Biosynthesis. Part 28.[1,2] Colchicine: definition of intermediates between *O*-methylandrocymbine and colchicine and studies on speciosine

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Labelled samples are prepared of demecolcine **3**, colchicine **4**, *N*-formyl-*N*-deacetylcolchicine **6** and *N*-deacetylcolchicine **7**, the last depending on a new method for its preparation from colchicine. Incorporation experiments with these compounds and with specifically labelled autumnaline **1** support the pathway **2** ⟶ **5** ⟶ **3** ⟶ **6** ⟶ **7** ⟶ **4** as the terminal sequence for the biosynthesis of colchicine.

The key intermediate *O*-methylandrocymbine **2** is isolated from *Colchicum autumnale* plants together with speciosine **14** and its *O*-acetyl derivative **15**; all three are first isolations from this plant. Speciosine **14** and *N*-methyldemecolcine **8** are shown to be formed *in vivo* largely from demecolcine **3** whereas *N*-formyldemecolcine **5** is the precursor of demecolcine and its *N*-formyl group is derived from C-3 of autumnaline. This discovery of a tropolone alkaloid which retains both carbons of the ethanamine bridge of **2** is important for future stereochemical work on the ring-expansion process.

Introduction

Previous studies[1,3] have established conclusively that (1*S*)-autumnaline **1** and *O*-methylandrocymbine **2** are key intermediates for the biosynthesis of the tropolone alkaloids demecolcine **3** and colchicine **4** in *Colchicum* plants (Scheme 1). This paper reports our studies of that part of the biosynthetic pathway which lies beyond *O*-methylandrocymbine **2**. Plausible intermediates have been synthesised in labelled and unlabelled form and their significance has been evaluated by both incorporation and trapping experiments.

It had already been demonstrated[1] that the *N*-methyl group of autumnaline **1**, and therefore also of *O*-methylandrocymbine **2**, is wholly retained as demecolcine **3** is biosynthesised but is entirely lost during the formation of colchicine **4**. Demecolcine **3** would therefore seem to lie before colchicine **4** on the biosynthetic pathway. Furthermore, the C-3 carbon atom of autumnaline **1** is eliminated[1] during the construction of both demecolcine **3** and colchicine **4**, a loss which could occur as formaldehyde *via* an intermediate containing the –N⁺Me=CH₂ moiety or as formic acid from a tropolone having the –N(Me)CHO group. The latter is *N*-formyldemecolcine **5** and our plans covered the possibility that this amide might be the last stable intermediate to retain the C-3 carbon atom of autumnaline **1**. A summary of the possible inter-relationships we planned to test is given in Scheme 2. Labelled samples of the tropolone alkaloids **3**–**7** were required for the necessary incorporation experiments. It is of interest that *N*-formyl-*N*-deacetylcolchicine **6** and *N*-deacetylcolchicine **7** have been isolated as minor alkaloids from *Colchicum autumnale*[4] and the formamide **5** was later identified in *Colchicum cornigerum*.[5]

Results and discussion

Samples of colchicine **4** and demecolcine **3** ³H-labelled at the 3-*O*-methyl group, were prepared by treatment of the naturally occurring phenolic alkaloids 3-demethylcolchicine[6,7] **10** and 3-demethyldemecolcine[6,7] **11** with diazomethane and tritiated water. Part of the labelled demecolcine was converted into
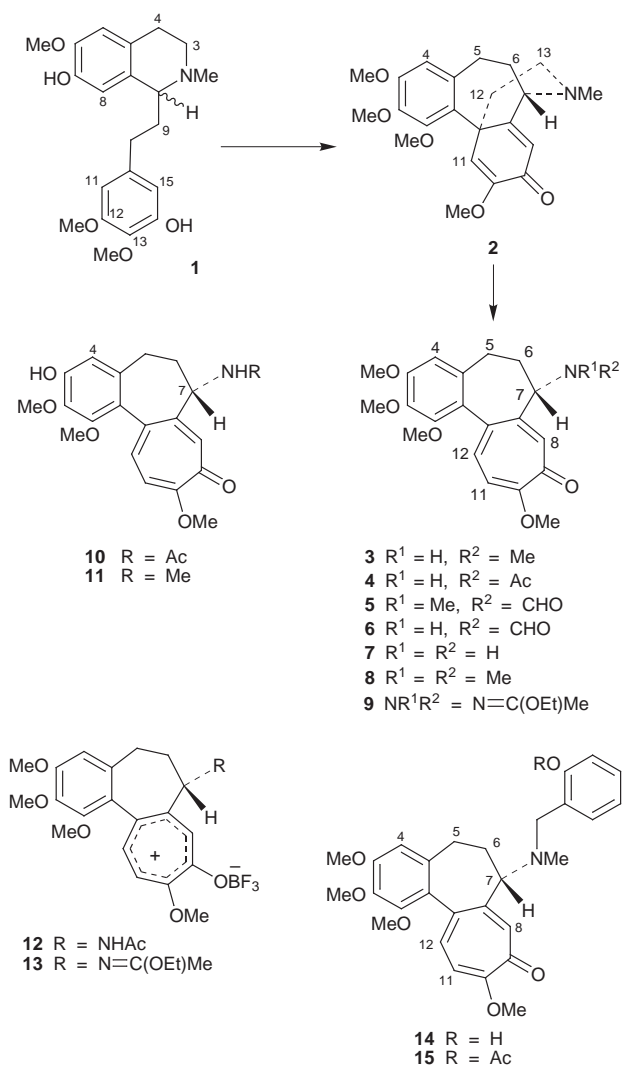
Scheme 1

Scheme 2
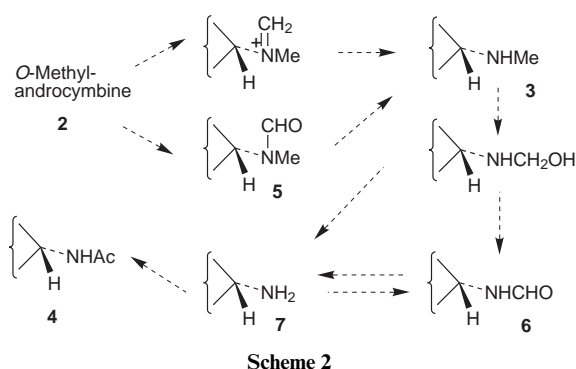
N-formyl[3-*O-methyl*-³H]demecolcine **5** by treatment with formic acid in pyridine.[8] *N*-Deacetylcolchicine **7** could not be prepared by direct hydrolysis of the *N*-acetyl group of colchicine **4** because the *O*-methyl group of the tropolone system is sensitive to both acidic and basic hydrolytic conditions.[9] Specific deacetylation was achieved by a new route *via* the imino ether **9** by treating colchicine **4** successively with (a) boron trifluoride–diethyl ether (1.1 equiv.) to protect the tropolone system against electrophilic attack by setting up the tropylium salt **12**, (b) triethyloxonium tetrafluoroborate (Meerwein's reagent) which then generated the imino ether **13** and thence **9**; methanolic hydrogen chloride[10] completed the cleavage of **9**. *N*-Deacetylcolchicine **7** was obtained in 68% yield based on unrecovered starting material and was isolated as its salt with (+)-tartaric acid. Treatment of the base **7** with formic acid in pyridine gave *N*-formyl-*N*-deacetylcolchicine **6**. Samples of the tropolones **6** and **7** in [3-*O-methyl*-³H]-labelled form were similarly prepared from the foregoing labelled colchicine.

The labelled compounds **3**–**7** were administered to *C. autumnale* and *C. byzantinum* plants and after a period for metabolism, demecolcine **3** and colchicine **4** were extracted and purified to constant specific acitivity. To ensure that the isolated alkaloids were uncontaminated by traces of highly radioactive initial precursor which had been used, a roughly equal quantity of radio-inactive *N*-formyldemecolcine **5** was added to the colchicine and demecolcine which had been isolated from the experiment involving labelled *N*-formyldemecolcine; the colchicine and demecolcine were then rigorously purified again. This same precaution was used for precursors **6** and **7**. The final results are recorded in Table 1 and in considering them, one must remember that the ratio of the amount of colchicine to that of demecolcine is high in *C. autumnale* and low in *C. byzantinum*. It can be seen that all five compounds **3**–**7** are incorporated by both plant species into colchicine **4** which thus appears to be the end-product of the biosynthetic pathway. In contrast, demecolcine **3** is either not formed or is formed very poorly relative to colchicine from compounds **6**, **7** and **4** and so these late stages of colchicine biosynthesis are not significantly reversible. Furthermore, the results in Table 1 indicate that the

difference in content of tropolones **3** and **4** in the two plant species arises from a weakly active *N*-demethylation process in *C. byzantinum* since the substances **6** and **7** were both incorporated into colchicine to about the same extent in both types of plant.

The results in Table 1 are in accord with the sequence **2** ⟶ **5** ⟶ **3** ⟶ **6** ⟶ **7** ⟶ **4**. However, further support for the intermediacy of the formamides **5** and **6** was sought to help assess the possibility that the true sequence is **2** ⟶ **3** ⟶ **7** ⟶ **4** and that the formamides **5** and **6** are simply undergoing hydrolysis by a non-specific enzyme to yield intermediates **3** and **7**. Accordingly, [*N-methyl*-¹⁴C]demecolcine (as **3**) was prepared from [¹⁴C]methyl iodide and a large excess of *N*-deacetylcolchicine **7** and the product was mixed with [3-*O-methyl*-³H]demecolcine to give a ³H : ¹⁴C ratio of 4.95. This doubly-labelled demecolcine was fed to *C. autumnale* plants and the total alkaloids were diluted with *N*-formyl-*N*-deacetylcolchicine **6** before separation. The recovered demecolcine **3** showed an unchanged ³H : ¹⁴C ratio whereas the *N*-formyl-*N*-deacetylcolchicine **6** showed a ratio of 19.4 (incorporation 0.29%). The colchicine **4** carried essentially no ¹⁴C activity (³H : ¹⁴C ratio 150; incorporation 0.62%) corresponding to complete loss of the *N*-methyl group in agreement with earlier findings.[1] It was essential that the formamide **6** should not be contaminated with colchicine **4** or a false ³H : ¹⁴C ratio would have been obtained. Therefore radio-inactive colchicine was added to the isolated formamide **6** and the separation was repeated; the original results were unchanged. The above ³H : ¹⁴C ratio for *N*-formyl-*N*-deacetyl-colchicine **6** corresponds to retention of *ca.* 25% of the *N*-methyl carbon of demecolcine **3** through to the amide carbonyl of formamide **6**. Evidently the latter may be formed either directly from demecolcine **3** or by formylation of *N*-deacetyl-colchicine **7** just as biological acetylation gives colchicine **4**. Its direct formation from demecolcine, however, adds weight to the view that the conversion **3** ⟶ **6** plays a significant role in the biosynthesis of colchicine.

Our interest in similar studies on the formamide **5** took us first into isolation work with the aim of identifying the intermediates lying beyond autumnaline **1** but retaining C-3 of this precursor. By injecting [3-¹⁴C]autumnaline (as **1**) into the plants, a considerable simplification was achieved since only intermediates standing before demecolcine **3** on the pathway will be labelled whereas the major alkaloids will be unlabelled. The plants were harvested after 1, 3 and 5 days (considerably shorter periods than the usual 14 days) and the total plant extract was fractionated; the neutral and water-soluble fractions were only weakly radioactive and were not examined further. The radioactive basic fraction contained four components by autoradiography. Two of these were phenolic but were not investigated further. A third corresponded chromatographically to *O*-methylandrocymbine **2** and so this fraction was diluted with radio-inactive dienone **2**; rigorous purification as the base and as the crystalline picrate gave material of constant activity. The incorporation of 1.2% established for the first time the presence of *O*-methylandrocymbine in *C. autum-*

Table 1 Incorporation of [3-*O-methyl*-³H]tropolones into demecolcine **3** and colchicine **4**

Tropolone precursor	Incorporation (%) in *C. autumnale*		Incorporation (%) in *C. byzantinum*	
	Demecolcine **3**	Colchicine **4**	Demecolcine **3**	Colchicine **4**
RN(Me)CHO **5**	0.004	10.7	15.2	3.8
RNHMe **3**	8.8[a]	13.8	30.5[a]	0.8
RNHCHO **6**	0.09	4.8	1.6	9.2
RNH₂ **7**	1.2	35.3	0.1	28.6
RNHCOCH₃ **4**	0.0	41.6[a]	0.3	53.8[a]

[a] These correspond to % recovered activity.

Table 2 Tracer experiments using *C. autumnale* (A) and *C. speciosum* (S) plants

Experiment	Plant	Precursor[a]	Incorporation (%) and [% retention ^{14}C]		
			RNMe$_2$ 8	Speciosine 14	RNMeCHO 5
1	S	[3-^{14}C, 12,13-*O-methyl*-^{3}H] Autumnaline 1	<10^{-3}	0.003 [24][b]	0.012 [88][b]
2	A	[3-^{14}C, 12,13-*O-methyl*-^{3}H] Autumnaline	c	c	0.18 [107]
3	A	[3-^{14}C, 12,13-*O-methyl*-^{3}H] Autumnaline	c	c	0.11 [103]
4	S and A	[3-^{14}C, 8,11,14-^{3}H] Autumnaline	0.0 (S) 0.01 (A) [25][b,d]	c	0.2 (A) [94][d]
5	S	[3-*O-methyl*-^{3}H] Demecolcine 3	0.8	1.0	0.02

[a] All samples of autumnaline were (1*RS*). [b] These values are of reduced accuracy because of low incorporations. [c] Not examined. [d] Allowance made for loss of one third of ^{3}H in the biosynthesis.[1]

nale plants. Only preliminary work has been done on the fourth base (see Experimental).

With this encouragement, a large-scale extraction was carried out on 250 *C. autumnale* plants to yield three pure bases. Two were identified by full spectroscopic comparison with authentic specimens as *O*-methylandrocymbine 2 (10 mg) and speciosine [2,11] 14 (5 mg) which is the major alkaloid of *Colchicum speciosum*.[11] The spectral properties of the third base (88 mg), *m/z* 519 were very similar to those of speciosine *m/z* 477 except for the absence of an O–H stretch in the IR spectrum, and the presence of an extra strong absorption at ν_{max} 1750 cm^{-1}. This evidence and the appearance of a singlet at δ 2.03 (3H) in the ^{1}H NMR spectrum showed that the new alkaloid is *O*-acetyl-speciosine 15. Mild alkaline hydrolysis afforded a phenol identical with natural speciosine 14.

The foregoing isolation work yielded important results but did not provide further information on the status of *N*-formyl-demecolcine 5 as an intermediate. A different tack was therefore taken which we expected to yield valuable information not only about *N*-formyldemecolcine but also about the mechanism of tropolone formation. This involved a study of hydrogen removal from C-13 and C-12 of *O*-methylandrocymbine 2 (corresponding to C-3 and C-4 of autumnaline 1) as it is converted into colchicine 4. This work is described in the two following papers.[12,13] Clearly though, the reactions at C-13 of *O*-methylandrocymbine can only be monitored if tropolone alkaloids can be found which retain C-13. The three substances which *a priori* seemed likely candidates are *N*-methyl-demecolcine [14] 8 (*C. cornigerum*), speciosine 14 (*C. speciosum* and *C. autumnale*) and *N*-formyldemecolcine 5 (*C. cornigerum*) isolated from the indicated species.

[3-^{14}C,12,13-*O-methyl*-^{3}H]Autumnaline [1] 1 was administered to both *C. autumnale* and *C. speciosum* plants to test whether C-13 of dienone 2, which is equivalent to C-3 of base 1, is retained in the above three alkaloids. A positive incorporation of tritium into the tropolones 5, 14 and 8 would demonstrate synthesis of these compounds in the plant, and importantly, if these substances showed an unchanged ^{14}C:^{3}H ratio then C-3 of autumnaline must be wholly retained during their biosynthesis.

In Expt. 1 (Table 2), the incorporation into *N*-methyl-demecolcine 8 was too low to permit any conclusion regarding retention of C-3. However, in *C. autumnale* (Expt. 4) a sufficiently active sample was obtained to show that only *ca.* 25% of the original ^{14}C activity at C-3 of autumnaline survived through to *N*-methyldemecolcine 8. A similar result (*ca.* 24% retention of C-3) was found for speciosine 14, Expt. 1, Table 2. Fortunately for later work, C-3 of autumnaline was retained, within experimental error, in *N*-formyldemecolcine 5 isolated from both *C. speciosum* and *C. autumnale*, Expts. 1–4, Table 2.

There was a small difference for Expt. 4 in that the ^{3}H-labels acting as internal standards were placed in the aromatic rings.[1] We conclude from these results that *N*-methyldemecolcine 8 and speciosine 14 are biosynthesised at least largely by alkylation of demecolcine 3, whereas *N*-formyldemecolcine 5 precedes demecolcine 3 on the biosynthetic pathway. This conclusion was supported by feeding [3-*O-methyl*-^{3}H]demecolcine 3 to *C. speciosum* when satisfactory incorporations were observed into *N*-methyldemecolcine 8 and speciosine 14 but essentially no incorporation into *N*-formyldemecolcine 5, Expt. 5, Table 2.

The specificity of incorporation of ^{14}C into *N*-formyl-demecolcine 5 was established by hydrolysis of the amide and conversion of the resultant formic acid into its *p*-bromophenacyl ester; a satisfactory yield of this ester could only be obtained with dimethylformamide (DMF) as solvent. This, however, introduced a complication in that the radio-active formic acid was diluted by traces of radio-inactive acid in the solvent and the isolated ester showed as expected no ^{3}H-activity but only 51% of the ^{14}C-specific activity of *N*-formyldemecolcine 5. Nevertheless, this result as it stands shows heavy specific labelling of the *N*-formyl group and the following argument makes it clear that all the ^{14}C-label at C-3 of autumnaline must be present in the *N*-formyl group of amide 5. If the alternative explanation is considered that only *ca.* 50% of the original activity of amide 5 resides in the *N*-formyl group, then the biosynthesis must have involved quantitative transfer of the remaining 50% of activity to other sites, since there is complete ^{14}C-retention from autumnaline through to the formamide 5. But it is known[1] that no ^{14}C-activity from C-3 of autumnaline is present in either demecolcine 3 or colchicine 4 so this explanation is eliminated. Further support came from subsequent work [12] in which even more rigorous purification of the DMF substantially reduced the dilution observed above.

N-Formyldemecolcine 5 is thus an excellent substance on which to study changes in the oxidation level at C-3 of autumnaline 1, corresponding to C-13 of *O*-methylandrocymbine 2, during the biosynthesis of the tropolone family of alkaloids including colchicine; these experiments are reported in Part 29 of this series.[2]

The results described here support the pathway 2 ⟶ 5 ⟶ 3 ⟶ 6 ⟶ 7 ⟶ 4 as being at least a major terminal sequence for the biosynthesis of colchicine in *Colchicum autumnale* and related plants.

Experimental

General
For general directions, see ref. 1.

Synthesis of radioactive precursors

[3-*O*-methyl-³H]Colchicine 4. Tritiated water (0.04 cm³; 0.05 Ci) was added to a solution of 3-demethylcolchicine **10** (20 mg) in dry dioxane (0.5 cm³); an equal quantity of tritiated water was allowed to equilibrate with ethereal diazomethane (0.5 cm³) and the two solutions were then mixed. After 4 h at 20 °C, and again after 8 h, further additions of ethereal diazomethane (0.5 ml) were made and after 20 h, the solution was evaporated. The residue was dissolved in methanol, the solvent evaporated and this procedure was repeated eight times. Radio-inactive colchicine (120 mg) was added to the final residue and crystallisation from ethyl acetate afforded [3-*O*-methyl-³H]colchicine **4** (111 mg; 2.5 µCi mg⁻¹) identified by comparison with authentic unlabelled material.

N-Deacetyl[3-*O*-methyl-³H]colchicine 7. A solution of the foregoing colchicine **4** (111 mg) in chloroform was passed through a column of alumina (Grade I) and evaporated to give dry colchicine. This in dry dichloromethane (5 cm³) was treated with redistilled boron trifluoride–diethyl ether (0.46 cm³) in dry dichloromethane (5 cm³), and with triethyloxonium tetrafluoroborate (210 mg). After 20 h at 20 °C, the solution was washed with saturated aqueous potassium carbonate (10 cm³), then water, dried and evaporated. Chromatography of the residue on neutral alumina (6 g) in 1:1 benzene–chloroform gave the imino ether **9** (25 mg) and unchanged [3-*O*-methyl-³H]colchicine (83 mg). The latter was recrystallised from ethyl acetate and used for incorporation experiments.

The imino ether **9** in saturated methanolic hydrogen chloride (1 cm³) was kept for 1.5 h at 20 °C, the solution was evaporated and the residue partitioned between dichloromethane (10 cm³) and saturated aqueous potassium carbonate. The organic phase was washed with water, dried and evaporated and the residue **7** (25 mg) was dissolved in hot ethanol (10 cm³) together with (+)-tartaric acid (11 mg) and radio-inactive deacetylcolchicine tartrate (27 mg). Concentration of the solution to *ca.* 5 cm³ afforded N-deacetyl[3-*O*-methyl-³H]colchicine tartrate (30.2 mg; 0.67 µCi mg⁻¹) mp 239–240 °C (lit.,[15] mp 242 °C). This and the other labelled alkaloids were finally identified by comparison with standard samples.[4,6,7,15,16]

A preparative run (with the late J. H. Clements) on colchicine (6 g) gave imino ether **9** (2 g) and colchicine (3.6 g) (76% yield based on unrecovered starting material). Methanolysis of the imino ether (450 mg) as above gave N-deacetylcolchicine (338 mg, 90%).

N-Formyl-N-deacetyl[3-*O*-methyl-³H]colchicine 6. The foregoing radioactive tartrate (14 mg) and a radio-inactive sample of the same compound (20 mg) were together dissolved in water (5 cm³). Addition of potassium hydrogen carbonate and extraction with chloroform (4 × 5 cm³) gave by evaporation a residue which was dissolved in dry pyridine (1 cm³). Formic acid (98%; 0.25 cm³) was added, the solution was heated at 60 °C for 3 h, then evaporated and the residue was crystallised twice from ethyl acetate to afford N-formyl-N-deacetyl[3-*O*-methyl-³H]colchicine **6** (9.4 mg; 0.29 µCi mg⁻¹), mp 261–263 °C (lit.,[16] mp 265 °C). A further sample of lower specific activity (14.4 mg; total activity 1.2 µCi) was obtained from the mother liquor after dilution with radio-inactive **6**.

[3-*O*-methyl-³H]Demecolcine 3. 3-Demethyldemecolcine **11** (21.8 mg) was treated with ethereal diazomethane in dry dioxane in the presence of tritiated water, as described above for **4** to give [3-*O*-methyl-³H]demecolcine (7.1 mg; 5.8 µCi mg⁻¹), identified by comparison with an authentic unlabelled sample.

[*N*-methyl-¹⁴C]Demecolcine 3. N-Deacetylcolchicine (108 mg: recovered from its tartrate) in dry acetonitrile (12 cm³) was treated with [¹⁴C]methyl iodide (2.5 mg; 0.5 mCi). The transfer of radioactive methyl iodide was made *in vacuo* and after the reaction mixture had been kept at 20 °C for 3 days in a sealed tube, radio-inactive methyl iodide was added (0.3 cm³ of a 1% v/v solution in acetonitrile). After a further 24 h, the solution was evaporated and the products were separated by PLC using benzene–ethyl acetate–diethylamine (5:4:1) on Kieselgel HK 254 to give [*N*-methyl-¹⁴C]demecolcine (8.2 mg; 46.2 µCi mg⁻¹) identified as in the previous preparation.

N-Formyl[3-*O*-methyl-³H]demecolcine 5. This was prepared from [3-*O*-methyl-³H]demecolcine **3** by treatment with formic acid in pyridine, as described above for **6**, mp 189–190 °C from methanol–diethyl ether (lit.,[16] mp 188 °C).

Administration of labelled precursors to the plants

Solutions of the radioactive precursors were prepared as follows:- colchicine **4** in water, autumnaline **1** and demecolcine **3** as hydrochloride salts in water; N-deacetylcolchicine **7** as the tartrate salt in water; N-formyldemecolcine **5** and N-formyl-N-deacetylcolchicine **6** in water containing 5–10% of dimethyl sulfoxide. These solutions were injected into the seed capsules of *Colchicum autumnale* or *Colchicum speciosum* in spring and for *Colchicum byzantinum* were transferred *via* a cotton wick into the corms.[3] Plants were normally harvested after two weeks.

Isolation of alkaloids from incorporation experiments

From *Colchicum autumnale* and *C. byzantinum*. The total alkaloidal fraction was chromatographed on a Celite partition column as earlier[3] and elution with wet 1:1 (v/v) ethyl acetate–light petroleum (bp 40–60 °C) gave the following fractions:- (*a*) N-methyldemecolcine contaminated by chlorophylls and lumicolchicines (75 cm³ of eluate); (*b*) demecolcine (next 150 cm³ of eluate); (*c*) N-formyldemecolcine (next 300 cm³). Further elution with neat ethyl acetate (150 cm³) gave (*d*) colchicine.

Fraction (*a*) was chromatographed on Grade I alumina in chloroform, and the purified N-methyldemecolcine was sublimed at 135 °C/10⁻⁴ mmHg and converted into the oxalate, mp 148–158 °C (decomp.) for crystallisation to constant specific activity from methanol–diethyl ether. Fraction (*c*) was crystallised from acetone–hexane. All four alkaloids were identified by chromatographic and spectroscopic comparison with authentic samples.

From *Colchicum speciosum*. The total mass of alkaloid (1.8 g) from ten flowering plants was obtained as described earlier for *C. autumnale*. Three reference alkaloids, speciosine (50 mg), N-methyldemecolcine (50 mg) and N-formyldemecolcine (50 mg) were added to the total alkaloid and the mixture was chromatographed on a Celite partition column in the usual way.[3] The alkaloids were eluted with 1:1 ethyl acetate–light petroleum (bp 60–80 °C) in the following order: (*a*) speciosine and N-methyldemecolcine (200 mg), (50 cm³ eluate); (*b*) demecolcine (770 mg), (next 650 cm³ eluate); (*c*) N-formyldemecolcine (55 mg), (next 800 cm³). The column was then stripped with ethyl acetate to give (*d*) colchicine (350 mg). Fraction (*a*) was fractionated by PLC (silica, 3% methanol in chloroform) to give speciosine (80 mg) and N-methyldemecolcine (52 mg).

Purification of colchicine and demecolcine

In most cases, fractions (*b*) and (*d*) obtained from the various plants above were sufficiently pure to be recrystallised directly to constant specific activity from dichloromethane–ethyl acetate. However, when the precursor being tested was a radio-active tropolone alkaloid, an extra purification step was included as follows. Fractions (*b*) and (*d*) were each mixed with 5–10 times the amount that had been administered of a radio-inactive sample of the compound that had been used. Demecolcine **3** was then separated from N-formyldemecolcine **5** by partition between dichloromethane and 0.5 M hydrochloric acid and from N-formyl-N-deacetylcolchicine **6** by partition chromatography as above. Colchicine was separated from demecolcine **3** by the usual partition chromatography, from N-formyldemecolcine **5** and from N-formyl-N-deacetylcolchicine **6** by PLC on silica using benzene–ethyl acetate–diethylamine–methanol (5:4:1:0.8) in combination with

crystallisation and from deacetylcolchicine **7** by partition between dichloromethane and 2% aqueous tartaric acid.

Investigation of the metabolism of [3-¹⁴C]autumnaline in *C. autumnale*

(1*RS*)-[3-¹⁴C]Autumnaline was fed as above to *C. autumnale* in spring and separate batches of plants were harvested after 1 day (A) and after 3 days (B). The alkaloids were isolated and separated by solvent partition as described above. The neutral and water soluble fractions were only weakly radioactive but the basic fractions were strongly radioactive and were analysed by TLC on silica using benzene–ethyl acetate–diethylamine–methanol, 5 : 4 : 1 : 0.8. The radioactive metabolites were located by a radioactivity scanner (Desaga 12-20) as highly active spots of R_f 0.17, 0.21 and 0.28 and a less active spot of higher R_f which corresponded to *O*-methylandrocymbine. The basic fraction from batch B (8 mg) was diluted with radio-inactive *O*-methylandrocymbine (50 mg) and subjected to PLC as above. The purified *O*-methylandrocymbine was converted into the picrate using picric acid (35 mg) in hot methanol, and the salt was recrystallised to constant specific activity (1.2% incorporation), mp 155–157 °C. For radio-assay, the free base was regenerated quantitatively from the picrate by passing a chloroform solution of the salt down a short column of basic alumina.

The basic fraction from batch A (20 mg) was partitioned between ethyl acetate and 0.1 M aqueous sodium hydroxide to give a non-phenolic fraction R_f 0.28 (16 mg) and a phenolic fraction (R_f 0.17, 0.21) (3 mg). The non-phenolic fraction was treated with acetic anhydride (0.2 cm³) in pyridine (1 cm³) for 16 h at 20 °C and a radioactive product of appreciably higher R_f value was obtained but no further work has been done on this product.

Isolation of minor alkaloids from *C. autumnale*

250 Plants were harvested in spring when the seed capsules were beginning to develop. The plants were extracted in the usual way[3] to give a total mass of alkaloid of 5 g. Part of this (2.86 g) was partitioned between diethyl ether (150 cm³) and 0.1 M tartaric acid (150 cm³), and the ethereal layer was further washed with 0.1 M tartaric acid (2 × 50 cm³) and water (2 × 25 cm³) before evaporation to give a neutral residue which was not investigated further. The aqueous phase was adjusted to pH 8 with saturated aqueous sodium hydrogen carbonate and extracted with diethyl ether (6 × 100 cm³) to yield the basic fraction (380 mg). Finally the aqueous phase was extracted with chloroform (6 × 100 cm³) to afford water-soluble materials (2.23 g).

The basic fraction (380 mg) in chloroform (50 cm³) and diethyl ether (200 cm³), was extracted with 2 M aqueous sodium hydroxide (4 × 30 cm³). Each alkaline extract was immediately acidified, then adjusted to pH 8 as above and extracted with chloroform (4 × 50 ml) to afford the phenolic bases (140 mg).

The organic phase after removal of phenols was washed with water (2 × 20 cm³) and evaporated to give the non-phenolic bases (220 mg) which were partitioned between 0.5 M phosphate buffer (pH 4.5) (4 cm³) and ethyl acetate (0.5 cm³). Celite (4 g) was added, followed by light petroleum (bp 60–80 °C) (1 cm³) and the mixture was shaken until homogeneous, then packed onto a column of Celite (60 g) which had previously been shaken with the lower layer from a mixture of 0.5 M phosphate buffer (60 cm³), ethyl acetate (1 dm³) and light petroleum (bp 60–80 °C) (1 dm³). Elution of the column with the upper organic phase afforded *O*-methylandrocymbine **2** (10 mg); *m/z* 385 (M⁺); $[a]_D^{24}$ −286 (*c* 0.079 in CHCl₃) {lit.,[17] $[a]_D^{24}$ −295 (*c* 0.127 in CHCl₃)}, further characterised as the picrate,[17] mp 153–154 °C. Its identity was established by full comparison with authentic material.[17]

A second basic product was shown to be O-*acetylspeciosine* **15** (88 mg) by the following data: v_{max}/cm⁻¹ 1750; λ_{max}/nm 213, 245, 252; δ_H 2.03 (3H, s, COCH₃), 2.23 (3H, s, NMe), 1.7–3.5 (6H, m, 3 × CH₂), 3.6 (1H, m, H-7), 3.62 (3H, s, 1-OMe), 3.89, 3.93 and 3.94 (each 3H, s, 3 × OMe), 6.53 (1H, s, H-4), 6.8–7.3 (3H, m, ArH), 6.75 and 7.26 (each 1H, d, *J* 11, H-11 and H-12), 7.6 (1H, m, ArH), 8.23 (1H, s, H-8); *m/z* 519 (M⁺, 28%), 371 (14), 370 (26), 342 (33), 207 (21), 107 (100).

A third fraction afforded speciosine **14** (4.8 mg) *m/z* 477 (M⁺, 14%), 371 (62), 356 (24), 207 (100), 107 (14), 106 (26) identified by full comparison with authentic material.[11] It is common experience to find some phenolic materials of substantial molecular weight in the non-phenolic fraction because of very favourable partition towards the organic phase.

Hydrolysis of *O*-acetylspeciosine

Aqueous 10% sodium hydroxide (0.1 cm³) was added to *O*-acetylspeciosine (15 mg) in methanol (1 cm³); the solution was kept at 20 °C for 16 h and then largely evaporated. Water (1 cm³) was added, followed by solid carbon dioxide, and the mixture was extracted with ethyl acetate to give speciosine (13.7 mg), mp 209–212 °C (lit.,[11] mp 211–214 °C), identified as above.

Hydrolysis of *N*-formyldemecolcine

N-Formyldemecolcine (30 mg) was heated for 20 h in concentrated sulfuric acid (0.2 cm³) and water (1 cm³) at 100 °C, then cooled and diluted with water. The distillate (400 cm³) from steam distillation was basified to phenolphthalein with 0.1 M aqueous lithium hydroxide and then evaporated. The residual solid, thoroughly dried under vacuum, was dissolved in dry DMF (0.5 cm³) and heated at 90 °C for 2.5 h with *p*-bromophenacyl bromide (100 mg). Evaporation of the dimethylformamide gave a solid; the part soluble in diethyl ether was purified by PLC on silica using benzene and the ester was eluted from the silica with anhydrous diethyl ether (400 cm³) to give a solid (16 mg), mp 97–98 °C (lit.,[18] mp 99 °C) from light petroleum (bp 60–80 °C). It was identified by full comparison with the authentic *p*-bromophenacyl formate. This sensitive ester decomposed during attempts to elute it from the silica using chloroform–methanol.

The aqueous solution after steam distillation was adjusted to pH 8 using saturated aqueous sodium hydrogen carbonate and extraction with chloroform yielded demecolceine (typically 40%), mp 133–135 °C from MeOH (lit.,[19] mp 133–135 °C) further identified by comparison with an authentic sample.

The radioactive *N*-formyldemecolcine from Experiment 4, Table 2, was diluted to the specific radioactivity of ¹⁴C $1.84 × 10^5$ and ³H $6.82 × 10^5$ (disintegrations per 100 s per mmol). Degradation as above yielded the *p*-bromophenacyl formate with specific activity (disintegrations per 100 s per mmol) of ¹⁴C $9.48 × 10^4$ (51%) and ³H $4.1 × 10^3$ (0.6%). The fall in specific activity is due to dilution effects (see text).

References

1 Part 27. E. McDonald, R. Ramage, R. N. Woodhouse, E. W. Underhill, L. R. Wetter and A. R. Battersby, *J. Chem. Soc., Perkin Trans. 1*, 1998, 2979.

2 Part of this work has appeared in preliminary form, 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., Perkin Trans. 1*, 1972, 1741.

4 H. Potěšilová, J. Hrbek and F. Šantavý, *Collect. Czech. Chem. Commun.*, 1967, **32**, 141.

5 A. R. Battersby, R. Ramage, A. F. Cameron, C. Hannaway and F. Šantavý, *J. Chem. Soc. (C)*, 1971, 3514.

6 F. Šantavý, *Collect. Czech. Chem. Commun.*, 1950, **15**, 552.

7 F. Šantavý and M. Talas, *Collect. Czech. Chem. Commun.*, 1954, **19**, 141.

8 *cf.* R. F. Raffauf, A. L. Farren and G. E. Ullyot, *J. Am. Chem. Soc.*, 1953, **75**, 3854.

9 J. W. Cook and J. D. Loudon, "The Alkaloids", ed. R. H. F. Manske and H. L. Holmes, vol. 2, Academic Press, New York, 1952, p. 261.

10 T. Oishi, M. Nagai and Y. Ban, *Tetrahedron Lett.*, 1968, 491 and 497.

11 V. V. Kiselev, *Zh. Obshch. Khim.*, 1956, **26**, 3218; R. Ramage, *Tetrahedron*, 1971, **27**, 1499.

12 R. N. Woodhouse, E. McDonald, R. Ramage and A. R. Battersby, *J. Chem. Soc., Perkin Trans. 1*, 1998, 2995.

13 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.

14 M. Saleh, S. El-Gangihi, A. El-Hamidi and F. Šantavý, *Collect. Czech. Chem. Commun.*, 1963, **28**, 3411.

15 A. Uffer, O. Schindler, F. Šantavý and T. Reichstein, *Helv. Chim. Acta*, 1954, **37**, 18.

16 F. Šantavý, R. Winkler and T. Reichstein, *Helv. Chim. Acta*, 1953, **36**, 1319.

17 A. R. Battersby, R. B. Herbert, L. Pijewska, F. Šantavý and P. Sedmera, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1736.

18 *Dictionary of Organic Compounds*, 4th edn., ed. J. R. A. Pollock and R. Stevens, Eyre and Spottiswood, London, 1965.

19 F. Šantavý, Z. Hoscalkova, R. Podivinsky and H. Potěšilová, *Collect. Czech. Chem. Commun.*, 1954, **19**, 1289.