Biosynthesis. Part XX.[1,2] Biosynthesis of c-Homoaporphines in *Kreysigia multiflora*

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Specifically ^{14}C-labelled 1-phenethylisoquinolines are administered to *Kreysigia multiflora* plants and the isolated alkaloids are degraded by an unambiguous sequence. The results show that the c-homoaporphine skeleton (1) is built from autumnaline (20), probably by *ortho–para* phenol coupling. Comment is made on the taxonomic interest of these findings in relation to the biosynthesis of colchicine (3) in *Colchicum autumnale*.

BIOGENETIC arguments which suggested the existence of substances with structures based upon the homoaporphine skeleton (1) were outlined in the preceding paper.[3] These compared the established biosynthetic pathway to morphine with the knowledge[4] that colchicine (3) is

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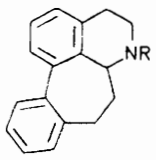
[1] Part XIX, A. R. Battersby, D. G. Laing, and R. Ramage, *J.C.S. Perkin I*, 1972, 2743.

[2] Preliminary publication, A. R. Battersby, P. Böhler, M. H. G. Munro, and R. Ramage, *Chem. Comm.*, 1969, 1066.

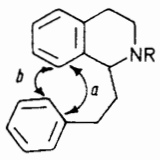
[3] A. R. Battersby, R. B. Bradbury, R. B. Herbert, M. H. G. Munro, and R. Ramage, preceding paper.

[4] A. R. Battersby, R. B. Herbert, E. McDonald, R. Ramage, and J. H. Clements, *J.C.S. Perkin I*, 1972, 1741, and references therein.

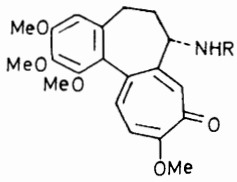
biosynthesised by subtle modification of the 1-phenethylisoquinoline skeleton (2) and that the dienone (6) is a key intermediate. The predictions were substantiated



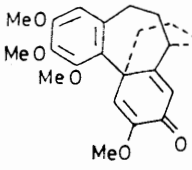
(1)



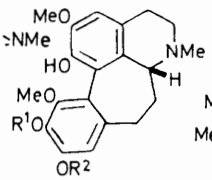
(2)

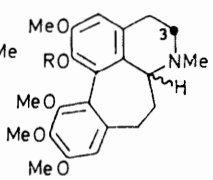


(3) R = Ac
(4) R = H
(5) R = CHO



(6)





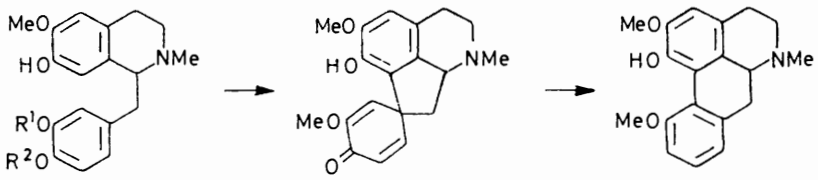
(7) R^1 = Me, R^2 = H (9) R = H
(8) R^1 = H, R^2 = Me (10) R = Me

when it was found [3] that (*R*)-floramultine, (*R*)-multifloramine, and (*RS*)-kreysigine from *Kreysigia multiflora* are homoaporphines with the structures (7)—(9), respectively.

trast, bulbocapnine [6] (15) and related systems [7] are built by direct coupling of reticuline (12), whilst glaucine (16) and its relatives are formed in *Dicentra eximia* [8] from norprotosinomenine (17), presumably *via* the dienone (18).

On this basis, it was decided to test couplings (*a*) and (*b*), as indicated on structure (2), for the biosynthesis of homoaporphines. Already our synthetic work in this series [3] had used the coupling (*a*) [(19) ⟶ (21)] followed by rearrangement of dienone (21) to give (*RS*)-multifloramine [as (8)]. Also, a possible pointer to coupling (*a*) being the natural one came from the occurrence of very small amounts of kreysiginone (22) in our plants.[3]

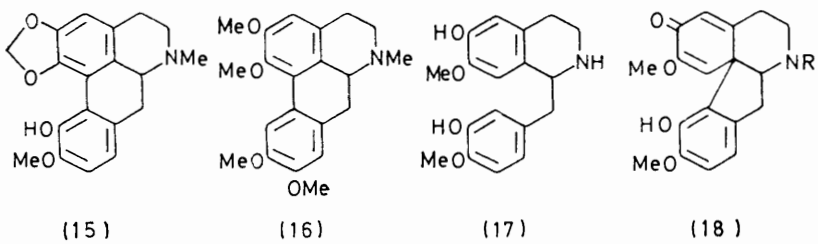
The following [3-^{14}C]diphenols were therefore synthesised for this work by standard methods [8]: (*RS*)-autumnaline (20), and the (*RS*)-isoquinolines (19) and (23). They were administered as aqueous solutions of the hydrochlorides to *Kreysigia multiflora* plants which after 2 weeks were worked up for alkaloids. The crude product was diluted with (*RS*)-multifloramine [as (8)] and then methylated with diazomethane to give (*RS*)-*O*-methylkreysigine (10). It was known that all three alkaloids (7)—(9) yield *O*-methylkreysigine (10) on methylation [3] and in this way a difficult separation on a minute scale was avoided. Rigorous chromatographic fractionation of base (10) followed by further purification *via* the picrate and picrolonate salts gave radiochemically



(11) R^1 = Me, R^2 = H
(12) R^1 = H, R^2 = Me

(13)

(14)



(15) (16) (17) (18)

Our planning of tracer studies on the homoaporphines was influenced by what was known about the biosynthesis of aporphines. Three different pathways to these substances have been recognised, all involving phenol coupling. Isothebaine (14) is formed [5] from orientaline (11) *via* the dienone (13) by dienol–benzene rearrangement of the dienol corresponding to the base (13). In contrast, bulbocapnine [6] (15) and related systems [7] are built by direct coupling of reticuline (12), whilst glaucine (16) and its relatives are formed in *Dicentra eximia* [8] from norprotosinomenine (17), presumably *via* the dienone (18).

On this basis, it was decided to test couplings (*a*) and (*b*), as indicated on structure (2), for the biosynthesis of homoaporphines. Already our synthetic work in this series [3] had used the coupling (*a*) [(19) ⟶ (21)] followed by rearrangement of dienone (21) to give (*RS*)-multifloramine [as (8)]. Also, a possible pointer to coupling (*a*) being the natural one came from the occurrence of very small amounts of kreysiginone (22) in our plants.[3]

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* There was a slight change in the low activity of the alkaloid from the isoquinoline (19) but this is of no importance since (19) is clearly not a precursor of the homoaporphines.

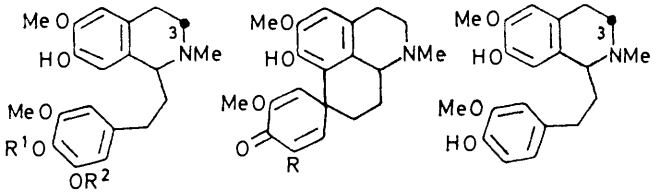
[5] A. R. Battersby, R. T. Brown, J. H. Clements, and G. G. Iverach, *Chem. Comm.*, 1965, 230; A. R. Battersby and T. H. Brown, *ibid.*, 1966, 170.

[6] G. Blaschke, *Arch. Pharm.*, 1970, **303**, 359.

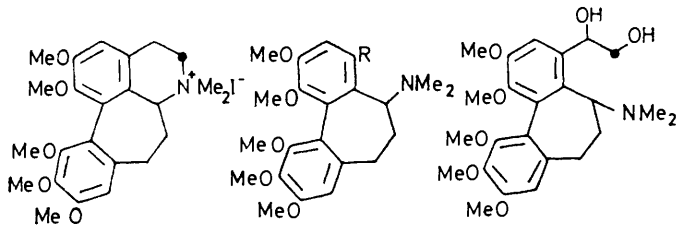
[7] E. Brochmann-Hanssen, C.-H. Chen, H.-C. Chiang, and K. McMurtrey, *J.C.S. Chem. Comm.*, 1972, 1269, and references therein.

[8] A. R. Battersby, J. L. McHugh, J. Staunton, and M. Todd, *Chem. Comm.*, 1971, 985.

from its conversion into autumnaline as happens in *Colchicum autumnale* plants.[4]



(19) R^1 = H, R^2 = Me (21) R = OMe (23)
(20) R^1 = Me, R^2 = H (22) R = H



(24) (25) R = CH=CH$_2$ (27)
(26) R = CHO

Specific degradation of the labelled *O*-methylkreysigine derived from [3-^{14}C]autumnaline (20) was carried out by a Hofmann elimination on the corresponding methiodide

Tracer experiments on *K. multiflora*

	Precursor, total activity fed, and specific activity of products (disint. per 100 s per mmol)		
	(RS)-Autumnaline (20)	(RS)-Base (23)	(RS)-Base (19)
	$5{\cdot}84 \times 10^7$ disint. per 100 s	$1{\cdot}98 \times 10^8$ disint. per 100 s	$1{\cdot}33 \times 10^8$ disint. per 100 s
Incorporation (%)	1·8	0·21	<0·014
O-Methylkreysigine (10); total activity	$1{\cdot}08 \times 10^6$ disint. per 100 s	$4{\cdot}24 \times 10^5$ disint. per 100 s	$1{\cdot}8 \times 10^4$ disint. per 100 s
O-Methylreysigine (10)	$4{\cdot}81 \times 10^5$	$2{\cdot}70 \times 10^6$	$1{\cdot}44 \times 10^5$
O-Methylkreysigine methiodide (24)	$4{\cdot}93 \times 10^5$	$2{\cdot}84 \times 10^6$	$1{\cdot}07 \times 10^5$
Methine (25)	$(4{\cdot}25 \times 10^5)$ [a]		
Formaldehyde dimedone deriv.	$3{\cdot}68 \times 10^5$		

[a] Minimum value since the amorphous methine was weighed as a gum.

(24). The resultant methine had structure (25) as shown by the presence in its n.m.r. spectrum of signals corresponding to three olefinic protons with the typical splitting pattern for a vinyl residue. Osmium tetraoxide converted the methine into the corresponding diol (27), which was cleaved (periodate) to give formaldehyde, isolated as its dimedone derivative. The molar activity of the formaldehyde corresponded to 77% of that originally present in the *O*-methylkreysigine (10), yet the major basic fragment, presumably of structure (26), retained only 2·5% of the original activity, which corresponds to *ca.* 97% of the original activity being located at the indicated position [see (27)]. It was apparent that additional radioinactive formaldehyde was being formed in the cleavage process (probably from an *N*-methyl group), so causing dilution of the radioactive product. The source of at least part of this dilution was determined by treating radioinactive *O*-methylkreysigine with [^{14}C]methyl iodide; the product was carried through the degradative sequence (24) → (25) → (27) → formaldehyde having molar activity equivalent to *ca.* 5% of that of the original methiodide. With the fact in mind that the methine (25) carries two equivalent *N*-methyl groups only one of which is labelled, an approximate correction can be applied to the result obtained earlier in the biosynthetic work. The final outcome is that the methiodide (24) carries at the indicated position 97% of the total activity, by difference, or at least 86% by isolation of the relevant carbon atom (with approximate correction for dilution).

Our results show that autumnaline (20) is incorporated specifically into the homoaporphine system and the simplest view is that a direct *ortho–para* mode of phenol coupling is operating. These basic findings lead to further interesting work on homoaporphines which we hope will be taken up by others.

There is considerable biosynthetic and taxonomic interest in the finding that the C-homoaporphine system is constructed from autumnaline (20). This base was first highlighted as the precursor of colchicine[4] (3) in *Colchicum autumnale*, and in *K. multiflora* (also in the Liliaceae family) one finds the tropolone alkaloids (3)—(5) co-occurring[3,9] with the homoaporphines. Whereas with high probability the homoaporphines arise by *ortho–para* coupling, it is certain[10] that *para–para* coupling is involved for the tropolones. Our results also indicate that the occurrence[3] of small amounts of kreysiginone (22) in *K. multiflora* is not connected with the main pathway to the homoaporphines.

EXPERIMENTAL

For general chemical and radiochemical directions see ref. 11.

Administration of Labelled Precursors to Kreysigia multiflora.—Each phenolic isoquinoline was dissolved as its hydrochloride in a minimal volume of water and 8 young shoots of *K. multiflora* were cut and immediately transferred to the solution. More water was added as uptake occurred until all the labelled material had been absorbed. The shoots were maintained in water for a total of 2 weeks.

Isolation and Purification of Radioactive O-Methylkreysigine.—The shoots were macerated in a blender in ethanol and the suspended plant material was extracted exhaustively in a column with cold ethanol. Removal of the solvent followed by partition of the residue between light petroleum and weakly acidic water (pH 3) gave an aqueous alkaloidal extract. This was basified (K_2CO_3) and extracted with chloroform, the extracts being dried and evaporated to give a gum (typically 50 mg). After addition of synthetic (*RS*)-multifloramine (20—50 mg), the mixture in methanol (2 ml) was treated with an excess of ethereal diazomethane for 1 day at 20°. This afforded crude *O*-methylkreysigine which was purified by p.l.c. on

[9] F. Šantavý, *Experientia*, 1967, **23**, 273.
[10] A. C. Barker, A. R. Battersby, E. McDonald, R. Ramage, and J. H. Clements, *Chem. Comm.*, 1967, 390.
[11] P. G. Strange, J. Staunton, H. R. Wiltshire, A. R. Battersby, K. R. Hanson, and E. A. Havir, *J.C.S. Perkin I*, 1972, 2364.

silica using 10% methanol in chloroform and the product was converted in ethanol into the picrate, m.p. 176—180°. Passage of a chloroformic solution of the picrate over a short column of alumina gave the free base which was fractionated by p.l.c. on silica using 5% diethylamine in acetone. The base was then converted into the *picrolonate*, m.p. 217—220° (from ethyl acetate–ether) (Found: C, 59·5; H, 5·9; N, 10·6. $C_{33}H_{37}N_5O_{10}$ requires C, 59·7; H, 5·6; N, 10·55%). For counting, accurately weighed samples of the picrate and picrolonate derivatives were passed in chloroform solution through alumina and the percolate was evaporated to dryness. The resultant free base was counted in the normal way.

Detection of Alkaloids on T.l.c. by Cerium(IV) *Sulphate*.—The alkaloids give different colour reactions on t.l.c. with a spray reagent made by treating (*dropwise*) a slurry of cerium(IV) sulphate (1 g) in water (65 ml) with concentrated sulphuric acid (35 ml). The colours are as follows:

	Immediate	After 16 h drying in air	After several days in air
Kreysigine (9)	Pale blue	Colourless	Colourless
Floramultine (7)	Pale green, purple outer zone	Green with pink outer zone	Green with pink outer zone
Multifloramine (8)	Dark green, red outer zone	Purple	Red-grey with green outer zone
Kreysiginine [12]	Blue	Colourless	Colourless

O-*Methylkreysigine Methiodide* (24).—*O*-Methylkreysigine (382 mg) in methanol (5 ml) and an excess of methyl iodide (500 mg) was kept at 20° for 16 h. Addition of ether precipitated the *methiodide* (334 mg), m.p. 247—248° (from methanol–ether) (Found: C, 53·05; H, 5·8; N, 2·75. $C_{24}H_{32}INO_5$ requires C, 53·2; H, 5·9; N, 2·6%).

[N-*methyl*-^{14}C]-O-*Methylkreysigine Methiodide* [*as* (24)].—*O*-Methylkreysigine picrolonate (105·8 mg) gave the free base (76 mg) by chromatography over alumina (3 g) in chloroform. The base was treated with [^{14}C]methyl iodide in ethyl acetate (0·5 ml) and after 30 min radioinactive methyl iodide (200 mg) was added. The radioactive methiodide which crystallised from solution over several hours was collected (61·8 mg); activity 1220 disint. per 100 s per mg. This product was diluted with radioinactive methiodide (202·8 mg) and recrystallised to constant activity, 379 disint. per 100 s per mg (244 mg).

Hofmann Degradation of O-*Methylkreysigine Methiodide*.—Moist silver oxide [from silver nitrate (600 mg) and sodium hydroxide (225 mg)] was added to a solution of the methiodide (300 mg) in 1 : 1 water–ethanol (30 ml) and the mixture was shaken for 1·25 h. After filtration (Celite), the solution was treated with sodium hydroxide (150 mg) and evaporated. The residue was heated at 100 °C for 3 h and then extracted with ether to afford a gum which was purified by p.l.c. on silica using 10% methanol in chloroform. The resultant methine (25) (7-dimethylamino-6,7-dihydro-1,2,3,10,11-pentamethoxy-8-vinyl-5*H*-dibenzo[*a*,*c*]cycloheptene) (208 mg) showed τ 7·91 (6H, NMe_2), 6·57 (3H, OMe), 6·37 (3H, OMe), 6·11 (6H, 2OMe), 6·09 (3H, OMe), 4·96 (1H, dd, *J* 2 and 10 Hz, olefinic), 4·63 (1H, dd, *J* 2 and 17 Hz, olefinic), 3·52 (1H, ArH), 2·98 (1H, ArH), and 2·01 (1H, dd, *J* 10 and 17 Hz, olefinic). The *methine methiodide*, prepared as usual from the methine and methyl iodide, had m.p. 232—236° (from methanol–ethyl acetate) (Found: C, 54·1; H, 6·05; N, 2·5. $C_{25}H_{34}INO_5$ requires C, 54·05; H, 6·1; N, 2·5%).

Hydroxylation of the Hofmann Product (25).—To a solution of the methine (500 mg) in ether (10 ml) was added osmium tetraoxide (450 mg) in ether (9 ml) containing pyridine (0·45 ml). There was an immediate precipitate and the mixture was kept for 16 h at 20°. The solid complex was collected, washed with ether (3 × 10 ml), and dried to give the osmate ester (1·00 g).

A solution of the complex 200 mg) in ethanol (15 ml) was mixed under nitrogen with a solution of sodium sulphite (0·8 g) in water (3 ml). The mixture was heated under reflux for 3 h, cooled, and filtered (Celite), and the filtrate was evaporated. Partition of the residue between ether (200 ml) and water (5 ml) and extraction of the aqueous layer with ether–chloroform (3 : 1; 100 ml) gave the crude diol (100 mg) from the combined organic solutions. In the radioactive series, this product was not purified further but in the trial runs it was separated into two components by p.l.c. over silica using 10% methanol–chloroform. The major product was the expected 8-(1,2-*dihydroxyethyl*)-7-*dimethylamino*-6,7-*dihydro*-1,2,3,10,11-*pentamethoxy*-5H-*dibenzo*[a,c]*cycloheptene* (27) (Found: M^+, 447·2245; $M^+ - H_2O$, 429·2140. $C_{24}H_{33}NO_7$ requires *M*, 447·2257; *M* $- H_2O$, 429·2151); $\nu_{max.}$ 3600 and 3450 cm^{-1}; τ 7·75 (3H, NMe), 7·68 (3H, NMe), 6·48 (3H, OMe), 6·32 (3H, OMe), 6·08 (6H, 2OMe), 3·45 (1H, ArH), and 3·15 (1H, ArH).

The minor product was not fully examined but it is probably the *N*-formyl-*N*-methyl analogue of the diol (27) since it lacks one *N*-methyl signal in its n.m.r. spectrum.

Periodate Cleavage of the Diol (27).—The diol (230 mg) was suspended in 0·2M-acetate buffer, pH 5 (13 ml), and sodium periodate (214 mg) in acetate buffer (5 ml) was added in three portions over 1·5 h with shaking. The mixture was poured into saturated aqueous arsenic(III) oxide (50 ml) and the solution at pH 5 was distilled directly into a solution of dimedone [210 mg in ethanol (5 ml) containing 2N-hydrochloric acid (1 drop)]. The dimedone solution was evaporated, the residue was extracted with chloroform, and the extract was purified by p.l.c. on silica using 10% methanol in chloroform to give formaldehyde dimedone derivative (32 mg; 22%), which was recrystallised to constant radioactivity from aqueous ethanol; m.p. 191° (lit., 188°), identical with authentic material.

We are indebted to Mr. K Mair (Royal Botanic Gardens, Sydney) and Sir George Taylor (Kew) for the supply of *K. multiflora* plants, to Mr. K. Hulme (Ness Botanic Garden) for cultivation of the plants, and to Dr. A. Brossi (F. Hoffmann–La Roche, Nutley, U.S.A.) for a gift of synthetic multifloramine. We also thank Roche Products Ltd. and the S.R.C. for financial support.

[3/2483 *Received*, 4*th December*, 1973]

[12] A. R. Battersby, M. H. G. Munro, R. B. Bradbury, and F. Šantavý, *Chem. Comm.*, 1968. 695.