H_{36}INO_4$ requires C, 62·9; H, 5·6%). 7-*Benzyloxy*-1-(3-*benzyloxy*-4-*methoxy*[α-^{14}C]*phenethyl*)-3,4-*dihydro*-6-*methoxy*-2-*methylisoquinolinium iodide* had m.p. 204° (from methanol–ether) (Found: C, 62·0; H, 5·7. $C_{34}H_{36}INO_4$,0·5H_2O requires C, 62·0; H, 5·7%). 7-*Benzyloxy*-1-(4-*benzyloxy*[α-^{14}C]*phenethyl*)-3,4-*dihydro*-6-*methoxy*-2-*methylisoquinolinium iodide* had m.p. 168° (from propan-2-ol) (Found: C, 63·7; H, 5·5; N, 2·2. $C_{33}H_{34}INO_3$ requires C, 64·0; H, 5·5; N, 2·3%). 7-*Benzyloxy*-1-(3,4-*dibenzyloxy*-5-*methoxy*[α-^{14}C]*phenethyl*)-3,4-*dihydro*-6-*methoxy*-2-*methylisoquinolinium iodide* had m.p. 141—143° (from acetone–ether) (Found: C, 64·2; H, 5·5. $C_{41}H_{42}INO_5$,0·5H_2O requires C, 64·4; H, 5·7%) 7-*Benzyloxy*-1-(4-*benzyloxy*-3,5-*dimethoxy*[α-^{14}C]*phenethyl*)-3,4-*dihydro*-6-*methoxy*-2-*methylisoquinolinium iodide* had m.p. 104—106° (from dichloromethane–ether) (Found: C, 61·3; H, 5·7; N, 2·0. $C_{35}H_{38}INO_5$ requires C, 61·9; H, 5·6; N, 2·0%).

Preparation of O-*Benzylated* 1,2,3,4-*Tetrahydro*-1-[α-^{14}C]*phenethylisoquinolines*.—All the reductions were carried out as follows.

(RS)-OO-*Dibenzyl*[9-^{14}C]*autumnaline*. A suspension of the corresponding isoquinoline methiodide (3·4 g) in dry methanol (50 ml) was treated in portions at 0° with sodium borohydride (250 mg). When the yellow colour had been discharged (*ca.* 1 h) a slight excess of 2N-hydrochloric acidwas

added and the methanol was evaporated off. The aqueous solution was made basic and extracted with dichloromethane to yield the *tetrahydroisoquinoline* (2·7 g), m.p. 86—87° (from aqueous methanol) (Found: C, 75·8; H, 7·3; N, 2·5. $C_{35}H_{39}NO_5$ requires C, 75·9; H, 7·1; N, 2·5%); $\lambda_{max.}$ 242 and 282 nm; τ 3·43 and 3·68 (both 2H, s, 4 × ArH), 4·93 (4H, s, 2 × O·CH_2Ph), 6·11 (9H, s, 3 × OMe), and 7·67 (3H, s, NMe).

(RS)-7-*Benzyloxy*-1-(4-*benzyloxy*-3-*methoxy*[α-^{14}C]*phenethyl*)-1,2,3,4-*tetrahydro*-6-*methoxy*-2-*methylisoquinoline* had m.p. 70—71° (from ether–cyclohexane) (Found: C, 78·0; H, 7·3; N, 2·7. $C_{34}H_{37}NO_4$ requires C, 78·0; H, 7·1; N, 2·7%). (RS)-7-*Benzyloxy*-1-(3-*benzyloxy*-4-*methoxy*[α-^{14}C]-*phenethyl*)-1,2,3,4-*tetrahydro*-6-*methoxy*-2-*methylisoquinoline* had m.p. 104—105° (from methanol) (Found: C, 77·9; H, 7·1; N, 2·6. $C_{34}H_{37}NO_4$ requires C, 78·0; H, 7·1; N, 2·7%). (RS)-7-*Benzyloxy*-1-(3,4-*dibenzyloxy*-5-*methoxy*-[α-^{14}C]*phenethyl*)-1,2,3,4-*tetrahydro*-6-*methoxy*-2-*methylisoquinoline* was characterised as the *oxalate*, m.p. 140° [from acetone–light petroleum (b.p. 40—60°)] (Found: C, 71·9; H, 6·1; N, 2·0. $C_{43}H_{45}NO_9$ requires C, 71·6; H, 6·3; N, 1·9%). (RS)-7-*Benzyloxy*-1,2,3,4-*tetrahydro*-6-*methoxy*-2-*methyl*-1-*phenethylisoquinoline* was characterised as the *picrate*, m.p. 190° (from methanol) (Found: C, 62·2; H, 5·2; N, 8·9. $C_{32}H_{32}N_4O_9$ requires C, 62·3; H, 5·2; N, 9·1%). (RS)-7-*Benzyloxy*-1-(4-*benzyloxy*-3,5-*dimethoxyphenethyl*)-1,2,3,4-*tetrahydro*-6-*methoxy*-2-*methylisoquinoline* was characterised as the *picrolonate*, m.p. 166—168° (from methanol, then ethyl acetate) (Found: C, 65·8; H, 5·8; N, 8·4. $C_{45}H_{47}N_5O_{10}$ requires C, 66·1; H, 5·7; N, 8·6%). (RS)-OO-*Dibenzyl*-N-*norautumnaline* was characterised as the *picrolonate*, m.p. 150—153° (from ethanol) (Found: C, 65·5; H, 5·6; N, 8·5. $C_{44}H_{45}N_5O_{10}$ requires C, 65·7; H, 5·6; N, 8·7%).

Catalytic O-Debenzylation of Labelled Substances to yield Phenolic Precursors.—The standard method used is illustrated in the following example; the resultant phenols were characterised as crystalline bases or salts. In the [^{14}C]-series, complete removal of the *O*-benzyl groups was demonstrated by n.m.r. analysis.

(RS)-[9-^{14}C]*Autumnaline* [as (3)]. A solution of the corresponding *OO*-dibenzyl ether (196 mg) in methanol (5 ml) and concentrated hydrochloric acid (0·04 ml) was shaken with hydrogen and 10% palladium–charcoal (20 mg). When uptake of hydrogen was complete (better than ±10% of theoretical), the solution was filtered and evaporated to yield (RS)-*autumnaline hydrochloride*, m.p. 235° (from methanol–ether) (Found: C, 61·7; H, 6·9; N, 3·7. $C_{21}H_{28}ClNO_5$ requires C, 61·5; H, 6·9; N, 3·4%). Standard recovery of the free base gave (RS)-*autumnaline*, m.p. 170—172° (from ethyl acetate) (Found: C, 67·3; H, 7·4; N, 3·8. $C_{21}H_{27}NO_5$ requires C, 67·5; H, 7·3; N, 3·8%); $\nu_{max.}$ 3550 cm^{-1}; $\lambda_{max.}$ 285 nm (log ε 3·59); τ 3·31 and 3·43 (both 1H, s, isoquinoline H-5 and H-8), 3·54 and 3·68 (both 1H, d, *J* 1 Hz, phenyl H-2 and H-6), 5·0br (2H, 2 × OH), 6·13 (9H, s, 3 × OMe), and 7·53 (3H, s, NMe).

(RS)-1,2,3,4-*Tetrahydro*-7-*hydroxy*-1-(4-*hydroxy*-3-*methoxy*[α-^{14}C]*phenethyl*)-6-*methoxy*-2-*methylisoquinoline* (23) was characterised as the *bisphenylurethane derivative*, m.p. 148—150° (from aqueous ethanol) (Found: C, 70·1; H, 6·0; N, 7·4. $C_{34}H_{35}N_3O_6$ requires C, 70·2; H, 6·1; N, 7·2%). (RS)-1,2,3,4-*Tetrahydro*-7-*hydroxy*-1-(4-*hydroxy*[α-^{14}C]*phenethyl*)-6-*methoxy*-2-*methylisoquinoline* (20) showed a double m.p., 98—100° then 150° (from chloroform–benzene) (Found: C, 72·6; H, 7·4. $C_{19}H_{23}NO_3$ requires C, 72·8; H, 7·4%). (RS)-1-(3,5-*Dimethoxy*-4-*hydroxyphenethyl*)-1,2,3,4-*tetrahydro*-7-*hydroxy*-6-*methoxy*-2-*methylisoquinoline* (14) was characterised as the *picrate*, m.p. 132—135° (from methanol–ethyl acetate) (Found: C, 57·4; H, 5·8; N, 10·2. $C_{31}H_{35}N_5O_{10}$,0·5EtOAc requires C, 57·8; H, 5·8; N, 10·3%). The presence of ethyl acetate of crystallisation was confirmed by n.m.r. spectroscopy.

Tritium labelling of the phenols (13), (14), and (19) was carried out in the standard way [11] by exchange with tritiated water, using a solution of the phenolic base in dimethylformamide.

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