Biosynthesis. Part 26.[1] Synthetic Studies on Structural Modification of Late Biosynthetic Precursors for Colchicine

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The latter part of the natural pathway to colchicine is known to involve conversion of a phenethyltetrahydroisoquinoline (2) by phenol oxidation into a dienone (*O*-methylandrocymbine) (4), the dienone subsequently undergoing ring-expansion to generate the tropolone system. Synthetic routes are here described which yield the isoquinoline and dienone structures in modified forms (16), (17), and (38), all having a *C*-methyl group introduced adjacent to the nitrogen atom. An alternative approach for synthesis of the dienone, involving conversion of a phenanthrene into a dibenzocycloheptene, is also explored.

Incorporation experiments on *Colchicium autumnale* plants using labelled isoquinolines (16a) and (17a) show that they are not converted enzymically into tropolone alkaloids nor is the former significantly cyclised in the plants to the *C*-methylated dienone (38). Clearly, at least one of the enzymes required for the late biosynthetic stages is highly sensitive to structural change close to the nitrogen atom.

The unravelling of the biosynthetic pathway to the tropolone colchicine [2] (1) in *Colchicum* species yielded a major surprise; no one had anticipated that colchicine would prove to be a modified isoquinoline alkaloid! By experiments starting in the mid-1950's, it was found that ring-A of colchicine (1) together with the three saturated carbons of ring-B were derived from L-phenylalanine,[3,4] this first being converted into cinnamic acid [3a,4] (see Scheme 1). Eventually, the origin of the tropolone ring-C was found to be a C(6)–C(1) unit derived from L-tyrosine.[4,5] Then the breakthrough came from the elucidation of the structure of androcymbine (3),[6] a dienone alkaloid from a plant related to the *Colchicum* family. This knowledge made it clear that autumnaline (2) and *O*-methylandrocymbine (4) should be intermediates on the pathway between cinnamic acid and tyrosine on the one hand and colchicine (1) on the other. In fact, strikingly high incorporations of radiolabelled forms of these substances (2) and (4) were achieved into colchicine [7,8] (10% and 15% incorporations, respectively). So far, no intermediates have been isolated to cast light on the mechanism of the fascinating oxidative process of ring-expansion (4)→(5). However, autumnaline (2) has been synthesized labelled with tritium at C-3 and C-4 and feeding experiments with these precursors established [9,10] that stereospecific removal of hydrogen was occurring from both carbon atoms. The remaining steps by which colchicine is produced through the specific sequence (5)→(6)→(7)→(8)→(1) have also been elucidated.[9]

To gain further insight into the conversion (4)→(5), we aimed to synthesise analogues of (2) and (4) which were substituted at one of the atoms directly involved, principally by methylation at C-3 of (2) and at C-13 of (4). Since several enzymic steps are required to convert the dienone (4) into the tropolone (5), it was conceivable because of differing substrate specificities of the enzymes, that new intermediates might accumulate which would shed fresh light on the ring-expansion mechanism. We first describe syntheses of the two diastereoisomers of the 3-methyl analogue of autumnaline (2) in specifically labelled form and of the corresponding 13-methyl analogue of the dienone (4).

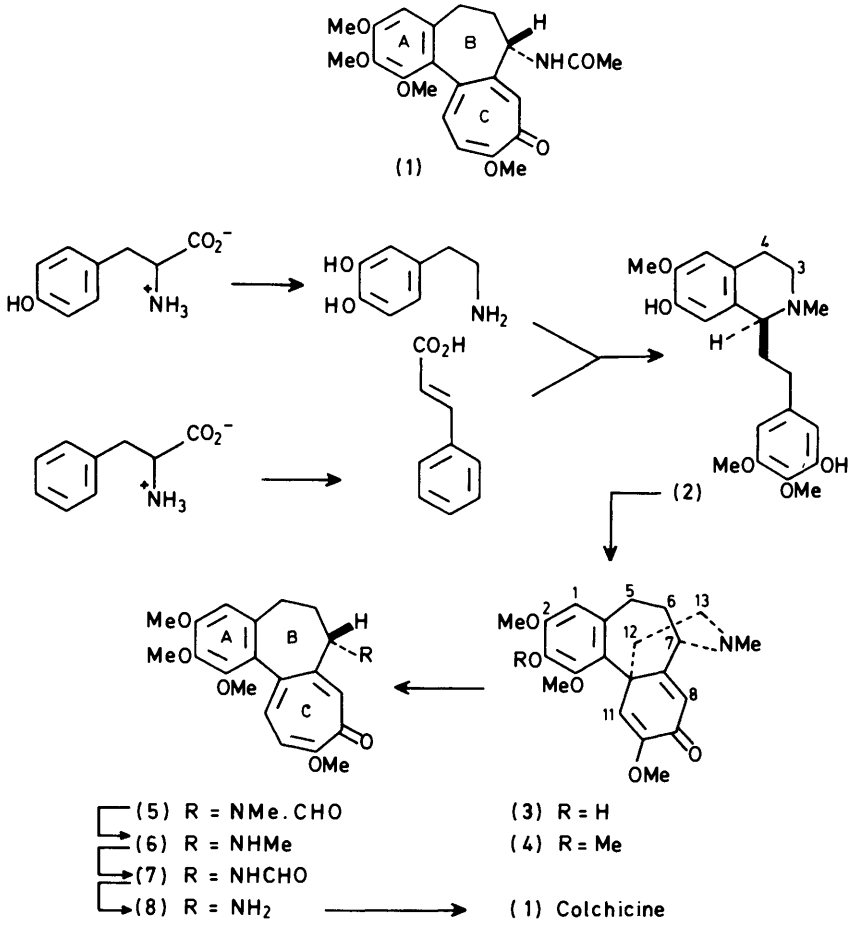
Synthesis of Radiolabelled 3-*Methylautumnalines* (16a) *and* (17a).—The synthetic route (Scheme 2) was based on that used for autumnaline (2) itself.[8] Thus condensation of *O*-benzylvanillin with nitroethane in a potassium acetate–methylamine hydrochloride buffer afforded the nitrostyrene (9) in 80% yield which appeared to be the *E*-isomer (δ 7.96, olefinic H). It was reduced with lithium aluminium hydride [11] to give the racemic amine, which was isolated as its hydrochloride (10) in 55% yield. The requisite 3-[(3-benzyloxy-4,5-dimethoxy)-phenyl]propionic acid (11a) was prepared as earlier [8] from 3-benzyloxy-4,5-dimethoxybenzaldehyde.[12]

Two-phase acylation of the base (10) with the chloride from the acid (11a) afforded the amide (12a) which was ring-closed with phosphorus oxychloride in dry acetonitrile. A single 3,4-dihydroisoquinoline was isolated in 80% yield as its methiodide (13a), which was reduced with sodium borohydride. A separable mixture of the diastereoisomers (14a) and (15a) was formed in high yield, the proportions being 5 : 1 (one enantiomer has been illustrated in each case). The two diastereoisomers had distinctive n.m.r. spectra, but a secure assignment of the relative stereochemistry at C-1 and C-3 was not possible. However, for studies described later, the closely related 3-methylated bases (14b) and (15b) were synthesized by the same procedure as above. In this case, it was established by *X*-ray analysis (see below) that the major isomer had the 1,3-*trans*-configuration. Thus, there can be no doubt that the same holds true for the relatives (14a) (the major isomer) and (15a) (the minor one). Hydrogenolysis of the isomers (14a) and (15a) gave the *trans*- and *cis*-3-methylautumnalines (16a) and (17a) which were isolated as their crystalline hydrochlorides.

In order to introduce a ^{14}C-label, [1-^{14}C]ethyl iodide was treated with a large excess of sodium nitrite in dimethyl sulphoxide–dimethylformamide, a solvent known to favour *N*- rather than *O*-alkylation.[13] The reaction was worked up by adding an excess of radioinactive ethyl iodide, giving nitro-[1-^{14}C]ethane in 15% radiochemical yield. Condensation of this material with *O*-benzylvanillin gave the nitro[1-^{14}C]-styrene (9) in Scheme 2, which was carried forward as previously described to give the *trans*- and *cis*-3-methyl[3-^{14}C]-autumnalines (16a) and (17a).

A reference label of tritium was introduced into the *trans*-isomer (16a) by heating the free base in dimethylformamide at 60 °C with tritiated water.[14] The carbon atoms *ortho* and *para* to the phenolic hydroxy groups exchange protium for tritium to give the labelling patterns shown as ' Labelled ' (16a) and it was this material carrying both ^{3}H and ^{14}C which was fed to the plants. The reasons for this double-labelling will become clear in the section on Incorporation Experiments.

Synthesis of Analogues of O-*Methylandrocymbine* (4).—It is evident from Scheme 1 that positions 3 and 4 of autumnaline (2) are biosynthetically equivalent to positions 13 and 12 of *O*-methylandrocymbine (4). Ideally, the synthesis of analogues



Scheme 1.

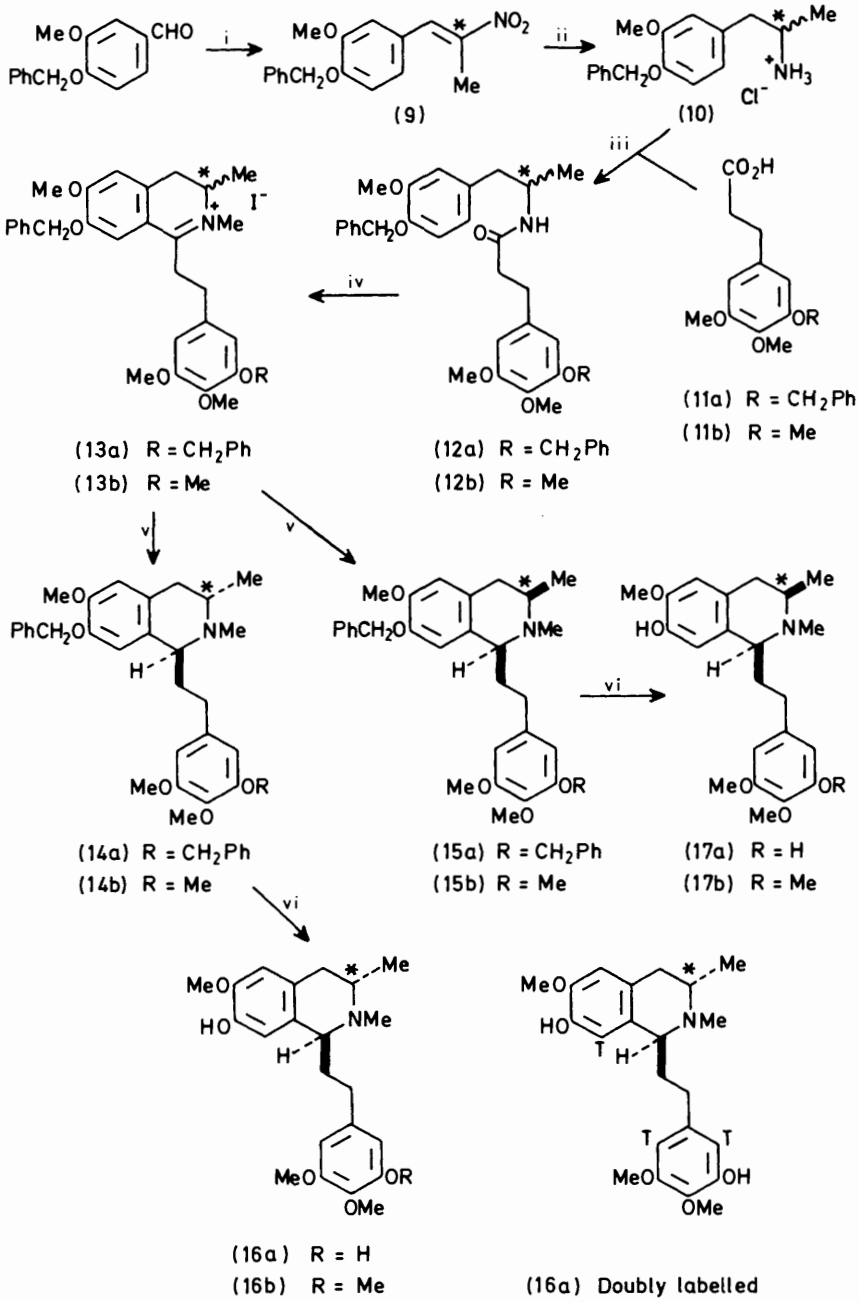
of the dienone (4) should allow introduction of the C-12/C-13 unit at a late stage; this would provide a range of substrates from a common intermediate (*cf.* other synthetic approaches [15] which had been described at the outset of our work).

Our target compound was therefore a correctly substituted dibenzocycloheptene (18), where R^1 (and possibly R also) is a suitable protecting group. The route to be explored for conversion of (18) into the dienones [as (4)], Scheme 3, involved the following steps. (i) Introduction of a C-2 unit by acylation or alkylation at the nitrogen atom to give (19); (ii) deprotection of the phenolic group; and (iii) base-catalysed ring-closure onto ring C of (19) involving aryl-[1,6]-participation.[16]

Condensation of 3,4,5-trimethoxybenzaldehyde with 3-benzyloxy-4-methoxyphenylacetic acid [17] (20) under Perkin conditions [18] afforded a 55—60% yield of the *Z*-α-carboxystilbene (21) (Scheme 4) which was converted into its methyl ester (22); the *Z*-stereochemistry from such condensations has been rationalised.[19] Oxidative closure of the stilbene (22) to the phenanthrene (23) was effected by photolysis [20] in the presence of iodine. The ester grouping conjugated with the stilbene double bond markedly slowed the reaction rate so that irradiation for 70 h was necessary to give a reproducible yield (25%) of the phenanthrene (23). This product was conveniently isolated as its complex with picric acid. The alternative structure (23a) for the phenanthrene, unlikely on steric grounds, was eliminated by the n.m.r. spectrum of the product which showed resonances for 5-H and 8-H as two *singlets* with different chemical shifts [*cf.* 7-H and 8-H for structure (23a)]. Reduction of the ester group by lithium aluminium hydride gave the alcohol (24) and hydrogenolysis under acidic conditions then cleaved both the allylic alcohol and the benzyl ether to give the phenolic 9-methylphenanthrene (25). The *O*-benzyl group was replaced to afford the fully protected system (26); the overall yield from (23) to (26) was 75%.

The stage was now set for cleavage of the phenanthrene 9,10-double bond. Ozonolysis [21] of (26) gave an unpromising mixture probably due to attack at the electron-rich rings A and C. However, osmium tetraoxide in benzene–pyridine yielded a complex which was reductively split [22] to yield the diol (27). This was readily cleaved by either sodium metaperiodate or lead(IV) acetate to form the ketoaldehyde (28) in 65% yield from (26). The desired aldol ring-closure of (28) to the dibenzotropone (29) proved difficult but was eventually achieved in 70% yield using potassium carbonate in aqueous ethanol at high dilution. This product (29) exhibited the characteristic low frequency carbonyl stretch of tropones, $\nu_{max.}(CHCl_3)$ 1635 cm^{-1}. Selective reduction of the olefinic residue was achieved over a rhodium catalyst, to form the ketone (30) in 80% yield.

To introduce the side chain, the ketone (30) was condensed with 2-aminoethanol and the crude product was reduced with sodium borohydride to give the amino-alcohol (31) which was characterised as the picrate salt. Reductive alkylation with formaldehyde–sodium borohydride then gave the *N*-methylamino-alcohol (32) in good yield. Conversion of the alcohol into the chloride proceeded smoothly with thionyl chloride and the chloro-hydrochloride (33) was obtained in over 90% yield. The *O*-benzyl group was readily removed by hydrogenolysis to give phenolic chloro-hydrochloride (34). The



Scheme 2. *Reagents:* i, $EtNO_2$; ii, $LiAlH_4$; iii, $(COCl)_2$ on (11), add (10) plus base; iv, $POCl_3$ then MeI on basic product; v, $NaBH_4$; vi, H_2–Pd

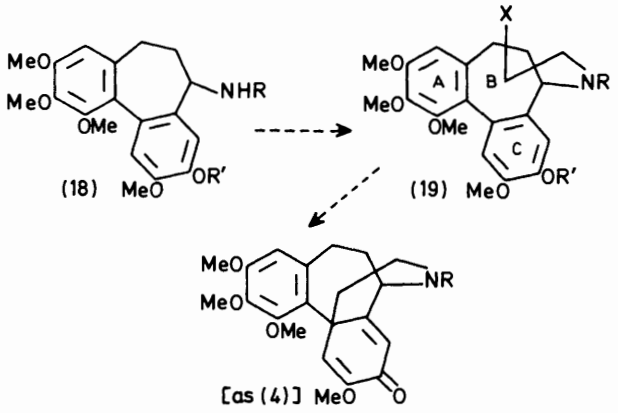
ketone (30) was also condensed with methylamine and the product reduced to afford the secondary base (35).

Many different reaction conditions and bases were studied for the possible ring-closure (34)→(4) but no dienone could be detected. A persistent by-product from these reactions showed *m/z* 328 corresponding to the olefin (36), which could be formed by Hofmann elimination after aziridinium ion formation as indicated in Scheme 5. It seemed that these problems might be overcome by blocking the nucleophilic nitrogen atom but attempts to convert the amino-alcohol (31) into its *N,O*-dimethanesulphonate gave in 70% yield the aziridine (37).

At this stage, our synthetic work took a different tack because an improved variation became available for the oxidative formation of dienones from mono-phenolic ethers which was successful in yielding *O*-methylandrocymbine [23] (4).

To construct *O*-methyl-13-methylandrocymbine (38) by this approach, the mono-phenolic *trans*-phenethyltetrahydroisoquinoline (16b) was required. This was synthesized as for the analogue (16a) by the sequence (10) + (11b)→(12b)→(13b)→(14b) + (15b)→(16b) + (17b). Here, the borohydride reduction of the methiodide (13b) afforded a 9 : 1 mixture of the 1,3-*trans* (14b) and 1,3-*cis* (15b) isomers, respectively. The stereochemical assignment was based on an *X*-ray structure determination of the minor isomer which proved to have the 1,3-*cis*-configuration [24] (15b).

Hydrogenolysis of the benzyl ethers (14b) and (15b) in the presence of hydrogen chloride gave the phenolic amine hydrochlorides (16b) and (17b). These were converted without separation into their borane complexes and oxidised with thallium(III) trifluoroacetate [23] to give a single dienone isolated



Scheme 3.

as its crystalline picrate in 14% yield. This product must by its quantity be the *trans* isomer (38).

Incorporation Experiments with Colchicum autumnale *Plants*.—In separate experiments, aqueous solutions of the hydrochlorides of *cis*-3-methyl[3-^{14}C]autumnaline [(17a), minor isomer] and *trans*-3-methyl[Ar-^{3}H,3-^{14}C]lautumnaline [(16a), major isomer; ^{3}H : ^{14}C ratio = 3.42 : 1], were injected into the capsules of *C. autumnale* plants. After a suitable period of growth, the plants were harvested and extracted, *N*-methylcolchicine (40) [25] being added as carrier prior to the isolation and separation of colchicine (1), demecolcine (6), *N*-formyldemecolcine (5), and *N*-methylcolchicine (40); the carrier material (40) was readily prepared by acetylation of demecolcine (6).

If the labelled 3-methylautumnaline isomers (16a) and (17a) undergo coupling and ring-expansion analogously to the steps (2)→(4)→(5) in Scheme 1, then the initial product will be ^{14}C-labelled 13-methyl-*O*-methylandrocymbine (38) or (39) ready for ring-expansion to give labelled *N*-methylcolchicine (40). Further progress along the biosynthetic pathway should involve hydrolysis of the ^{14}C-labelled *N*-acetyl group to yield demecolcine (6) devoid of ^{14}C-activity but still carrying the ^{3}H-label from the 1,3-*trans*-isomer (16a). In the event, none of the alkaloids was radioactive from feeding the *cis*-base (17a), but the *trans*-isomer (16a) yielded a radioactive demecolcine fraction (1.3×10^{-3} % incorporation, ^{3}H : ^{14}C = 2.30 : 1). In parallel feeding experiments, labelled autumnaline (2) gave the normal high incorporations in the 5—10% range.

The extremely low level of ' incorporation ' into the demecolcine fraction (3—4 orders of magnitude less than normal) pointed against this being a true biosynthetic conversion of precursor into product. Moreover, the labelled material in this fraction contained ^{14}C-activity whereas demecolcine (6) should be devoid of this label (see above). One possibility was that the demecolcine fraction might be carrying 13-methyl-*O*-methylandrocymbine (38) resulting from a successful, but highly inefficient, phenol oxidation step but rejection of the resultant dienone by the enzyme(s) necessary for the ring-expansion process. Accordingly, the radioactive demecolcine fraction was mixed with unlabelled (±)-13-methyl-*O*-methylandrocymbine (38), available from the foregoing synthesis, and the dienone was then reisolated and rigorously purified. It was radioinactive.

The foregoing synthetic and biosynthetic studies show that at least one of the late enzymic steps in the conversion of autumnaline (2) into colchicine (1) is highly sensitive to structural change close to the nitrogen atom. Similar experiments on position 4 of the precursor (2) [corresponding to C-12 of the intermediate dienone (4)] would be of considerable interest.

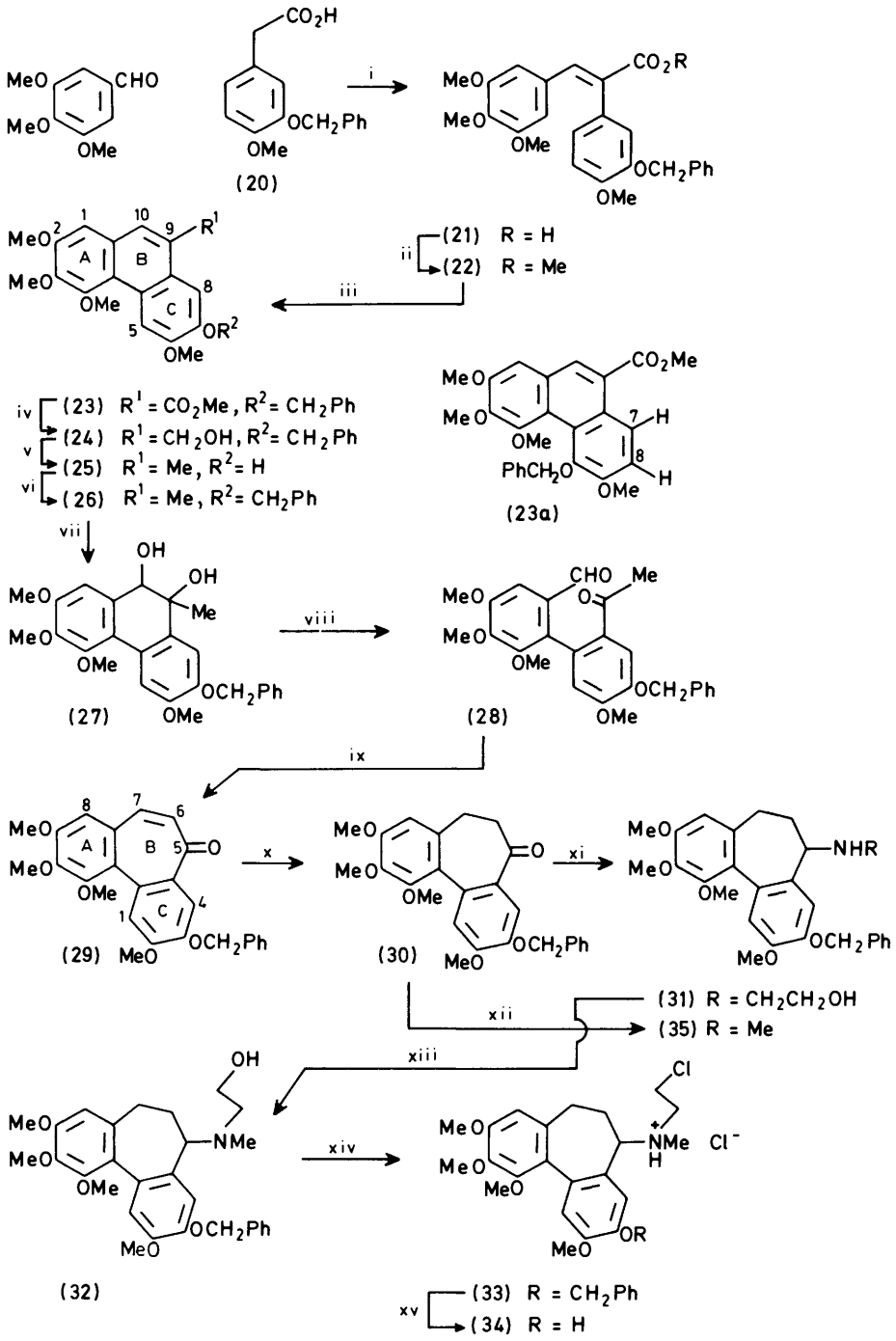
Experimental

Organic extracts were washed finally with saturated brine and dried over anhydrous magnesium or sodium sulphate prior to rotary evaporation at below 40 °C under reduced pressure. M.p.s were determined on a Kofler hot-stage. T.l.c. (qualitative) was carried out on plates coated with Kieselgel F_{254} (Merck), and preparative t.l.c. on 20 × 20 × 0.1 cm plates with the same coating. Alumina for column chromatography was normally grade III neutral. Unless otherwise stated, u.v. spectra were recorded on Unicam SP 800 and 8000 instruments for solutions in 95% ethanol, i.r. spectra for solutions in chloroform on a Perkin-Elmer 257 spectrometer, and n.m.r. spectra for solutions in deuteriochloroform on Varian HA 100 or XL 100 spectrometers. Mass spectra were determined by direct insertion into A.E.I. MS 9 or MS 902 machines. Radioactive samples were assayed as detailed in Part 24,[12b] and the synthesis of labelled compounds is described only where the procedure differed from that used in the unlabelled series. Cultivation of *Colchicum autumnale* plants and the isolation and separation of the alkaloids were carried out as described in Part 6.[4]

E-2-(4-*Benzyloxy*-3-*methoxyphenyl*)-1-*methyl*-1-*nitroethene* (9).—*O*-Benzylvanillin (5 g, 21 mmol) and redistilled nitroethane (1.75 g, 23 mmol) in ethanol (20 ml) were stirred with potassium acetate (1 g) and methylamine hydrochloride (1 g) at 20 °C for 16 h. The resultant mixture was partitioned between water (30 ml) and chloroform (2 × 25 ml), the total organic extract was washed once with water and then evaporated. The residue was recrystallised from ethanol to afford the yellow *nitrostyrene* (4.95 g, 81%), m.p. 90—91 °C (Found: C, 68.15; H, 5.75; N, 4.4. $C_{17}H_{17}NO_4$ requires C, 68.2; H, 5.7; N, 4.7%); $\nu_{max.}$ 1 650, 1 600, 1 580, 1 510, and 1 320 cm^{-1}; $\lambda_{max.}$ 214, 243, and 354 nm; δ 2.40 (3 H, s, CH_3C=C), 3.83 (3 H, s, OCH_3), 5.12 (2 H, s, OCH_2Ar), 6.91 (3 H, s, ArH), 7.0—7.52 (5 H, m, ArH), and 7.96 (1 H, s, CH=CNO_2); *m/z* 299 (M^+) and 253 ($M^+ - NO_2$).

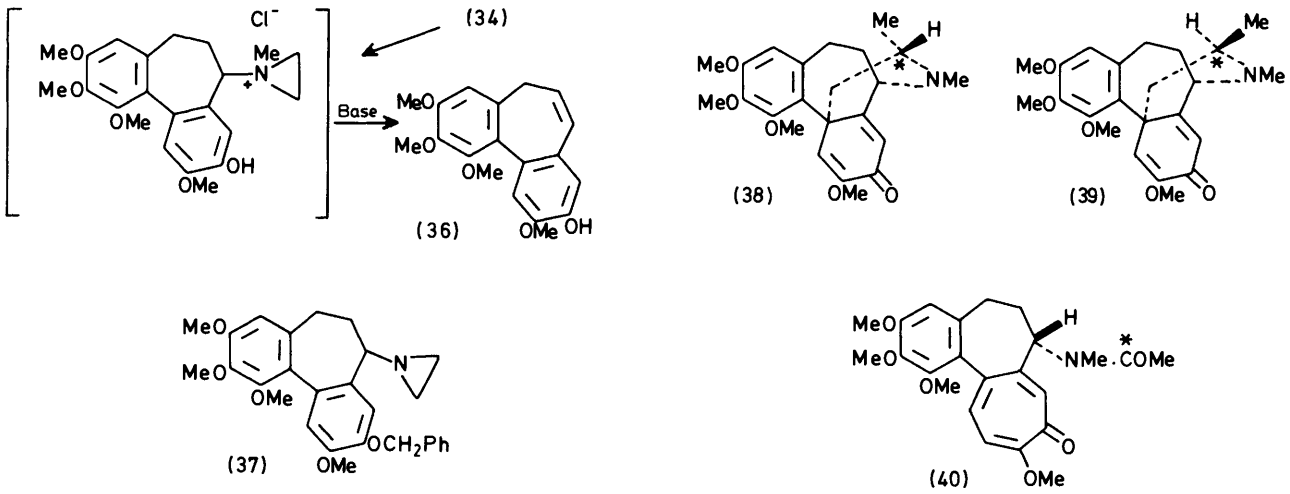
2-*Amino*-1-(4-*benzyloxy*-3-*methoxyphenyl*)*propane Hydrochloride* (10).—A solution of the nitrostyrene (9) (15 g, 50 mmol) in dry tetrahydrofuran (150 ml) was added dropwise during 0.5 h to a well-stirred suspension of lithium aluminium hydride (5.43 g) in dry tetrahydrofuran (375 ml) at gentle reflux under nitrogen.[11] The mixture was then stirred and heated at reflux for a further 2 h, cooled, and worked up by cautious addition of dilute aqueous sodium hydroxide.[26] The precipitated solids were filtered off and subjected to Soxhlet extraction with ether (120 ml). The total organic solutions were evaporated to give the amine (13.2 g) which was dissolved in dry ether (200 ml); gaseous hydrogen chloride was bubbled through the solution until no further precipitation occurred. The solid was collected and recrystallised from ethanol–ethyl acetate to give prisms of the *amine hydrochloride* (9.83 g, 62%), m.p. 182—183 °C (Found: C, 66.35; H, 7.4; Cl, 11.7; N, 4.5. $C_{17}H_{28}NO_2$·HCl requires C, 66.35; H, 7.15; Cl, 11.55; N, 4.55%); $\nu_{max.}$ 3 200—2 300br, 1 600, 1 585, and 1 505 cm^{-1}; $\lambda_{max.}$ 218, 233, and 281 nm; δ (CD_3OD) 1.26 (3 H, d, *J* 6 Hz, CH_3CH), 2.83 (2 H, d, *J* 8 Hz, ArCH_2CH), 3.40—3.60 (1 H, m, CH_2CHCH_3), 3.83 (3 H, s, OCH_3), 5.05 (2 H, s, OCH_2Ph), 6.60—7.02 (3 H, m, ArH), and 7.1—7.5 (5 H, m, ArH); *m/z* 271 (M^+ − HCl), and 256 (M^+ − HCl − CH_3).

N-[(2-(4-*Benzyloxy*-3-*methoxy*)-1-*methylphenethyl*]-3-(3-*benzyloxy*-4,5-*dimethoxyphenyl*)*propionamide* (12a).—3-(3-



Scheme 4. *Reagents:* i, Ac_2O, NaOAc, heat; ii, MeOH, H_2SO_4; iii, *hv*, I_2; iv, $LiAlH_4$; v, H_2–Pd, AcOH; vi, $PhCH_2Cl$, K_2CO_3, KI; vii, OsO_4 then reduce; viii, $NaIO_4$ or $Pb(OAc)_4$; ix, K_2CO_3, H_2O–EtOH; x, H_2–Rh; xi, $H_2NCH_2CH_2OH$ then $NaBH_4$; xii, $MeNH_2$, $NaBH_4$; xiii, CH_2O, $NaBH_4$; xiv, $SOCl_2$; xv, H_2–Pd

Benzyloxy-4,5-dimethoxyphenyl)propionic acid (11a) was prepared in two steps from 3-benzyloxy-4,5-dimethoxybenzaldehyde [12] as described previously.[8] This acid (1.6 g, 5.2 mmol) in dry benzene (30 ml) was treated with oxalyl chloride (1 ml) and dimethylformamide (2 drops). After 2 h at 20 °C, the solution was evaporated and the residue was twice re-evaporated from benzene (10 ml). The resulting acid chloride was dissolved in dry dichloromethane (10 ml) and added dropwise to a vigorously stirred mixture of water (100 ml) and methylene chloride (100 ml) containing sodium hydrogen carbonate (1.2 g) and the amine hydrochloride (10) (1.5 g, 5.2 mmol). After 0.5 h, the organic phase was separated and washed successively with dilute aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water. Evaporation of the organic phase afforded the title *amide* (2.5 g, 91%), m.p. 110—111 °C (from ethyl acetate) (Found: C, 74.1; H, 6.9; N, 2.4. $C_{35}H_{39}NO_6$ requires C, 73.8; H, 6.85; N, 2.45%); $\nu_{max.}$ 3 420, 1 665, 1 595, and 1 515 cm^{-1}; $\lambda_{max.}$ 209, 233, and 278 nm; δ 1.02 (3 H, d, *J* 6 Hz, CH_3CH), 2.16—2.93 (6 H, m, 3 × CCH_2C), 3.9—4.32 (1 H, m, $CHNH$), 3.78 (3 H, s) and 3.80 (6 H, s, 3 × OCH_3), 5.06 (4 H, s, 2 × OCH_2-Ar), 5.18—5.26 (1 H, br d, D_2O exchanged, NH), 6.26—6.84



Scheme 5.

(5 H, m, ArH), and 7.10—7.50 (10 H, m, ArH); *m/z* 569 (M^+).

1-[2-(3-*Benzyloxy*-4,5-*dimethoxyphenyl*)*ethyl*]-7-*benzyloxy*-6-*methoxy*-2,3-*dimethyl*-3,4-*dihydroisoquinolinium Iodide* (13a).—The amide (12a) (0.5 h, 0.88 mmol) in dry redistilled acetonitrile (25 ml) was heated at reflux for 0.5 h with freshly distilled phosphorus oxychloride (0.5 ml). The cooled solution was evaporated to dryness, eventually under high vacuum to remove all phosphorus oxychloride. The residue in chloroform (10 ml) was shaken with 2M-aqueous potassium hydroxide (50 ml) and ether (30 ml). The separated upper layer was washed with water (3 × 20 ml) and evaporated to give an oil which in ethyl acetate (10 ml) was treated with methyl iodide (1 ml) and kept at 0 °C overnight. Yellow crystals of the *methiodide* were then collected (0.49 g, 80%), m.p. 115—120 °C (from acetone–ethyl acetate) (Found: C, 62.0; H, 6.0; N, 1.95; I, 18.2. $C_{30}H_{40}INO_5$ requires C, 62.3; H, 5.8; N, 2.0; I, 18.3%), $\nu_{max.}$ 1 600, 1 590, 1 555, 1 525, and 1 500 cm^{-1}; $\lambda_{max.}$ 229, 251, 310, and 357 nm (shifting in base to 226, 258 and 309sh nm); δ (CD_3OD) 1.29 (3 H, d, *J* 6 Hz, $CHCH_3$), 2.8—3.2 (4 H, m, 2 × CH_2Ar), 3.46—3.62 (2 H, m, CCH_2C=N), 3.72, 3.74, and 3.78 (9 H, 3 s, 3 × OCH_3), 3.98 (3 H, s, NCH_3), 4.1—4.4 (1 H, m, CHN), 5.05 and 5.09 (4 H, 2 s, 2 × OCH_2Ph), 6.50 and 6.60 (2 H, 2 d, *J* 1 Hz, ArH), 7.07 and 7.22 (2 H, 2 s, ArH), and 7.26—7.60 (10 H, m, ArH); *m/z* 566 ($M^+ - I$).

trans-1-[2-(3-*Benzyloxy*-4,5-*dimethoxyphenyl*)*ethyl*]-7-*benzyloxy*-6-*methoxy*-2,3-*dimethyl*-1,2,3,4-*tetrahydroisoquinoline* (14a) *and the Corresponding* cis-*Isomer* (15a).—The methiodide (13a) (300 mg, 0.43 mmol) was dissolved in warm ethanol (10 ml) and to the solution at 20 °C was added sodium borohydride (20 mg). The mixture was stirred for 0.25 h (then colourless), excess reagent was destroyed by dropwise addition of 2M-hydrochloric acid, and the solution was basified using 2M-aqueous sodium hydroxide. Most of the ethanol was evaporated and the residue was partitioned between water (10 ml) and chloroform (10 ml). The organic phase was washed once with water (5 ml), then evaporated to give the diastereoisomeric amines (245 mg, 100%) as a gum. The isomers were separated by p.l.c. on 8 alumina plates which were eluted with ether; extraction of the appropriate bands with hot chloroform afforded the trans-*isomer* (194 mg), R_F 0.40; *picrate*, m.p. 141—142.5 °C (from methanol–ethyl acetate) (Found: C, 63.4; H, 5.5; N, 6.8. $C_{42}H_{44}N_4O_{12}$ requires C, 63.3; H. 5.5; N, 7.0%). The *cis*-isomer (34 mg), R_F 0.50, *picrolonate*, m.p. 181—182 °C (from ethanol–ethyl acetate) (Found: C, 66.1; H, 5.9; N, 8.3. $C_{46}H_{49}N_5O_{10}$ requires C, 66.4; H, 6.0, N, 8.4%). The trans-*isomer*, *free base* had $\nu_{max.}$ 1 590 and 1 510 cm^{-1}; $\lambda_{max.}$ 213, 235sh, and 283 nm; δ 1.19 (3 H, d, *J* 6 Hz, $CHCH_3$), 1.58—2.08 (2 H, m, $C{-}CH_2{-}C$), 2.28 (3 H, s, NCH_3), 2.50—2.72 (4 H, m, 2 × CCH_2Ar), 3.10—3.56 (2 H, m, 2 × NC*H*), 3.82 (9 H, s, 3 × OCH_3), 5.07 and 5.09 (4 H, 2 s, 2 × OCH_2Ph), 6.40, 6.42, 6.53, and 6.57 (4 H, 4 s, ArH), and 7.10—7.68 (10 H, m, ArH); *m/z* 567 (M^+) and 552 ($M^+ - CH_3$). The cis-*isomer*, *free base* showed δ 1.20 (3 H, d) and 2.33 (3 H, s) with other spectral data virtually identical.

trans-7-*Hydroxy*-1-[2-(3-*hydroxy*-4,5-*dimethoxyphenyl*)-*ethyl*]-6-*methoxy*-2,3-*dimethyl*-1,2,3,4-*tetrahydroisoquinoline* (16a) *and the Corresponding* cis-*Isomer* (17a).—The 1,3-*trans* isomer of the dibenzyl ether (14a) (194 mg, 0.34 mmol) in methanol (10 ml) containing 3M-aqueous hydrochloric acid (0.2 ml) was shaken with 5% palladium on charcoal (50 mg) and hydrogen under ambient conditions until the theoretical uptake was just exceeded; the solution was then filtered through Celite. The filtrate and hot methanol washings (10 ml) were evaporated to give the diphenolic amine hydrochloride (145 mg, quant.), m.p. 224—226.5 °C (decomp.) (from methanol–ether). The *hydrobromide* (saturated aqueous sodium bromide added to an aqueous solution of the hydrochloride) had m.p. 247—248 °C (decomp.) (Found: C, 56.6; H, 6.4; Br, 16.8; N, 3.0. $C_{22}H_{30}BrNO_5$ requires C, 56.4; H, 6.4; Br, 17.05; N, 3.0%). The free base recovered from the hydrobromide showed $\nu_{max.}$ 3 530br, 1 595, and 1 510 cm^{-1}; $\lambda_{max.}$ 210, 235sh, and 283 nm, shifting in base to 213, 250sh, 290, and 310sh nm; δ 1.20 (3 H, d, *J* 6 Hz, $CHCH_3$), 1.82—2.16 (2 H, m, CCH_2C), 3.32 (3 H, s, NCH_3), 3.44—3.82 (4 H, m, 2 × CCH_2Ar), 3.18—3.62 (2 H, m, 2 × C*H*N), 3.82 (9 H, s, 3 × OCH_3), 5.34 (2 H, br s, D_2O exchanged, 2 × OH), 6.32 and 6.44 (2 H, 2d, *J* 2 Hz, ArH ring C), and 6.51 and 6.61 (2 H, 2 s, ArH ring A); *m/z* 387 (M^+), 386, 385 ($M^+ - H$, $M^+ - 2H$ respectively), and 372 ($M^+ - CH_3$).

The cis-*isomer* (17a) was similarly obtained; the free base was distinguishable by showing a signal at δ 1.24 (3 H, s). The free bases (16a) and (17a) were obtained by partition of the appropriate hydrochloride between saturated aqueous sodium hydrogen carbonate and methylene chloride (4 portions).

Synthesis of the Radiolabelled trans- *and* cis-*Isomers* (16a) *and* (17a).—Iodo[1-^{14}C]ethane (1 mCi) was transferred *in vacuo* from a ' break-seal ' ampoule into a mixture containing radioinactive iodoethane (120 mg), sodium nitrite (400 mg),

and dry dimethyl sulphoxide (2 ml) cooled in liquid nitrogen. The vacuum was released and the mixture allowed to warm to 20 °C; it was then stirred for 1 h. The nitro[1-^{14}C]ethane produced was transferred *in vacuo* into a mixture of *O*-benzylvanillin (100 mg), anhydrous potassium acetate (20 mg), methylamine hydrochloride (20 mg), and ethanol (0.5 ml); the enclosed reaction mixture was then stirred at 20 °C for 16 h. Excess radioinactive nitroethane was added and stirring was continued until no aldehyde was visible by t.l.c. Work-up as described previously gave the nitro[1-^{14}C]styrene (9), labelled as shown in Scheme 2; total activity 0.15 mCi. Subsequent steps were carried out as described in the radioinactive series, giving finally the isomeric tetrahydro[3-^{14}C]-isoquinolines (16a) and (17a) as their hydrochlorides. The unlabelled 1,3-*trans*-isomer was tritiated by dissolving the base recovered from the hydrochloride (5 mg) in dimethylformamide (2 ml) in a tube containing tritiated water (300 mCi). The tube was sealed and kept for 17 days at *ca.* 60 °C, when the solution was evaporated and revaporated from methanol (5 × 10 ml) to remove excess of tritiated water and exchangeable tritium. This base was reconverted into its hydrochloride, which was mixed with [3-^{14}C]-labelled material and recrystallised to a constant ^{3}H : ^{14}C ratio to give the 1,3-*trans*-[Ar-^{3}H, 3-^{14}C]isoquinoline (16a) as its hydrochloride; ^{3}H : ^{14}C ratio = 3.42 : 1. The combined total ^{14}C-activity for the 1,3-*trans*-, and 1,3-*cis*-isomers, as their hydrochlorides corresponded to an overall radiochemical yield of 44% from the radiolabelled nitrostyrene (9).

Z-1-(3-*Benzyloxy*-4-*methoxyphenyl*)-1-*carboxy*-2-(3,4,5-*trimethoxy*)*phenylethene* (21).—3,4,5-Trimethoxybenzaldehyde [27] (8.2 g, 41 mmol) and 3-benzyloxy-4-methoxyphenylacetic acid [17] (20) (11.5 g, 41 mmol) were heated at reflux with anhydrous potassium acetate (4.1 g) in acetic anhydride (33 ml) for 16 h.[18] The cooled mixture was poured with stirring into 2M-sulphuric acid (100 ml) and the stirred mixture was heated at reflux for 0.5 h. After the mixture had cooled, the product was extracted into chloroform (3 × 50 ml), and the total extract was washed with water (3 × 50 ml). The residual oil crystallised from ether (150 ml), and the solid was collected, washed with ether, and dried to give the *cis-stilbene* (11.05 g, 58%), m.p. 178—179 °C (from ethyl acetate) (Found: C, 68.85; H, 5.9. $C_{20}H_{26}O_7$ requires C, 69.3; H, 5.8%); $\nu_{max.}$ 3 200—2 700, 1 680, and 1 610 cm^{-1}; $\lambda_{max.}$ 215, 233, and 303 nm; δ 3.56 (6 H, s, 2 × OCH_3), 3.84 and 3.89 (6 H, 2 s, 2 × OCH_3), 5.10 (2 H, s, OCH_2Ph), 6.36 (2 H, s, ArN), 6.92 (3 H, ArH), 7.2—7.5 (5 H, m, ArH), 7.82 (1 H, s, CH=C), and 11.5 (1 H, br s, D_2O exchanged, CO_2H); *m/z* 450 (M^+) and 406 ($M^+ - CO_2$).

Z-1-(3-*Benzyloxy*-4-*methoxyphenyl*)-1-*methoxycarbonyl*-2-(3,4,5-*trimethoxyphenyl*)*ethene* (22).—The foregoing acid (21) (5 g) was heated at reflux in methanol (100 ml) with concentrated sulphuric acid (4 ml) for 2 h; the mixture was then cooled to 0 °C. The resultant crystals were collected and washed with cold solvent to give the *methyl ester* (4.75 g, 92%), m.p. 129—130 °C (from methanol) (Found: C, 69.8; H, 5.8. $C_{27}H_{28}O_7$ requires C, 69.8; H, 6.0%); $\nu_{max.}$ 1 705 and 1 615 cm^{-1}; $\lambda_{max.}$ 230 and 310 nm; δ 3.50 (6 H, s, 2 × OCH_3), 3.71, 3.77, and 3.85 (9 H, 3 s, 3 × OCH_3), 5.05 (2 H, s, OCH_2Ph), 6.28 (2 H, s, ArH), 6.7—6.9 (3 H, m, ArH), 7.1—7.45 (5 H, m, ArH), and 7.68 (1 H, s, CH=C); *m/z* 464 (M^+), 433 ($M^+ - OCH_3$), and 432 ($M^+ - HOCH_3$, with *m** at 403).

7-*Benzyloxy*-2,3,4,6-*tetramethoxy*-9-*methoxycarbonyl*-*phenanthrene* (23).—The ester (22) (5 g, 10.8 mmol) was dissolved in redistilled tetrahydrofuran (11) containing iodine (0.5 g, *ca.* 2 mmol) and a little solid ferrous sulphate. The solution, in a flask open to the atmosphere, was irradiated with a 125-W medium-pressure mercury lamp in a quartz sheath, the whole apparatus being enclosed in aluminium foil. The progress of the reaction was monitored by t.l.c. and by u.v. (appearance of a strong maximum at 270 nm). After 70 h, the solution was removed and concentrated to *ca.* 50 ml; chloroform (100 ml) was then added and the mixture shaken with 1% aqueous sodium thiosulphate solution (100 ml) and water (3 × 100 ml). The residue (6.5—7.0 g) from the organic phase was mixed with picric acid (7 g) in ethanol (28 ml). The red solid which separated (6.5 g) was recrystallised from ethanol to give the picric acid complex (2.5 g) of (23). This material in chloroform was chromatographed on Spence 'H' alumina (80 g) and eluted with chloroform, the fluorescent fractions containing phenanthrene being collected. The recovered *phenanthrene methyl ester* (1.25 g, 25%) had m.p. 155—156 °C (from ethyl acetate) (Found: C, 69.8; H, 5.7. $C_{27}H_{26}O_7$ requires C, 70.1; H, 5.6%); $\nu_{max.}$ 1 710 and 1 610 cm^{-1}; $\lambda_{max.}$ 220, 245sh, 271vs, 292sh, 326, and 380sh nm; δ 3.96—4.10 (15 H, 4 s, 5 × OCH_3), 5.33 (2 H, s, OCH_2Ph), 7.13 (1 H, s, 1-H), 7.3—7.7 (5 H, m, ArH, benzyl ether), 8.30 (1 H, s, 10-H), 8.64 (1 H, s, 8-H), and 9.22 (1 H, s, 5-H); *m/z* 462 (M^+) and 431 ($M^+ - OCH_3$).

7-*Benzyloxy*-9-*hydroxymethyl*-2,3,4,6-*tetramethoxyphenanthrene* (24).—The ester (23) (200 mg, 0.4 mmol) in dry tetrahydrofuran (15 ml) was treated cautiously with lithium aluminium hydride (40 mg) at 0—5 °C. The solution was allowed to regain room temperature and after 0.5 h the excess of hydride was destroyed by cautious dropwise addition of water. (On larger-scale runs, basic work-up [26] proved more convenient.) The filtered solution (Celite) and hot chloroform washings were evaporated to afford the *alcohol* (178 mg, 96%), m.p. 169—170 °C (from chloroform–ethyl acetate) (Found: C, 71.9; H, 6.15. $C_{26}H_{26}O_6$ requires C, 71.9; H, 6.0%); $\nu_{max.}$ 3 480br and 1 610 cm^{-1}; $\lambda_{max.}$ 215, 261, 282, 309, 340, and 354 nm; δ 1.60 (1 H, br s, D_2O exchanged, OH), 3.98, 4.00, 4.05, and 4.10 (12 H, 4 s, 4 × OCH_3), 5.0 (2 H, s, CCH_2OH), 5.35 (2 H, s, OCH_2Ph), 7.05 (1 H, s, 1-H), 7.2—7.7 (7 H, m, 8-H + 10-H + ArH of benzyl ether), and 9.20 (1 H, s, 5-H); *m/z* 434 (M^+) and 432 ($M^+ - 2$ H).

7-*Hydroxy*-2,3,4,6-*tetramethoxy*-9-*methylphenanthrene* (25).—The preceding alcohol (24) (2.3 g, 5.3 mmol) was suspended in acetic acid (110 ml) and hydrogenated under ambient conditions using 5% palladised charcoal (0.9 g). Complete dissolution was gradually obtained, and when uptake of hydrogen was complete, the solution was filtered (Celite); the solids were then washed with chloroform (100 ml). Evaporation of the total chloroform solution, eventually at 0.1 mmHg, gave the *phenol* (1.75 g, 100%), m.p. 151.5—152.5 °C (from methanol) (Found: C, 69.3; H, 6.2. $C_{19}H_{20}O_5$ requires C, 69.5; H, 6.1%); $\nu_{max.}$ 3 540 and 1 610 cm^{-1}; $\lambda_{max.}$ 220, 260, 281, 310, 342, and 355, shifting in base to 273, 292sh, 310sh, 353, and 367 nm; δ 2.60 (3 H, s, C=CCH_3), 3.96, 3.97, 3.98, and 4.05 (12 H, 4 s, 4 × OCH_3), 6.97 (1 H, s, 1-H), 7.33 (1 H, s, 10-H), 7.47 (1 H, s, 8-H), and 9.10 (1 H, s, 5-H); *m/z* 328 (M^+), 327 (w, $M^+ - H$), and 313 ($M^+ - CH_3$, with *m** at 299).

7-*Benzyloxy*-2,3,4,6-*tetramethoxy*-9-*methylphenanthrene* (26).—A stirred solution of the phenol (25) (3.8 g, 11.5 mmol) in redistilled butan-2-one (125 ml) was heated at reflux with redistilled benzyl chloride (2 g), anhydrous potassium carbonate (2.1 g), and potassium iodide (100 mg) for 16 h. The mixture was then filtered through Celite; the solids were washed with chloroform (50 ml) and the total solution on evaporation gave the crude product (4.9 g). Recrystallisation from ethyl acetate gave the *benzyl ether* (4.3 g, 88%) as needles,

m.p. 174—175 °C (Found: C, 74.5; H, 6.3. $C_{26}H_{26}O_5$ requires C, 74.6; H, 6.4%); $\nu_{max.}$ 1 610 cm^{-1}; $\lambda_{max.}$ 215, 263, 282, 311, 342, and 360 nm; δ 2.51 [3 H, s, C=C(CH_3)], 3.94 (3 H, s), 3.98 (6 H, s), and 4.06 (3 H, s, 4 × OCH_3), 5.31 (2 H, s, OCH_2Ph), 6.96 (1 H, s, 1-H), 7.21—7.62 (7 H, m, 8-H + 10-H + ArH of benzyl ether), and 9.18 (1 H, s, 5-H); *m/z* 418 (M^+), 403 (w, $M^+ - CH_3$), and 327 ($M^+ - C_7H_7$, with *m** at 256).

7-*Benzyloxy*-2,3,4,6-*tetramethoxy*-9,10-cis-*dihydroxy*-9-*methyl*-9,10-*dihydrophenanthrene* (27).—The 9-methylphenanthrene (26) (1.15 g, 2.75 mmol) in benzene (10 ml) and dry pyridine (5 ml) was treated with fresh osmium tetraoxide (1 g) and the flask was stoppered and sealed. After 4 weeks some solid had separated; the mother-liquors were poured into n-hexane (20 ml) and the precipitated solids were collected, washed with ether, and dried *in vacuo* (combined wt. *ca.* 2.5 g). A suspension of this solid in water–ethanol (1 : 4; 500 ml) was stirred and heated at reflux with anhydrous sodium sulphite (50 g) [27] for 8 h. The mixture was cooled and filtered (Celite), the collected precipitate being washed with ethanol (100 ml). The bulk of the ethanol was evaporated and the residue (*ca.* 50 ml) was extracted with dichloromethane (5 × 70 ml) to yield the *diol* which slowly solidified (1.25 g, 100%); t.l.c. showed traces of faster-moving yellow materials but this product was sufficiently pure to use in the next step; m.p. 135—136 °C (from methanol) (Found: C, 68.5; H, 5.9%. $C_{26}H_{28}O_7$ requires C, 69.0; H, 6.2%); $\nu_{max.}$ 3 600—3 200s, br and 1 595 cm^{-1}; $\lambda_{max.}$ 217, 238, 284, and 302 nm; δ 2.15 [3 H, s, C(OH)CH_3], 2.56 (2 H, br s, D_2O exchanged, 2 × OH), 3.77 (3 H, s), 3.88 (6 H, s), and 3.91 (3 H, s, 4 × OCH_3), 4.18 (1 H, s, ArCHOH), 5.19 (2 H, s, OCH_2Ph), 6.74 (1 H, s, 1-H), 7.20—7.56 (6 H, m, 8-H + ArH of benzyl ether), and 8.01 (1 H, s, 5-H); *m/z* 452 (M^+), 450 ($M^+ - 2$ H), 434 ($M^+ - H_2O$), and 361 ($M^+ - C_7H_7$).

2-*Acetyl*-4-*benzyloxy*-2′-*formyl*-4′,5,5′,6′-*tetramethoxybiphenyl* (28).—The preceding diol (27) (1.25 g, 2.75 mmol) in dry benzene (40 ml) was stirred at 20 °C for 1 h with lead(IV) acetate [22] (1.25 g). The suspension was filtered (Celite) and the collected precipitate was washed with benzene (20 ml); the combined organic solutions were then washed with water (3 × 50 ml). Evaporation gave a product which was recrystallised from methanol to give needles of the *keto-aldehyde* [0.80 g, 64% overall yield from (26)], m.p. 117—118.5 °C (Found: C, 69.5; H, 6.4. $C_{26}H_{26}O_7$ requires C, 69.3; H, 6.7%); $\nu_{max.}$ 2 750w, 1 680, 1 675, and 1 590 cm^{-1}; $\lambda_{max.}$ 236 and 278 nm; δ 2.14 (3 H, s, ArCOCH_3), 3.54, 3.83, 3.89, and 3.91 (12 H, 4 s, 4 × OCH_3), 5.19 (2 H, s, OCH_2Ph), 6.64 (1 H, s, 3-H), 7.14—7.54 (7 H, m, 6-H + 3′-H + ArH of benzyl ether), and 9.51 (1 H, s, ArCHO); *m/z* 450 (M^+), 421 ($M^+ - CHO$), and 407 ($M^+ - COCH_3$, with *m** at 368).

3-*Benzyloxy*-2,9,10,11-*tetramethoxy*-5H-*dibenzo*[a,c]*cyclohepten*-5-*one* (29) *.—The preceding keto-aldehyde (28) (400 mg, 0.93 mmol) in 1 : 1 aqueous ethanol (20 ml) was heated at reflux and stirred for 0.5 h with anhydrous potassium carbonate (0.4 g). The mixture was diluted with water (10 ml) and the cooled solution extracted with chloroform (2 × 20 ml); the organic phase was washed once with water and evaporated. The resultant yellow solid (390 mg) was chromatographed on Spence H alumina (50 g), the fractions containing the yellow band being pooled and evaporated to give the *dibenzocycloheptenone* (295 mg, 73%), m.p. 148—149 °C (from methanol) (Found: C, 71.9; H, 5.6. $C_{26}H_{24}O_6$ requires C, 72.2; H, 5.5%); $\nu_{max.}$ 1 635 and 1 595 cm^{-1}; $\lambda_{max.}$ 227, 235, and 279 nm; δ 3.41 (3 H, s), 3.92 (6 H, s), and 3.98 (3 H, s, 4 × OCH_3), 5.23 (2 H, s, OCH_2Ph), 6.78 (1 H, s, ArH ring A), 6.51 (1 H, d, *J* 12 Hz), and 7.14 (1 H, d, *J* 12 Hz, ArCH=CHCO), 7.3—7.6 (6 H, m, ArH), and 7.60 (1 H, s, ArH ring C); *m/z* 432 (M^+), 404 (M^+ − CO, with *m** at 378), and 341 ($M^+ - C_7H_7$).

3-*Benzyloxy*-2,9,10,11-*tetramethoxy*-6,7-*dihydro*-5H-*dibenzo*[a,c]*cyclohepten*-5-*one* (30).—The dibenzocycloheptenone (29) (548 mg, 1.3 mmol) in ethyl acetate (40 ml) was shaken with hydrogen and 5% rhodium on charcoal (25 mg) until the theoretical uptake of hydrogen (28 ml) was complete. The catalyst was filtered off (Celite), washed with ethyl acetate, and the combined solutions evaporated; recrystallisation of the residue from methanol afforded the *ketone* (440 mg, 80%), m.p. 137—138 °C (Found: C, 71.9; H, 6.25. $C_{26}H_{26}O_6$ requires C, 71.9; H, 6.0%); $\nu_{max.}$ 1 665 and 1 600 cm^{-1}; $\lambda_{max.}$ 226, 230sh, 255, 285sh to 380 nm; δ 2.5—3.1 (4 H, m, CH_2CH_2), 3.48 (3 H, s), 3.89 (6 H, s), and 3.91 (3 H, s, 4 × OCH_3), 5.19 (2 H, s, OCH_2Ph), 6.60 (1 H, s, ArH ring A), and 7.1—7.6 (7 H, m, ArH); *m/z* 434 (M^+), and 343 ($M^+ - C_7H_7$, with *m** at 271).

3-*Benzyloxy*-5-(2-*hydroxyethylamino*)-2,9,10,11-*tetramethoxy*-6,7-*dihydro*-5H-*dibenzo*[a,c]*cycloheptene* (31).—The ketone (30) (100 mg, 0.23 mmol) in n-butanol (15 ml) was heated at reflux for 6 h with 2-aminoethanol (200 mg). The solution was evaporated and the residue in ethanol (10 ml) was stirred at 20 °C for 0.5 h with sodium borohydride (20 mg). Excess of reagent was destroyed by dropwise addition of 2M-hydrochloric acid after which the solution was basified with 10% aqueous sodium hydroxide. Addition of water (20 ml) and extraction with chloroform (2 × 10 ml) gave extracts which were washed with water (10 ml) and evaporated. The product was isolated as its *picrate* from ethanol (144 mg, 88%), m.p. 196—197 °C (Found: C, 57.7; H, 5.1; N, 7.9. $C_{34}H_{36}N_4O_{13}$ requires C, 57.6; H, 5.1; N, 7.9%). Passage of a chloroform solution of the picrate through alumina gave the *amino-alcohol* (31); $\nu_{max.}$ 3 600—3 200br and 1 600 cm^{-1}; $\lambda_{max.}$ 223, 266, and 285sh nm; δ 1.90—2.60 (8 H, m, 6 H on D_2O exchange, NCH_2C + 2 × CCH_2C + NH + OH), 3.10—3.58 (3 H, m, OCH_2C + NCHAr), 3.60 (3 H, s), and 3.88 (9 H, s, 4 × OCH_3), 5.26 (2 H, s, OCH_2Ph), 6.56 (1 H, s, ArH ring A), 7.07 (2 H, d, *J* 2 Hz, ArH ring C), and 7.23—7.56 (5 H, m, ArH of benzyl ether); *m/z* 479 (M^+) and 434 ($M^+ - CH_2CH_2OH$).

3-*Benzyloxy*-5-[2-*hydroxyethyl*(*methyl*)*amino*]-2,9,10,11-*tetramethoxy*-6,7-*dihydro*-5H-*dibenzo*[a,c]*cycloheptene* (32).—The amino-alcohol (31) (40 mg, 0.09 mmol) in boiling absolute ethanol (5 ml) was treated with aqueous formaldehyde (0.5 ml) and acetic acid (5 drops). The solution was cooled to 20 °C, stirred, and treated with sodium borohydride [1] (50 mg) for 0.5 h. Work-up was as described in the preparation of (31) and gave the chromatographically homogeneous tertiary amino-alcohol (32) as a gum (41 mg, 100%); *picrate*, m.p. 181—183 °C (from ethanol) (Found: C, 58.2; H, 5.5; N, 7.7. $C_{35}H_{38}N_4O_{13}$ requires C, 58.2; H, 5.3; N, 7.8%). The *free base*, recovered as above from the picrate, showed $\nu_{max.}$ 3 600—3 200br, 1 600, and 1 585sh cm^{-1}; $\lambda_{max.}$ 226, 265, and 290sh nm; δ 2.16 (3 H, s, NCH_3), 2.0—2.8 (7 H, m, 6 H after D_2O exchange, NCH_2C + ArCHC + CCH_2C + OH), 3.04—3.25 (1 H, m, CHN), 3.59 (3 H, s, OCH_3), 3.52—3.70 (2 H, m, OCH_2C), 3.87 and 3.91 (9 H, 2 s, 3 × OCH_3), 5.23 (2 H, s, OCH_2Ph), 6.55 (1 H,

* Throughout the Experimental section names based on ' dihydrodibenzocycloheptene ' have been used; in earlier papers the names for compounds of this type were based on ' dibenzocycloheptadiene '. The former names are in accord with the IUPAC rules of organic nomenclature.

s, ArH ring A), 7.02 (1 H, s, ArH ring C), and 7.18—7.60 (6 H, m, ArH); *m/z* 493 (M^+), 462 ($M^+ - CH_2OH$), and 419 [$M^+ - N(CH_3)CH_2CH_2OH$].

3-*Benzyloxy*-5-[2-*chloroethyl*(*methyl*)*amino*]-2,9,10,11-*tetramethoxy*-6,7-*dihydro*-5H-*dibenzo*[a,c]*cycloheptene Hydrochloride* (33).—The preceding amino-alcohol (32) (20 mg, 0.022 mmol) in anhydrous tetrahydrofuran (2 ml) was treated with purified thionyl chloride (2 drops), and kept at 20 °C, protected from moisture, for 2 h. The solution was evaporated and then re-evaporated from ether (1 ml), eventually under high vacuum to yield the *chloride hydrochloride* as a labile gum (22 mg, 95%); $\nu_{max.}$ 1 600 and 1 510 cm^{-1}; $\lambda_{max.}$ 225, 267, and 286sh nm; δ 3.75 (3 H, s, $\overset{+}{N}CH_3$), 5.49 (2 H, s, OCH_2Ph), with other signals poorly resolved; *m/z* 513, 511 (1 : 3, M^+ for protonated base for ^{37}Cl, ^{35}Cl respectively), 422, and 420 (1 : 3, $M^+ - C_7H_7$).

5-[2-*Chloroethyl*(*methyl*)*amino*]-2,9,10,11-*tetramethoxy*-6,7-*dihydro*-5H-*dibenzo*[a,c]*cyclohepten*-3-*ol Hydrochloride* (34).—The hydrochloride (33) (22 mg, 0.021 mmol) in methanol (2 ml) was hydrogenated in the presence of 5% palladium on charcoal (5 mg) until 1 equiv. H_2 had been absorbed. The catalyst was filtered off (Celite) and washed with methanol, and the filtrate was evaporated to give the labile *phenolic chloride* as a yellow gum (16 mg, 87%); $\nu_{max.}$ 1 595 and 1 510 cm^{-1}; $\lambda_{max.}$ 224, 269, and 300 shifting in base to 224, 293, and 310 nm; δ 3.85 (3 H, s, $\overset{+}{N}CH_3$), no OCH_2Ph signals, other signals poorly resolved; *m/z* 423, 421 (1 : 3, M^+ for protonated base for ^{37}Cl, ^{35}Cl respectively), and 329 [$M^+ - N(Me)CH_2CH_2Cl$].

Cyclisation Studies on the Phenolic Chloride.—The phenolic chloride hydrochloride (34) was treated with base under various conditions in attempts to effect closure to the dienone system using for each experiment 10—20 mg of (34): (i) sodium hydroxide or ethoxide in ethanol; (ii) sodium hydride in tetrahydrofuran or dimethylformamide; (iii) potassium t-butoxide in t-butyl alcohol. In no case could the desired dienone (4) be detected by its characteristic i.r. or u.v. spectra. From (iii), *ca.* 60% of a phenolic material was obtained which showed olefinic resonances and *m/z* 328 as required for (36). In support of this structure, the amino-alcohol (31) (58 mg, 0.12 mmol) in dry dichloromethane (5 ml) was stirred with 2,6-dimethylpyridine (40 mg) and methanesulphonyl chloride (30 mg) at 0 °C. The mixture was warmed to 20 °C and then kept with exclusion of moisture for 16 h. The solution was diluted to 10 ml with dichloromethane and then washed with 2M-hydrochloric acid (2 × 5 ml), saturated aqueous sodium hydrogen carbonate (5 ml), and water (5 ml). Evaporation of the organic phase gave crude product, which in ether was passed down an alumina column (5 g); the eluate gave, on evaporation, the *aziridine* (37) (40.5 mg, 70%); $\nu_{max.}$ 1 600 and 1 510 cm^{-1}; δ 2.31 (4 H, br s, 2 × NCH_2), 2.63 (2 H, t, *J* 5 Hz, $ArCH_2$), 3.30—3.88 (3 H, m, CH_2CH), 3.61 (3 H, s), and 3.90 (9 H, s, 4 × OCH_3), 5.22 (2 H, s, OCH_2Ph), 6.56, 7.04, and 7.16 (3 H, 3s, ArH), and 7.24—7.58 (5 H, m, ArH of benzyl ether); *m/z* 461 (M^+), 418 ($M^+ - NC_2H_5$), and 370 ($M^+ - C_7H_7$). The peak of *m/z* 418 corresponds to the *O*-benzyl ether of the olefin (36).

3-*Benzyloxy*-2,9,10,11-*tetramethoxy*-5-*methylamino*-6,7-*dihydro*-5H-*dibenzo*[a,c]*cycloheptene* (35).—A solution of the ketone (30) (50 mg, 0.12 mmol) in n-butanol (2.5 ml) was saturated with methylamine gas and sealed in a thick-walled glass tube. The tube was kept at 120 °C for 16 h after which the contents were evaporated and the residue was dissolved in ethanol (2 ml) and reduced with sodium borohydride as described for the preparation of (31). The crude product was chromatographed on Spence H alumina, with ether as eluant, to give the *methylamine* as a gum (21.5 mg, 40%); $\nu_{max.}$ 3 400w, 1 600, and 1 510 cm^{-1}; $\lambda_{max.}$ 225, 265, and 285sh nm; δ 1.65 (2 H, m, CCH_2C), 2.22 (3 H, s, NCH_3), 2.35 (2 H, m, $ArCH_2C$), 3.10—3.80 (2 H, m, 1 H, on D_2O exchange, CH and NH), 3.60 (3 H, s), and 3.88 (9 H, s, 4 × OCH_3), 6.59, 7.09, and 7.17 (3 H, 3s, ArH), and 7.30—7.70 (5 H, m, ArH); *m/z* 449 (M^+), 418 ($M^+ - CH_3NH_2$) (Found: M^+, *m/z* 449.2201. $C_{27}H_{32}NO_5$ requires *M*, *m/z* 449.2210).

N-[2-(4-*Benzyloxy*-3-*methoxy*)-1-*methylphenethyl*]-3-(3,4,5-*trimethoxyphenyl*)*propionamide* (12b).—3-(3,4,5-Trimethoxyphenyl)propionic acid [28] (11b) (1.5 g, 6.25 mmol) was converted into its acid chloride and coupled with the amine (10) as described for the preparation of (12a) to give the *amide* (2.6 g, 83%), m.p. 125—126 °C (from ethyl acetate) (Found: C, 70.85; H, 7.2; N, 2.8. $C_{29}H_{35}NO_6$ requires C, 70.6; H, 7.1; N, 2.8%); $\nu_{max.}$ 3 320, 1 635, 1 590, 1 535, and 1 510 cm^{-1}; $\lambda_{max.}$ 208, 235sh and 278 nm; δ 0.98 (3 H, d, *J* 6 Hz, $CHCH_3$), 2.16—2.94 (6 H, m, 2 × CH_2Ar + $CH_2C{=}O$), 3.72 (3 H, s) and 3.77 (9 H, s, 4 × OCH_3), 3.90—4.30 (1 H, m, CHNH), 5.03 (2 H, s, OCH_2Ph), 5.20 (1 H, br m, D_2O exchanged, NH), 6.28—6.78 (5 H, m, ArH), and 7.15—7.43 (5 H, m, ArH of benzyl ether); *m/z* 493 (M^+).

7-*Benzyloxy*-6-*methoxy*-2,3-*dimethyl*-1-(3,4,5-*trimethoxyphenethyl*)3,4-*dihydroisoquinolinium Iodide* (13b).—The amide (12b) (5 g, 10.1 mmol) was converted [as for (12a)→(13a)] into the *methiodide* (5.4 g, 88%), m.p. 105—110 °C (from acetone–ethyl acetate) (Found: C, 58.5; H, 6.05; N, 2.1. $C_{30}H_{36}INO_5$ requires C, 58.4; H, 5.8; N, 2.3%); $\nu_{max.}$ 1 600, 1 590, 1 560, 1 520, and 1 510 cm^{-1}; $\lambda_{max.}$ 207, 249, 310, and 356, shifting in base to 207, 259, and 305sh nm; δ 1.26 (3 H, d, *J* 6 Hz, $CHCH_3$), 2.60—3.06 (4 H, m, 2 × $ArCH_2C$), 3.40—3.70 (2 H, m, CCH_2N^+), 3.73 (3 H, s), 3.76 (6 H, s) and 3.80 (3 H, s, 4 × OCH_3), 3.97 (3 H, s, $\overset{+}{N}CH_3$), 4.10—4.54 (1 H, m, $CCH\overset{+}{N}$), 5.11 (2 H, s, OCH_2Ph), 6.40 (2 H, s, ArH ring C), 6.87 (1 H, s) and 7.11 (1 H, s, ArH ring A), and 7.20—7.52 (5 H, m, ArH of benzyl ether); *m/z* 490 (M^+ for cation) and 475 ($M^+ - CH_3$).

trans-7-*Benzyloxy*-6-*methoxy*-2,3-*dimethyl*-1-(3,4,5-*trimethoxyphenethyl*)-1,2,3,4-*tetrahydroisoquinoline* (14b) *and the Corresponding* cis-*Isomer* (15b).—Reduction of the methiodide (13b) (100 mg, 0.16 mmol) was as for the conversion (13a)→(14a) and (15a) to give the diastereoisomeric amines (75 mg, 94%), which were separated as before. The *trans*-isomer (54 mg), R_F 0.60 (alumina–ether), m.p. 58—62 °C (from methanol), formed a *picrate*, m.p. 144—146 °C (from ethanol) (Found: C, 60.1; H, 5.5; N, 7.6. $C_{36}H_{40}N_4O_{12}$ requires C, 60.0; H, 5.6; N, 7.8%). The recovered free base showed $\nu_{max.}$ 1 590 and 1 500 cm^{-1}; $\lambda_{max.}$ 215, 235sh, and 282 nm; δ 1.08 (3 H, d, *J* 6 Hz, $CHCH_3$), 1.60—2.08 (2 H, m, CCH_2), 2.20 (3 H, s, NCH_3), 2.36—2.66 (4 H, m, 2 × $ArCH_2C$), 3.00—3.46 (2 H, m, 2 × CHN), 3.71 (12 H, s, 4 × OCH_3), 4.98 (2 H, s, OCH_2Ph), 6.28 (2 H, s, ArH ring C), 6.46 (2 H, s, ArH ring A), and 7.10—7.40 (5 H, m, ArH of benzyl ether); *m/z* 491 (M^+), 400 ($M^+ - C_7H_7$). The *cis*-isomer (6 mg), R_F 0.70 (alumina–ether), m.p. 102—103.5 °C (from ether–light petroleum), exhibited δ 1.11 (3 H, d, *J* 6 Hz, $CHCH_3$) and 2.25 (3 H, s, NCH_3), being otherwise very similar to the *trans*-isomer in spectral characteristics. The *cis*-isomer crystallised well as single needles suitable for an *X*-ray analysis [24] which established the 1-H, 3-H *cis*-configuration.

trans-7-*Hydroxy*-6-*methoxy*-2,3-*dimethyl*-1-(3,4,5-*trimethoxyphenethyl*)-1,2,3,4-*tetrahydroisoquinoline* (16b) *and the Corresponding* cis-*Isomer* (17b).—The *trans*-isomer (14b) (229 mg, 0.47 mmol) was hydrogenated [as for (14a)→(16a)] to give the trans-*phenolic amine hydrochloride* (178.5 mg, 98%). Partition of the salt between aqueous sodium hydrogen carbonate and ethyl acetate gave the free base, which formed a *picrate*, m.p. 141.5—143 °C (from ethanol) (Found: C, 55.4; H, 5.4; N, 9.0. $C_{29}H_{34}N_4O_{12}$ requires C, 55.2; H, 5.4; N, 8.9%). The recovered free base showed $\nu_{max.}$ 3 530, 1 590, and 1 500 cm^{-1}; $\lambda_{max.}$ 215, 240sh, and 284 nm shifting in base to 218, 255sh, and 302 nm; δ 1.20 (3 H, d, *J* 7 Hz, $CHCH_3$), 1.70—2.20 (2 H, m, CCH_2C), 2.32 (3 H, s, NCH_3), 3.40—3.85 (4 H, m, 2 × $ArCH_2C$), 3.04—3.64 (2 H, m, 2 × CHN), 3.81 (12 H, s, 4 × OCH_3), 4.3—5.1 (1 H, br s, D_2O exchanged, OH), 6.43 (2 H, s, ArH ring C), 6.53 (1 H, s), and 6.62 (1 H, s, ArH ring A); *m/z* 401 (M^+). The *cis*-isomer, prepared similarly, was distinguished by δ 1.25 (3 H, d, *J* 7 Hz, $CHCH_3$) and 3.37 (3 H, s, NCH_3), the other spectral data being very similar to those of the *trans*-isomer.

7,13-trans-13-*Methyl*-O-*methylandrocymbine* (38).—The mixture of isomers (16b) and (17b) as the hydrochlorides (603.5 mg, 1.36 mmol) containing 90% of the *trans*-isomer was converted into the free base by partitioning between saturated aqueous sodium hydrogen carbonate (20 ml) and chloroform (4 × 10 ml). Evaporation of the organic phase gave the isomeric free bases (560 mg, 100%) which were dissolved in chloroform (13.5 ml), and the solution cooled to 0 °C and stirred while 1M-diborane in tetrahydrofuran (2.2 ml) was added dropwise.[23] Stirring was continued for 0.5 h after which the solution was evaporated under reduced pressure and the residual gum chromatographed on silica gel (5 g) with chloroform (180 ml) as eluant; the eluate gave the purified amine-borane complex (477 mg, 87%). This in anhydrous dichloromethane (136 ml) in a dry box under nitrogen was treated with thallium(III) trifluoroacetate (3.2 g). The flask was sealed, protected from light, and stirred at 20 °C for 20 h. Solvents were evaporated and the residue in the minimum volume of 5% methanol–chloroform was chromatographed on silica gel, with the same eluant (480 ml). The residue from the eluate was heated with anhydrous sodium carbonate (530 mg) in methanol (54 ml) for 4 h, after which the solvent was evaporated and the residue partitioned between water (30 ml) and chloroform (5 × 10 ml). Evaporation of the chloroform gave the free base (325 mg) as a gum and this was triturated with warm ether (5 × 20 ml); the soluble material (140 mg) was chromatographed on silica gel (5.5 g), first with chloroform as eluant and then with 5% methanol–chloroform. Evaporation of the eluates gave material (125 mg) which on treatment with picric acid in ethanol yielded the *dienone* as its *picrate* (98 mg, 14%), m.p. 174.5—177.5 °C (from ethanol–chloroform) (Found: C, 55.1; H, 5.0; N, 9.1. $C_{29}H_{32}N_4O_{12}$ requires C, 55.4; H, 5.1; N, 8.9%). The free base recovered from the picrate as in earlier cases showed $\nu_{max.}$ 1 665, 1 635, 1 610, 1 595, and 1 495 cm^{-1}; $\lambda_{max.}$ 212, 240sh, and 274sh nm; δ 1.09 (3 H, d, *J* 6 Hz, $CHCH_3$), 1.20—1.60 (2 H, m, CCH_2C), 2.31 (3 H, s, NCH_3), 1.60—2.55 (3 H, m, CCHN + CCH_2C), 2.62—3.15 (3 H, m, CHN + $ArCH_2C$), 3.60 (3 H, s), 3.80 (6 H, s), and 4.00 (3 H, s, 4 × OCH_3), 6.27 (1 H, s), and 6.31 (1 H, s, 2 olefinic H, ring C), and 6.79 (1 H, s, ArH); *m/z* 399 (M^+), and 384 ($M^+ - CH_3$, with *m** at 370).

N-*Acetyldemecolcine or* N-*Methylcolchicine* (40).[25]—Demecolcine (123 mg, 0.32 mmol), isolated from *C. byzantium* in the usual way,[4] was heated at reflux in dry benzene (2 ml) with redistilled acetic anhydride (0.1 ml) for 2 h. The cooled solution was basified with aqueous ammonia, then water (5 ml) was added and the mixture was extracted with chloroform (3 × 5 ml). The extracted material on recrystallisation from ethyl acetate gave *N*-acetyldemecolcine (112 mg, 82%), m.p. 230—232 °C (lit.,[25] m.p. 231—232 °C); $\nu_{max.}$ 1 640, 1 615, 1 590, and 1 560 cm^{-1}; $\lambda_{max.}$ 208, 248, and 351 nm; δ 2.11 (3 H, s, $COCH_3$), 2.0—2.8 (4 H, m, CH_2CH_2), 3.27 (3 H, s, NCH_3), 3.69, 3.90, 3.93, and 3.98 (12 H, 4 s, 4 × OCH_3), 4.84—5.14 (1 H, m, CHN), 6.54 (1 H, s, ArH ring A), 6.78 (1 H, d, *J* 11 Hz), and 7.16 (1 H, d, *J* 11 Hz, ArH ring C), and 7.31 (1 H, narrow d, ArH ring C); *m/z* 413 (M^+).

Incorporation Experiments and Isolation of Alkaloids.—Aqueous solutions of the hydrochlorides of the *cis*-tetrahydro[3-^{14}C]isoquinoline (17a) and of the corresponding *trans*-[Ar-^{3}H, 3-^{14}C]-base (16a) were fed to *C. autumnale* plants by direct injection into the capsules (0.5 mg hydrochloride per injection). After 14 days, the plants were harvested and worked up as previously described,[4] with the exception that *N*-acetyldemecolcine (40) (80 mg) was added at the beginning of the work-up. The order of elution of alkaloids from the partition column was: (i) demecolcine (6), (ii) *N*-acetyldemecolcine (40), (iii) *N*-acetyldemecolcine (40) + colchicine (1), and (iv) colchicine (1). *N*-Acetyldemecolcine and colchicine in the mixed fractions were separated by preparative t.l.c. (alumina–chloroform). All three alkaloids isolated from feeding the 1,3-*cis*-[3-^{14}C]-system (17a) were radioinactive. From the feeding of the *trans*-[Ar-^{3}H,3-^{14}C]-isomer (16a), however, the demecolcine (6) isolated (25 mg) had a total activity of 8.84×10^{-4} μCi, constant on recrystallisation, corresponding to an incorporation of 1.33×10^{-3}%, with a ^{3}H : ^{14}C ratio of 2.30 : 1. The other alkaloids were radioinactive. Admixture of the isolated demecolcine with 13-methyl-*O*-methylandrocymbine (38), followed by preparative t.l.c., led to complete retention of radioactivity by the re-isolated demecolcine. The dienone (38), after separation from the plate and conversion into its picrate was radioinactive.

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References

1 Part 25, A. R. Battersby, R. C. F. Jones, A. Minta, A. P. Ottridge, and J. Staunton, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2030.
2 Reviews: *Pure Appl. Chem.*, 1967, **14**, 117; W. C. Wildman and B. A. Pursey in 'The Alkaloids,' ed. R. H. F. Manske, Academic Press, New York, 1968, vol. 11, p. 407.
3 (*a*) A. R. Battersby and J. J. Reynolds, *Proc. Chem. Soc.*, 1960, 346; (*b*) E. Leete and P. E. Nemeth, *J. Am. Chem. Soc.*, 1960, **82**, 6055; (*c*) E. Leete, *ibid.*, 1963, **85**, 3666.
4 A. R. Battersby, R. Binks, J. J. Reynolds, and D. A. Yeowell, *J. Chem. Soc.*, 1964, 4257.
5 (*a*) A. R. Battersby, R. Binks, and D. A. Yeowell, *Proc. Chem. Soc.*, 1964, 86; (*b*) A. R. Battersby and R. B. Herbert, *ibid.*, 1964, 260; (*c*) A. R. Battersby, T. A. Dobson, D. M. Foulkes, and R. B. Herbert, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1730.
6 (*a*) A. R. Battersby, R. B. Herbert, L. Pijewska, and F. Santavy, *Chem. Commun.*, 1965, 228; (*b*) A. R. Battersby, R. B. Herbert, L. Pijewska, F. Santavy, and P. Sedmera, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1736.
7 A. R. Battersby, R. B. Herbert, E. McDonald, R. Ramage, and J. H. Clements, *Chem. Commun.*, 1966, 603.

8 A. R. Battersby, R. B. Herbert, E. McDonald, R. Ramage, and J. H. Clements, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1741.
9 A. R. Battersby, A. C. Barker, E. McDonald, R. Ramage, and J. H. Clements, *Chem. Commun.*, 1967, 390; A. C. Barker, Ph.D. Thesis, University of Liverpool, 1967.
10 R. N. Woodhouse and P. W. Sheldrake, Ph.D. Theses, University of Cambridge, 1971 and 1974, respectively.
11 *Cf.* H. Finkelstein, *J. Am. Chem. Soc.*, 1951, **73**, 550.
12 (*a*) L. Jurd, *J. Am. Chem. Soc.*, 1969, **81**, 4606; (*b*) A. R. Battersby, R. C. F. Jones, R. Kazlauskas, C. W. Thornber, S. Ruchirawat, and J. Staunton, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2016.
13 N. Kornblum, H. O. Larson, R. K. Blackwood, D. D. Mooberry, E. P. Oliveto, and G. E. Graham, *J. Am. Chem. Soc.*, 1956, **78**, 1497.
14 G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
15 (*a*) T. Kametani, M. Koizumi, K. Shishido, and K. Fukumoto, *J. Chem. Soc. C*, 1971, 1923; (*b*) T. Kametani, Y. Satoh, S. Shibuya, M. Koizumi, and K. Fukumoto, *J. Org. Chem.*, 1971, **36**, 3733.
16 *inter alia*, (*a*) S. Masumune, *J. Am. Chem. Soc.*, 1961, **83**, 1009; (*b*) R. S. Atkinson and A. S. Dreiding, *Helv. Chim. Acta*, 1967, **50**, 23; (*c*) E. J. Corey, N. N. Girotra, and C. T. Mathew, *J. Am. Chem. Soc.*, 1969, **91**, 1557, and refs. therein.
17 A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.
18 G. N. Walker, *J. Am. Chem. Soc.*, 1954, **76**, 3999.
19 H. Zimmermann and L. Ahramjian, *J. Am. Chem. Soc.*, 1959, **81**, 2086.
20 Review: E. Blackburn and C. V. Timmons, *Q. Rev. (London)*, 1969, **23**, 482.
21 P. S. Bailey, *Org. Synth.*, 1960, **41**, 41.
22 J. W. Cook, J. D. Loudon, J. Jack, J. MacMillan, and G. Buchanan, *J. Chem. Soc.*, 1951, 1397.
23 M. Schwarz, B. Rose, and B. Vishnuvajjala, *J. Am. Chem. Soc.*, 1973, **95**, 612.
24 O. Kennard and Z. Shakked, *Acta Crystallogr., Ser. B*, 1977, **33**, 615.
25 A. D. Cross, F. Santavy, and B. Trived, *Collect. Czech. Chem. Commun.*, 1963, **28**, 3402.
26 L. F. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' vol. I, p. 1180.
27 R. Criegee, *Annalen*, 1936, **75**, 522.
28 H. Rapoport and J. E. Campion, *J. Am. Chem. Soc.*, 1951, **73**, 2239.

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