Tetrahedron Vol. 46, No. 20, pp. 7119-7138, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

The Biosynthesis of the Phenethylisoquinoline Alkaloid Colchicine. Early and Intermediate Stages.[1]

Richard B. Herbert,* Abdullah E. Kattah, and Eric Knagg

School of Chemistry, The University, Leeds LS2 9JT

(*Received in UK* 11 *July* 1990)

Abstract : Experiments with ^{14}C- and ^{3}H-labelled compounds in *Colchicum byzantinum* and *C. autumnale* show that in the early stages of biosynthesis the major pathway to colchicine (7) and demecolcine (6) is (13)→(15)→(16)→(19). Condensation of the aldehyde (19) with dopamine affords a set of phenethylisoquinolines which are precursors for the alkaloids (6) and (7), and (21) is identified as the first of these followed by (22) and then (1); (39) is, by contrast, also an exellent alkaloid precursor; it is deduced that neither of two phenethylisoquinolines [as (37) and (38)], which contain a side-chain double bond, are involved in alkaloid biosynthesis.

Introduction

Colchicine is a neutral alkaloid with interesting and useful biological properties and it is found chiefly in species of *Colchicum*.[2] The unique structure (7) with its strange tropolone ring meant that at one time colchicine was viewed as "*sui generis* and no structural relation with other alkaloids can be recognised"[3]. Then it was shown through the assignment of the structure (4) to androcymbine[4] and proved through appropriate and rigorous feeding experiments with *Colchicum autumnale* and *C. byzantinum* that colchicine is a modified phenethylisoquinoline [as (3)] and the late stages of biosynthesis are as shown in part in Scheme 1.[5-7] (*S*)-Autumnaline (3) and *O*-methylandrocymbine (5) are proven, key intermediates.

It is, with exceptions, now a common observation[9,10] for the biosynthesis of very many diffrent alkaloids that a key step involves the condensation of an aldehyde with an amine, *e.g.* the biosynthesis of benzylisoquinoline alkaloids involves the enzyme-catalysed condensation of dopamine (8) with 4-hydroxyphenylacetaldehyde (9) yielding (*S*)-norcoclaurine (10) which is the precursor then for the whole family of benzylisoquinoline alkaloids.[9] We report here the results of experiments which show that the phenethylisoquinoline skeleton, as exemplified in demecolcine (6) and colchicine (7), is formed also by the condensation of an aldehyde (19) with an amine [dopamine (8)].[1]

Results of Biosynthetic Experiments

The tropolone ring of colchicine (7) is formed from the aromatic nucleus of tyrosine (11) plus C-3 of the sidechain and biosynthesis proceeds by way of dopamine (8). Ring a of (7) together with C-5, -6, and -7 derive from phenylalanine by way of cinnamic acid (13)[6,7,11]. Beyond cinnamic acid, negative results with

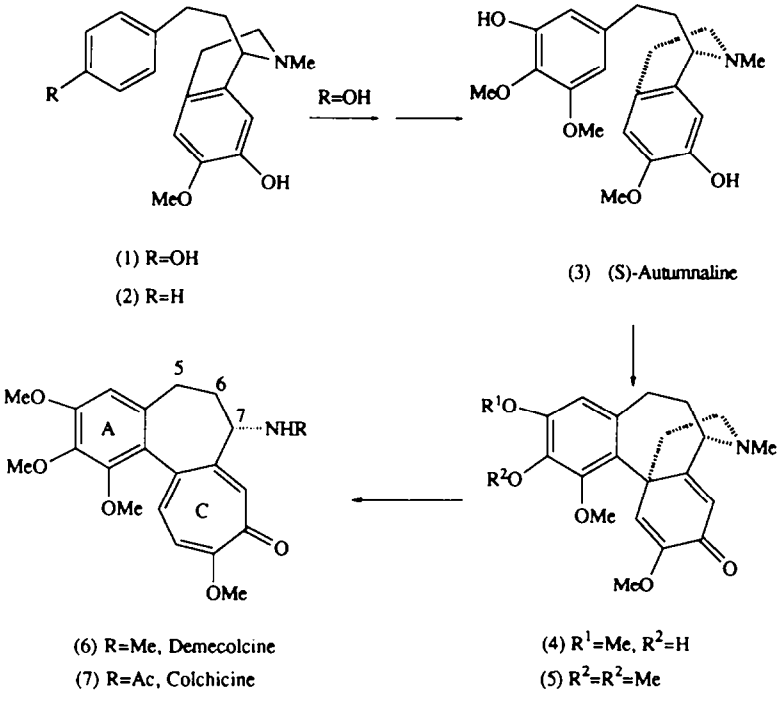
cinnamic acid derivatives oxygenated on the aromatic ring [as (12)] indicated that something else (reduction?) happened before hydroxylation of the aromatic ring.[5] We examined this in our first experiments which were with (24), (26), (28) and (30).

The following, which relates to the experiments described in this paper, is to be noted : (i) mixtures of precursors, where one compound bore a ^{14}C-label and the other a ^{3}H-label, were used generally in order to provide the most accurate comparison of incorporation efficiencies, by testing the incorporation in the same plant tissue; (ii) the aldehydes were deliberately labelled with tritium on their carbonyl groups so that incorporation would only be observed if the aldehyde did not suffer oxidation (to a carboxylic acid) during biosynthesis; (iii) because of their insolubility in water the aldehydes were fed as aqueous solutions of their

Table 1. Incorporation of early precursors into colchicine (7) in *C. byzantinum*

	Precursor	^{3}H:^{14}C	Colchicine % incorporation
1.	(28)	4.4	0.1
	+ (24)[a]		0.12
2.	(30)	4.4	0.08
	+ (26)[a]		0.04
3.	(31)	2.7	0.09
	+ (24)[b]		0.02
4.	(29)	3.0	0.01
	+ (25)[b]		0.001[e]
5.	(32)	5.2	0.03
	+ (27)[b]		0.01
6.	(23)[c]	-	0.07[f]
7.	(31)[c]	-	1.05[c] & 0.06[d]
8.	(29)[c]	-	0.015
9.	(32)	5.3	0.05
	+ (27)[d]		0.01
10.	(29)	3.1	0.02
	+ (25)[d]		0.001[e]

All feeds were carried out during the autumn flowering period. The letters a to d refer to separate sets of feeding experiments : a and b, wick feed to whole plants; c and d : feed to tissue slices. e : (25) was also found previously to be a very poor colchicine precursor[5]; f: similar results were obtained for this precursor in whole plants.[12]



(1) R=OH
(2) R=H

(3) (S)-Autumnaline

(6) R=Me, Demecolcine
(7) R=Ac, Colchicine

(4) R^1=Me, R^2=H
(5) R^2=R^2=Me

Scheme 1

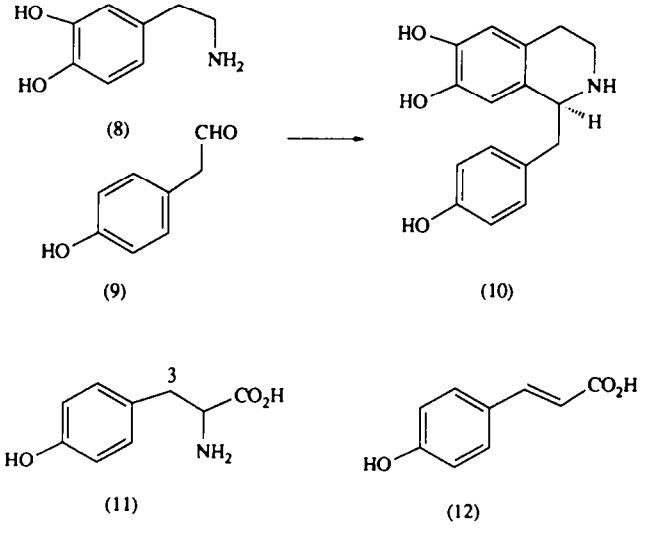


Table 2. Incorporation of isoquinoline precursors into colchicine (7) and demecolcine (6).

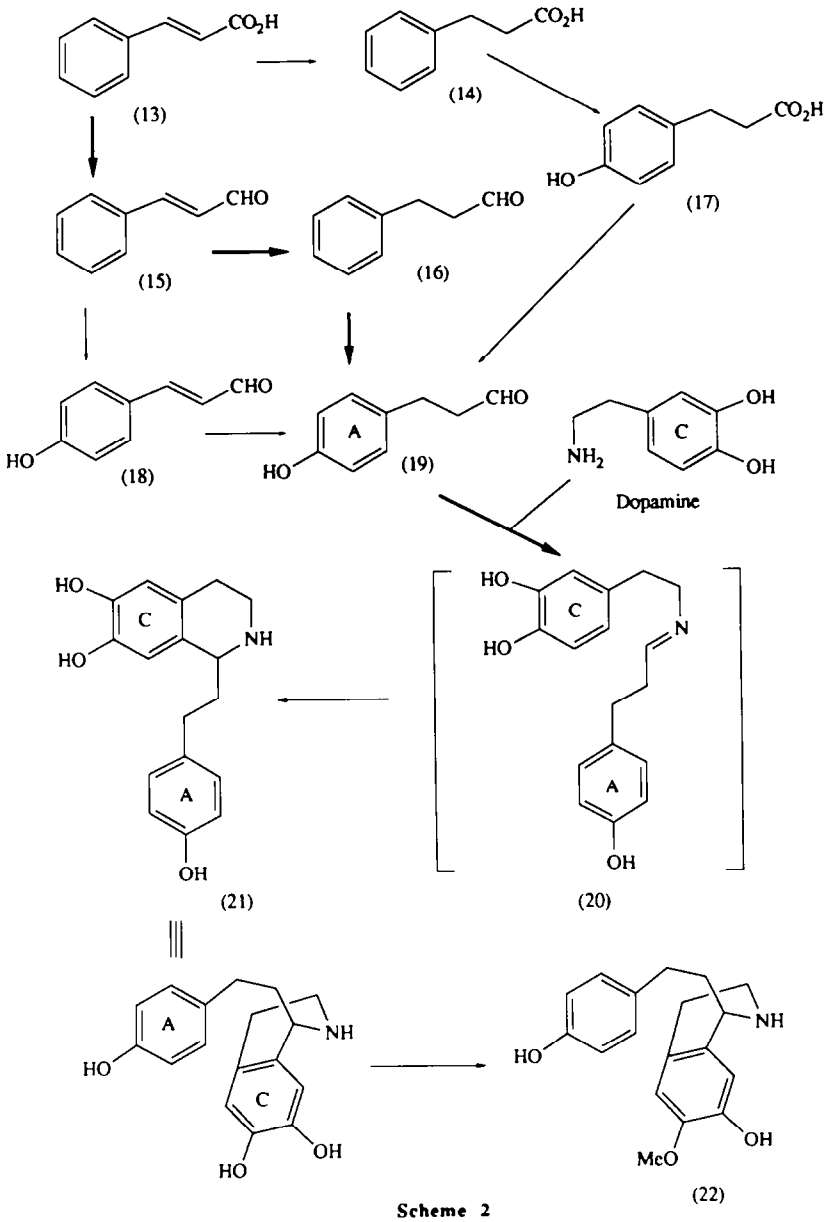
Precursor	% Incorp. into (7)	% Incorp. into (6)	% Total
1. (33)[b]	0.15	-	0.15
2. (34)[a]	0.01	0.06	0.17
3. (35)(^{3}H:^{14}C = 3.8)[c]	0.50 (^{3}H:^{14}C = 2.3)	0.03 (^{3}H:^{14}C = 3.3)	0.53
4. (36)[a]	0.02	0.01	0.03
5. (34)	0.035	0.025	0.06
+ (38) (^{3}H:^{14}C=3.4)[d]	0.015 (^{3}H:^{14}C=8.45)	0.01 (^{3}H:^{14}C=10.1)	0.025
6. (37)[a]	0.001	0.003	0.004
7. (39)[c]	0.46	0.05	0.51

The letters refer to separate sets of feeding experiments : a : autumn wick feeding to *C. byzantinum*; b : autumn feeding to slices of *C. byzantinum*; c : spring feeding by injection into seed capsules of *C. autumnale*; d : wick feeding to *C. byzantinum* in spring. All precursors were racemic.

bisulphite-addition compounds. As can be seen (Table 1) the strategy of using these addition compounds was successful here (also in another case[13]) and is potentially applicable in other biosynthetic experiments involving aldehyde precursors.

It can be seen from Table 1 that the ^{3}H:^{14}C ratio of the precursor mixture in experiment 1 is closely similar to the ratio in the isolated colchicine. This indicates that cinnamic acid [(13)=(24)] and cinnamaldehyde (3-phenylprop-2-enal) [(15)=(28)] are equally good precursors for colchicine (7); retention of tritium shows that biosynthesis is from cinnamic acid (13) *via* cinnamaldehyde (15). The results of exeriment 2 show that dihydrocinnamaldehyde (3-phenylpropanal) [(16)=(30)] is incorporated at a similar level to the precursors in experiment 1, and that it is a significantly better precursor than dihydrocinnamic acid (3-phenylpropionic acid) [(14)=(26)]. Results of further experiments with the four hydroxy-compounds (25), (27), (29), and (31), and also (32) (Table 1, experiments 3-5, 7-10) allow us to build upon the foregoing findings to deduce a major pathway (Scheme 2, thickened arrows) for the biosynthesis of colchicine (7) as involving (13)→(15)→(16)→(19); alternative minor routes are also implied by the results (Scheme 2, normal arrows). Final resolution of these stages in the biosynthetic pathway must now depend on evidence with isolated enzymes.

As an alternative to wick-feeding corms of *C. byzantinum* during flowering in the autumn, we found incubation in the dark of slices of corm tissue flooded with water to be similarly effective for the incorporation of precursors (Table 1, experiments 6-10). Some, generally less satisfactory, results were obtained with wick-feeding in spring and one of these results is given in Table 2 (experiment 5). The best incorporations[5] of


CO2H
(13)
CO2H
(14)
CO2H
HO
(17)
CHO
(15)
CHO
(16)
CHO
HO
(18)
CHO
A
HO
(19)
OH
C
NH2
OH
Dopamine
HO
C
HO
NH
A
OH
(21)
HO
C
HO
N
A
OH
(20)
A
HO
NH
C
HO
OH
HO
NH
HO
OH
MeO
(22)

Scheme 2

precursors have been obtained by injecting into seed capsules of *C. autumnale* in the spring. To our exasperation no seed capsules were produced for several years, that is until the spring of 1989. The good results recorded as experiments 3 and 7 (Table 2) are the very satisfying outcome of being able to use seed capsules in feeding experiments (*cf.* ref 5).

The first phenethylisoquinoline to be identified thus far[5] has been (1); (2) is not involved in biosynthesis[5] and this is consistent with the extent of oxygenation in early precursors which is illustrated in Scheme 2. Hypothetically condensation of dopamine with the aldehyde (19) gives (21) *via* (20). The results obtained with the unstable trihydroxyisoquinoline [(21)=(33)] (see Table 2) validated this hypothesis and further results (Table 2) locate [(22)=(34)/(35)] as a precursor reasonably btween (21) and [(1)=(36)].

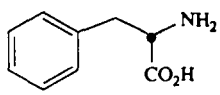
Results with (38), where direct comparison with [(34)=(22)] was obtained, and with (37) confirm that the cinnamyl double bond is reduced before formation of the phenethylisoquinoline skeleton. The excellent incorporation of (39) indicates that *N*-methylation of (22) [which yields (1)] need not happen before further aromatic oxygenation occurs. Although fairly poor, the incorporation of (32) (Table 1, experiment 5) is also to be noted; similarly the incorporation of (39) which is similar to that of (35) (both fed under similar conditions; Table 2.) The result for (39) indicates that *N*-methylation need not occur straight after the formation of (22), *i.e.* may occur after further aromatic hydroxylation and *O*-methylation.

In the experiment with (35), labelled with ^{3}H (at C-1) and ^{14}C, the isolated alkaloids showed some loss of tritium had occurred. This had previously been observed in the incorporation into colchicine of autumnaline (3), and is attributed to redox processes beyond this intermediate.[15] This means that in all of our precursors where tritium was present at C-1 or equivalent (aldehyde carbonyl group) the incorporations of precursors measured are underestimates. (This does not affect the conclusions). This loss of tritium also accounts for the change in ratio for (35) (Table 2, experiment 3) and indicates that [(35)=(22)] is an intact precursor for (6) and (7).

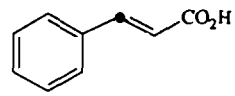
The combined results now indicate the course of biosynthesis for colchicine (7) and demecolcine (6) in *Colchicum* sp. (Liliaceae) illustrated in Schemes 2 and 1. It is interesting to note that *Sceletium* alkaloids which are found in species of *Sceletium* belonging to the Aizoaceae family are formed in the early stages in a not too dissimilar way to the *Colchicum* alkaloids, *i.e.* 3-(4-hydroxyphenyl)propanal (19) is involved as a precursor and a likely intermediate is (40)[13] which compares with (20) in *Colchicum* biosynthesis. Perhaps ingenuously, it seems as if it is the essential lack of one aryl hydroxy group in *Sceletium* biosynthesis which prevents isoquinoline ring formation and results in quite different alkaloids in, it is true, unrelated families.[14]

Synthesis of precursors

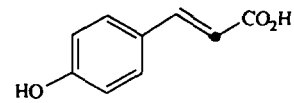
Possible synthetic routes to the aldehydes we required for feeding experiments were circumscribed by the requirement that each of them be prepared in labelled form with tritium on the carbonyl group and that, as is usual with labelled compounds, the label should be introduced as late in the synthetic sequence as possible.



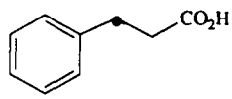
(23) ●=^{14}C



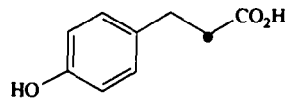
(24)



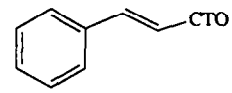
(25)



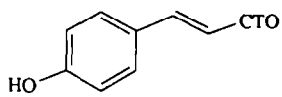
(26) o=^{14}C



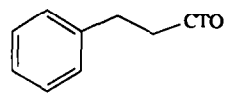
(27)



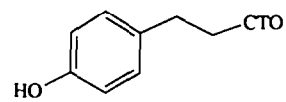
(28)



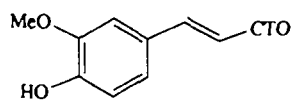
(29)



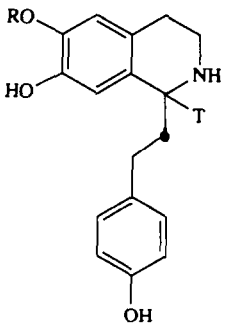
(30)



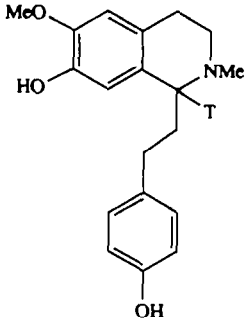
(31)



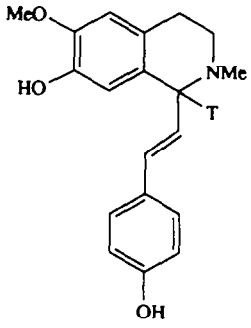
(32)



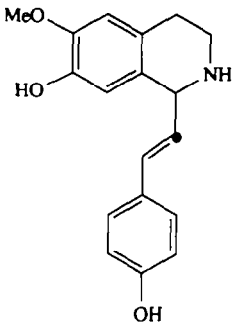
(33) R=H, ●=^{12}C
(34) R=Me, ●=^{12}C
(35) R=Me, ●=^{14}C



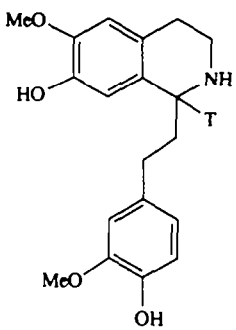
(36)



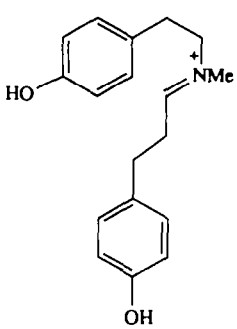
(37)



(38)



(39)



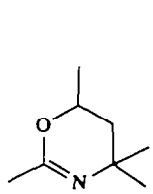
(40)

The three propenals (28), (29) and (32) were prepared by simple adaptation of an excellent general method.[16] Condensation of benzaldehyde with the anion of the oxazine (41) gave (42) which afforded (15) after reduction with sodium borohydride followed by oxalic acid hydrolysis. After checking that hydrogen isotope would only be located on the carbonyl group by preparing the deuteriated aldehyde using sodium borodeuteride, reduction of (42) was carried out with a limited amount of sodium borotritiide followed by excess sodium borohydride. The tritiated aldehyde (28) was obtained in satisfactory yield.

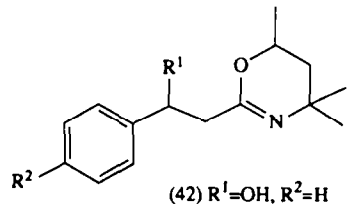
The same approach was used for the preparation of the aldehydes (29) and (32). In both cases the phenolic function was protected as the MEM derivative which was advantageously removed during the last step which involves oxalic acid hydrolysis. In the case of (29) the methyl ether was first tried but demethylation of the aldehyde product with boron tribromide or trimethylsilyl chloride and sodium iodide gave multi-component mixtures; use of a tetrahydropyranyl ether protecting group gave poor yields in the condensation with the oxazine anion.

The propanal (16) could not be prepared by hydrogenation of (15) (*cf.* ref. 17). An alternative approach was, however, successful. This involved the sodium-liquid ammonia reduction, under scrupulously dry conditions, of (42) to give (43) with imine double bond still intact. Reduction with sodium borohydride and hydrolysis afforded the aldehyde (16). Reduction with sodium borodeuteride provided a check on the reaction course and the tritiated aldehyde (30) was simply prepared. Similar sodium-liquid ammonia reduction of (44), however, failed, and alternative approach was again necessary. The method used[18] involves condensation of an acid chloride with 1,3-propanedithiol to give a salt [as (46)] which on reduction and hydrolysis affords an aldehyde [as (19)]. *p*-Hydroxybenzaldehyde as its MEM-derivative was not a successful starting material for this route because the protecting group was lost on later, attempted acid chloride [as (45)] formation. However, *p*-benzyloxybenzaldehyde was a successful substitute leading by conventional means through to the acid chloride (45). Condensation with 1,3-propanedithiol afforded the salt (46) with advantageous loss of the protecting group. Reduction with sodium borohydride [sodium borotritiide for the tritiated aldehyde (31)] and hydrolysis gave the aldehyde (19). This route also gave a better yield of (16) than the one described above *via* the oxazine.

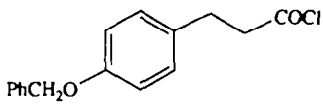
The routes to the phenethylisoquinolines used in the feeding experiments were essentially following published procedure. Amides [as (49)] were prepared, however, using dicyclohexylcarbodiimide and the *N*-methyl derivative (36) was prepared from (34) by reaction with formaldehyde followed by sodium borohydride (*cf.* ref. 19). Debenzylation of the dibenzyl ethers of (37) and (38), to give the dihydroxyisoquinolines (37) and (38) proved difficult but was successfully achieved with boron trifluoride etherate plus ethanethiol.[20]



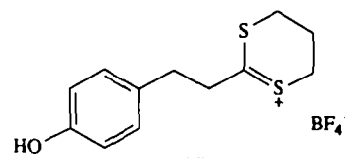
(41)



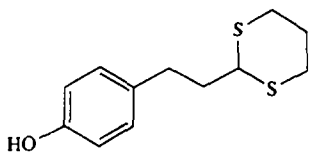
(42) R^1=OH, R^2=H
(43) R^1=R^2=H
(44) R^1=OH, R^2=O-MEM



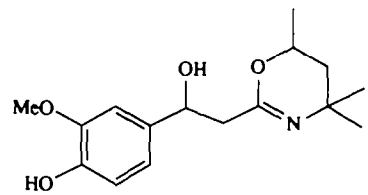
(45)



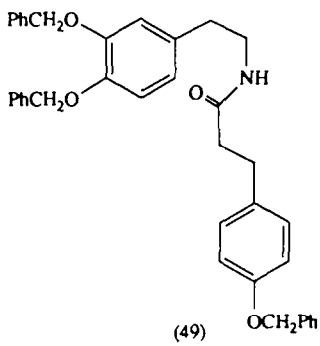
(46)



(47)



(48)



(49)

Experimental

General : For general directions see ref. 13.

Methods of feeding to *C. autumnale* and *C. byzantinum*

C. byzantinum : Corms were lifted from the ground during the autumn flowering period and aqueous solutions of precursors were fed *via* a wick inserted into the corm. After complete absorption of the aqueous solution the plants were kept for 12-14 days prior to the isolation of alkaloids. Tissue slices were prepared simply by cutting a clean corm into slices. The slices were kept at 25° in water in a petri dish in the dark. Precursor solutions were simply distributed over the slices, which were then incubated for 4-5 days before isolation of alkaloids.

C. autumnale : Precursor solutions were injected into the hollow seed capsules in late spring. The complete plants were lifted after 12-14 days and the alkaloids were isolated.

Aqueous solutions of precursors were prepared as follows : Acids were dissolved in very dilute aq. NaOH and the pH was then adjusted to 7-8. Amines were dissolved in very dilute aq. HCl and the pH was then adjusted to 6-7. Aldehydes were dissolved in a minimum volume of ether and an excess of saturated aq. sodum bisulphite was added. The ether was removed *in vacuo* and water was added to give a complete solution; pH 6-7, adjusted if necessary.

Isolation of colchicine and demecolcine

Plant material was macerated in ethanol and kept in the dark for 2 days with occasional agitation. The solvent was removed *in vacuo* and the residue was taken up in chloroform and extracted thrice with 1 M hydrochloric acid. The organic layer was kept for the isolation of colchicine. The aqueous extracts were combined and the emulsion discharged on a rotary evaporator. The mixture was filtered and the filtrate was basified with sodium carbonate, then extracted five times with chloroform. The combined extracts were dried and the solvent was removed *in vacuo*. The residue was purified by column chromatography eluting with $CHCl_3$ through to 2% MeOH in $CHCl_3$. The demecolcine which was obtained as a pale yellow solid was recrystallized from ethyl acetate. The demecolcine was identified by its m.p. (186°C, as lit[21]) and ^1H n.m.r. spectrum : δ 7.71 (1H,s), 7.25 (1H, d, J=10.5Hz), 6.82 (1H, d, J=10.5Hz), 6.55 (1H, s), 4.00 (3H, s), 3.91 (6H), 3.62 (3H, s), 3.28 (1H), 2.55-2.28 (4H, m), 2.21 (3H, s).

The organic solution containing the colchicine was evaporated *in vacuo*. The colchicine was then isolated as described previously[22] with further purification after alumina chromatography by use of column chromatography on Kieselgel with $CHCl_3$ through to 2% MeOH in chloroform. The colchicine (m.p. 156°C, from EtOAc) was identical with an authentic sample (Aldrich Chemical Co.). Radioactive samples of colchicine and demecolcine were recrystallized to constant activity.

[1-^{3}H]-3-Phenylprop-2-enal (28) and [1-^{3}H]-3-phenylpropanal (30).

The unlabelled aldehyde (15) was prepared following a published procedure.[16] *[1-^{2}H]-3-Phenylprop-2-enal* was prepared using $NaBD_4$ to reduce the intermediate dihydro-1,3-oxazine (42). The tritiated compound (28) was prepared as follows : To (42) (150 mg 0.6 mmol) in thf (0.5 ml) at pH7 and -30° to -40°C (bath temp.) (correct pH and temperature is critical for success) was added sodium borotritiide (1mg, 15.5 mCi) and the reaction mixture was stirred at the above temperature and pH for 2h. Sodium borohydride (25 mg, 0.65 mmol) was added in three batches over 15 min and the reaction was run for 1.5h as before. Water (5ml) was added and the solution was basified (aq. KOH) then extracted with ether. The extract was dried (K_2CO_3) and the solvent was removed *in vacuo*. The residue, a pale yellow oil (140 mg, 93%) was refluxed in aq. (2 ml) oxalic acid (200 mg) under nitrogen for 1 h. Water (10 ml) was added and the mixture was subjected to steam

distillation. The milky distillate was extracted with ether, the extract was dried and the solvent was removed *in vacuo* to give the pure tritiated aldehyde (28) (26 mg, 34%, 440 μCi).

[1-^{3}H]-3-Phenylpropanal (30) was prepared from the dihydro-1,3-oxazine (43) which was synthesized as follows: Liquid ammonia (15 ml) (dried over sodium wire) was condensed on to a solution of the oxazine (42) (200mg) in dry thf. Sodium wire (approx. 0.04 g) was then added slowly over 2h until the mixture remained deep blue. Evaporation of the ammonia was followed by cautious addition of crushed ice. The solution was extracted with ether, the extracts wre dried (Na_2CO_3) and the solvent was removed *in vacuo*. A pale yellow oil which was *5,6-dihydro-2-(2-phenylethyl)-4,4,6-trimethyl-1,3-oxazine* (43) was obtained. ν_{max} (film) 2975,1660 (C=N), 1500, 1450 cm^{-1}; δ 7.45-7.1 (5H, m), 4.1 (1H, m), 2.9 (2H, m), 2.45 (2H, m), 1.7 (2H, m), 1.3-1.1 (9H); m/z 231.16199 (M$^+$, $C_{15}H_{21}NO$ requires m/z 231.16231), 154, 131, 105, 91.

After checking the reduction (i) with $NaBH_4$ to give (16) after hydrolysis [ν_{max}(film) 1725 cm^{-1}; δ 9.85 (1H, t, J = 1.5 Hz), 7.5-7.2 (5H, m), 3.1-2.6 (4H, m)] and (ii) with $NaBD_4$ to give the C-1 deuteriated aldehyde, reduction to give the tritiated aldehyde (30) was carried out in a manner closely similar to that described above for (28) except that the product was extracted from the final aqueous solution rather than be subjected to steam distillation which resulted in a lowered yield (22 mg from 275 mg, 444 μCi).

[3-^{14}C]-3-Phenylpropionic acid (26) was prepared quantitatively by hydrogenation (PtO_2, EtOH, atmospheric pressure, room temp.) of commercial [3-^{14}C]cinnamic acid (24).

[1-^{3}H]-3-(4-Hydroxyphenyl)prop-2-enal (29) and [1-^{3}H]-3-(4-hydroxyphenyl)propanal (31).

Reaction of MEM protected *p*-hydroxybenzaldehyde with dihydro-oxazine following the method[16] referred to above gave (44) (78%) [ν_{max}(film) 3300, 1660, 1610, 1510 cm^{-1}; δ 7.32 (2H, d, J = 9Hz), 7.01 (2H, d, J = 9Hz), 5.25 (2H, s) 4.95 (1H, t, J = 6 Hz), 4.15 (1H, m), 3.9-3.7 (2H, m), 3.7-3.5 (2H, m) 3.35 (3H, s), 2.6-2.3 (2H, m), 2.0-1.1 (12H, unresolved); m/z 351.2040 (M$^+$, $C_{19}H_{29}NO_5$ requires 351.20456), 333, 262, 89, 59.

Standard reduction with sodium borohydride gave the dihydro-derivative of (44) [ν_{max}(film) 3350, 1610, 1510 cm^{-1}; δ 7.28 (2H, d, J= 9Hz), 7.0 (2H, d, J = 9Hz), 5.3 (2H, s), 4.8 (2H, m), 4.45 (2H, m), 4.1 (1H, m), 3.9-3.7 (2H, m), 3.6-3.4 (2H, m), 3.37 (3H, s), 3.1 (2H, broad), 2.7-2.4 (4H, m), 1.9-1.1 (11H, unresolved) and oxalic acid hydrolysis afforded 3-(4-hydroxyphenyl)prop-2-enal (18). The tritiated aldehyde (29) (40%, 8.01 mCi, 1.10 mCi mmol^{-1}) was prepared from (44) as described above for (16) from (43) (421 mg). After hydrolysis, the residue obtained on evaporation of the ether extract was purified by column chromatography ($CHCl_3$) to give pure *[1-^{3}H]-3-(4-hydroxyphenyl)prop-2-enal* (29). δ 9.65 (1H, d, J = 8 Hz), 7.6-7.3 (3H, m), 6.95 (2H, d, J = 9 Hz), 6.6 (1H, dd, J = 14 and 8 Hz); m/z 148.05275 (M$^+$, $C_9H_8O_2$ requires 148.05243).

The aldehyde (19) was prepared from 4-hydroxybenzaldehyde : *O*-benzylation (benzyl chloride, KI, Na_2CO_3 in Me_2CO, 14h reflux : 90%) was followed by a standard Doebner reaction (malonic acid, pyridine, piperidine, 1h, steam bath : 75%), hydrogenation (H_2, Pt : 89%) and the formation of the acid chloride (45) (oxalyl chloride, dmf, CH_2Cl_2, 2h). This acid chloride was reacted with 1,3-propanedithiol in boron trifluoride etherate following closely the method of Stahl[18] to give *2-[2-(4-hydroxyphenyl)ethyl]-1,3-dithian-2-ylium tetrafluoroborate* (46) which upon reduction with sodium borohydride (*cf.* ref. 18) gave the corresponding *dithian* (65%) (47). δ 7.06 (d, J = 9Hz), 6.75 (d, J = 9 Hz), 5.75 (1H, OH), 3.95 (1H, t, J = 6Hz), 2.8 (6H, m), 2.05 (4H, m); m/z 240.06459 (M^+, $C_{11}H_{16}S_2O$ requires 240.06426). This was converted into *3-(4-hydroxyphenyl)ethylpropanal* (19) (47%). δ 9.8 (1H, t, J = 1.5 Hz), 7.06 (2H, d, J = 9Hz), 6.75 (2H, d, J = 9Hz), 3.0-2.6 (4H, m); m/z 150.06795 (M^+, $C_9H_{10}O_2$ requires 150.06808). The tritiated aldehyde (31) (18.7 mCi mmol^{-1}) was prepared using sodium borotritiide.

[2-^{14}C]-p-Hydroxycinnamic acid (25) and [2-^{14}C]-3-(4-hydroxyphenyl)propionic acid (27).

The acid (25) (58%, m.p. 214°C, 531 μCi mmol^{-1}) was prepared from *p*-hydroxy-benzaldehyde and [2-^{14}C]malonic acid (commercial disodium salt converted first into the free acid) in a standard Doebner reaction. Hydrogenation (H_2, Pt) gave (27) (537 μCi mmol^{-1}).

[1-^{3}H]-3-(4-Hydroxy-3-methoxyphenyl)prop-2-enal (32).

Vanillin was converted into its MEM derivative (1.1 equiv. NaH in dry thf, then 1 equiv. methoxymethyl chloride 1h. room temp.) which gave (48) (79%) following the published oxazine method.[16] Reduction with sodium borohydride followed by oxalic acid hydrolysis (*cf* ref 16) gave the aldehyde [as (32)]. δ 9.65 (1H, d, J = 8Hz), 7.45 (1H, d, J = 16 Hz), 7.2-6.9 (3H), 6.65 (1H, dd, J = 8 and 16Hz), 3.95 (3H, s); m/z 178.06367 (M^+, $C_{10}H_{10}O_3$ requires 178.06299). Use of sodium borotritiide, as described for similar compounds above, afforded the tritiated aldehyde (32) (30%, 4.63 mCi mmol^{-1}).

N-[2-(3,4-Dibenzyloxyphenyl)ethyl]-3-(4-benzyloxyphenyl)propionamide (49).

Condensation of 3,4-dibenzyloxybenzaldehyde with nitromethane (amylamine, 36h) gave the nitrostyrene which was reduced ($LiAlH_4$) to 2-(3,4-dibenzyloxyphenyl)ethylamine.[23] This compound (0.61g, 1.84 mmol) and *p*-benzyloxypropionic acid[5] (standard Doebner reaction and hydrogenation) (0.47g, 1.84 mmol) were dissolved in dry dichloromethane (10ml). DCC (0.57g, 2.75 mmol) was added. The mixture was stirred at room temp. for 5 h. and then filtered. The filtrate was washed with sat. aq. sodium bicarbonate and was then dried. The solvent was removed *in vacuo* and the residue was chromatographed. ($CHCl_3$:MeOH, 99:1). The *propionamide* (49) was recrystallized from EtOAc (0.46g, 45%) mp 157-158°C. ν_{max} (nujol) 3265, 1630 cm^{-1}; δ(400 MHz) 7.46-7.25 (15H, m), 7.08 (2H, d, J = 9Hz), 6.88 (2H, J = 9Hz), 6.85 (1H, d, J = 8Hz), 6.73 (1H, d, J = 2Hz), 6.58 (1H, dd, J = 2 and 8Hz), 5.26 (1H, t, J = 2Hz), 5.13 (4H, s), 5.02 (2H, s), 3.40 (2H, q, J =

6Hz), 2.86 (2H, t, J = 8Hz), 2.63 (2H, t, J = 6Hz), 2.34 (2H, t, 8Hz); m/z 571 (M^+), 316, 225, 91, 44. Found: C, 80.15; H, 6.6; N, 2.65%. $C_{38}H_{37}NO_4$ requires : C, 79.85; H, 6.47; N, 2.45%.

N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-3-(4-benzyloxyphenyl)propionamide

This compound was prepared (72%) as described for (49), from *p*-benzyloxypropionic acid and 1-amino-2-(4-benzyloxy-3-methoxyphenyl)ethane,[24] m.p. 149-151° (lit.[5] 149.-150°) m/z 495.23917 (M^+, $C_{32}H_{33}NO_4$ requires 495.24094).

N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-4'-benzyloxycinnamide

This compound was prepared (47%), as described for (49), from *p*-benzyloxycinnamic acid[25] (standard Doebner) and 1-amino-2-(benzyloxy-3-methoxyphenyl)ethane[24], m.p. 190°C (lit.[26] 187-189°C). Found : C, 77.4; H, 6.45; N, 3.3%. Calc. for $C_{32}H_{31}NO_4$: C, 77.9; H, 6.28; N, 2.84%.

N-[2-(4-Benzyloxy-3-methoxyphenyl)ethyl]-4'-benzyloxy-3-'-methoxycinnamide

This compound was prepared (46%), as described for (49), from 4'-benzyloxy-3'-methoxycinnamic acid[27] and 1-amino-3-(benzyloxy-3-methoxyphenyl)ethane,[24] m.p. 160-162°C (lit.[27] 159°C). Found : C, 75.2; H, 6.5; N, 3.3%. Calc. for $C_{33}H_{33}NO_5$: C, 75.7; H, 6.3; N, 2.7%. ν_{max} (nujol) 3300, 1642, 1612 cm^{-1}; δ (400Mz), 7.53 (1H, d, J = 16Hz), 7.46-7.27 (10H,m), 7.02 (1H, d, J=1.5Hz), 6.99 (1H, dd, J=8Hz and 1.5Hz), 6.85 (1H, d, J=8Hz), 6.83 (1H, d, J=8Hz), 6.76 (1H, d, J=1.5Hz), 6.67 (1H, dd, J=8Hz and 1.5Hz), 6.17 (1H, d, J=16Hz), 5.54 (1H, t, J=5.5Hz, NH), 5.19 (2H, s), 5.14 (2H, s), 3.91 (3H, s), 3.88 (3H, s), 3.62 (2H, q, J=6.5Hz), 2.82 (2H, t, J=7Hz); m/z, 523 (M^+, 1.9%), 416 (7%), 240 (18%), 149 (7%), 91 (100%).

1-[2-(4-Benzyloxyphenyl)ethyl]-6,7-dibenzyloxy-1,2,3,4 tetrahydroisoquinoline

The amide (49) (0.44g, 0.78 mmol) was suspended in dry acetonitrile (15 ml). The stirred mixture was heated to reflux. Then phosphorus oxychloride (0.5ml) was added dropwise. The reflux was continued for a further 1h. The solution was evaporated thoroughly to dryness under high vacuum to remove the excess of $POCl_3$. The residue was dissolved in chloroform (10ml), shaken with 2M KOH (20ml) and ether (50ml). The separated upper layer was washed with water (2x10ml), and evaporated *in vacuo* to give an oil. This was dissolved in ethanol (8ml), then sodium borohydride (20mg) was added. The mixture was stirred at room temperature for 30 min. The excess reagent was destroyed by dropwise addition of 2M HCl. The reaction mixture was basified with 2M NaOH. Most of the ethanol was removed *in vacuo*. The residue was partitioned between water (15ml) and chloroform (25ml). The organic layer was washed with water (2x10ml). The solvent was removed *in vacuo*. The residue was purified by column chromatography eluting with $CHCl_3$:CH_3OH:conc. NH_3 (96:4:4 drops/100ml) to give the required isoquinoline (0.34g, 78%). δ 7.60-7.27 (15H, m), 7.15 (2H, d, J=9Hz), 6.95 (2H, d, J=9Hz), 6.71 (1H, s), 5.14 (4H), 5.07 (2H, s), 3.91 (1H, t, J=6Hz), 3.33-2.96 (2H, m),

2.96-2.58(4H, m), 2.15-1.91 (2H, m); m/z 555.27557 (M^+, $C_{38}H_{37}NO_3$ requires 555.277326), 344 (18%), 91 (100%).

1-[2-(4-Hydroxyphenyl)ethyl]-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolinium hydrochloride

To 1-[2-(4-benzyloxyphenyl)ethyl]-6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline (100 mg, 0.169mmol) in methanol (10ml) was added Pd/C (30mg; 10%) and two drops of conc. HCl. Then the mixture was hydrogenated at room temperature and high pressure 150lb/in^2) for 5h. The solution was then filtered through celite and the filtrate was evaporated *in vacuo*. The crude product was purified by column chromatography eluting with methanol to give the required isoquinoline (52mg, 95%). δ (CD_3OD), (400MHz) 7.05 (2H, d, J=9Hz), 6.70 (2H, d, J=9Hz), 6.55 (1H, s), 6.49 (1H,s), 3.87 (1H, q, J=4Hz), 3.24-3.16(1H, m), 2.94-2.86 (1H, m), 2.77-2.55 (4H, m), 2.11-1.90 (2H, m). Acetylation (Ac_2O, Py, 100°, 2h) gave the triacetate: m/z 453.17915 ($C_{25}H_{27}NO_7$ requires 453.17874).

[1-^{3}H]-1-[2-(4-Benzyloxyphenyl)ethyl]-6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline.

This tritiated compound (95mg, 75μCi, 98%) was prepared from (49) (100mg, 0.175 mmol) and tritiated sodium borohydride in the same way as described for the unlabelled compound, except that sodium [^{3}H]-borohydride (0.4 mCi, approx. 0.1 mg) was added and the mixture was stirred for 30 min then inactive sodium borohydride (20mg) was added and the mixture was stirred for a further 30 min before isolation.

[1-^{3}H]-1-[2-(4-Hydroxyphenyl)ethyl]-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (33).

This tritiated precursor (33) (46mg, 0.16mmol, 75 μCi, 100%) was prepared from [1-^{3}H]-1-[2-(4-benzyloxyphenyl)ethyl]-6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline (89.7mg, 0.16mmol, 75 μCi) in the same way as described for the unlabelled compound.

1-[2-(4-Benzyloxyphenyl)ethyl]-7-benzyloxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline.

This compound (93%) was prepared from the corresponding amide above in the same way as described for 1-[2-(4-benzyloxyphenyl)ethyl]-6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline. ν_{max} (film), 3300 (NH) cm^{-1}; δ 7.55-7.20 (10H, m), 7.08 (2H, d, J=9Hz), 6.89 (2H, d, J=9Hz), 5.03 (4H, d), 3.84 (4H, s, OMe, and H-1), 3.30-2.90 (2H,m), 2.90-2.50 (4H, m), 2.07-1.73 (2H, m); m/z, 479 (M^+ was not visible), 268, 91.

1-[2-(4-Hydroxyphenyl)ethyl]-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinolinium hydrochloride

This compound (52%) was prepared by hydrogenolysis (method : above) of the foregoing isoquinoline. δ (CD_3OD), 7.23 (2H, d, J=9Hz), 6.86 (2H, d, J=9Hz), 6,86 (1H, s), 6.79 (1H, s), 4.39 (1H, t, J=8Hz), 3.97 (3H, s), 3.75-2.50 (6H, m), 2.50-2.00 (2H, m); m/z 299.15146 (M^+, $C_{18}H_{21}NO_3$ requires 299.15213), 192, 178, 43.

For the prepration of tritiated material, *i.e.* (34) (2.03 mCi mmol^{-1}), sodium borotritiide was substituted in part for sodium borohydride in the reaction of the corresponding amide with $POCl_3$ followed by $NaBH_4$ (above). ^{14}C-labelled material, *i.e.* [as (35)] (320 μCi mmol^{-1}) was prepared by substituting [2-^{14}C]malonic acid for unlabelled material in the Doebner reaction which gives *p*-benzyloxycinnamic acid. The double bond, in this case, was retained through to the last synthetic step (method below) when the benzyl groups were removed by hydrogeneolysis together with saturation of the double bond in *1-[(1-^{14}C)-2-(4-benzyloxyphenyl)vinyl]-7-benzyloxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline* to give (35). Isoquinoline (38) was also prepared from this compound by using the debenzylation method described below (BF_3.Et_2O/EtSH).

[1-^{3}H]-1-[2-(4-Hydroxyphenyl)ethyl]-7-hydroxy-6-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline.

[1-^{3}H]-1-[2-(4-Hydroxyphenyl)ethyl]-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (30mg, 0.1mmol, 181 μCi) was dissolved in dry methanol (0.7 ml). Formaldehyde (0.2ml) was then added. The mixture was refluxed for 45 min. After cooling excess sodum borohydride was added carefully. The mixture was stirred for 1.5h, then one drop of 1M HCl was added to destroy any excess $NaBH_4$, and the mixture was basified with conc. aq. ammonia. Most of the ethanol was removed *in vacuo*. The residue was partitioned between water (10ml) and chloroform (25ml). The aqueous layer was re-extracted with chloroform (2x25ml). The extracts were combined with the above organic layer and dried. The solvent was removed *in vacuo* and the residue was purified by column chromatography eluting with $CHCl_3$:MeOH:conc,NH_3 (92ml:8ml:8drops) to give the required product (36) (17mg, 0.045mmol, 102 μCi, 54%). δ 6.98 (2H, d, J=9Hz), 6.67 (2H, d, J=9Hz), 6.63 (1H, s), 6.53 1H, s), 5.10 (2H, broad s), 3.85 (3H, s), 3.42 (1H, t, J=5Hz), 3.30-2.50 (6H, m), 2.44 (3H, s), 2.20-1.83 (2H, m); m/z 313.1671 (M$^+$, $C_{19}H_{23}No_3$ requires 313.16778), 192, 177, 120, 107.

1-[2-(4-Benzyloxyphenyl)vinyl]-7-benzyloxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline.

This compound (92.5%) was prepared from the corresponding amide (above) using $POCl_3$ and then sodium borohydride using the method described above for 1-[2-(4-benzyloxyphenyl)ethyl]-6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline. δ 7.5-7.10 (12H, m), 6.93 (2H, d, J=9Hz), 6.64 (2H, s), 6.48 (1H, d, J=16Hz), 6.03 (1H, q, J=8Hz), 5.06 (4H, d, J=4Hz), 4.47 (1H, d, J=8Hz), 3.86 (3H, s), 3.30-2.94 (2H, m), 2.90-2.60 (2H, m), 2.17 (1H, s); m/z 477.22808 (M$^+$, $C_{32}H_{31}NO_3$ requires 477.23038), 386, 268, 91.

1-[2-(4-Hydroxyphenyl)vinyl]-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline.

1-[2-(4-Benzyloxyphenyl)vinyl)-7-benzyloxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (60mg, 0.125mmol) was dissolved in ethanethiol (1ml). Boron trifluoride etherate (0.6ml) was then added. The mixture was stirred at O°C for 3.5h under nitrogen. Then water (1.5ml) was added. The mixture was stirred for 15min than basified with conc. aq. ammonia, and extracted with ether (3x20ml) (a few drops of methanol were added to dissolve the gum which was formed during the basification process). The extracts were

combined and dried over $NaSO_4$. The solvent was removed *in vacuo* and the residue was purified by column chromatography eluting with $CHCl_3$:MeOH:conc.NH_3 (80ml:20m:20drops) to give the product which was re-purified by h.p.l.c. [reverse phase Polymer Laboratories PLRP-S100 column with CH_3CN:H_2O (1:1)] to give the required compound (16mg, 0.053mmol, 43%). δ 7.78 (2H, d, J=9Hz), 7.20-6.50 (5H, m), 6.14 (1H, q, J=8Hz), 4.65 (1H, d, J=8Hz), 3.82 (3H, s), 3.30-2.70 (4H, m). m/z 297.13584 (M^+, $C_{18}H_{19}NO_3$ requires 297.13649), 178, 107.

^{14}C-labelled material, (38), was prepared similarly (see above).

1-[2-(4-Benzyloxyphenyl)vinyl]-7-benzyloxy-6-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline.

N-[2-(4-benzyloxy-3-methodyphenyl)ethyl]-4'-benzyloxycinnamide (206mg, 0.42mmol) was suspended in dry acetonitrile (16ml). The mixture was heated to reflux, then $POCl_3$ (0.5ml) was added dropwise. The mixture was refluxed for a further 1 h, then evaporated to dryness, eventually under high vacuum to remove all the excess of $POCl_3$. The residue in $CHCl_3$ (15ml) was shaken with 2M KOH (20ml), and ether (45ml). The upper layer was washed with water (15ml), and dried over Na_2SO_4. The solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (10ml). Iodomethane (0.6ml) was then added. The mixture was kept at O°C for 18h. The solvent was removed *in vacuo*. The residue was dissolved in ethanol (25ml). Sodium borohydride (100mg) was then added. The mixture was stirred for 45min. The excess of $NaBH_4$ was destroyed by dropwise addition of 2M HCl. The mixture was basified with 2M NaOH. Most of the ethanol was removed *in vacuo*. The residue was partitioned between water (15ml) and $CHCl_3$ (25ml). The organic layer was washed with water (2x10ml) and dried. The solvent was removed *in vacuo*. The residue was purified by column chromatography eluting with $CHCl_3$:MeOH:conc.NH_3 (97ml:3ml:3drops) to give the required isoquinoline (50%). δ 7.50-7.02 (12H, m), 6.93 (2H, d, J=9Hz), 6.42 (1H, d, J=16Hz), 6.61 (2H), 5.86 (1H, dd, J=16Hz and 8Hz), 5.02 (4H), 3.83 (3H, s), 3.68 (1H, d, J=8Hz), 3.20-2.30 (4H, m), 2.40 (3H, s); m/z 491.24681 (M^+, $C_{33}H_{33}NO_3$ requires 491.24603), 400, 282, 91.

1-[2-(4-Hydroxyphenyl)vinyl]-7-hydroxy-6-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline.

This compound was obtained in 76% yield after column chromatography by debenzylation (BF_3.Et_2O/EtSH) of the dibenzyl ether above. The method was as for 1-[2-(4-hydroxyphenyl)ethyl)vinyl]-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (as (38)] except that the reaction procedure was carried out twice for complete debenzylation (total : 9h). δ 7.00 (2H, d, J=9Hz), 6.58 (2H, d, J=9Hz), 6.60 (1H, s), 6.50 (1H, s), 6.39 (1H, d, J=16Hz), 5.77 (2H, broad s), 5.77 (1H, dd, J=16Hz and 8Hz), 3.77 (1H, d, J=8Hz), 3.77 (3H, s), 3.25-2.50 (4H, m), 2.42 (3H, s); m/z 311.15114 (M^+ $C_{19}H_{21}NO_3$ requires 311.15213), 266, 192, 177, 107.

[1-^{3}H]-1-(2-(4-Hydroxyphenyl)vinyl)-7-hydroxy-6-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline (37).

This tritiated material (14.1 mCi mmol^{-1}) was prepared as described above for unlabelled material. Tritium was introduced through partial replacement of sodium borohydride by sodium borotritiide following cyclisation of the amide.

1-[2-(4-Benzyloxy-3-methoxyphenyl)vinyl]-7-benzyloxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline.

This isoquinoline (89%) was prepared from the corresponding amide (0.3g, 0.59mmol) using $POCl_3$ (0.6ml), and then sodium borohydride (0.15g) in the same way as described above for 1-[2[(4-benzyloxyphenyl)ethyl]-6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline, except that the product was recrystallised from ethyl acetate, m.p. 118-120°C. Found: C, 77.95; H, 6.65; N, 2.85. $C_{33}H_{33}NO_4$ requires C, 78.1; H, 6.5; N, 2.76%. δ (400Mz), 7.47-7.12 (10Hz, m), 6.94 (1H, d J=1.5Hz), 6.84 (1H, d, J=8Hz), 6.80 (1H, dd, J=1.5Hz and 8Hz), 6.63 (2H, s), 6.43 (1H, d, J=16Hz), 6.06 (1H, q, J=8Hz), 5.18 (2H, s), 5.05 (2H, d J=7.5Hz), 4.49 (1H, d, J=8Hz), 3.89 (3H, s), 3.87 (3H, s), 3.30-3.00 (2H, m), 2.90-2.65 (2H, m); m/z, 507 (M^+), 416, 268, 91.

1-[2-(4-Hydroxy-3-methoxyphenyl)ethyl]-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline.

To 1-[2-(4-benzyloxy-3-methoxyphenyl)vinyl]-7-benzyloxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (122mg, 0.24mmol) in dry methanol (25ml) was added 2 drops of conc. HCl, and Pd/C 10% (150mg). The mixture was hydrogenated at room temperature and high pressure (200lb/in^2) for 4.5h, then filtered through celite. The filtrate was basified with conc. ammonia. The solvent was removed *in vacuo*. The residue was purified by column chromatography eluting with $CHCl_3$:MeOH:conc.NH_3 (80ml:20ml:20drops) to dive the required compound (73mg, 92%), m.p. 97-100°C (from ethyl acetate). δ (CD_3OD) (400MHz) 6.82 (1H, d, J=1.5Hz), 6.72 (1H, d, J=8Hz), 6.73 (1H, s), 6.69 (1H, dd, J=1.5Hz and 8Hz), 6.64 (1H, s), 4.20 (1H, q, J=4Hz), 3.85 (3H, s), 3.83 (3H, s), 3.48-3.16 (2H, m), 3.01-2.82 (2H, m), 2.77-2.62 (2H, m), 2.26-2.05 (2H, m); m/z 329.16239 (M^+, $C_{19}H_{23}NO_4$ requires 329.16270), 178, 137.

[1-^{3}H]-1-[2-(4-Hydroxy-3-methoxyphenyl)ethyl]-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (39).

This material (1.79 mCi mmol^{-1}) was prepared as for the unlabelled compound with partial substitution of sodium borotritiide for sodium borohydride after cyclisation of the amide.

Acknowledgements

We thank Mr K Redshaw and his colleagues, Department of Pure & Applied Biology, University of Leeds for raising plants for us. We thank Dr G.P. Owen, Orac Ltd., for literature searches. We are grateful, for scholarships, to SERC (E.K.) and to the University of Aleppo, Syria (A.E.K.).

References

1. Part of this work has appeared in preliminary form : Herbert, R.B., Knagg, E. Tetrahedron Lett., **1986**, 27, 1099-1102.

2. Capraro, H.D.; Brossi, A., in "The Alkaloids", ed. A. Brossi, Academic Press, Orlando, Florida, **1984**, Vol. 23, pp. 1-70; and earlier volumes in the series.

3. Robinson, R., "The Structural Relations of Natural Products", Oxford University Press, Oxford, **1955**.

4. Battersby, A.R.; Herbert, R.B.; Pijewska, L.; Šantavý, F., Sedmera, P. J. Chem. Soc., Perkin Trans. 1, **1972**, 1736-1740.

5. Battersby, A.R.; Herbert, R.B.; Mcdonald, E., Ramage, R., Clements, J.H. J. Chem. Soc., **1972**, 1741-1746.

6. Herbert, R.B., in "The Chemistry and Biology of Isoquinoline Alkaloids", ed. J.D. Phillipson, M.F. Roberts and M.H. Zenk, Springer-Verlag, Berlin, **1985**, pp. 213-228.

7. Herbert, R.B., in "Rodd's Chemistry of Carbon Compounds", second edn. ed. S. Coffey, Elsevier, Amsterdam, **1980**, Vol. IV part L, pp. 291-455.

8. Kappadia, G.J.; Rao, G.S, Leete, E.; Fayez, M.B.E.; Vaishnav, Y.N.; Fales, H.M. J. Am. Chem. Soc., **1970**, 92, 6943-6951; Herbert, R.B. and Mann, J. J. Chem. Soc., Perkin Trans. 1, **1982**, 1523-1525.

9. Loeffler, S.; Stadler, R.,; Nagakura, N.; Zenk, M.H., J. Chem. Soc., Chem. Commun., **1987**, 1160-1163; refs. cited.

10. Hedges, S.H.; Herbert, R.B.; Knagg, E.; Pasupathy, V. Tetrahedron Lett., **1988**, 29, 807-810; refs. cited.

11. Battersby, A.R.; Dobson, T.A.; Foulkes, D.M.; Herbert, R.B. J. Chem. Soc., Perkin Trans 1, **1972**, 1730-1736.

12. Leete, E. J. Am. Chem. Soc., **1963**, 85, 3666-3669.

13. Herbert, R.B.; Kattah, A.E. Tetrahedron Lett., **1989**, 30, 141-144; also preceding paper.

14. Hutchinson, J. "The Families of Flowering Plants", third edn., Oxford University Press, London, **1973**.

15. Barker, A.C.; Battersby, A.R.; Mcdonald, E.; Ramage, R.; Clements, J.H., J. Chem. Soc., Chem. Commun., **1967**, 390-392.

16. Meyers, A.I.; Nabeya, A.; Adickes, H.W.; Politzer, I.R.; Malone, G.R.; Kovelesky, A.C.; Nolen, R.L.; Portnoy, R.C. J. Org. Chem., **1973**, 38, 36-56.

17. Rylander, P.N.; Himselstein, N. U.S. Patent, **1968**, 3, 372, 199; Weidlich, H.A.; Meyer-Delius, M. Berichte, **1941**, 74, 1195-1212.

18. Stahl, I. Chem. Ber., **1985**, 118, 3166-3171.

19. Sondengam, B.L.; Hentchoya Hémo, J.; Charles, G. Tetrahedron Lett., **1973**, 14, 261-3.

20. Fuji, K.; Ichikawa, K.; Node, M.; Fujita, E. J. Org. Chem., **1979**, 44, 1661-1664.

21. Southon, I.W., Buckingham, J. "Dictionary of Alkaloids", Chapman and Hall, London, **1989**.

22. Battersby, A.R.; Binks, R.; Reynolds, J.J; Yeowell, D.A., J. Chem. Soc., **1964**, 4257-4268.

23. Cava, M.P.; Buck, K.T., Tetrahedron, **1969**, 25, 2795-2805.

24. Finkelstein, J. J. Am. Chem. Soc., **1951**, 73, 550-553.

25. Joshi, B.S., Viswananthan, N.; Balakrishnan, V.; Gawad, D.H.; Ravindranath, K.R., Tetrahedron, **1979**, 35, 1665-1671.

26. Bobbitt, J.M.; Noguchi, I.; Ware, R.S.; Chiong, K.N.; Huang, S.J. J. Org. Chem., **1975**, 40, 2924-2928.

27. Chiji, H., Giga, T.; Izawa, M., Kiriyama, S. Agric. Biol. Chem., **1984**, 48, 1653-1654.