# Biosynthesis. Part 27.[1,2] Colchicine: studies of the phenolic oxidative coupling and ring-expansion processes based on incorporation of multiply labelled 1-phenethylisoquinolines

**Edward McDonald,[a] Robert Ramage,†,[a] Robert N. Woodhouse,[a] Edward W. Underhill,[c] Leslie R. Wetter[c] and Alan R. Battersby*,[a]**

[a] *University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW*
[b] *Robert Robinson Laboratories, University of Liverpool, Liverpool, UK L69 3BX*
[c] *Prairie Regional Laboratory, National Research Council, Saskatoon, Canada*

**Earlier research from our group had proved that the biosynthesis of colchicine and its tropolonoid relatives involves the oxidative ring-closure of a 1-phenethyltetrahydroisoquinoline, autumnaline, followed by extensive modification of the cyclised product. These steps pose many mechanistic and stereochemical questions which are set out in the Introduction. The sequel then provides the answers based on the results from a series of incorporation experiments on *Colchicum* plants involving multiply labelled forms of autumnaline and its biological precursors. These multiply labelled samples required the synthesis of eleven differently labelled tetrahydroisoquinolines; the methods used to introduce the labels are described. Autumnaline is shown to be present in *Colchicum autumnale* plants and labelled forms of some precursors of it are synthesised and studied. Taken together, the findings allow further definition of a substantial part of the biosynthetic pathway to colchicine and its relatives.**

## Introduction

Colchicine **4** and demecolcine **3** are examples of tropolone alkaloids isolated from various *Colchicum* species of plants. Inspection of these structures had earlier shown no obvious relationship to any of the other known families of plant alkaloids; they appeared to be "odd men out". Then the surprising discovery was made[3] that colchicine **4** and demecolcine **3** are biosynthesised by extensive enzymic modification of a 1-phenethylisoquinoline, autumnaline **1**, which in racemic [9-$^{14}$C]-labelled form (as **1a**), was efficiently incorporated by *Colchicum* plants into both **4a** and **3a** labelled exclusively at C-6, Scheme 1. A key intermediate lying further along the pathway was shown[3] to be the dienone *O*-methylandrocymbine **2**, clearly arising from autumnaline **1** by phenol oxidation[4,5] and *O*-methylation. The phenolic coupling has recently been shown to involve a cytochrome P-450 enzyme.[6] With the major features of the pathway thus defined, the way was open to study the intervening stages between autumnaline **1** and colchicine **4**. This paper and the three[7–9] that accompany it, describe research which has defined in considerable detail the remarkable pathway used to construct these tropolones.

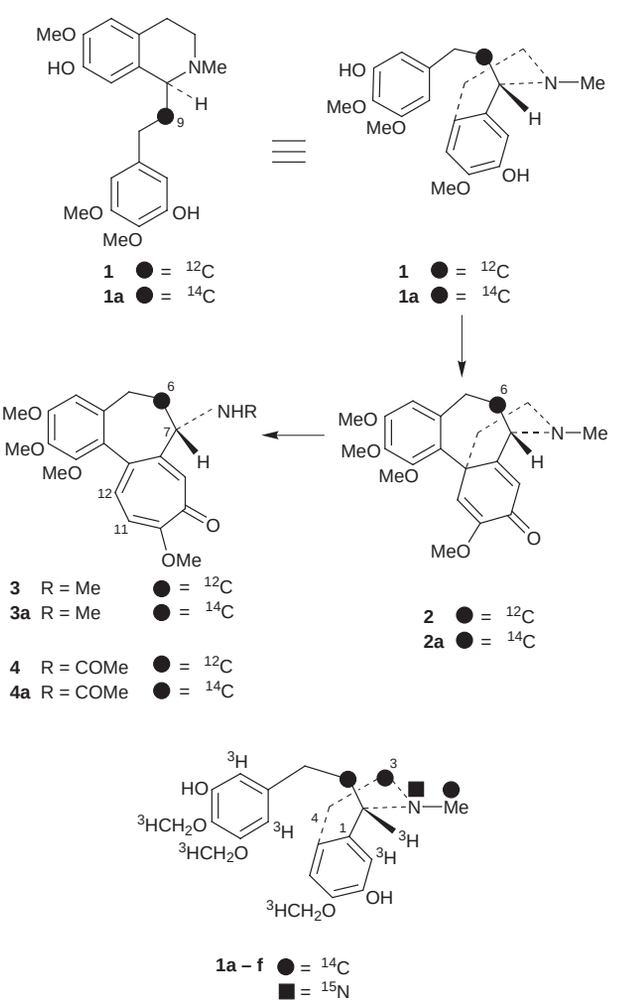
This work required the use of multiply labelled precursors **1a**–**f** and Scheme 1 shows the eleven labels needed for the experiments in this paper. They were not used as a single undecatuply labelled sample but were grouped into convenient sets to produce answers to the following main questions.

(a) Is the nitrogen atom of autumnaline **1** retained in demecolcine **3** and colchicine **4**?

(b) What are the fates of C-3 and the *N*-methyl group of **1**?

(c) Are the *O*-methyl groups of autumnaline **1** retained in colchicine **4**?

(d) Does the oxidative coupling of **1** occur in a *para–para* fashion as adumbrated in Scheme 1 or does the pendant aryl ring rotate leading to *ortho–para* coupling?




**Scheme 1**

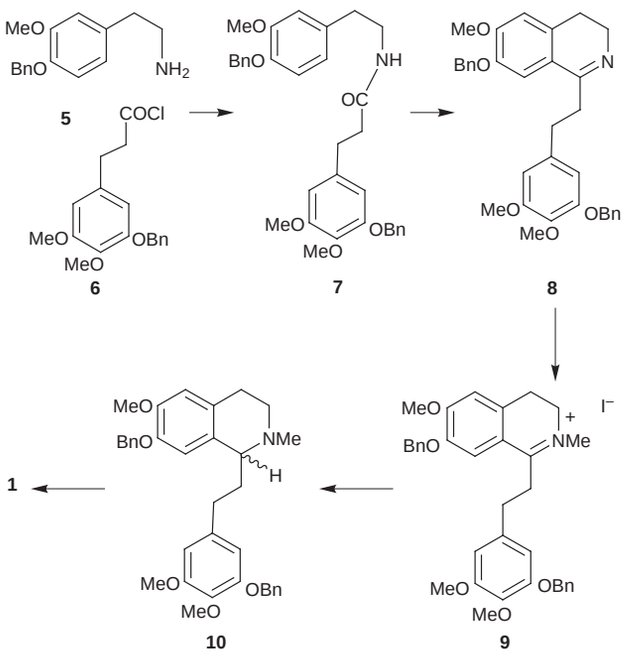
† Present address: Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh, UK EH9 3JJ.

(e) Is the (1*S*)-enantiomer of **1** (which is illustrated) preferred over the (1*R*)-isomer as the precursor of colchicine **4** and is the C-1 hydrogen atom retained?

## Results and discussion

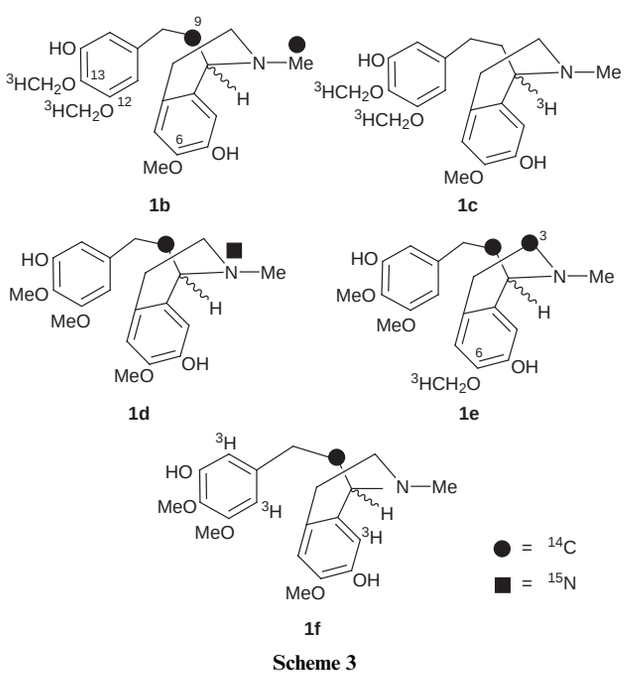
**Synthesis of labelled samples of racemic autumnaline (as 1)**

The synthetic route to (1*RS*)-autumnaline (as **1**), outlined in Scheme 2, had been developed earlier[3] and all the intermediates

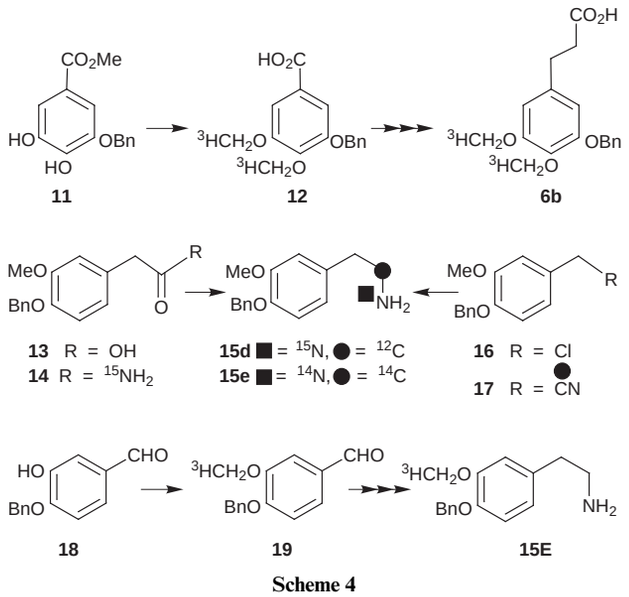



**Scheme 2**

and the final product had been characterised. Thus it is only necessary here to report how the various labels were introduced into **1** since the present materials could be securely identified by comparison with the earlier standard samples. The differently labelled forms of racemic autumnaline **1a**–**f** that were synthesised are collected in Scheme 3, and Scheme 4 shows the routes to the labelled building blocks.




**Scheme 3**




**Scheme 4**

**(1*RS*)-[*N-methyl*-$^{14}$C,9-$^{14}$C,12,13-*O-methyl*-$^{3}$H]Autumnaline 1b**

Methyl 3-*O*-benzylgallate[10] **11** was treated with diazomethane in the presence of tritiated water to give, after hydrolysis of the ester, the [*O*,*O-methyl*-$^{3}$H]-labelled acid **12**. Rosenmund reduction of the acid chloride from **12** as for a closely related case[3] gave the corresponding aldehyde which was converted into **6b** from which the correspondingly labelled *O*,*O*-dibenzylautumnaline was prepared.[3]

The unlabelled dihydroisoquinoline intermediate[3] **8** reacted with [$^{14}$C]methyl iodide to yield the [*N-methyl*-$^{14}$C] salt **9b** which was reduced with borohydride to **10b** *en route* to *O*,*O*-dibenzyl-[*N-methyl*-$^{14}$C]autumnaline. These two samples of protected autumnaline were mixed in appropriate known proportions with *O*,*O*-dibenzyl[9-$^{14}$C]autumnaline[3] to afford the protected form of the required autumnaline **1b**, Scheme 3. In this case and for all those that follow, the multiply labelled *O*,*O*-dibenzylautumnaline was carefully purified to constant specific activity (and constant $^{3}$H : $^{14}$C ratio where both isotopes were present). Then hydrogenolysis in the presence of hydrochloric acid gave autumnaline, **1b** in the present example, as its hydrochloride which was further purified. The specific activity and $^{3}$H : $^{14}$C ratio where appropriate, were then checked again before the incorporation experiments with *Colchicum* plants.

**(1*RS*)-[1-$^{3}$H,12,13-*O-methyl*-$^{3}$H]Autumnaline 1c**

An unlabelled sample of the dihydroisoquinolinium salt **9** was reduced with sodium borotritiide to afford (1*RS*)-*O*,*O*-dibenzyl[1-$^{3}$H]autumnaline **10c** which was mixed with (1*RS*)-*O*,*O*-dibenzyl[12,13-*O-methyl*-$^{3}$H]autumnaline **10b** synthesised above. Hydrogenolysis then gave the triply labelled autumnaline **1c**.

**(1*RS*)-[9-$^{14}$C,$^{15}$N]Autumnaline 1d**

The acid chloride derived from the phenylacetic acid **13** reacted in a two-phase system with aqueous $^{15}$N-ammonia having 96.15 atom% $^{15}$N; an excess of acid chloride was used so that the conversion of $^{15}NH_3$ into the amide **14** was essentially quantitative. Reduction of **14** with lithium aluminium hydride then gave the [$^{15}$N]phenethylamine **15d** for conversion into *O*,*O*-dibenzyl[$^{15}$N]autumnaline. This was mixed with the *O*,*O*-dibenzyl derivative of [9-$^{14}$C]autumnaline **1a** and converted as above into the [9-$^{14}$C,$^{15}$N]autumnaline **1d**.

**(1*RS*)-[3,9-$^{14}$C,6-*O-methyl*-$^{3}$H]Autumnaline 1e**

Treatment of the benzyl chloride **16** with potassium [$^{14}$C]-cyanide afforded the nitrile[11] **17**. Catalytic hydrogenation over a rhodium catalyst then allowed selective reduction of the nitrile without cleavage of the *O*-benzyl group to give the

**Table 1** Incorporation of labelled precursors into colchicine **4** and demecolcine **3** by *Colchicum* plants

| Expt. and precursor numbers | Plants | Sites of labelling | Relative radioactivity at each site of precursor ($^3$H : $^{14}$C ratio) | Relative radioactivity retained by colchicine **4** from corresponding sites of precursor ($^3$H : $^{14}$C ratio) | Incorporation (%) | |
|---|---|---|---|---|---|---|
| | | | | | Colchicine **4** | Demecolcine **3** |
| 1 **1a** | *C. autumnale* | 9-$^{14}$C | [a] | [a] | 6.5 | — |
| 2 **1b** | *C. autumnale* | *N*-$^{14}$CH$_3$ : 9-$^{14}$C : 12,13-[$^3$H]OMe | 0.98 : 1 : 9.9 (5.0) | 0.08 : 1 : 9.9 (9.2) | 10.1 | 0.81 |
| 3 **1b** | *C. byzantinum* | *N*-$^{14}$CH$_3$ : 9-$^{14}$C : 12,13-[$^3$H]OMe | 0.98 : 1 : 9.9 (5.0) | [a] (9.7) | 0.02 | 1.2 |
| 4 **1e** | *C. autumnale* | 3-$^{14}$C : 9-$^{14}$C : 6-[$^3$H]OMe | 1.3 : 1 : 9.5 (4.1) | 0.2 : 1 : 9.5 (7.9) | 6.3 | [a] |
| 5 **1e** | *C. byzantinum* | 3-$^{14}$C : 9-$^{14}$C : 6-[$^3$H]OMe | 1.3 : 1 : 9.5 (4.1) | 0.1 : 1 : 9.5 (8.6) | 0.14 | 0.87 |
| 6 **1f** | *C. autumnale* | Ar-$^3$H : 9-$^{14}$C | 4.4 : 1 (4.4) | 3.1 : 1 (3.1) | 3.5 | [a] |
| 7 **1d** | *C. autumnale* | $^{15}$N : 9-$^{14}$C | See text | See text | | [a] |
| 8 **1c** | *C. autumnale* | 1-$^3$H : 12,13-[$^3$H]OMe | 22.1 : 1 | 17.1 : 1 | 8.2 | [a] |
| 9 (1*S*)-**1c** | *C. byzantinum* | (1*S*)-1-$^3$H : 12,13-[$^3$H]OMe | 8.25 : 1 | 5.3 : 1 | 0.53 | 1.4 |
| 10 (1*R*)-**1c** | *C. byzantinum* | (1*R*)-1-$^3$H : 12,13-[$^3$H]OMe | 8 : 25 : 1 | [a] | <0.015 | <0.007 |
| 11 **32** | *C. autumnale* | 9-$^{14}$C | [a] | [a] | 6.9 | [a] |
| 12 | *C. autumnale* | [3-$^{14}$C]Phenylalanine | [a] | [a] | 2.45 | 0.01 |
| 13 **27g** | *C. autumnale* | 9-$^{14}$C : 6-[$^3$H]OMe | 1 : 7.7 | 1 : 7.6 | 1.7 | [a] |
| 14 **27h** | *C. autumnale* | 9-$^{14}$C : 12,14-$^3$H | 1 : 2.3 | 1 : 0 | 3.8 | [a] |
| 15 **28i** | *C. autumnale* | 9-$^{14}$C : 6,12-[$^3$H]OMe | 1 : 3.1 : 6.8 (9.9) | 1 : 3.8 : 7.2 (11.0) | 4.0 | [a] |

[a] Not relevant or not determined.

[1-$^{14}$C]phenethylamine **15e**. This was converted as usual into *O*,*O*-dibenzyl[3-$^{14}$C]autumnaline.

The phenol[12] **18** was *O*-methylated by treatment with [$^3$H]-methyl iodide under basic conditions to yield *O*-benzyl[*O*-*methyl*-$^3$H]vanillin **19**. This was converted by standard steps[11] into the [3-*O*-*methyl*-$^3$H]phenethylamine **15e** ready to be elaborated to give the correspondingly labelled *O*,*O*-dibenzylautumnaline. This product and the one from the foregoing paragraph were mixed in known proportions with [9-$^{14}$C]-labelled material to give a product from which the triply labelled autumnaline **1e** was obtained by hydrogenolysis.

### (1*RS*)-[9-$^{14}$C,8,11,15-$^3$H]Autumnaline 1f

Exchange of protium by tritium at the aromatic positions *ortho* and *para* to the phenolic hydroxy groups of autumnaline was achieved by heating the free base (as **1**) with tritiated water in dimethylformamide for 4 days. A long heating period was used to allow complete exchange at all three positions.[13] Addition of an appropriate amount of [9-$^{14}$C]autumnaline (as **1a**) completed the preparation of this precursor **1f**.

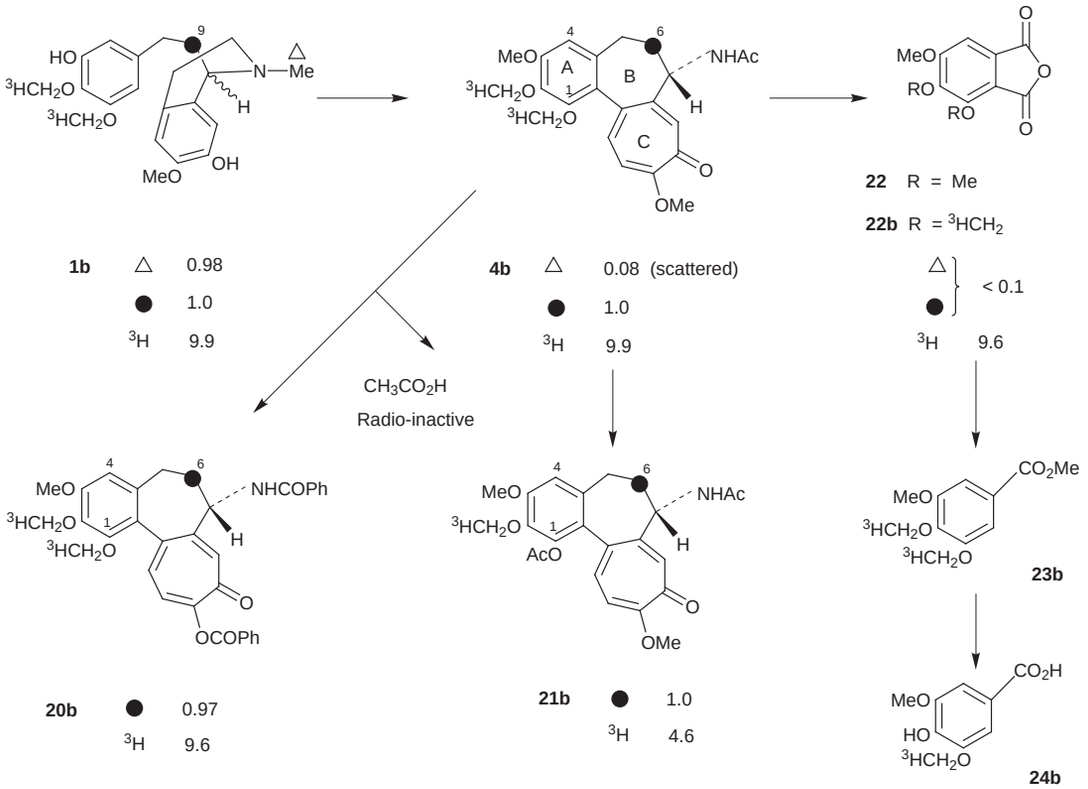
### Incorporation experiments on *Colchicum* plants

The various multiply labelled forms of (1*RS*)-autumnaline **1a**–**f** as hydrochlorides were usually injected as an aqueous solution into the seed capsules of *Colchicum* plants. However, some experiments carried out at times that did not coincide with capsule formation involved introduction of the labelled precursor into corms of the plants by a wick. Both techniques have been fully described.[3] Colchicine **4** and demecolcine **3** were isolated from the plants normally two weeks later by methods already reported in detail,[3] as have the purification procedures that ensured radiochemical purity of the alkaloids. The results are collected in Table 1 together with incorporation values. For the latter, it should be noted that colchicine **4** is the major alkaloid of *C. autumnale* whereas it is demecolcine **3** that predominates in *C. byzantinum*.

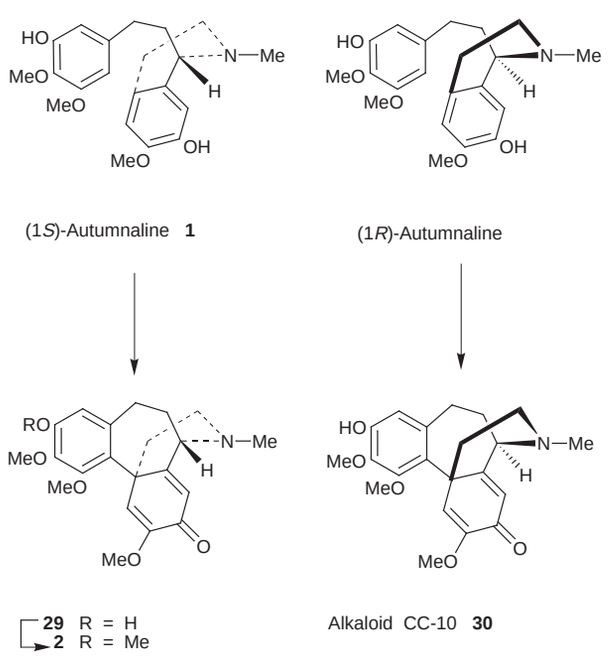
Usually many precursors were studied at the same time and it was important to confirm that the plants were biosynthesising colchicine **4** and its relatives satisfactorily. For each such set of experiments a known labelled form of autumnaline was used (or alternatively, a labelled form of phenylalanine) to act as a standard. Expt. 1, Table 1, where **1a** was used is an example of such a check.

Experiments 2 and 3 involving the quadruply labelled autumnaline **1b** provided a large amount of important information about the biosynthesis of tropolone alkaloids. *C. autumnale* plants were used in Expt. 2 and they gave a high incorporation of the precursor into colchicine **4b** which had a much higher $^3$H : $^{14}$C ratio than the autumnaline **1b**. The change corresponded to loss of 92% of the $^{14}$C-activity present in the *N*-*methyl*-$^{14}$C group of **1b** relative to the stable skeletal label at C-9 of **1b** which appears at C-6 of colchicine **4b**. This result really means that the *N*-methyl group of **1b** has been totally lost in the formation of colchicine **4b**, not 92% of it. The small difference from 100% loss is probably due to non-specific reincorporation of a little $^{14}$C-activity by metabolism of the one-carbon $^{14}$C-fragment that had been eliminated, as observed[7,14] in other examples; other cases of such feed-back will appear later in this paper. The $^3$H : $^{14}$C ratio also demonstrated that the two $^3$H-labelled *O*-methyl groups are entirely retained in the biosynthesised colchicine **4b** and this conclusion will receive further support later. Retention of the *O*-methyl groups was also important for future studies by allowing labelling at these sites to be used for the internal standard as an alternative to $^{14}$C-labelling at C-9 of **1b** in Expt. 2. The relative molar specific activities for Expt. 2 and for the further degradations of the isolated colchicine **4b** are presented in Scheme 5 where now the two $^{14}$C-labels are given different symbols.

Hydrolysis of the colchicine **4b** from Expt. 2 afforded radioinactive acetic acid (isolated as its *p*-bromophenacyl ester) and deacetylcolchiceine which was benzoylated to form the crystalline derivative **20b**. The $^3$H : $^{14}$C ratio in **20b** was essentially unchanged from that of **4b** proving that the *O*-methyl group of the tropolone nucleus was unlabelled and thus that scrambling of the *O*-methyl groups had not occurred. In addition, the *O*-methyl group at C-1 of **4b** was specifically demethylated with tin(IV) chloride and acetyl chloride[15] to give 1-acetyl-1-demethylcolchicine **21b** which retained 46% of the $^3$H-activity present in **4b**. The $^1$H NMR methoxy signal at high field shown in the spectrum of colchicine **4** is characteristic[16] of the *O*-methyl group at C-1 which lies between the two aromatic rings. This signal was not present in the spectrum of **21b** which pinpoints the site of the specific demethylation. These results prove




**Scheme 5**




**Scheme 6**

that the two labelled *O*-methyl groups on ring A of colchicine **4b** are located as illustrated. It follows that *para–para* coupling has occurred in the oxidative ring-closure of autumnaline **1** to yield the dienone **29** ready for *O*-methylation to form *O*-methylandrocymbine **2**, Scheme 6; confirmation will be adduced later in the paper. This same conclusion on the direction of coupling has also been reached recently from a different angle.[17]

Further support for some of the foregoing deductions came from oxidative degradation of the labelled colchicine **4b** to the anhydride **22b**, Scheme 5. This retained 95% of the $^{3}H$ present in **4b**. It was diluted with unlabelled anhydride to provide sufficient material for acid catalysed decarboxylation[18] to the known tris-*O*-methylgallic acid isolated as its methyl ester **23b** followed by selective demethylation and hydrolysis using concentrated sulfuric[19] acid to form syringic acid **24b**. The latter retained 48% of the $^{3}H$-activity present in **23b** thus establishing rigorously the site of the second [*O-methyl*-$^{3}H$] group in **22b** and so also in colchicine **4b**. The data in Scheme 5 interlock to make all the foregoing conclusions beyond doubt.

The same labelled precursor **1b** was also used for Expt. 3 but now with *C. byzantinum* plants. The $^{3}H:^{14}C$ ratio for colchicine, Table 1, confirmed the foregoing findings in that 96% of the $^{14}C$-labelled *N*-methyl group had been eliminated. In contrast, the $^{3}H:^{14}C$ ratio of 5.3 for demecolcine **3b** corresponded within experimental error to complete retention of the *N*-methyl group. When this result was considered alongside the entire loss of the *N*-methyl group from colchicine, it strongly suggested that demecolcine **3** precedes colchicine **4** on the biosynthetic pathway; this topic will be carried further in the following paper.[7]

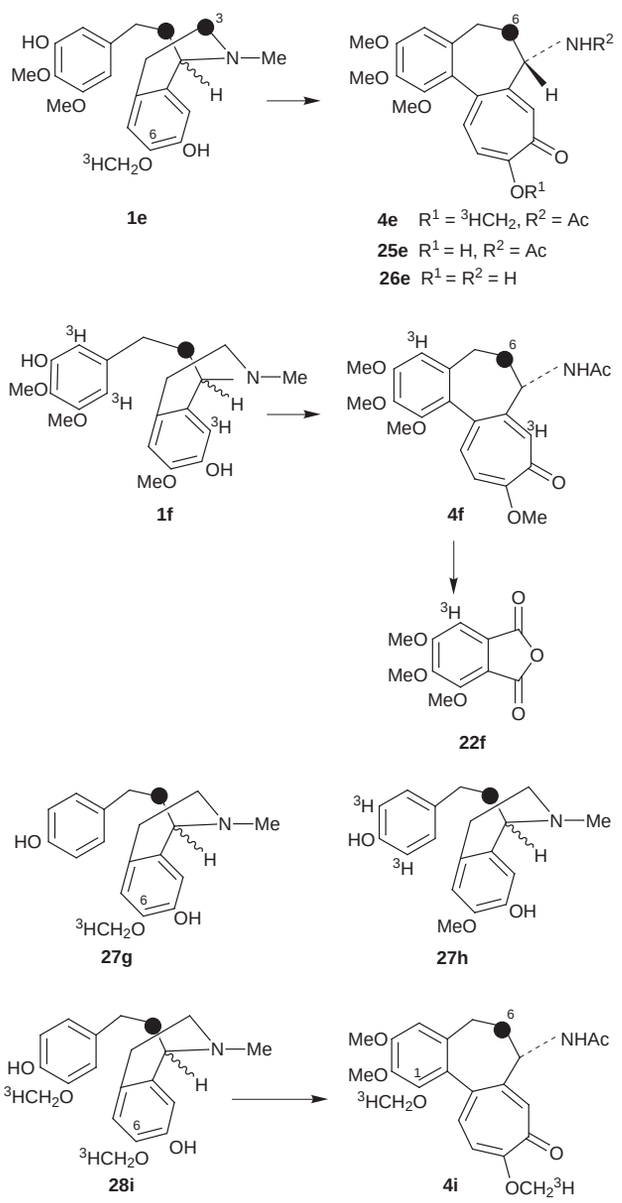
The colchicine **4e** isolated from Expt. 4 using the precursor **1e** showed a $^{3}H:^{14}C$ ratio of 7.9 compared with the starting ratio in **1e** of 4.1. This corresponds to the loss of *ca.* 80% of the $^{14}C$ located at C-3 in autumnaline **1e**. By inspection, this carbon atom cannot be transferred in any straightforward way into colchicine **4e** and the foregoing result demonstrates the loss of most of it. Degradation of the colchicine **4e** to colchiceine **25e**, deacetylcolchiceine **26e**, Scheme 7, and the anhydride **22,** Scheme 5, proved that the remaining 20% of $^{14}C$-activity was scattered over the molecule, mainly in the *O*-methyl groups together with a trace in the *N*-acetyl group. The relative radioactivities of the various degradation products are collected in Table 2. Here again there had been some reincorporation of activity from an eliminated fragment. In addition, the foregoing degradations proved that the $^{3}H$-labelled *O*-methyl group at C-6 of **1e** is fully retained in the colchicine **4e** formed; all this $^{3}H$ activity was lost when **4e** was converted into colchiceine **25e**.

Expt. 5 involved the same precursor **1e** now with the alternative *Colchicum* species. The $^{3}H:^{14}C$ ratio in the isolated colchicine was 8.6 whereas that in **1e** was 4.1, a result that confirms the foregoing findings. However, the loss of the $^{14}C$-activity

**Table 2** Degradation of radioactive samples of colchicine to determine labelling pattern

| Compound | Relative radioactivity in **1** and at corresponding sites of the products Expt. 4 | Relative radioactivity in **1** and at corresponding sites of the products Expt. 15 |
|---|---|---|
| Autumnaline **1** | 3-$^{14}$C : 9-$^{14}$C : 6-[$^{3}$H]OMe | 9-$^{14}$C : 6-[$^{3}$H]OMe : 12-[$^{3}$H]OMe |
| | 1.3 : 1.0 : 9.5 | 1.0 : 3.1 : 6.8 |
| Colchicine **4** | 0.2 : 1.0 : 9.5 | 1.0 : 3.8 : 7.2 |
| Colchiceine **25** | 1.1 : 0 | 0.96 : 0 : 6.5 |
| Deacetylcolchiceine **26** | 1.1 : 0 | [a] |
| Anhydride **22** | 0.07 : <0.002 | [a] |
| *p*-Bromophenacylacetate | 0.01 : 0 | [a] |
| 1-Acetyl-1-demethylcolchicine **21** | [a] | 1.06 : 3.8 : 0 |

[a] Not examined.




**Scheme 7**

from C-3 of **1e** on conversion into colchicine **4e** was *ca.* 90% with presumably less scattered feedback of $^{14}$C than in Expt. 4.

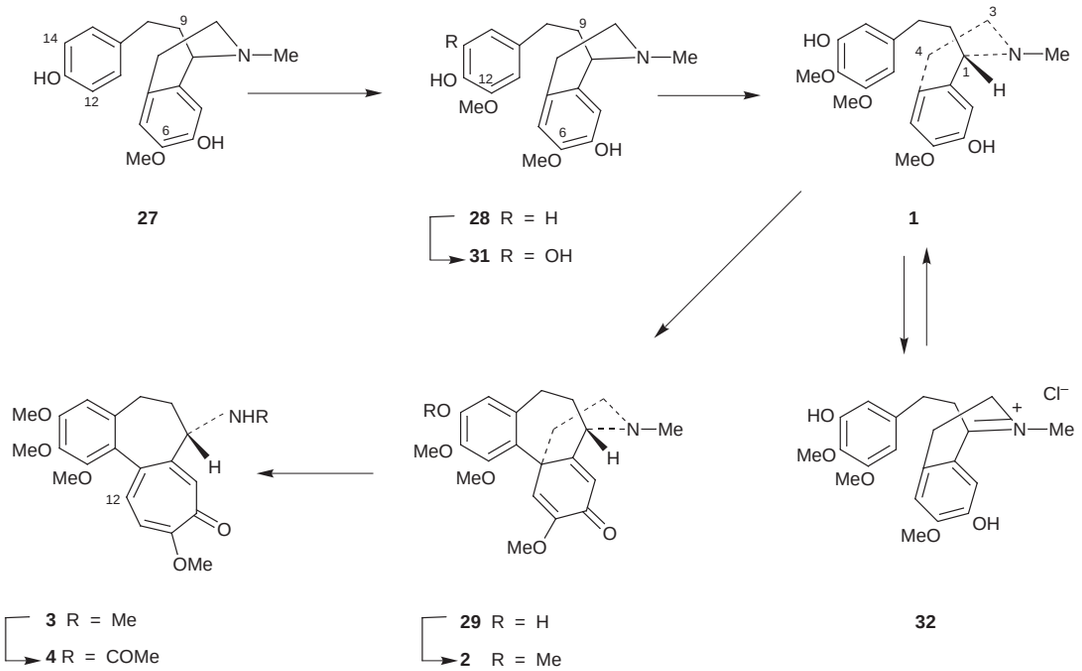
The aim of Expt. 6 was to study the fate of three of the protons of the aromatic rings of autumnaline **1f**. The biosynthesised colchicine **4f** showed a $^{3}$H : $^{14}$C ratio that was 70% of that in **1f**. The 30% loss of $^{3}$H fits the expected result of 33% loss, Scheme 6. In addition, 52% of the specific molar activity of the colchicine **4f** was retained in the derived anhydride **22f**. Thus the oxidative ring-closure of **1f** to **4f** shows no surprises with two of the protons investigated being unaffected and the third being lost.

The final study of this set focused on the nitrogen atom of autumnaline by using **1d** that was $^{15}$N-labelled together with the internal $^{14}$C-standard at C-9, Expt. 7. All the determinations of the $^{15}$N abundances were carried out in Canada by mass spectrometry. First, the $^{15}$N abundance of the precursor was determined using the amide (as **7**), Scheme 2, from which the [$^{15}$N]autumnaline was synthesised. The amide was first accurately diluted to give material in which the $^{15}$N abundance was suitable for the mass spectrometry and then subjected to Kjeldahl digestion to release the nitrogen as ammonia for analysis. Six mass spectrometric determinations were carried out to establish the $^{15}$N atom% abundance of the undiluted amide to be 96.15, a value that holds good for the derived autumnaline. Addition of the small amount of [9-$^{14}$C]autumnaline **1a** reduced the $^{15}$N abundance of **1d** to 89.35 atom% with a specific $^{14}$C-activity of 78.2 μCi mmol$^{-1}$. The specific $^{14}$C-activity of the isolated colchicine **4d** was 0.498 μCi mmol$^{-1}$ corresponding to a dilution of 157 times with endogenous unlabelled colchicine present in the plants. Degradation of the colchicine to ammonia as before followed by analysis showed the $^{15}$N abundance to be 0.592 atom%. This corresponds to a dilution of 151 times during the biosynthesis. Bearing in mind the various dilutions, radio-assays and mass spectrometric analyses involved in this experiment, the value of 151 is in remarkable agreement with the $^{14}$C-standard of 157. There can be no doubt that the nitrogen atom of autumnaline is entirely retained in the colchicine biosynthesised from it.

Taken together, the foregoing results provide clear answers to all the questions (a) to (d) posed in the Introduction. The studies aimed at answering the remaining stereochemical question (e) will now be described.

### Studies with (1*S*)- and (1*R*)-enantiomers of autumnaline

Colchicine **4** has the (7*S*)-configuration[20] which corresponds to (1*S*)-autumnaline **1**. It was important to determine whether this (1*S*)-enantiomer was the preferred precursor of colchicine **4** or whether a rapid redox process of the type **1** $\rightleftharpoons$ **32** in Scheme 8 would allow the plants to use both the (1*S*)- and (1*R*)-isomers of autumnaline. We first examined this possible redox process in Expt. 8 using (1*RS*)-autumnaline **1c**. Note that the *O-methyl*-$^{3}$H groups are now being used as the internal standard, shown to be a reliable one by Expt. 2. They carried 1 part of the $^{3}$H with 22.5 parts being at C-1; this was known from the amounts of the two separately labelled materials that were mixed to yield **1c**. The isolated colchicine **4c** was degraded to the anhydride **22c** having $^{3}$H-activity showing that in **4c** 1 part of the total $^{3}$H was carried by the *O*-methyl groups of ring A and 17.1 parts remained at C-7. This corresponds to 76% retention of $^{3}$H from C-1 of **1c** through to colchicine **4c**. The conclusion was that though the redox process appeared to be occurring to some extent, it was not so rapid as to frustrate the experiments with resolved forms of autumnaline.




**Scheme 8**

(1*RS*)-*O*,*O*-Dibenzylautumnaline **10** was resolved using *O*,*O*-dibenzoyltartaric acid of which both enantiomers are available.[21] The salt from the (+)-acid yielded the isoquinoline showing $[a]_D$ +5.3 in pyridine and this value was unchanged by further recrystallisations of the salt. Similarly, use of the (−)-acid finally afforded the isoquinoline having $[a]_D$ −5.3. These two products showed ORD curves that were mirror images and the correlation between the sign of the ORD and the absolute configuration of the sample had earlier been established.[22] It could thus be deduced that the (−)-*O*,*O*-dibenzylautumnaline has the (1*S*)-configuration which by debenzylation afforded (1*S*)-autumnaline **1** whilst the (+)-base is the precursor of (1*R*)-autumnaline (enantiomer of **1**).

The labelled material for resolution was prepared by mixing accurately known weights of (1*RS*)-*O*,*O*-dibenzyl[1-$^3$H]-autumnaline and (1*RS*)-*O*,*O*-dibenzyl[12,13-*O*-*methyl*-$^3$H]-autumnaline, both of known $^3$H-specific activity. This material was resolved as before, then debenzylated and the (−)-(1*S*)-autumnaline and (+)-(1*R*)-autumnaline were introduced into *C. byzantinum* corms, Expts. 9 and 10, respectively. The incorporation levels for colchicine **4c** and demecolcine **3c**, Table 1, showed that whereas (1*S*)-autumnaline was a good precursor, the (1*R*)-enantiomer was a very poor one. Thus there is indeed a correct match between the absolute configurations of the precursor (1*S*)-autumnaline **1** and colchicine **4**.

Oxidation of the colchicine from Expt. 9 to the anhydride **22c** showed that the ratio of $^3$H at C-1 to $^3$H in the *O*-methyl groups had fallen from 8.25:1 in (1*S*)-autumnaline to 5.32:1 in the biosynthesised colchicine. This demonstrated that *ca.* 35% of the $^3$H at C-1 of (1*S*)-autumnaline had been lost during the biosynthetic process. A redox interconversion **1** ⟺ **32**, Scheme 8, is the most plausible explanation; a similar loss from a tetrahydroisoquinoline had earlier been observed during research on the biosynthesis of morphine[23] and was rationalised in the same way. Support for this interpretation in the present case came by showing that 1,2-dehydro[9-$^{14}$C]autumnaline (as **32**, Scheme 8) is an efficient precursor of colchicine **4**, Expt. 11, Table 1. The higher level of incorporation of the dehydro system (as **32**) into colchicine as compared with the levels from (1*S*)-autumnaline in Expt. 9 is simply because Expt. 11 was run at the best time with the best plants, *i.e.* in the spring into the seed capsules of *C. autumnale*.

**Isolation of autumnaline 1 from *Colchicum autumnale* plants**

By this stage, the evidence was overwhelming in support of (1*S*)-autumnaline **1** as the precursor of *O*-methylandrocymbine **2** on the biosynthetic pathway to demecolcine **3** and colchicine **4**. Indeed, (1*S*)-autumnaline had been isolated from *C. cornigerum* plants[24] and we wished to show that this intermediate is also present in *C. autumnale*, the species mainly used for the experiments in this paper. We expected that the amount of it in the plants would be small and therefore planned to produce some labelled autumnaline by introducing (2*RS*)-[3-$^{14}$C]phenylalanine into the plants, Expt. 12; this amino acid is an established precursor of ring A and the three sp$^3$ carbons of ring B of colchicine.[14] The labelled autumnaline from Expt. 12 was isolated by adding unlabelled (1*RS*)-autumnaline hydrochloride (as **1**) to the crude plant extract followed by re-isolation of the hydrochloride and rigorous purification. It was strongly $^{14}$C-labelled demonstrating that autumnaline was present in the plants. Such a dilution analysis is only reliable if a second derivative is prepared and purified. Here, the autumnaline was converted by diazomethane into *O*,*O*-dimethylautumnaline for multiple recrystallisation of its picrate. The molar specific activity was unchanged from that of the autumnaline hydrochloride.

The strength of this approach lies in the fact that though it is just conceivable that a minor radiochemical impurity might by chance (a) be inseparable chromatographically from the larger amount of added unlabelled carrier material and then, in addition (b) co-crystallise at a constant ratio, the chances are infinitesimal that both could happen again for a second different derivative and at the *same* constant proportion.

**Studies on precursors of (1*S*)-autumnaline 1**

The racemic forms of **27**, **28** and **31**, Scheme 8, had been synthesised earlier[3] with $^{14}$C-labelling at the illustrated C-9 site. The first two gave good incorporations into colchicine **4** and demecolcine **3** relative to those from (1*RS*)-autumnaline and the third gave a satisfactory, but lower, level; possibly, it was thought,[3] because of its susceptibility to oxidation. These results indicated that (1*S*)-autumnaline **1** is preceded on the pathway by the sequence **27** ⟶ **28** ⟶ **31** ⟶ **1**. The word "indicated" is used rather than "proved" because it is conceivable that the hydroxylations and *O*-methylations might occur in a

slightly different order. Nevertheless, the order indicated is not at variance with our general experience in the field and therefore further labelling studies were undertaken.

The labelled precursors synthesised were **27g**, **27h** and **28i** in Scheme 7. Both bases had been synthesised earlier[3] in [9-$^{14}$C]-labelled form and the same routes were used here. All the required building blocks were available from the foregoing studies save one, 4-hydroxy[3,5-$^{3}$H]benzaldehyde, which was prepared as for earlier phenols by exchange with tritiated water *ortho* to the phenolic hydroxy group of 4-hydroxybenzaldehyde. From this was built[3] 4-hydroxy[3,5-$^{3}$H]phenylpropionic acid for the synthesis of **27h**.

These labelled precursors were introduced into *C. autumnale* plants as usual and the colchicine was isolated, Expts. 13–15, Table 1. Expt. 13 showed that **27g** is a specific precursor of colchicine **4g** and that its *O*-methyl group is entirely retained. The colchicine from Expt. 14 had satisfactorily incorporated the $^{14}$C-label but was devoid of tritium showing that in the two hydroxylation steps *ortho* to the phenolic group needed to reach autumnaline **1**, no "NIH-shift"[25] of tritium had occurred. Finally, the complete retention of $^{3}$H from **28i** through to colchicine **4i** in Expt. 15 demonstrated that both *O*-methyl groups had been retained in the final product. In addition, the colchicine **4i** was degraded and the results in Table 2 show that the labelled *O*-methyl group on the phenethyl ring of **28i** appears at C-1 of colchicine **4i**, Scheme 7. This result provides a second proof that *para–para* coupling occurs in the oxidative step that converts autumnaline **1** into the dienone **29** *en route* to *O*-methylandrocymbine **2**.

## Summary and conclusions

The foregoing results taken together proved that demecolcine **3** and colchicine **4** are biosynthesised from (1*S*)-autumnaline **1** by the steps illustrated in Scheme 8; the (1*R*)-enantiomer of **1** is an ineffective precursor. Support has been given to earlier indications[3] that the bases **27**, **28** and **31** lie on the biosynthetic pathway before (1*S*)-autumnaline **1**. Tritium at C-1 of (1*S*)-autumnaline is largely retained in the biosynthesised colchicine but some loss occurs probably by the redox process **1** $\rightleftharpoons$ **32** illustrated in Scheme 8. The nitrogen atom of **1** is retained through to colchicine **4** but the *N*-methyl group of **1** is lost; in contrast, this *N*-methyl group is fully retained in demecolcine **3**.

All three *O*-methyl groups of **1** are entirely retained in both tropolones **3** and **4** and their demonstrated disposition in colchicine **4** proves that the oxidative coupling occurs in a *para–para* fashion to generate isoandrocymbine **29** ready for *O*-methylation to give the key dienone, *O*-methylandrocymbine **2**. The occurence in *C. cornigerum*[24] of **30**, the enantiomer of **29,** interlocks with our present findings since **30** is almost certainly formed by *para–para* coupling of (1*R*)-autumnaline, Scheme 6. The expected loss of one aryl hydrogen atom occurs in the coupling step **1** $\longrightarrow$ **29**. It was already known[14] that C-4 of autumnaline appears as C-12 in the tropolone ring of colchicine **4**. The present studies show that C-3 is lost but, interestingly, as a C-1 unit that can to a small extent be re-used by the plants especially for building *O*-methyl groups. Finally, results were gained suggesting that demecolcine **3** precedes colchicine **4** on the pathway and this is included in Scheme 8; firm experimental evidence on this point appears in the following paper.[7]

That colchicine **4** is biosynthesised as illustrated in Scheme 8 was a complete surprise and this knowledge opened the way to further studies both enzymic, referred to earlier, and by the labelling described here. The research exploring the intermediates between the dienone **2** and colchicine **4** are outlined in Part 28 whilst Parts 29 and 30 describe those on the mechanism of the ring expansion that generates the tropolone nucleus.

## Experimental

General directions are as previously published;[1] in addition, light petroleum refers to the fraction bp 60–80 °C. The work in the present paper involved the synthesis mainly of autumnaline (as **1**), but also of some relatives, in many differently labelled forms. All the synthetic routes have been fully described[3] so the sequel covers just the preparation of the labelled units from which **1** and its relatives were built.

### 3-Benzyloxy-4,5-di[O-*methyl*-$^{3}$H]methoxybenzoic acid 12

Tritiated water (1 drop, *ca.* 100 mCi) was added to methyl 3-*O*-benzylgallate[10] (1.1 g) in dioxane (7 cm$^3$) and the solution was treated at room temperature with ethereal diazomethane (2 equiv., 20 cm$^3$). After 12 h, more diazomethane solution (20 cm$^3$) was added and the solution was kept at 0 °C for 24 h. The ester of **12** obtained by evaporation was hydrolysed by heating under reflux for 2 h with potassium hydroxide (0.45 g) in ethanol (25 cm$^3$). Water (25 cm$^3$) was added and most of the ethanol evaporated before acidification with sulfuric acid. Extraction with ethyl acetate gave the acid **12** (1.07 g, 93%), mp 169–171 °C (lit.,[10] mp 170 °C), total activity 11.8 mCi.

### 4-Benzyloxy-3-methoxyphenyl[$^{15}$N]acetamide 14

A solution of (4-benzyloxy-3-methoxyphenyl)acetic acid[23] (368 mg) in benzene (20 cm$^3$) was treated with oxalyl chloride (400 mg) and dimethylformamide (20 mg). When effervescence had ceased, the solvent was evaporated and the crude acid chloride dissolved in dichloromethane (10 cm$^3$). To this stirred solution was added saturated aqueous sodium hydrogen carbonate (3 cm$^3$) and [$^{15}$N]ammonium chloride (52 mg). After stirring the mixture at room temperature for 3 h, the organic layer was separated, washed with water and dried. Evaporation of the solvent and crystallisation of the residue from ethyl acetate–light petroleum gave the [$^{15}$N]amide **14** (270 mg, 100% based on [$^{15}$N]ammonium chloride), mp 133 °C (lit.,[26] mp 133–134 °C).

### 4-Benzyloxy-3-methoxyphenethyl[$^{15}$N]amine 15d

The foregoing [$^{15}$N]amide **14** (270 mg) was added to a stirred suspension of lithium aluminium hydride (390 mg) in anhydrous diethyl ether (20 cm$^3$) and heated under reflux for 4 h. After 16 h at room temperature, the complex was decomposed with saturated aqueous sodium potassium tartrate. The mixture was filtered and the filtrate evaporated to afford a gum which on treatment with hydrogen chloride in diethyl ether–methanol gave the [$^{15}$N]amine **15d** as its hydrochloride (90 mg, 31%), mp 175 °C (lit.,[27] mp 176–178 °C).

### 4-Benzyloxy-3-methoxyphenyl[1-$^{14}$C]acetonitrile 17

Potassium [$^{14}$C]cyanide (3.6 mg, *ca.* 2 mCi) and potassium [$^{12}$C]cyanide (12 mg) were added to dimethylformamide (25 cm$^3$) and stirred for 1 h with gentle heating. (4-Benzyloxy-3-methoxyphenyl)methyl chloride[11] (141 mg) in dimethylformamide was added and the mixture stirred for 21 h at room temperature. Potassium cyanide (27 mg) was added and the mixture stirred for a further 24 h. After addition of water (50 cm$^3$), the solution was extracted with benzene (5 × 50 cm$^3$), the extracts were washed with water, dried and evaporated. The residue crystallised from diethyl ether–light petroleum to give the [$^{14}$C]-nitrile **17** (132 mg, 99%), mp 66 °C (lit.,[11] 68–69 °C), 2.1 mCi.

### 4-Benzyloxy-3-methoxyphenyl[1-$^{14}$C]ethylamine 15e

The foregoing [$^{14}$C]nitrile **17** (132 mg) in dry ethanol (6 cm$^3$) containing 2 M hydrochloric acid (0.05 cm$^3$) was added to pre-reduced 5% rhodium on carbon (154 mg) in dry ethanol (4 cm$^3$) and shaken with hydrogen for 26 h. The catalyst was filtered off, the filtrate was evaporated and the residue crystallised from methanol–diethyl ether to give the amine **15d** as its hydrochloride (92 mg, 61%), mp 175 °C, 1.23 mCi.

**O-Benzyl[*O-methyl*-$^3$H]vanillin 19** †

3-Hydroxy-4-benzyloxybenzaldehyde (48 mg) was dissolved in methanol (4 cm$^3$) containing potassium hydroxide (7.6 mg). [$^3$H]Methyl iodide (12 mg, 12.5 mCi) was distilled into the solution using a sealed vacuum system. The mixture was then heated in a sealed tube at 70 °C for 64 h. The contents of the tube were added to methanol (4 cm$^3$) containing methyl iodide (4 cm$^3$) and heated under reflux for 7 h. The residue from evaporation was fractionated on alumina using benzene as eluent to give an oil (38 mg) which was mixed with unlabelled *O*-benzylvanillin (254 mg) for recrystallisation from methanol to yield the labelled aldehyde (227 mg, 10.3 mCi). More unlabelled *O*-benzylvanillin (216 mg) was added to the mother liquors and recrystallisation afforded additional labelled material (217 mg, 2.1 mCi).

**(1*RS*)-[8,11,15-$^3$H]Autumnaline**

Typically, (1*RS*)-autumnaline (*ca.* 50 mg), recovered from its hydrochloride, was heated at 100 °C in an evacuated sealed tube for 4 days with triethylamine (40 mg) and dimethylformamide (0.5 cm$^3$) containing tritiated water (*ca.* 50 mCi). The tube was flushed with nitrogen during the repeated evacuation process before sealing. The contents of the tube were evaporated to dryness at high vacuum and then freed from readily exchangeable tritium (*e.g.* on the OH groups) by repeatedly dissolving the residue in methanol followed by evaporation. The final residue was converted as usual[3] into the crystalline hydrochloride (typically 40 mg, 3–5 mCi) of (1*RS*)-[8,11,15-$^3$H]autumnaline.

**4-Hydroxy[3,5-$^3$H]benzaldehyde**

4-Hydroxybenzaldehyde (200 mg) and triethylamine (70 mg) in dimethylformamide (1 cm$^3$) containing tritiated water (20 mCi) were heated at 100 °C in a sealed tube for 100 h essentially as in the foregoing experiment. Evaporation of the solvent, removal of readily exchangeable tritium as above and sublimation of the residue followed by crystallisation of the sublimate from ethyl acetate–light petroleum gave the tritiated aldehyde (120 mg, 1 mCi).

***O*,*O*-Dibenzyl-1,2-dehydro[N-*methyl*-$^{14}$C]autumnaline iodide (as 9)**

The *O*,*O*-dibenzyl-3,4-dihydroisoquinoline[3] **8** (104 mg) in dry ethyl acetate (0.5 cm$^3$) was kept at 0 °C for 2 weeks in a sealed tube with [$^{14}$C]methyl iodide (0.27 mCi) and then at room temperature for a further 2 weeks. The contents of the tube were then mixed with excess methyl iodide (1 cm$^3$) and kept at 0 °C for 16 h. Evaporation gave the crystalline (*N-methyl*-$^{14}$C)methiodide (as **9**) (80 mg, 0.24 mCi) in 89% radiochemical yield, identified by comparison with an authentic sample.[3]

**(1*RS*)-*O*,*O*-Dibenzyl[1-$^3$H]autumnaline**

A radioinactive sample of the foregoing iodide[3] (36 mg) in dry dimethyl sulfoxide (2 cm$^3$) was treated at room temperature for 4 days with just less than 1 mol equiv. of sodium borotritiide (0.524 mg, 10 mCi). An excess of sodium borohydride (10 mg) was then added to complete the reduction and after 15 h, water was added and the product was extracted into 3:1 diethyl ether–chloroform. The extracts were washed with water, dried and evaporated, the residue being crystallised from methanol to afford (1*RS*)-*O*,*O*-dibenzyl[1-$^3$H]autumnaline (20 mg, 6.1 mCi). Dilution of the mother liquors with the same unlabelled isoquinoline (42 mg) gave on crystallisation a further crop (39 mg, 2.6 mCi). Total radiochemical yield 87%.

**Resolution of (1*RS*)-*O*,*O*-dibenzylautumnaline**

Separate portions of racemic *O*,*O*-dibenzylautumnaline[3] **10** were warmed in a suitable solvent with a slight excess of the following acids: (+)-*O*,*O*-dibenzoyltartaric acid, (+)-*O*,*O*-di-*p*-toluoyltartaric acid, (+)-camphor-10-sulfonic acid, *N*-acetylleucine and abietic acid. The only suitable crystalline salt was the *O*,*O*-dibenzoyltartrate.

**(1*R*)-(+)-*O*,*O*-dibenzylautumnaline**

Racemic *O*,*O*-dibenzylautumnaline (111 mg) in ethyl acetate (3 cm$^3$) was warmed with a solution of (+)-*O*,*O*-dibenzoyltartaric acid (72 mg) in methanol (3 cm$^3$). Diethyl ether was added and the solution was cooled to yield a crystalline salt, $[\alpha]_D$ +50.1 (*c* 0.12 in methanol). The base liberated from this salt by treatment with aqueous sodium hydrogen carbonate showed $[\alpha]_D$ +4.2 (*c* 0.85 in pyridine). The specific rotation varies with solvent: $[\alpha]_D$ in pyridine > benzene ~ methanolic HCl > methanol. After three recrystallisations of the salt, the rotation reached $[\alpha]_D$ +59.7 (*c* 0.26 in methanol) and the liberated free base showed $[\alpha]_D$ +5.3 (*c* 0.92 in pyridine). Further recrystallisation of the salt had no effect on these values, mp 153–154.5 °C (Found: C, 69.7; H, 5.9. $C_{53}H_{53}NO_{13}$ requires C, 69.8; H, 5.9%). The released free base showed ORD (MeOH) $\lambda_{max}$/nm (Φ) 229 (+10 600), 247 (−4900), 273 (0), 292 (−1270) corresponding to a (1*R*)-tetrahydroisoquinoline.

**(1*S*)-(−)-*O*,*O*-Dibenzylautumnaline**

Racemic *O*,*O*-dibenzylautumnaline (111 mg) in ethyl acetate (3 cm$^3$) was warmed with a solution of (−)-*O*,*O*-dibenzoyltartaric acid (72 mg) in ethanol (3 cm$^3$) as above until part of the ethanol had evaporated. Diethyl ether was added and the crystalline salt which precipitated was recrystallised three times from ethanol–diethyl ether causing the rotation of the liberated free base to rise from $[\alpha]_D$ −4.0 to a maximum of $[\alpha]_D$ −5.3 (in pyridine). ORD (MeOH) $\lambda_{max}$/nm (Φ) 226 (−6780), 246 (+5280), 265 (0), 290 (+1510) corresponding to a (1*S*)-tetrahydroisoquinoline.

***O*,*O*-Dibenzyl-1,2-dehydro[9-$^{14}$C]autumnaline chloride**

A solution of the corresponding iodide[3] (226 mg) in 1:1 aqueous methanol (15 cm$^3$) was added to a column of IRA-400 resin (25 g) in the chloride form. Slow elution with water gave, after evaporation of the eluate, the chloride salt (142 mg, 73%), mp 92 °C (Found: C, 67.2; H, 6.5. $C_{35}H_{38}ClNO_5 \cdot 2H_2O$ requires C, 67.3; H, 6.8%). $\lambda_{max}$/nm (log ε) 249 (4.24), 310 (3.95) and 363 (3.95).

**1,2-Dehydro[9-$^{14}$C]autumnaline chloride 32**

A solution of the foregoing chloride salt (13 mg) in methanol (0.1 cm$^3$) was heated at 160 °C for 2.5 h with concentrated hydrochloric acid (1 cm$^3$) while a stream of nitrogen was passed through the solution. Complete evaporation of the solution and crystallisation of the residue from ethanol–ethyl acetate gave the phenolic chloride **32** (5 mg, 55%), mp 194–197 °C; $\lambda_{max}$/nm (after addition of KOH) 211 (220), 253 (275), 312 (324), 371 (435).

**Degradations of labelled alkaloids**

The degradations shown in Scheme 5 have been described earlier[3,14] or they closely followed published methods. The specific *O*-demethylation **4b** ⟶ **21b** was carried out as follows.

**1-Acetyl-1-demethoxycolchicine 21b.** Tin(IV) chloride (0.5 cm$^3$) was added dropwise to a solution of colchicine (500 mg) in dry dichloromethane (5 cm$^3$) and acetyl chloride (1.5 cm$^3$) at 0 °C. The resultant mixture of a red solution and an orange oil was stirred at room temperature for 30 h and then poured into ice and water (25 cm$^3$). The mixture was made homogeneous by addition of acetic acid and then extracted with dichloromethane. The combined extracts were washed with aqueous sodium hydrogen carbonate, dried and evaporated. Crystallisation of the residue by warming with ethyl acetate gave 1-acetyl-1-demethoxycolchicine (173 mg, 36%), mp 238–240 °C (from

‡ With Dr M. Todd.

acetone) (lit.,[15] mp 243 °C); $\lambda_{max}$/nm (log $\varepsilon$) 239 (4.53) 348 (4.30); $\nu_{max}$/cm$^{-1}$ 1675, 1750, 3300; $\delta_H$ 1.94 (3H, s, NAc), 2.22 (3H, s, OAc), 3.85, 3.91, 3.98 (each 3H, s, 3 × OMe), 4.68 (1H, br, NH), 6.68 (1H, s, H-1), 6.79 and 7.13 (each 1H, d, *J* 10 Hz, H-11 and H-12), 7.60 (1H, s, H-8); *m*/*z* 427 ($M^+$, 427).

**1-Demethoxycolchicine**

The foregoing acetate was treated at room temperature for 5 h with 0.1 M aqueous methanolic sodium hydroxide. After evaporation of most of the methanol, extraction with dichloromethane removed most of the non-phenolic material. Acidification of the aqueous layer with sulfuric acid followed by extraction with dichloromethane then gave 1-demethoxycolchicine, mp 269 °C [lit.,[15] reports mp 355 °C (error for 255 °C?)] (Found: C, 65.5; H, 6.1; N, 3.8. $C_{21}H_{23}NO_6$ requires C, 65.5; H, 6.0; N, 3.6%); $\lambda_{max}$/nm (log $\varepsilon$) 212 (4.38), 244 (4.49), 354 (4.24) shifting to 366 on addition of alkali; $\nu_{max}$(Nujol)/cm$^{-1}$ 1665, 3240; *m*/*z* 385 ($M^+$, 385).

**Methyl 3,4,5-trimethoxybenzoate (as 23b)**

Trimethoxyphthalic anhydride (52 mg), prepared by ferricyanide oxidation[18] of colchicine, was heated with concentrated hydrochloric acid (2 cm$^3$) at 100 °C for 14 h and then kept at room temperature for 8 h. Extraction with ethyl acetate gave a crude acidic fraction (49 mg) which was esterified by heating in 7% $BF_3$–MeOH (2 cm$^3$) at reflux for 3 h. After evaporation, the residue was partitioned between chloroform and water, the crude ester from the chloroform being chromatographed on grade III alumina (5 g). Elution with chloroform gave methyl 3,4,5-trimethoxybenzoate (20 mg), mp 83 °C (from methanol) (lit.,[28] 82.5 °C). The radioactive sample **22b** was degraded in the same way to afford **23b** for radio-assay (see text).

**Syringic acid (as 24b)**

The foregoing ester (100 mg) was heated at 45 °C in concentrated sulfuric acid (4 cm$^3$) for 6 h under nitrogen. After being kept at room temperature for a further 12 h, the solution was added to ice and water (20 cm$^3$) and extracted with ethyl acetate to give syringic acid (91 mg), mp 204 °C from aqueous methanol (lit.,[19] mp 204–206 °C); $\lambda_{max}$/nm 272 shifting to 300 on addition of potassium hydroxide; $\nu_{max}$/cm$^{-1}$ 1690, 3540; $\delta_H(CD_3OD)$ 3.84 (6H, s, 2 × OMe), 7.35 (2H, s, ArH). The radioactive material **23b** was hydrolysed and decarboxylated in the same way to yield **24b** for radio-assay (see text).

**Detection of autumnaline in *Colchicum autumnale* plants**

The plants from Expt. 12 were worked up by the methods fully described in Part 28[7] with addition of (1*RS*)-autumnaline hydrochloride (103 mg) to the initial ethanolic extracts of the plants. The partition column was run as usual to the point where elution with ethyl acetate had removed the colchicine. The partition column was then eluted with 1:9 methanol:chloroform to afford a fraction containing the autumnaline. The material from this fraction was fractionated by chromatography on grade III alumina using 1:1 benzene:chloroform. The fractions containing pure autumnaline were combined and their contents converted into the hydrochloride (51 mg) by treatment with ethanolic hydrogen chloride. This was recrystallised as usual to constant specific activity ($7.7 \times 10^4$ disintegrations per 100 s per mg). A solution in methanol (1 cm$^3$) of the base recovered from all the foregoing hydrochloride was treated with a large excess of ethereal diazomethane. After 1 day, a solution of acetic acid in diethyl ether was added dropwise until the yellow colour was just discharged. The ethereal solution was extracted thrice with 0.1 M aqueous sodium hydroxide to remove remaining traces of phenolic material then washed with water. The residue from evaporation of the ether was treated in methanol with a slight excess of picric acid and the resultant picrate of the known *O,O*-dimethyl ether[29] was multiply recrystallised. The free base was recovered by passing a solution of a weighed quantity of the picrate in chloroform through a short column of alumina. Radio-assay of this base showed it to have essentially the same specific molar activity as that of the foregoing hydrochloride.

## Acknowledgements

Grateful acknowledgement is made to the SRC and SERC for the award of studentships (to E. McD and R. N. W.). We also thank Mr J. K. Hulme (Ness Botanic Garden) and Mr J. Symonds (University Botanic Gardens, Cambridge) for cultivation of the *Colchicum* plants, Dr M. Todd for carrying out the indicated experiment and the EPSRC and Roche Products for financial support.

## References

1 Part 26. A. R. Battersby, E. McDonald and A. V. Stachulski, *J. Chem. Soc.*, *Perkin Trans. 1*, 1983, 3053.
2 A small part of this work has been briefly reported, A. C. Barker, A. R. Battersby, E. McDonald, R. Ramage and J. H. Clements, *J. Chem. Soc.*, *Chem. Commun.*, 1967, 390.
3 A. R. Battersby, R. B. Herbert, E. McDonald, R. Ramage and J. H. Clements, *J. Chem. Soc.*, *Chem. Commun.*, 1966, 603; *J. Chem. Soc.*, *Perkin Trans. 1*, 1972, 1741.
4 D. H. R. Barton and T. Cohen, "*Festschr. A. Stoll*", Birkhauser, Basle, 1957, p. 117.
5 A. R. Battersby in "*Oxidative Coupling of Phenols*", eds. W. I. Taylor and A. R. Battersby, Marcel Dekker, New York, 1967, p. 119; E. McDonald in "*Chemistry of Heterocyclic Compounds*, *Part 1 Isoquinolines*", ed. G. Grethe, Wiley, New York, 1981, p. 38.
6 A. Nasreen, M. Rueffer and M. H. Zenk, *Tetrahedron Lett.*, 1996, **37**, 8161.
7 A. C. Barker, D. R. Julian, R. Ramage, R. N. Woodhouse, G. Hardy, E. McDonald and A. R. Battersby, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 2989.
8 R. N. Woodhouse, E. McDonald, R. Ramage and A. R. Battersby, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 2995.
9 P. W. Sheldrake, K. E. Suckling, R. N. Woodhouse, A. J. Murtagh, R. B. Herbert, A. C. Barker, J. Staunton and A. R. Battersby, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 3003.
10 L. Jurd, *J. Am. Chem. Soc.*, 1959, **81**, 4606.
11 A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.
12 Y. Sekine, C. Creveling, M. Bell and A. Brossi, *Helv. Chim. Acta*, 1990, **73**, 426.
13 G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
14 A. R. Battersby, R. Binks, J. J. Reynolds and D. A. Yeowell, *J. Chem. Soc.*, 1964, 4257.
15 Roussel-UCLAF, Neth. Pat. Appl., 6 412 619 (Cl. 507c) (*Chem. Abstr.*, 1965, **63**, 13 339).
16 V. Delaroff and P. Rathle, *Bull. Soc. Chim. Fr.*, 1965, 1621.
17 A. Nasreen, H. Gundlach and M. H. Zenk, *Tetrahedron Lett.*, 1997, 7357.
18 E. Leete and P. Nemeth, *J. Am. Chem. Soc.*, 1960, **82**, 6055.
19 M. T. Bogert and B. B. Coyne, *J. Am. Chem. Soc.*, 1929, **51**, 569.
20 H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, 1955, **38**, 2030.
21 C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, 1933, **55**, 2605.
22 A. R. Battersby, I. R. C. Bick, W. Klyne, J. P. Jennings, P. M. Scopes and M. J. Vernengo, *J. Chem. Soc.*, 1965, 2239.
23 A. R. Battersby, D. M. Foulkes and R. Binks, *J. Chem. Soc.*, 1965, 3323 and refs. therein.
24 A. R. Battersby, R. Ramage, A. F. Cameron, C. Hannaway and F. Šantavý, *J. Chem. Soc.* (*C*), 1971, 3514.
25 G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop and S. Udenfriend, *Science*, 1967, **157**, 1524.
26 D. H. Hey and L. C. Loba, *J. Chem. Soc.*, 1954, 2246.
27 J. M. Bobbitt and T.-T. Chou, *J. Org. Chem.*, 1959, **24**, 1106.
28 *Dictionary of Organic Compounds*, Supplement 1, Chapman and Hall, London, 1983, p. 559.
29 A. Brossi, J. Van Burik and S. Teitel, *Helv. Chim. Acta*, 1968, **51**, 1965.

*Paper* 8/03850C
*Received* 21*st May* 1998
*Accepted* 3*rd July* 1998