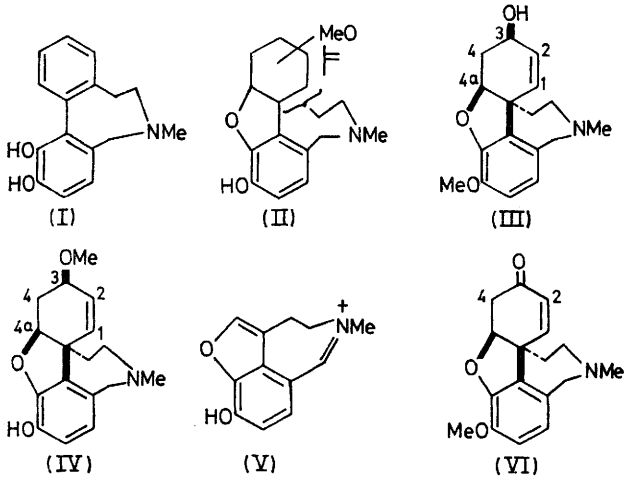
Structure and Biosynthesis of Chlidanthine

By **J. G. Bhandarkar** and **G. W. Kirby,*** Chemistry Department, University of Technology, Loughborough

The structure of the phenolic *Amaryllidaceae* alkaloid, chlidanthine, has been determined by *NO*-dimethylation to give (−)-galanthamine methiodide, which was in turn prepared from (−)-galanthamine *via* (−)-epigalanthamine. The relative stereochemistry of the pair of epimeric, allylic alcohols derived from Pummerer's ketone has been determined. The conversion of these alcohols into allylic chlorides with thionyl chloride and with trisdimethylaminophosphine and carbon tetrachloride is described. Biosynthetic conversion of tritiated galanthamine and narwedine into chlidanthine has been observed in *Chlidanthus fragrans* Herb.

CHLIDANTHINE was first isolated from *Chlidanthus fragrans* Herb. by Boit,[1] who established the molecular formula, $C_{17}H_{21}NO_3$, and identified the principal functional groups. Treatment with hydrobromic acid gave apogalanthamine (I), therby establishing the partial structure (II) for the alkaloid and suggesting a close relationship to galanthamine (III), which occurs in the same plant.[2] Recently[3] a resemblance has been noted between the n.m.r. spectra of chlidanthine and galanthamine though no details were given. We propose the structure and stereochemistry (IV) for chlidanthine and present evidence for its biosynthetic derivation from galanthamine.

Accurate mass measurements on chlidanthine and *O*-acetylchlidanthine, $\nu_{max.}$ 1765 cm.$^{-1}$, confirmed Boit's molecular formula. In the mass spectrum of chlidanthine the molecular ion, *m/e* 287, formed the base peak (100%). A peak at *m/e* 202 (37%), $C_{12}H_{12}NO_2$, may be tentatively assigned to the ion (V) since the spectra of galanthamine (III), epigalanthamine [C-3 epimer of (III)], and narwedine (VI) showed strong peaks (re-

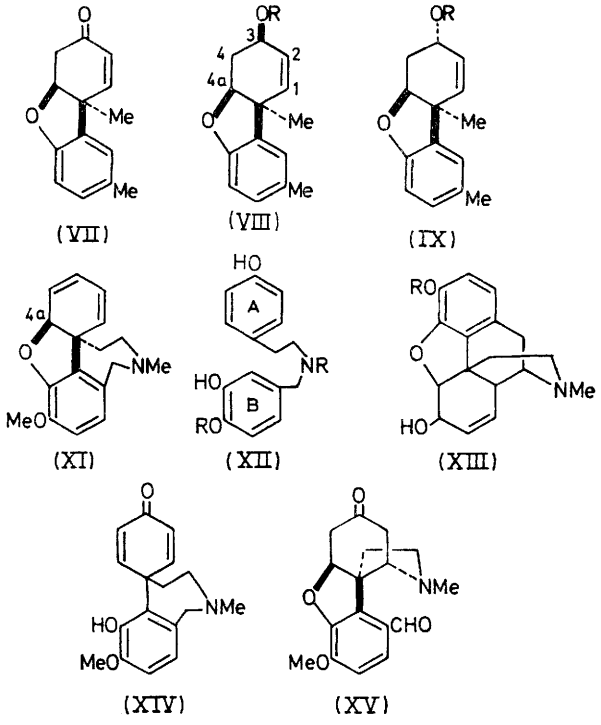


spectively 36, 65, and 19% of the corresponding base peaks) at *m/e* 216 and not at *m/e* 202. The n.m.r. spectrum of *O*-acetylchlidanthine confirmed the gross structural features of the molecule: τ ($CDCl_3$) 3·17 and 3·42 (*J* 8·2 Hz, aromatic protons), 3·80 (d, *J* 11·0 Hz, H-1), 4·02 (q, *J* 11·0 and 3·4 Hz, H-2), 5·43 (distorted t, *J ca.* 3 Hz, H-4a), 5·86 and 6·32 (q, *J* 15·7 Hz, $ArCH_2$), 6·65 (s, alkyl-OMe), 7·62 (s, MeN), and 7·76 (s, MeCO). The superficial resemblance of this spectrum to that of galanthamine was not taken as reliable evidence for relative configuration: in galanthamine the conformation of the cyclohexene ring is defined by hydrogen bonding between the hydroxy-group and the ether oxygen[4,5] whereas in the corresponding ether or its C-3 epimer flattening of the cyclohexene ring might well take place. We decided to compare directly the methyl ether methiodides of chlidanthine and galanthamine. Initial experiments with galanthamine were frustrated by the insolubility of the metho-salts, which formed very rapidly, and the reluctance of the hydrogen-bonded hydroxy-group to undergo methylation. Since both alkaloids were in short supply, studies were carried out first with the alcohols derived from Pummerer's ketone (VII).[6]

Reduction of Pummerer's ketone [racemate of (VII)] with lithium aluminium hydride gave two alcohols. Elution of the mixture from alumina gave first an oily alcohol (VIII; R = H), $\nu_{max.}$ (CCl_4) 3560 cm.$^{-1}$, and second a crystalline alcohol (IX; R = H), $\nu_{max.}$ (CCl_4) 3610 cm.$^{-1}$. The crystalline alcohol had been obtained previously[7] from the Meerwein-Ponndorf reduction of Pummerer's ketone. The observation of intramolecular hydrogen bonding in (VIII; R = H) and not in (IX; R = H) defined the relative configuration and emphasised a structural analogy[4] with galanthamine (III) and epigalanthamine respectively. Support for an intramolecular hydrogen bond in (VIII; R = H) came from the n.m.r. spectrum of a solution in deuteriochloroform. The proton at C-3 gave a multiplet, τ 5·86, of at least 8 lines (separation of extreme lines 23·1 Hz). Addition of deuterium oxide caused collapse of this signal into a broad quartet (separation 12·9 Hz), indicating a CH–OH coupling constant of 10·2 Hz. This assignment was supported by a doublet, τ 7·6, for the hydroxylic proton, although the high-field component appeared only as a shoulder on the $ArCH_3$ singlet. However, in the spectrum of a solution in hexadeuteriodimethyl sulphoxide, the hydroxylic proton gave a doublet, τ 5·33 (*J* 5·8 Hz), the coupling now being characteristic[8] of a freely rotating hydroxy-group. The

[1] H.-G. Boit, *Chem. Ber.*, 1956, **89**, 1129.
[2] H.-G. Boit and H. Ehmke, *Chem. Ber.*, 1957, **90**, 57.
[3] W. Döpke and H. Dalmer, *Naturwiss.*, 1965, **52**, 61.
[4] D. H. R. Barton and G. W. Kirby, *J. Chem. Soc.*, 1962, 806. D. J. Williams and D. Rogers, *Proc. Chem. Soc.*, 1964, 357.
[6] R. Pummerer, D. Melamed, and H. Puttfarcken, *Ber.*, 1922, **55**, 3116.
[7] V. Arkley, F. M. Dean, A. Robertson, and P. Sidisunthorn, *J. Chem. Soc.*, 1956, 2322.
[8] O. L. Chapman and R. W. King, *J. Amer. Chem. Soc.*, 1964, **86**, 1256.

larger value observed for the solution in deuteriochloroform is explicable [9] if rotation of the hydroxy-group is prevented by intramolecular hydrogen bonding. Acetylation of the alcohols was carried out with acetic anhydride–pyridine: methylation was achieved in good



yield with silver oxide in neat methyl iodide. In the n.m.r. spectra of the alcohol (IX; R = H), the acetate (IX; R = Ac), and the methyl ether (IX; R = Me), the methylene protons at C-4 gave multiplets of fourteen lines, τ *ca.* 7·1—8·5. The line spacings and chemical shifts were closely similar for all three compounds, thus providing a useful criterion for stereochemistry at C-3 in this series (see later). The alcohol (IX; R = H) was converted into the epimeric methyl ether (VIII; R = Me) indirectly in the following way. Treatment of the alcohol in benzene or chloroform with thionyl chloride, in the presence or absence of pyridine, gave an oily chloro-compound. This material appeared, from its n.m.r. spectrum, to consist solely of the allylic chloride (IX; RO = Cl), produced with retention of configuration at C-3. The spectrum showed thirteen of the C-4 methylene signals (the fourteenth was obscured by a methyl signal, τ 7·74) characteristic of this configuration. Treatment of the allylic chloride with sodium methoxide in methanol gave the methyl ether (VIII; R = Me). Examination (n.m.r.) of the crude reaction mixture showed the presence of a minor (*ca.* 10%) constituent, probably the epimer (IX; R = Me). The displacement of halogen had therefore occurred, as expected, largely with inversion at C-3. The same allylic chloride (IX; RO = Cl) was obtained, also stereospecifically, from the reaction of the alcohol (VIII; R = H) with trisdimethylaminophosphine and carbon tetrachloride. This reagent is known [10] to convert alcohols into the corresponding chloro-compounds with inversion of configuration. In contrast, treatment of the alcohol (VIII; R = H) with thionyl chloride or (IX; R = H) with the phosphorus reagent gave mixtures of chloro-derivatives: one component of each mixture was recognised (n.m.r.) as the compound (IX; RO = Cl) but the other components were not identified.

These preliminary studies indicated a route for the indirect methylation of galanthamine. Epimerisation [11] of galanthamine (III) with hot mineral acid gave epigalanthamine [C-3 epimer of (III)]. This was treated with thionyl chloride and the total product was treated with sodium methoxide in methanol. Chromatographic separation gave two compounds. One was a crystalline elimination product assigned, on the basis of u.v., n.m.r., and mass spectra, the constitution (XI). The other, oily product appeared (t.l.c. and n.m.r. spectrum) homogeneous, and was judged by its n.m.r. spectrum and method of preparation to be galanthamine methyl ether. The oily product reacted with methyl iodide in methanol to give, in good yield, a highly crystalline methiodide. Methylation of chlidanthine with dimethyl sulphate and alkali followed by addition of potassium iodide gave a methiodide having physical constants, including optical rotation, the same as those of galanthamine methyl ether methiodide. Chlidanthine therefore has the structure and absolute stereochemistry (IV).

The biosynthesis of galanthamine (III) from a variety of norbelladine derivatives (XII) is well documented.[12] In particular, the derivative (XII; R = Me) is a precursor of galanthamine. The biosynthesis of chlidanthine (IV) could involve cyclisation of a derivative of the type (XII) with two hydroxy-groups on ring B, thus by-passing the route to galanthamine. Alternatively, chlidanthine could be derived from galanthamine by methylation and demethylation (not necessarily in this order). Late-stage demethylation seemed an attractive possibility, having an analogy in the conversion [13] of codeine (XIII; R = Me) into morphine (XIII; R = H). To test this point and to confirm the stereochemistry of chlidanthine, biosynthetic experiments were carried out with *Chlidanthus fragrans* plants. Narwedine (VI) is known [4] to racemise in hydroxylic solvents by reversible ring opening to the dienone (XIV). In this way hydrogens at C-2 and C-4 can be exchanged for deuterium or tritium. (±)-[^{3}H]Narwedine was prepared in tritiated methanol and reduced [4]

[9] E. W. Garbisch, *J. Amer. Chem. Soc.*, 1963, **85**, 1696.
[10] D. Brett, I. M. Downie, J. B. Lee, and M. F. S. Matough, *Chem. and Ind.*, 1969, 1017.
[11] G. W. Kirby and H. P. Tiwari, *J. Chem. Soc.*, 1964, 4655, and references cited.
[12] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J. Chem. Soc.*, 1963, 4545.
[13] A. R. Battersby and B. J. T. Harper, *Tetrahedron Letters*, 1960, No. 27, 21; F. R. Stermitz and H. Rapoport, *J. Amer. Chem. Soc.*, 1961, **83**, 4045.

to give (±)-[^{3}H]galanthamine and (±)-[^{3}H]epigalanthamine. These three compounds were fed separately, by the wick method, to vigorously growing *C. fragrans* plants in summer. Two sets of experiments were performed, the first allowing 2 days and the second 7 days for metabolism. In both sets, conversion of galanthamine but not of epigalanthamine into chlidanthine was observed (see Table), supporting the proposed relative stereochemistry for the last alkaloid. Additionally, narwedine was efficiently (7·7%) converted (7-day experiment) into galanthamine, thus demonstrating the long suspected [14] precursor–product relationship of these compounds. No suitable degradative sequence was available for the location of tritium in small quantities of labelled chlidanthine. However, useful evidence that biosynthesis in *C. fragrans* had occurred without 'scrambling' of tritium was obtained by examination of the galanthamine derived from (±)-[^{3}H]narwedine. Oxidation [4] of the galanthamine [relative molar activity (r.m.a.) 1·00] with chromic acid and chromatography of the product gave inactive (±)-narwedine (r.m.a. <0·01). The tritium must therefore have resided at C-2, C-4, or C-3, location at the last position being, however, inherently unlikely. The keto-aldehyde (XV) (r.m.a. 0·32) was isolated as a by-product of this oxidation. The partial retention of tritium in this material supports the idea [15] that the keto-aldehyde is formed partly racemic from the oxidation of galanthamine.

Incorporations (%) of precursors into chlidanthine

	(±)-Galanthamine	(±)-Epigalanthamine	(±)-Narwedine
2-Day experiment	1·08	<0·005	
7-Day experiment	0·081	<0·009	0·069

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage apparatus. N.m.r. spectra were run at 60 MHz for solutions in deuteriochloroform. Mass spectra were measured with A.E.I. MS 9 and MS 12 spectrometers, with an ionising potential of 70 ev, by direct insertion of samples. Radioactive compounds were counted with a Beckmann Instruments Inc. type CPM-100 liquid scintillation spectrometer.

Chlidanthine.—The total bases (1·5 g.) isolated [16] from resting *Chlidanthus fragrans* Herb. bulbs were treated with a small amount of ethanol. The crystalline mass (0·15 g.) which slowly separated was triturated with 2N-sodium hydroxide. Filtration and neutralisation of the filtrate with hydrochloric acid followed by addition of sodium hydrogen carbonate gave a precipitate of crude chlidanthine. Crystallisation from methanol gave plates (70 mg.), m.p. 240—242° (lit.,[1] 238—239°), $[\alpha]_D$ −135 ± 5° (*c* 0·2 in EtOH) (lit.,[1] −140°), $\lambda_{max.}$ 287 (ε 2960), $\lambda_{infl.}$ 230 nm. (ε 9770) in ethanol shifting to $\lambda_{max.}$ 300 (ε 3350), $\lambda_{infl.}$ 245 nm. (ε 9080) upon addition of sodium hydroxide, *m/e* 287·150 (M^+, $C_{17}H_{21}NO_3$ requires *M*, 287·152) 212·080 ($C_{14}H_{12}O_2$ requires 212·084) and 202·084 ($C_{12}H_{12}NO_2$ requires 202·087). Acetylation (acetic anhydride–pyridine) gave the corresponding *O*-acetyl derivative, $\nu_{max.}$ ($CHCl_3$) 1765 cm.$^{-1}$, *m/e* 329·162 ($C_{19}H_{12}NO_4$ requires *M*, 329·163).

Reduction of Pummerer's Ketone (with Dr. H. P. Tiwari).—Pummerer's ketone (VII) [6] (1·0 g.) in ether (30 ml.) was added to lithium aluminium hydride (1·0 g.) in ether (30 ml.). After 6 hr. stirring at room temperature the excess of lithium aluminium hydride was decomposed with water and the ether-soluble products were separated by chromatography on grade III alumina (10 g.). Elution with benzene–ethyl acetate (9 : 1) gave first the oily *alcohol* (VIII; R = H) (Found: C, 77·4; H, 7·35. $C_{14}H_{16}O_2$ requires C, 77·75; H, 7·5%) and then the alcohol (IX; R = H), which crystallised from light petroleum (b.p. 60—80°) as needles, m.p. 80—81° (lit.,[7] 80°) (Found: C, 77·5; H, 7·4%). The oily alcohol slowly crystallised but could not conveniently be recrystallised. The i.r. spectra of the alcohols (10 mg.) (see text) were measured for solutions in carbon tetrachloride (15 ml.) in 40 mm. cells.

Methylation of the Alcohols (VIII and IX; R = H).—The alcohols (50 mg.) were heated overnight under reflux in methyl iodide (15 ml.) in the presence of freshly prepared silver oxide (500 mg.). The products were purified by chromatography on grade III alumina. The *methyl ether* (VIII; R = Me), after sublimation (80—120°/1·5 mm. Hg), had m.p. 41° (Found: C, 78·2; H, 7·8. $C_{15}H_{18}O_2$ requires C, 78·2; H, 7·9%). The isomeric *methyl ether* (IX; R = Me) crystallised from light petroleum (b.p. 60—80°) as plates, m.p. 90° (Found: C, 78·3; H, 8·0%).

Acetylation of the Alcohols (VIII and IX; R = H).—Acetylation with acetic anhydride–pyridine at room temperature gave products which were purified by chromatography and sublimation. The *acetate* (VIII; R = Ac) had m.p. 67° (Found: C, 74·1; H, 7·4. $C_{16}H_{18}O_3$ requires C, 74·4; H, 7·0%); the *acetate* (IX; R = Ac) had m.p. 63° (Found: C, 74·3; H, 6·8%).

N.m.r. Spectra of Derivatives (IX).—The spectra showed fourteen lines attributable to the methylene protons at C-4. The equality of various spacings suggested that a first-order interpretation was appropriate. Even if this is not so, presentation of the spectra in first-order terms [τ values (|*J*|Hz)] as follows is convenient ('*e*' and '*a*' represent, respectively, equatorial and axial): (IX; R = H), H_e 7·39 (4·5, 13·7), H_a 8·24 (2·9, 13·7, 9·8); (IX; R = Me), H_e 7·39 (5·1, 13·7), H_a 8·25 (2·9, 13·7, 9·6); (IX; R = Ac), H_e 7·40 (4·6, 14·2), H_a 8·12 (2·8, 14·2, 9·5).

Reaction of the Alcohol (IX; R = H) *with Thionyl Chloride*.—The alcohol (IX; R = H) (70 mg.) in benzene (5 ml.) was treated with thionyl chloride (0·5 ml.) at room temperature for 1 hr. The solvent was evaporated off and traces of thionyl chloride were removed by repeated addition and evaporation of benzene. The residue was chromatographed on grade III alumina (10 g.), elution with benzene giving the allylic chloride (IX; RO = Cl) (58 mg.) as an oil which appeared homogeneous (t.l.c. and n.m.r. spectrum). The n.m.r. spectrum confirmed the configuration at C-3 (see before). Thirteen of the fourteen C-4 methylene bands were observable, giving the following apparent, first-order terms [τ values (|*J*| Hz)]: H_e 7·35 (5·1, 13·9), H_e 7·86 (3·3, 14·2, 9·3). This material was heated under reflux in methanol containing an excess of sodium

[14] D. H. R. Barton and T. Cohen, 'Festschrift Arthur Stoll,' Birkhäuser, Basle, 1957, p. 117.

[15] J. G. Bhandarkar and G. W. Kirby, *J. Chem. Soc.* (*C*), 1970, 592.

[16] *Cf.* H. M. Fales, L. D. Giuffrida, and W. C. Wildman, *J. Amer. Chem. Soc.*, 1956, **78**, 4145.

methoxide for 4 hr. Chromatography of the product on alumina gave the methyl ether (VIII; R = Me) (48 mg.), having n.m.r. and i.r. spectra identical with those of the ether prepared directly from the alcohol (VIII; R = H). Examination (n.m.r.) of the total reaction mixture before chromatography showed the presence (*ca.* 10%) of a second methyl ether, presumably (IX; R = Me). Qualitatively similar results were obtained when the alcohol (IX; R = H) was treated with thionyl chloride in chloroform in the presence or absence of pyridine.

Reaction of the Alcohol (VIII; R = H) *with Trisdimethylaminophosphine and Carbon Tetrachloride*.—The alcohol (VIII; R = H) (190 mg.) in chloroform (2 ml.) and carbon tetrachloride (2 ml.) was treated [10] with trisdimethylaminophosphine (0·5 ml.) in chloroform (3 ml.) with stirring at −35°. The temperature of the mixture was allowed to rise slowly to 20°. After 2 hr. the solvent was evaporated off and the residue was chromatographed (as before) to give the allylic chloride (IX; RO = Cl) (90 mg.) and the alcohol (VIII; R = H) (30 mg.).

Galanthamine Methyl Ether Methiodide.—(−)-Epigalanthamine was obtained [11] by epimerisation of (−)-galanthamine in hot dilute hydrochloric acid prepared from the concentrated acid (2 ml.) and water (98 ml.). Our earlier description [11] of this acid as '2% hydrochloric acid' is ambiguous. (−)-Epigalanthamine (50 mg.) in chloroform (5 ml.) containing thionyl chloride (0·1 ml.) was kept at room temperature for 2 hr. The solvent was evaporated off at room temperature and traces of thionyl chloride were removed from the crystalline residue by repeated addition and evaporation of chloroform. The residue was heated under reflux for 5 hr. in dry methanol (5 ml.) containing N-methanolic sodium methoxide (0·6 ml.). The methanol was evaporated off and the residue was extracted with chloroform. The extract (40 mg.) was chromatographed on grade III alumina (8 g.) with benzene, benzene–ethyl acetate (9 : 1), and benzene–ethyl acetate (8 : 2) as eluants. The early fractions gave the diene (XI) (12 mg.), m.p. 94—96°, $\lambda_{max.}$ (EtOH) 261 nm. (ε 4035), τ 3·33 (s, aromatic protons), 3·8 (m, 4 vinyl protons), 5·12 (d, *J* 5·3 Hz, H-4a), 5·78 and 6·28 (q, *J* 14·6, $ArCH_2N$), 6·12 (s, MeO), and 7·60 (s, MeN), *m/e* 269 (M^+). Later fractions gave galanthamine methyl ether (10 mg.) as an oil. Treatment with methyl iodide (0·05 ml.) in methanol (0·5 ml.) at room temperature gave (−)-*galanthamine methyl ether methiodide* which separated as fine needles (12 mg.), m.p. 259° (decomp.). Crystallisation from methanol gave material, m.p. 274°, $[\alpha]_D$ −90° (*c* 0·105 in methanol) (Found: C, 50·95; H, 6·05; N, 3·15. $C_{19}H_{26}INO_3$ requires C, 51·4; H, 5·9; N, 3·2%).

Methylation of Chlidanthine.—A solution of chlidanthine (25 mg.) in N-sodium hydroxide (0·5 ml.) was shaken vigorously at 0° with dimethyl sulphate (0·1 ml.) until a clear solution was obtained. Methylation was continued by addition, at intervals, of three further quantities of 10N-sodium hydroxide (0·03 ml.) and dimethyl sulphate (0·03 ml.). After 4 hr. saturated aqueous potassium iodide was added to effect crystallisation of (−)-galanthamine methyl ether methiodide (10 mg.), m.p. and mixed m.p. 274—276°, $[\alpha]_D$ −87° (*c* 0·106 in MeOH). The i.r. spectrum (KBr) of this material was identical with that of the methiodide prepared (see before) from galanthamine.

Labelling of Precursors.—Tritiated methanol was prepared from tritiated water (200 mCi/ml.) and magnesium methoxide. (±)-Narwedine was labelled by heating in this solvent under reflux. Correspondingly labelled (±)-galanthamine and (±)-epigalanthamine were obtained by reduction [4] of the (±)-narwedine with lithium aluminium hydride.

Biosynthetic Experiments.—Aqueous solutions of precursors (pH *ca.* 6) were fed to *Chlidanthus fragrans* plants, growing in early summer, through wicks of untreated cotton passed through the fleshy leaves near soil level. Periods of 2 and 7 days were allowed for metabolism. The total bases (see before) were separated preparatively on Merck alumina PF_{254} plates developed with ethyl acetate–methanol (95 : 5): typical R_F values are galanthamine 0·46, epigalanthamine 0·33, lycorine 0·26, and chlidanthine 0·13. To effect further purification, chlidanthine was mixed with inactive precursor and the mixture was separated by a second chromatographic run. Final purification was achieved by crystallisation, and the radiochemical purity was checked by use of the derived methyl ether methiodide. The results shown in the Table.

We thank the Whitehall Foundation for financial support, the S.R.C. for provision of a scintillation spectrometer and mass spectrometer, and the Physico-Chemical Measurements Unit (Aldermaston) for accurate mass measurements.

[9/2123 *Received, December 12th,* 1969]