Biosynthesis. Part XXII.[1,2] The Origin of Chelidonine and of Other Alkaloids derived from the Tetrahydroprotoberberine Skeleton

By **Alan R. Battersby,* James Staunton,** and **Hugh R. Wiltshire,** The University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW
R. John Francis and **Robert Southgate,** The Robert Robinson Laboratories, University of Liverpool, Liverpool L69 3BX

The major benzophenanthridine alkaloid chelidonine (14) is shown to be biosynthesised in *Chelidonium majus* plants from (*S*)-reticuline (2) *via* (*S*)-scoulerine (4) and (*S*)-stylopine (7), and evidence consistent with a dihydroisoquinoline intermediate [*e.g.* (11)] is presented. Similar though less extensive evidence is provided for the same pathway to the aromatic systems, *e.g.* sanguinarine (13). Protopine (10) is shown to be formed *in vivo* from (*S*)-scoulerine (4) and (*S*)-stylopine (7). Studies have been made of the late biosynthetic stages beyond scoulerine and stylopine and the role of isocorypalmine (6) and canadine (21) in the biosynthesis of narcotine (3) and berberine in the opium poppy has been investigated.

The results from ^{3}H labelling at four different sites in the various precursors limit the possible mechanisms which can be considered for the enzymic transformations revealed by this research. Unexpected increases in ^{3}H : ^{14}C ratio (up to 20%) are discussed.

Extensive degradations of the labelled alkaloids have been carried out to establish quantitatively the level of radioactivity at the multiple sites. The syntheses for the many specifically labelled substances used in this study are described.

CHELIDONINE (14) is the most abundant member of the benzophenanthridine class of alkaloids; it occurs in *Chelidonium majus* together with more highly oxidised members, *e.g.* sanguinarine (13). Stylopine (7), the subject of the preceding paper, is also present in this plant and there is a satisfying economy to the view [3] that the benzophenanthridine bases arise by modification of the tetrahydroprotoberberine (berbine) skeleton. The necessary changes, without regard to mechanistic considerations at present, are C–N fission at *a* in structure (7) and formation of a new C–C bond between C-6 and C-13 (Scheme 1). This paper describes our biosynthetic studies on chelidonine and its relatives and the opportunity has been taken to investigate the natural pathways to several other alkaloids which occur in *C. majus* plants, especially protopine (10). Some related experiments with *Papaver somniferum* are also covered.

† No single numbering scheme has become established for chelidonine and its relatives, so one based on the biosynthetic origin of the skeleton is used here (*cf.* current practice in the indole alkaloid series).

[1] Part XXI, A. R. Battersby, R. J. Francis, M. Hirst, E. A. Ruveda, and J. Staunton, preceding paper.

[2] For preliminary accounts of part of this work, see A. R. Battersby, R. J. Francis, E. A. Ruveda, and J. Staunton, *Chem. Comm.*, 1965, 89; A. R. Battersby, R. J. Francis, M. Hirst, R. Southgate, and J. Staunton, *ibid.*, 1967, 602.

Chelidonine (14), *Sanguinarine* (13), *and Chelerythrine* (24).—Earlier work [4] had shown the incorporation of [2-^{14}C]tyrosine and 3,4-dihydroxy[α-^{14}C]phenethylamine into chelidonine; the former precursor labelled C-6 and C-14 of chelidonine † and the latter C-6 only. The carbon skeleton is thus built from two C_6–C_2 units derivable from tyrosine. We favoured the intermediacy of stylopine (7) and, drawing upon the knowledge of alkaloid biosynthesis [5,6] available then, selected reticuline [(1) and (2)] as the most probable precursor of both stylopine [1] (7) and chelidonine (14). Multiply labelled (−)-(*R*)- and (+)-(*S*)-reticuline, (1) and (2), respectively, had been synthesised and the extent of labelling at each site had been determined; [1] 76% of the ^{14}C activity resides at ■, 11% at ▲, and 13% at ●. These labelled reticulines were administered to *C. majus* plants and experiments 1 and 2 (Table 1) show (+)-(*S*)-reticuline (2)

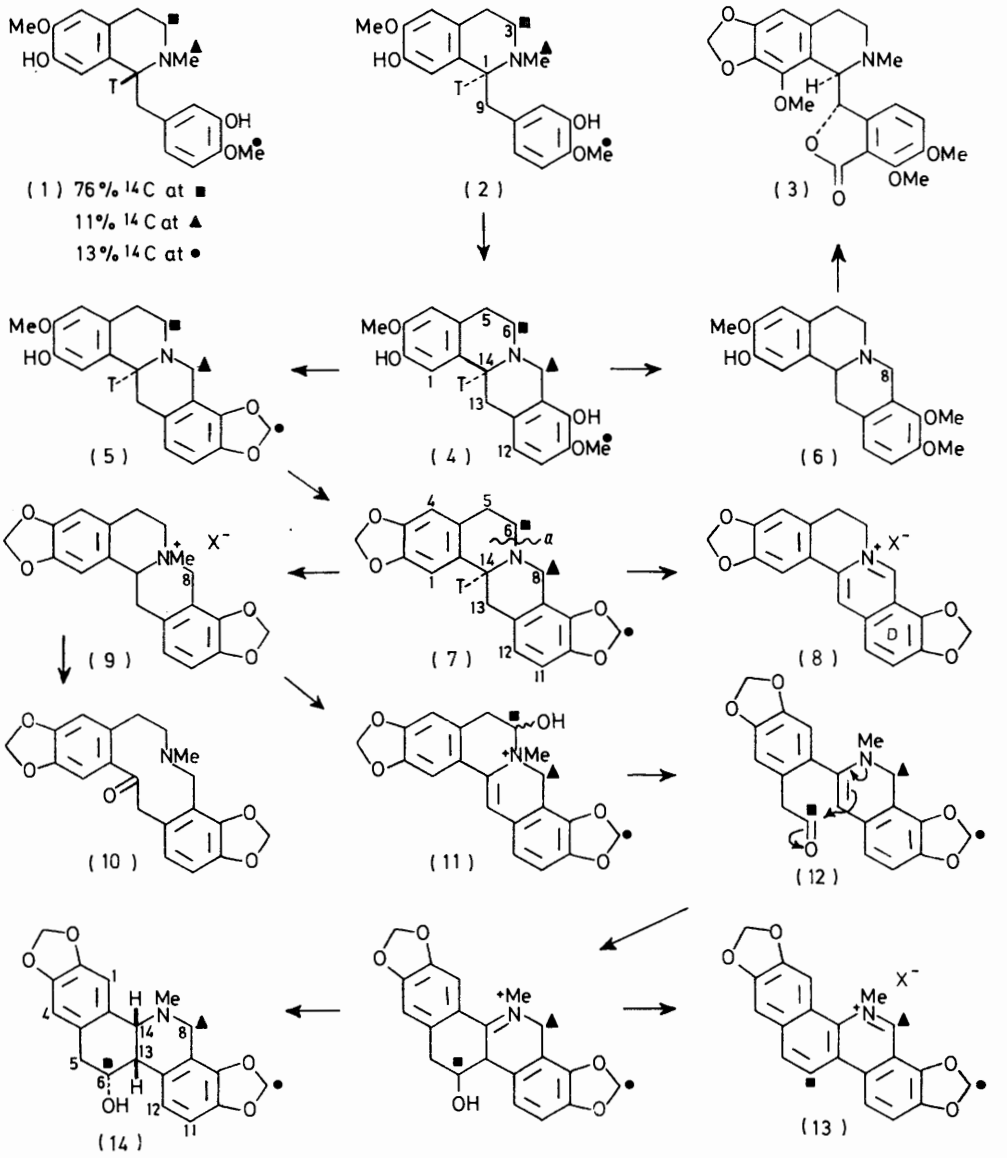
[3] R. B. Turner and R. B. Woodward, in 'The Alkaloids,' ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1953, vol. III, p. 54; R. Robinson, 'The Structural Relations of Natural Products,' Clarendon Press, Oxford, 1955, p. 89.

[4] E. Leete, *J. Amer. Chem. Soc.*, 1963, **85**, 473; E. Leete and S. J. B. Murrill, *Tetrahedron Letters*, 1964, 147.

[5] A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189.

[6] D. H. R. Barton, Hugo Müller Lecture, *Proc. Chem. Soc.*, 1963, 293.

to be an effective precursor of chelidonine, the incorporation being *ca.* 50 times higher than that of the (—)-(*R*)-form (1). The discrimination between the two enantiomers of reticuline was also striking for incorporation into protopine (10), with the (+)-form being **38** times more efficient than the (—)-form. This result for protopine agrees with that of the Imperial College group,[7] who found that (+)-(*S*)-reticuline was incorporated 13 times better into protopine than was the (—)-isomer in *Dicentra spectabilis* plants.



SCHEME 1 The illustrated labelling is for experiment 1, Table 1

Degradation of the labelled chelidonine (14) from experiment 1, Table 1, was carried out as in Scheme 2, which is largely based on the work of Gadamer *et al*;[8] the modifications which were introduced to allow small quantities to be degraded are given in the Experimental section. Hofmann degradation of *O*-acetylchelidonine methiodide gave mainly the methine (15), together with some of the naphthalene derivative (16); the latter was the sole product when the initial mixture was heated in benzene with toluene-*p*-sulphonic acid. Further methylation followed by Emde degradation yielded the nitrogen-free system (17), which was oxidised (Kuhn–Roth), and the resultant acetic acid was degraded to methylamine. Oxidation of chelidonine with permanganate gave two phthalic acids, isolated as their *N*-ethylimides,[4] and one of them (19) was further degraded with hot phosphoric acid to release formaldehyde from the methylenedioxy-group. The activities of the various products from Scheme 2 are collected in Table 2 and they show that the proportions of activity at the three ^{14}C-labelled sites are

[7] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *J. Chem. Soc.*, 1965, 6379.

[8] J. Gadamer, H. Dieterle, A. Stichel, M. Theissen, and K. Winterfeld, *Arch. Pharm.*, 1924, **262**, 249.

77% at ■, 9·4% at ▲, and 14% at ●. (+)-(*S*)-Reticuline (2) is thus proved to be specifically incorporated into chelidonine to give a labelling pattern qualitatively and quantitatively in agreement with biosynthetic conversion

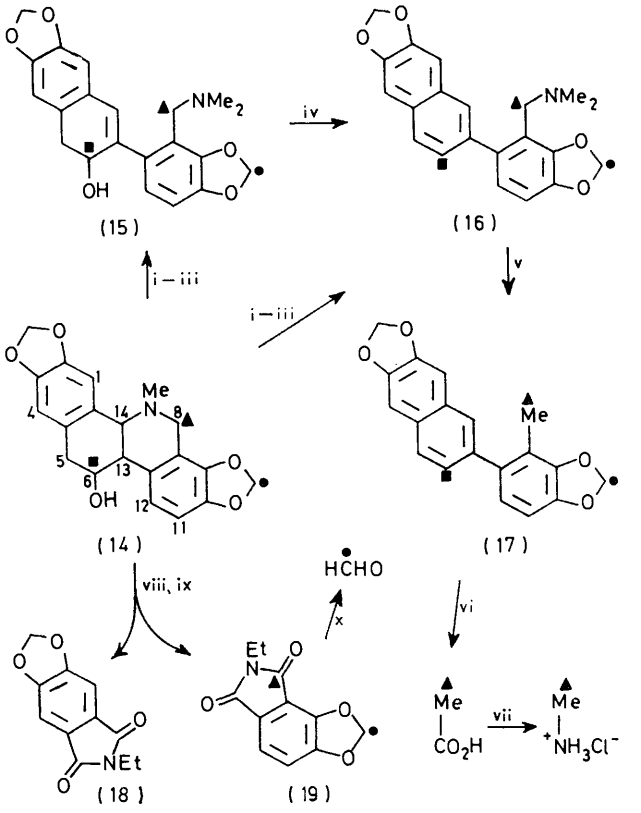
TABLE 1

Tracer experiments on *Chelidonium majus* plants

Expt. no.	Precursor	Incorporation (and ^{3}H-retention) (%) Chelidonine	Other alkaloids
1	(+)-(*S*)-[1-^{3}H, 3-^{14}C, N-*methyl*-^{14}C, 4′-O-*methyl*-^{14}C]Reticuline (2)	0·50 (0)	Protopine 0·19 (0)
2	(−)-(*R*)-[1-^{3}H, 3-^{14}C, N-*methyl*-^{14}C, 4′-O-methyl-^{14}C]Reticuline (1)	0·01	Protopine 5×10^{-3}
3	(−)-(*S*)-[1, 12-^{3}H]Scoulerine (4)	1·5	Sanguinarine 0·2 Chelerythrine 0·005
4	(−)-(*S*)-[8-^{3}H]Stylopine (7)	0·8	Stylopine [1] 1·8 Coptisine [1] 0·08
5	(+)-(*S*)-[3-^{14}C, N-*methyl*-^{3}H]-Reticuline [a]	0·41 (108)	
6	(−)-(*R*)-[3-^{14}C, N-*methyl*-^{3}H]-Reticuline [a]	0·053 (89) [b]	
7	(+)-(*S*)-[3-^{14}C, 9-^{3}H]-Reticuline [a]	0·58 (82)	
8	(−)-(*R*)-[3-^{14}C, 9-^{3}H]-Reticuline [a]	0·029 (84) [b]	
9	(−)-(*S*)-[6-^{14}C, 14-^{3}H]-Scoulerine (4)	0·61 (<1)	Protopine 0·92 (<1)
10	(+)-(*R*)-[6-^{14}C, 14-^{3}H]-Scoulerine	0·014	Protopine 0·04
11	(*RS*)-[5-^{3}H, 6-^{14}C]Scoulerine	1·2 (123)	(*RS*)-Stylopine [1] 0·3 (111)
12	(*RS*)-[8-^{14}C]Nandinine [c] (20)	0·03	Stylopine 0·02 Protopine 0·05
13	(*RS*)-[8-^{3}H, N-*methyl*-^{14}C]-Stylopine methochloride [c] (9)	0·2 (116)	Protopine 1·4 (112)
14	(*RS*)-[8-^{14}C]Isocorypalmine (6)		Allocryptopine 2·0

[a] Carried out by Dr. M. Hirst. [b] Reduced accuracy because of low activity. [c] The incorporations from a parallel feeding of [8-^{14}C] scoulerine were 0·6% into chelidonine, 0·3% into stylopine, and 0·6% into protopine.

dimethylformamide. Only the iminium system was reduced with labelling at C-8 and the reduction was completed by addition of a protic solvent (methanol) and an excess of borohydride.†



SCHEME 2 Reagents: i, Ac_2O; ii, MeI; iii, hot KOH; iv, *p*-$MeC_6H_4{\cdot}SO_3H$; v, Na–Hg; vi, CrO_3–H_2SO_4; vii, HN_3; viii, $KMnO_4$; ix, $EtNH_2$; x, hot H_3PO_4.

Experiment 3, Table 1, shows (−)-(*S*)-scoulerine to be well incorporated into chelidonine and that it is also converted into the aromatic systems of sanguinarine (13)

TABLE 2

Degradation of labelled chelidonine (14)

Relative molar activities or (^{3}H : ^{14}C ratios) and [expected values]

Source Table 1	*O*-Acetyl-chelidonine	*N*-Ethyl-3,4-methylenedioxyphthalimide (19)	*N*-Ethyl-4,5-methylenedioxyphthalimide (18)	Formaldehyde derivative	The naphthalene (16)	Emde product (17)	Sodium acetate	Methylamine hydrochloride
Expt. 1	100 [100]	23 [24]	<2 [0]	13·6 [13]	104 [100]	104 [100]	10·5 [11]	10·2 [11]
Expt. 3	100 [100]	52 [*ca.* 50]	47 [*ca.* 50]					
Expt. 4	100 [100]	0 [0]	0 [0]					
Expt. 7 [a]	(9·8 : 1) [b]				(0·09 : 1) [0]			

[a] Carried out by Dr. M. Hirst. [b] The corresponding methiodide had ^{3}H : ^{14}C ratio 9·5 : 1.

of the tetrahydroprotoberberine skeleton [*e.g.* (7)] into the benzophenanthridines (13) and (14); see Scheme 1.

Direct evidence for the protoberberine–benzophenanthridine relationship came from feeding the plants with (−)-(*S*)-[1, 12-^{3}H]scoulerine [1] (4) and with (−)-(*S*)-[8-^{3}H]stylopine (7). The latter was prepared by reduction of coptisine (8) with borotritiide in anhydrous and chelerythrine (24). (−)-(*S*)-Stylopine was proved to be a good precursor of chelidonine by experiment 4, which also demonstrated the formation of coptisine (8) from stylopine (7). The vigorous basic conditions used

† The major labelling site is undoubtedly C-8 but some labelling at C-14 cannot be excluded; any protonation of the intermediate dihydrocoptisine in the first step would allow further introduction of tritium.

to label (−)-(*S*)-[1,12-^{3}H]scoulerine (4) should bring about equal exchange at C-1 and C-12. These labels should then reside at C-1 and C-12 of chelidonine (14) and oxidative degradation should yield the two phthalic acids of equal activity (Schemes 1 and 2). Experiment 3, Table 2, shows that the results are in accord with this expectation. Similarly, the chelidonine from experiment 4 should be labelled at C-8 and, in the absence of scatter of labelling, the two phthalic acids should be radioinactive; this was found to be so (Table 2).

The following statements can be made about experiments 5—8: (*a*) the far greater efficiency of (+)-(*S*)-reticuline (2) than of (−)-isomer (1) for the biosynthesis of chelidonine is confirmed; (*b*) since the same precursor used in experiment 5 was incorporated into stylopine (7) with no more than 5% loss of tritium,[1] it follows that C-8 is unaffected during the further transformation into chelidonine; (*c*) tritium at C-9 of (+)-(*S*)-[3-^{14}C, 9-^{3}H]-reticuline (2) is *retained* through to stylopine [1] (7) whereas there is significant loss in the subsequent conversion into chelidonine (experiment 7). The loss, however, is less than the 50% removal which is characteristic of a stereospecific biochemical process.[9,10] Two explanations of this observation are possible; either the loss of hydrogen from C-13 of stylopine is a non-enzymic non-stereospecific process, subject to an appreciable kinetic isotope effect, or a stereospecific reaction has in fact occurred (with 50% loss of tritium *over that step*) but the ^{3}H : ^{14}C ratio has been raised by loss of ^{14}C-labelled material along competing pathways (*cf.* ref. 1). This ambiguity has been resolved by use of stereospecifically labelled substrates and will be described in an accompanying paper; [11] experiment 7 is instructive in showing what care is necessary in the interpretation of results from multiply labelled substrates which are *mixtures* of labelled molecules.

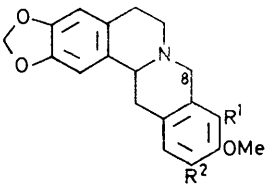
The tritium retained in chelidonine from the [9-^{3}H]-reticuline (experiment 7) should reside at C-13. Proof was obtained by carrying the labelled chelidonine through the first steps of Scheme 2. There was no loss of ^{3}H activity until the methine (15) was formed with essentially complete removal of tritium (results in Table 2).

Experiments 9 and 10, Table 1, are important in confirming that (−)-(*S*)-[6-^{14}C, 14-^{3}H]scoulerine [1] (4) is a good precursor of chelidonine and in showing that (+)-(*R*)-scoulerine [enantiomer of (4)] is, for practical purposes, ineffective [incorp. *ca.* 2% of that of (−)-isomer]. This means that (*RS*)-scoulerine can be used interchangeably with (−)-(*S*)-scoulerine in later work. Complete loss of tritium from the chiral centre of scoulerine (4) as it is transformed into chelidonine was again observed (*cf.* experiment 1). This tritium loss is in keeping with the suggested dihydroisoquinoline intermediate (11) in Scheme 1 which allows the new carbon–carbon bond to be formed by enamine chemistry [see (12)].

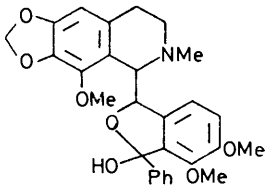
[9] D. Arigoni and E. L. Eliel, *Topics Stereochem.*, 1969, **4**, 127.
[10] A. R. Battersby, *Accounts Chem. Res.*, 1972, **5**, 148.
[11] A. R. Battersby, B. J. Bircher, C. Fuganti, and H. R. Wiltshire, *J.C.S. Perkin I*, 1975, 1162.

The conversion of stylopine into chelidonine involves fission of the C(6)–N bond with loss of at least one hydrogen atom from C-6 and possibly also from C-5. In order to select among various possible mechanisms, (*RS*)-[5-^{3}H]scoulerine and (*RS*)-[6-^{3}H]scoulerine [as (4)] were synthesised as follows.

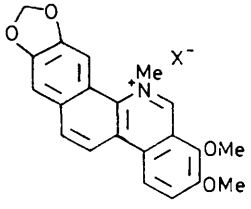
The route basically involved preparation of the [α-^{3}H]- and [β-^{3}H]-phenethylamines (29) and (27). The latter was readily made by proton exchange between the amide (26) and tritiated methanol in the presence of magnesium methoxide, the labelled amide then being reduced with lithium aluminium hydride. Preparation



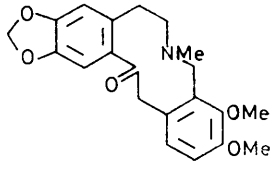
(20) R^1 = OH, R^2 = H
(21) R^1 = OMe, R^2 = H
(22) R^1 = H, R^2 = OH



(23)



(24)



(25)

of the [α-^{3}H]isomer proved difficult but was accomplished by exchanging the protons of nitromethane against tritiated water followed by condensation with *O*-benzylvanillin. The resultant nitrostyrene (28) was reduced as for the amide.

Specificity of labelling was established by diluting a small sample of each amine followed by degradation as in Scheme 3; the results are in Table 3. These show, in addition, a small kinetic isotope effect in the Hofmann elimination. The two amines were next converted as usual [12] into (*RS*)-[5-^{3}H]- and (*RS*)-[6-^{3}H]-scoulerine, to which was added (*RS*)-[6-^{14}C]scoulerine [1] as internal standard. Further, the intermediate (*RS*)-[3-^{3}H]-nor-

TABLE 3

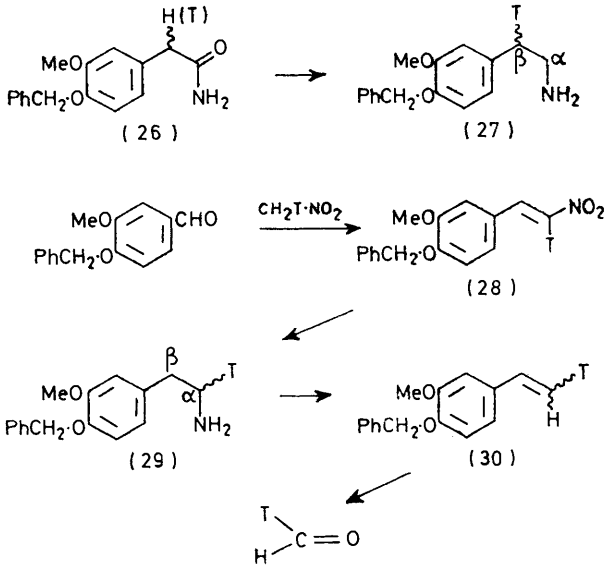
Degradation of [^{3}H]phenethylamines (27) and (29)

Starting material	Specific activity (disint. per 100 s per mmol) [and relative molar activity]			
	Amine	Amine methiodide	Styrene	Formaldehyde derivative
[α-^{3}H]Amine (29)	$2{\cdot}64 \times 10^7$ [100]		$2{\cdot}78 \times 10^7$ [105]	$2{\cdot}70 \times 10^7$ [102]
[β-^{3}H]Amine (27)	$1{\cdot}83 \times 10^8$ [100]	$1{\cdot}74 \times 10^8$ [95]	$1{\cdot}19 \times 10^8$ [65]	Inactive [0]

[12] A. R. Battersby, R. Southgate, J. Staunton, and M. Hirst, *J. Chem. Soc.* (*C*), 1966, 1052.

reticuline was resolved,[12] the configurational purities of the products were shown [13] to be virtually 100%, and the two enantiomers were then converted into (−)-(*S*)-[6-^{3}H]scoulerine (4) and (+)-(*R*)-[6-^{3}H]scoulerine [enantiomer of (4)].

The results from experiments with the three samples of [6-^{3}H]scoulerine will be covered in a forthcoming paper [14]



SCHEME 3

in which the stereospecificity of hydrogen removal from C-6 of scoulerine and stylopine is elucidated. Experiment 11, which made use of (*RS*)-[5-^{3}H, 6-^{14}C]scoulerine, showed good incorporations into both chelidonine and stylopine and the ^{3}H : ^{14}C ratio was increased in both cases. This is indicative of the sequence scoulerine (4) ⟶ stylopine (7) ⟶ chelidonine (14), in which scoulerine and stylopine are also being converted into other materials by steps which include at the outset attack at the C(5)–H bond. Such an oxidation will result in the remaining stylopine having an increased ^{3}H : ^{14}C ratio. This rise in ratio is of considerable interest but the major conclusion from experiment 11 is that C-5 is not involved in the conversion of stylopine into chelidonine.

Two final studies (experiments 12 and 13) were planned to provide information about the sequence over the late stages of biosynthesis. There are two possible intermediates (5) and (20) between scoulerine (4) and stylopine (7), depending on which methylenedioxy-group is formed first. We have synthesised (*RS*)-[8-^{14}C]-nandinine (20) from the corresponding 1-benzylisoquinoline and labelled formaldehyde in a way analogous to the route used for scoulerine.[12] Methylation of nandinine with diazomethane gave (*RS*)-[8-^{14}C]canadine (21) for use in later experiments. It can be seen from experiment 12, Table 1, that nandinine (20) is not effective as a precursor of chelidonine, stylopine, or protopine. We believe that the alternative (5) is the true intermediate and we hope a study of it will be taken up by others; no additional work is planned here.

Moving one further stage along the pathway, a possible intermediate beyond stylopine is the *N*-metho-derivative of stylopine (9); this has recently been isolated from *Glaucium corniculatum*.[15] To ensure that incorporation values from feeding this compound to the plants should be unambiguous, it was synthesised by standard methods as [*N-methyl*-^{14}C, 8-^{3}H]stylopine methochloride. Experiment 13, Table 1, shows that, relative to the parallel feeding of (*RS*)-[8-^{14}C]scoulerine, the incorporation into chelidonine is low and the one into protopine is high, but in both cases the ^{3}H : ^{14}C ratio was close to that of the precursor. These results show that stylopine methochloride is being incorporated without degradation and so indicate that it lies on the pathway to both protopine (10) and chelidonine (14).

These experiments in sum largely define the biosynthetic pathway to chelidonine (14) and sanguinarine (13) as illustrated in Scheme 1. Further study will be necessary to determine the precise sequence of steps by which *N*-methylation, dehydrogenation, and ring cleavage occur [*i.e.* around intermediate (11)].

Protopine (10), *Allocryptopine* (25), *Berberine, and Narcotine* (3).—Results relevant to the biosynthesis of protopine in *C. majus* have largely been covered in earlier paragraphs. Mention should be made here of the satisfactory incorporation of (−)-(*S*)-[6-^{14}C, 14-^{3}H]-scoulerine (4) into protopine (experiment 9, Table 1), with complete loss of tritium, and the very low incorporation of the (+)-(*R*)-isomer (experiment 10). Dilution of the total alkaloidal material from *C. majus* plants, which had metabolised (*RS*)-[8-^{14}C]scoulerine, with both protopine and dihydroprotopine (10; CHOH in place of CO) followed by re-isolation and rigorous purification, gave protopine of specific activity $1{\cdot}15 \times 10^5$ disint. per 100 s per mg and dihydroprotopine of activity 7 disint. per 100 s per mg. These values point against dihydroprotopine being an intermediate though it is accepted that at least one other explanation of this result could be offered.

A route to protopine in accord with all our data and earlier work [7] is as follows: two C_6–C–C units by several stages ⟶ (+)-(*S*)-reticuline (2) ⟶ (−)-(*S*)-scoulerine (4) ⟶ (5) ⟶ (−)-(*S*)-stylopine (7) ⟶ (9) ⟶ protopine (10).

The closely related allocryptopine (25) is strongly labelled from (*RS*)-[8-^{14}C]isocorypalmine (6) (experiment 14, Table 1); this precursor was synthesised by modification of the route developed [12] for scoulerine (see Experimental section). A reasonable biosynthetic pathway to allocryptopine thus only requires a switch by *O*-methylation at stage (4) of the sequence given for protopine to generate isocorypalmine (6) and so forward *via* canadine (21) to allocryptopine (25).

[13] A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.

[14] A. R. Battersby, J. Staunton, R. Southgate, and M. C. Summers, in preparation.

[15] V. Novak, L. Dolejo, and J. Slavik, *Coll. Czech. Chem. Comm.*, 1972, **37**, 3346.

The biosynthetic route to narcotine (3) has been tracked [16] as far as (−)-scoulerine (4) and the results in Table 4 allow an extension to be made. (*RS*)-[8-^{14}C]-Isocorypalmine (6) is incorporated well (experiment 3) and also (*RS*)-[8-^{14}C]canadine (21) (experiment 2). Values for a parallel experiment with labelled (*RS*)-scoulerine are included to provide a baseline. The narcotine from experiment 5, which had lost essentially all the tritium from the precursor (as expected), was degraded as earlier [16] *via* phenylnarcotine (23) to benzoic acid which extracts only the lactonic carbonyl group from narcotine. The phenylnarcotine and benzoic acid carried, respectively, 104 and 103% of the original activity of narcotine; the incorporation of (*RS*)-[8-^{14}C]-canadine into narcotine thus takes place without randomisation.

TABLE 4

Tracer experiments on *Papaver somniferum* plants

Expt. no.		Incorporation or [^{3}H-retention] (%) Narcotine (3)	Berberine [8; $(MeO)_2$ for CH_2O_2]	Canadine (21)	Protopine (10)	Allocryptopine (25)
1	(*RS*)-[8-^{14}C]Scoulerine [as (4)]	1·1	*a*	0·04	0·04	*a*
2	(*RS*)-[8-^{14}C]Canadine (21)	1·2	0·02	*a*	*a*	0·2
3	(*RS*)-[8-^{14}C]Isocorypalmine (6)	4·6 [b]	0·06	*a*	*a*	*a*
4	(*RS*)-[8-^{14}C]Nandinine (20)	0·009	<0·002	<0·008	<0·002	*a*
5	(*RS*)-[8-^{3}H, 8-^{14}C]Canadine (21)	Ratio 0·3 : 1	Ratio 7·4 : 1	Ratio 17·5 : 1	*a*	Ratio 14·0 : 1
	^{3}H : ^{14}C ratio 16·0 : 1	(2)	[46]	[109]		[88]

[a] Not examined. [b] A feeding during same season of (*RS*)[8-^{14}C]scoulerine gave 4·0% incorporation into narcotine.

These results lead to the view that in the opium poppy (−)-(*S*)-scoulerine (4) is *O*-methylated to form (−)-isocorypalmine (6), which is converted into narcotine (3). (−)-(*S*)Canadine (21) probably stands on the pathway between isocorypalmine and narcotine but further studies are necessary to confirm canadine's role.

The ^{3}H retentions for experiment 5, Table 4, are of interest, especially the retention in berberine (8; dimethoxy in place of methylenedioxy on ring D) of *ca.* half the original ^{3}H activity. This points to a stereospecific enzymic process operating at C-8 for the aromatisation of canadine (21). Incidentally, the loss of essentially all the tritium during the formation of narcotine is consistent with the ^{3}H label having been located specifically at C-8 of the precursor.

The rapier of stereospecific labelling with tritium has been used to probe more deeply into the mechanism of some of the enzymic conversions revealed by the present work; these studies are described in an accompanying paper [11] and in later ones (*e.g.* ref. 14).

EXPERIMENTAL

For general directions and details on work with *C. majus* plants see ref. 1.

Hofmann Degradation of Chelidonine (14).—Chelidonine (70—300 mg) was shaken for 1 h at 20 °C with acetic anhydride (3 ml) and anhydrous sodium acetate (100 mg). The mixture was treated with water (20 ml), then basified with ammonia or sodium carbonate, and the *O*-acetyl derivative was extracted with ethyl acetate; m.p. 162—163° (from ethanol) (lit.,[8] 161—163°); t.l.c. on silica, R_F 0·8.

This product (207 mg) was heated under reflux for 12 h with methyl iodide (10 ml) and the crystals (288 mg) which separated from the cooled mixture were run in ethanol through Amberlite IRA-400 resin (OH^- form). The residue from evaporation of the percolate in ethanol (20 ml) was mixed with sodium hydroxide (1 g) and water (3 ml) and the solution was heated under reflux for 2 h. After addition of water (150 ml), extraction with 3 : 1 ether–chloroform gave the crude methine (185 mg), which was either repeatedly crystallised from aqueous ethanol to give the pure methine, m.p. 146—147° (lit.,[8] 145—146°), or dehydrated without purification. For this, the crude methine in benzene (50 ml) was heated under reflux for 15 min with toluene-*p*-sulphonic acid (50 mg), an excess of aqueous potassium carbonate was then added, and the naphthalene (16) was extracted with 3 : 1 ether–chloroform. The product (113 mg) from ethanol had m.p. 143—144° (lit.,[8] 145—146°). (Found: C, 72·6; H, 5·5. Calc. for $C_{21}H_{19}NO_4$: C, 72·2; H, 5·5%).

2,3-*Methylenedioxy*-6-(2-*methyl*-3,4-*methylenedioxyphenyl*)-*naphthalene* (17).—the foregoing base (16) (193 mg) was warmed with acetone (2 ml) and methyl iodide (5 ml) for a few min. The crystals were collected and passed in ethanol over Amberlite IRA-400 resin (Cl^- form). Evaporation of the percolate left the methochloride, which in water (20 ml) was treated at 70 °C with 3% sodium amalgam (0·5 g) for ½ h and then was kept at 20 °C for 6 h more with the addition of the amalgam (0·5 g) every 2 h. The mercury was removed and the aqueous mixture was extracted with ether to give the (methylphenyl)naphthalene (67 mg), m.p. 136—137° (lit.,[8] 142—143°) (from ethanol) (Found: C, 74·3; H, 4·9. Calc for $C_{19}H_{14}O_4$: C, 74·45; H, 4·6%).

Kuhn–Roth Degradation of the Naphthalene (17).—The foregoing product (58 mg) was heated under reflux for 2 h with chromium trioxide (5·6 g), water (33 ml), and sulphuric acid (7 ml), and the resultant acetic acid was steam-distilled and titrated with 0·05N-sodium hydroxide. After the solution had been basified to pH 11, it was evaporated to small volume, sodium azide (40 mg) was added, and the solution was evaporated completely. Polyphosphoric acid (2 ml) was added to the residue and the mixture was heated at 100 °C for 2 h. Water was added, the solution was basified with strong potassium hydroxide, and the methylamine was steam-distilled into 2N-hydrochloric acid, from which the hydrochloride was obtained by evaporation; from ethanol–ether the salt (10 mg) had m.p. 226° (Found: C, 17·9; H, 9·05. Calc. for CH_5N,HCl: C, 17·8; H, 9·0%).

N-*Ethyl*-3,4-*methylenedioxyphthalimide* (19) *and* N-*Ethyl*-

[16] A. R. Battersby, M. Hirst, D. J. McCaldin, R. Southgate, and J. Staunton, *J. Chem. Soc.* (*C*), 1968, 2163.

4,5-methylenedioxyphthalimide (18).—Chelidonine (420 mg), was dissolved in 0·1N-sulphuric acid (100 ml) and treated with aqueous sodium carbonate until the solution had become slightly turbid. This was vigorously stirred while aqueous potassium permanganate (2·5 g in 100 ml) was added over 2 h, and the mixture was then warmed to 70 °C for ½ h before being decolourised with sodium disulphite, concentrated to half volume, acidified with hydrochloric acid, and continuously extracted with ether for 2 days. The residue from the ether was dissolved in aqueous ethylamine (5 ml; 30%) and the solution was evaporated to dryness. The residue was heated at 160° for 5 min prior to sublimation (120°; 0·11 mmHg). The sublimate was dissolved in 1 : 1 benzene–light petroleum (b.p. 60—80°) and chromatographed on an alumina column (8 g) in that solvent and then in pure benzene. The 4,5-methylenedioxy-isomer (20 mg) was eluted first and was crystallised from ethanol and sublimed as before; m.p. 166—167° (lit., 166—167°). The 3,4-methylenedioxy-compound (30 mg) was eluted second and was treated in the same way; m.p. 124—125° (lit., 124—125°).

Formaldehyde Derivative.—The 3,4-methylenedioxy-imide (19) (25 mg) was heated with phosphoric acid (15 ml) at 140 °C for 3 h, the formaldehyde produced being swept with nitrogen into dimedone (100 mg) in ethanol (1 ml) and water (5 ml). Water (50 ml) was then added to the acidic solution, which was boiled until half of the solvent had been removed, the distillate being passed into the same dimedone solution. More water (20 ml) was added to the reaction mixture and distillation was repeated; after 2 h, the formaldehyde derivative was collected and recrystallised from ethanol; m.p. 193—194° (Found: C, 70·1; H, 8·1. Calc. for $C_{17}H_{24}O_4$: C, 69·8; H, 8·3%).

(RS)-[8-^{3}H]*Stylopine* [*as* (7)].—Sodium borotritiide (1·12 mg; 1·1 mCi) was added to a solution of coptisine chloride (30 mg) in dry dimethylformamide (4 ml) and the mixture was kept for 2 h at 18 °C. After addition of an excess of sodium borohydride (20 mg) and methanol (4 ml), the colour was rapidly discharged (15 min) and water (20 ml) was then added. Extraction with ether gave (*RS*)-[8-^{3}H]stylopine (19 mg), m.p. 219—220° (from ethanol) (total activity 0·17 mCi).

4-Benzyloxy-3-methoxy[β-^{3}H]*phenethylamine* (27).—4-Benzyloxy-3-methoxyphenylacetamide [17] (400 mg), magnesium methoxide (350 mg), and tritiated water (0·1 ml; 100 mCi) were heated under reflux in dry tetrahydrofuran (30 ml) for 24 h. After acidification with N-acetic acid (150 ml), the amide was extracted with chloroform and crystallised (370 mg) from aqueous ethanol, and the dried product was heated under reflux in ether (50 ml) with lithium aluminium hydride (0·4 g) for 2 h. The excess of hydride was destroyed with saturated aqueous sodium potassium tartrate and the solid was collected and washed with ether. Evaporation of the combined ethereal solution gave a residue which was dissolved in the minimum of ethanolic hydrogen chloride, and the amine hydrochloride (118 mg; 9·4 mCi) was precipitated with ether.

4-Benzyloxy-3-methoxy[α-^{3}H]*phenethylamine* (29).—A solution of nitromethane (20 mg), potassium hydroxide (18 mg), tritiated water (0·05 ml; 250 mCi), and ethanol (0·05 ml) in dimethylformamide (0·5 ml) was kept at 20 °C for ½ h before addition of *O*-benzylvanillin (120 mg) in dimethylformamide (0·5 ml). After a further ½ h, the solution was added to an excess of 2N-hydrochloric acid and the mixture was extracted with chloroform. The residue from the chloroform crystallised from methanol to give the [α-^{3}H]nitrostyrene (50 mg; 7 mCi), m.p. 122—123° (lit.,[18] 122—123°).

Reduction of this product with lithium aluminium hydride as for the methylenedioxy-analogue below gave the [α-^{3}H]phenethylamine, isolated as the hydrochloride (*cf.* above), m.p. 175—177° (lit., [19] m.p. 176—178°).

Both the foregoing amines were identical with an authentic radioinactive sample.

Degradation of the [α-^{3}H]- *and* [β-^{3}H]-*Phenethylamines* (29) *and* (27).—A solution of the tritiated phenethylamine (250 mg) in aqueous 40% formaldehyde (5 ml) was adjusted to pH 7 with formic acid and heated at 85 °C for 4 h. The solvents were evaporated off and the residue was dissolved in methanol (5 ml) and heated under reflux with methyl iodide (2 ml) for 1 h before being concentrated and diluted with ether to give the methiodide (166 mg), m.p. 180—181° (lit.,[20] 180—18·5°).

The methiodide (120 mg) was vigorously stirred with aqueous 30% sodium hydroxide (10 ml) for 1 h at 95 °C, the mixture was cooled, and the resultant styrene was extracted with ether. The extracted gum crystallised (31 mg) from methanol–water; m.p. 46—47°. Ozone was bubbled through a solution of the styrene (25 mg) in methylene chloride (10 ml) at −78° for 10 min, after which the excess of oxidant was blown off with nitrogen and the solution was evaporated. The residual ozonide was heated under reflux with water (10 ml), zinc dust (200 mg), and a crystal of silver nitrate for ½ h before the addition of more water (10 ml) and distillation; the distillate was collected in 1 : 4 ethanol–water (6 ml) containing dimedone (100 mg). When half the water had been distilled off, more water (10 ml) was added and distillation to half volume was repeated. The precipitated dimedone derivative (13 mg) was recrystallised from ethanol; m.p. 193—194° (see Table 3 for specific activities) (Found: C, 70·1; H, 8·1. Calc. for $C_{17}H_{24}O_4$: C, 69·8; H, 8·3%).

(RS)-*Nor-reticuline* [*as* (1; NH *in place of* NMe)], (RS)-[3-^{3}H]*Nor-reticuline, and* (RS)-[4-^{3}H]*Nor-reticuline, and Resolution of the Last.*—Earlier methods [20] were considerably improved with respect to yield as follows. 3-Benzyloxy-4-methoxyphenylacetic acid (9·4 g) in dry ether (400 ml) was treated with thionyl chloride (20 ml) and dimethylformamide (1 ml) for 2 h before evaporation of the solvents. The residue in dry ether (200 ml) was added over ½ h to a vigorously stirred mixture of ether (200 ml), water (100 ml), potassium hydroxide (12 g), and 4-benzyloxy-3-methoxyphenethylamine hydrochloride (10·6 g). After a further 2 h stirring, the amide (16·3 g, 92%) was collected, washed with water, and dried; m.p. 136·5° (from ethanol) (lit.,[21] 140·5—141·5°) (sufficiently pure for use in the next stage); $\nu_{max.}$ 3400 and 1650 cm^{-1}; $\lambda_{max.}$ 280 and 231 nm; τ 2·5—2·8 and 3·3—3·7 (16H, m, aryl H), 4·7br (1H, NH), 5·01 (4H, s, CH_2O), 6·15 and 6·19 (each 3H, s, MeO), 6·69 (2H, s, CH_2CO), and 6·54 and 7·5 (each 2H, t, CH_2); *m/e* 511 (M^+, 0·3%), 421 (0·15), 299 (31), 208 (23), 137 (14), and 91 (100).

[17] D. H. Hey and L. C. Loba, *J. Chem. Soc.*, 1954, 2246.
[18] N. A. Lange and W. E. Hambourger, *J. Amer. Chem. Soc.*, 1931, **53**, 3865.
[19] J. M. Bobbitt and Tsu-Tah Chai, *J. Org. Chem.*, 1959, **24**, 1106.
[20] M. Tomita and T. Nakano, *J. Pharm. Soc. Japan*, 1952, **72**, 1256.
[21] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.

The amide (155 mg) in dry toluene (20 ml) was heated at 100 °C for 1 h with freshly distilled phosphoryl chloride (0·5 ml), before evaporation of the solvents. The residue was dissolved in ethanol (4 ml) and concentrated hydrochloric acid (0·1 ml); ether precipitated the dihydroisoquinoline base hydrochloride (144 mg), m.p. 203—204° (lit.,[21] 201—203°); $\nu_{max.}$ 1640 cm^{-1}; $\lambda_{max.}$ 360, 307, 280, and 228 nm (ε 3410, 6860, 7160, and 24,800), shifting to 310, 276, and 228 nm (ε 6250, 9950, and 30,100) with base; τ 2·6—2·9 and 3·3—3·55 (15H, m, aryl H), 4·93 (4H, s, OCH_2), 5·66 (2H, s, CH_2·C=N), 6·15 and 6·31 (each 3H, s, MeO), and 6·3 and 7·3 (each 2H, m, CH_2); *m/e* 493 (M^+, 2%), 402 (25), 312 (15), and 91 (100).

The imine hydrochloride in the minimum of methanol was treated with an excess of sodium borohydride until there was no u.v. absorption above 315 nm. The solvent was evaporated off, the residue was partitioned between water and ethyl acetate, and the concentrated organic solution was acidified with ethereal hydrogen chloride to give (*RS*)-*OO*-dibenzylnor-reticuline hydrochloride [12] (95%), m.p. 161·5—162° (from methanol–ether).

The [α-^{3}H]- and [β-^{3}H]-phenethylamines (29) and (27) prepared above were converted, respectively, into (*RS*)-[3-^{3}H]- and (*RS*)-[4-^{3}H]-nor-reticuline in the foregoing way; the (*RS*)-*OO*-dibenzyl[3-^{3}H]nor-reticuline was resolved as earlier.[12] That complete resolution had been achieved was proved by correlation [13] of the (*R*)- and (*S*)-[3-^{3}H]nor-reticulines so obtained with *N*-benzoyltetrahydropapaverine as follows.

A solution of (−)-*OO*-dibenzylreticuline (50 mg) in acetone (0·5 ml) was shaken with benzoyl chloride (0·5 ml) and aqueous 2N-potassium hydroxide (2·5 ml). The mixture was diluted with water and the precipitate, dissolved in benzene, was run through alkaline alumina. Evaporation of the percolate gave (+)-*N*-benzoyl-*OO*-dibenzylnor-reticuline (56 mg), $[\alpha]_D^{26}$ +75° (*c* 0·96 in $CHCl_3$). This amide in methanol (10 ml) was shaken with hydrogen and 10% palladised charcoal (15 mg) until uptake ceased (2 mol. equiv.). Evaporation of the filtered solution left *N*-benzoylnor-reticuline (35 mg), which in methanol (3 ml) was treated for 20 h with an excess of ethereal diazomethane. The product was purified by p.l.c. to give (+)-*N*-benzoyltetrahydropapaverine, m.p. and mixed m.p. 155—156°, identical (i.r.) with an earlier sample,[13] $[\alpha]_D^{25}$ +77° (*c* 1·03 in EtOH). The previous sample [13] was further fractionated and showed $[\alpha]_D^{25}$ +74° (*c* 0·82 in EtOH).

The four samples of labelled nor-reticuline were then converted [12] into (*RS*)-[5-^{3}H]scoulerine, (*RS*)-[6-^{3}H]scoulerine, (−)-(*S*)-[6-^{3}H]scoulerine, and (+)-(*R*)-[6-^{3}H]scoulerine.

(+)-(S)- *and* (−)-(R)-[N-methyl-^{3}H, 3-^{14}C]*Reticulines*.—7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxyisoquinoline (383 mg) from above was treated in ethyl acetate (10 ml) with [^{3}H]methyl iodide (25 mCi) using a vacuum line. After a week at 20 °C the solvents were removed by freeze-drying and a solution of methyl iodide (2 ml) in ethyl acetate (10 ml) was added. Two days later, the methiodide was collected (404 mg), suspended in propan-2-ol (12 ml), and stirred with sodium borohydride (50 mg) for 72 h. After dilution with water, the labelled *OO*-dibenzylreticuline was extracted with chloroform as a gum (315 mg), yielding a picrate which was recrystallised (168 mg). The recovered base (101 mg; 6·2 mCi) was mixed with (*RS*)-*OO*-dibenzyl[3-^{14}C]reticuline [21] and the mixture resolved and debenzylated as earlier.[16] (+)-(*S*)-*OO*-Dibenzylreticuline had m.p. 91—92° (lit.,[13] 90—93°), $[\alpha]_D$ +43·4° (*c* 1·0 in $CHCl_3$); (−)-(*R*)-*OO*-dibenzylreticuline had m.p. 91—93°, $[\alpha]_D$ −42·6° (*c* 1·0 in $CHCl_3$); the activities of (+)-(*S*)-reticuline hydrochloride for feeding were ^{3}H 0·66 mCi and ^{14}C 0·06 mCi, and the (−)-*R*-isomer had ^{3}H 0·44 mCi and ^{14}C 0·04 mCi. The configurational purities of the final products and their precise ^{3}H : ^{14}C ratio were determined by conversion with diazomethane into the corresponding laudanosine enantiomers followed by dilution analysis as described earlier.[16] The (+)-(*S*)-isomer was 98% configurationally pure and the value for the (−)-(*R*)-isomer was 96%.

(RS)-[8-^{14}C]*Nandinine* (20), (RS)-[8-^{14}C]*Canadine* (21), *and* (RS)-[8-^{14}C]*Scoulerine* [as (4)].—Lithium aluminium hydride (35 g) was suspended in dry tetrahydrofuran (350 ml), and 3,4-methylenedioxy-β-nitrostyrene [22] (55 g), largely dissolved in tetrahydrofuran (660 ml), was added over 2 h to the boiling suspension. The undissolved styrene was then washed in with more dry tetrahydrofuran (120 ml) during ½ h and, after cooling, the excess of hydride was destroyed by the careful addition of a saturated aqueous solution of Rochelle salt. The inorganic solid was collected and was boiled with benzene (1 l) for 1 h, the inorganic salts were filtered off, and the solutions in tetrahydrofuran and benzene were combined and evaporated. The residue in ethyl acetate (80 ml) was acidified with ethereal hydrogen chloride to give the amine hydrochloride (45·9 g, 80%), which was recrystallised from methanol (6 ml g^{-1}) and ether (10 vol) to give pure material (34·7 g, 60%), m.p. 207—210° (lit.,[22] 210°); $\lambda_{max.}$ 282 and 230 nm; τ (free base) *ca.* 3·35 (3H, m, aryl H), 4·11 (2H, s, OCH_2O), 7·12 and 7·35 (each 2H, m, CH_2), and 8·48 (2H, s, NH_2); *m/e* 165 (M^+, 27%), 136 (100), 135 (40), and 77 (20).

3-*Benzyloxy*-4-*methoxy*-N-(3,4-*methylenedioxyphenethyl*)-*phenylacetamide*.—The amide with 3-benzyloxy-4-methoxyphenylacetic acid was prepared (67%) as for the case described above; m.p. 135—136° (from propan-2-ol) (Found: C, 71·65; H, 6·2; N, 3·2. $C_{25}H_{25}NO_5$ requires C, 71·6; H, 6·0; N, 3·3%); $\nu_{max.}$ 3420 and 1670 cm^{-1}; $\lambda_{max.}$ 287 and 233 nm; τ 2·5—2·8 (5H, m), 3·15—3·65 (6H, m, aryl H), 4·17 (2H, s, OCH_2O), 4·65br (1H, s, NH), 4·94 (2H, s, CH_2O), 6·17 (3H, s, MeO), 4·63 (2H, s, CH_2CO), and 6·73 and 7·47 (each 2H, t, CH_2); *m/e* 419 (M^+, 10%), 329 (3), 149 (81), and 91 (100). This product (5·13 g) was dissolved in hot acetonitrile (50 ml) and heated under reflux with freshly distilled phosphoryl chloride (7 ml) for 15 min. The residue from evaporation was dissolved in 95% ethanol (50 ml) containing concentrated hydrochloric acid (1 ml) and vigorously stirred as ether (10 vol) was added to precipitate 1-(3-*benzyloxy*-4-*methoxybenzyl*)-3,4-*dihydro*-6,7-*methylenedioxyisoquinoline hydrochloride* (5·08 g. 91%), m.p. 152—155° (from ethanol) (Found: C, 67·5; H, 6·1; N, 2·9. $C_{25}H_{24}ClNO_4$,0·5C_2H_5OH requires C, 67·75; H, 5·9; N, 3·0%); $\nu_{max.}$ 1660 cm^{-1}; $\lambda_{max.}$ 360, 304, 285, and 230 nm (ε 5000, 5000, 5050, and 17,500), shifted to 317, 280, and 225 nm (ε 6220, 6390, and 25,200) with base; τ 2·5—3·3 (10H, m, aryl H), 3·85br (1H, NH^+), 3·95 (2H, s, OCH_2O), 4·82 (2H, s, CH_2O), 5·59 (2H, s, $CH{=}N^+$), 6·21 (3H, s, MeO), *ca.* 6·15 (q, CH_2 of ethanol and CH_2 of isoquinoline), 7·21 (2H, t, CH_2), and 8·78 (t, CH_3 of ethanol); *m/e* 401 (*M*, 8%), 310 (100), 239 (8), 168 (28), 96 (89), and 91 (61).

The above imine hydrochloride was reduced as usual with sodium borohydride to give 1-(3-*benzyloxy*-4-*methoxy*-

[22] D. H. R. Barton, D. S. Bhakuni, G. M. Chapman, and G. W. Kirby, *J. Chem. Soc.* (*C*), 1967, 2134.

benzyl)-1,2,3,4-*tetrahydro*-6,7-*methylenedioxyisoquinoline*, isolated as the *hydrochloride*, m.p. 220—223° (from ethanol) (Found: C, 68·05; H, 5·6; N, 3·2. $C_{25}H_{26}ClNO_4$ requires C, 68·25; H, 5·9; N, 3·2%); $\lambda_{max.}$ 288 and 233 nm; *m/e* 403 (M^+, 0·3%), 402 (0·25), 401 (0·25), 312 (3·5), 227 (2·2), 176 (100), and 91 (13).

This was debenzylated as for related cases [15] and the phenol was isolated as the hydrochloride, $\nu_{max.}$ 3500 cm^{-1}; $\lambda_{max.}$ 287 and 233 nm, shifted by base to 292 and 237 nm.

A solution of the above phenolic isoquinoline salt (73·5 mg) in methanol (7·5 ml) and water (15 ml) at pH 9 was treated with 3·6% aqueous formaldehyde (0·05 ml) and after the solution had been stirred under nitrogen for 2·5 h, aqueous [^{14}C]formaldehyde (0·5 mCi) was added. After 2 days, aqueous 40% formaldehyde (2 ml) was added and the mixture was stirred for 16 h and then extracted with chloroform. The extract was separated into (*RS*)-[8-^{14}C]-nandinine (20) and the isomeric phenol (22) by p.l.c. on silica in ether; the less polar band of nandinine was eluted with 10% methanol in ether and half the resultant base was converted into the hydrochloride and recrystallised from methanol–ether (yield 16·3 mg; 0·1 mCi); *m/e* 325 (M^+, 100%), 176 (100), 150 (50), and 135 (12).

The remaining half was treated with an excess of ethereal diazomethane for 24 h to yield (*RS*)-[8-^{14}C]canadine, isolated as the hydrochloride (13·1 mg; 0·074 mCi) (from methanol–ether); *m/e* 339 (M^+, 51%), 174 (24), 169·5 (2), 164 (100), and 149 (74). This product was further identified by comparison with authentic (*RS*)-canadine prepared from berberine.

(With Dr. C. Fuganti) (*RS*)-Nor-reticuline hydrochloride (102 mg) was converted essentially as above, by using [^{14}C]formaldehyde (0·5 mCi), but at pH 6—7, into (*RS*)-[8-^{14}C]scoulerine, which was isolated by chromatography on alumina [12] and converted immediately into the hydrochloride (35 mg; 0·15 mCi) (from methanol–ether). The picrate (from ethanol) had m.p. 205—207°; *m/e* for base 327 (M^+, 45%), 178 (100), 176 (30), 163·5 (1), 150 (57), and 135 (34).

(RS)-[8-^{3}H]*Stylopine* [*as* (5)] and (RS)-[8-^{3}H]*Canadine* (21).—These were prepared together for convenience in using a minute amount of borotritiide. A mixture of berberine and coptisine (48 mg isolated from *C. majus*) in stirred dimethylformamide (10 ml) was treated with sodium borotritiide (0·91 mg; 7·45 mCi). After 16 h, an excess of potassium borohydride was added, and after 2 h more water (30 ml) was added. Extraction with ether gave the two bases, which were separated by p.l.c. on silica in 3 : 7 ether–benzene to give (from ethanol) (*RS*)-[8-^{3}H]canadine (13·5 mg; 1·3 mCi) and (*RS*)-[8-^{3}H]stylopine (2·8 mg; 0·6 mCi), which were identical with authentic samples.

(RS)-[8-^{3}H, N-*methyl*-^{14}C]*Stylopine Methochloride* (9; X = Cl).—The foregoing labelled stylopine was diluted with unlabelled material (15 mg), and as a solution in ethyl acetate (5 ml) was treated with [^{14}C]methyl iodide (0·5 mCi), added by vacuum line. After 24 h at 20 °C, a large excess (0·5 ml) of inactive methyl iodide was added and the mixture was kept at 20 °C for 24 h. The crystals which separated were passed in 1 : 1 water–ethanol over Amberlite IRA-400 resin (5 g; Cl^- form) and the column was washed with the same solvent (100 ml). Evaporation of the eluate gave the doubly labelled (*RS*)-stylopine methochloride (34 μCi ^{14}C; 590 μCi ^{3}H).

N-(4-*Benzyloxy*-3-*methoxyphenethyl*)-3-*hydroxy*-4-*methoxyphenylacetamide*.— 3-Benzyloxy-4-methoxyphenylacetic acid (5 g) in glacial acetic acid (100 ml) was hydrogenated at 18 °C over 10% palladised charcoal (500 mg) until uptake (414 ml) was complete (80 min). After filtration, the solution was evaporated and the residue triturated with water to give the phenol (3·05 g, 88%), m.p. 128—130° (from water) (lit.,[23] 122·5—124·5°); $\nu_{max.}$ 3550 and 1715 cm^{-1}; $\lambda_{max.}$ 280 and 227sh nm, shifting to 294 and 243 nm with alkali; τ [$(CD_3)_2SO$] 1·1br (1H, OH), 3·1—3·5 (3H, m, aryl H), 6·25 (3H, s, MeO), and 6·61 (2H, s, CH_2); *m/e* 182 (M^+, 12%), 138 (14), and 137 (100).

This acid (1 g) was suspended in dry ether, and oxalyl chloride (1 ml) and dimethylformamide (2 drops) were added. After 1 h the solution was evaporated and the residue in dichloromethane (100 ml) was added dropwise to a vigorously stirred mixture of 4-benzyloxy-3-methoxyphenethylamine (1·62 g) in ether (80 ml), water (80 ml), and sodium hydrogen carbonate (1·6 g). Twenty minutes after the addition was complete, the layers were separated, the aqueous solution was re-extracted with methylene chloride and the combined solutions of the amide were washed with dilute hydrochloric acid and water. The product (2 g, 86%) from the organic solution was triturated with 1 : 1 ether–propan-2-ol to give the *amide* (1·6 g, 69%), m.p. 111—113° (from propan-2-ol) (Found: C, 71·0; H, 6·2; N, 3·7. $C_{25}H_{27}NO_5$ requires C, 71·3; H, 6·4; N, 3·3%); $\nu_{max.}$ 3570 and 1670 cm^{-1}; $\lambda_{max.}$ 280 and 230sh nm, shifting to 298sh, 285, and 230 nm with alkali; τ [$(CD_3)_2CO$] 2·12 (1H, t, NH), 2·5—2·8 (5H, m), 3·05—3·5 (6H, m, aryl H), 4·97 (2H, s, OCH_2), 6·26 (6H, s, MeO), 6·56 (4H, m, CH_2·CO·CH_2), and 7·3 (2H, m, CH_2); *m/e* 421 (M^+, 9%), 240 (33), 150 (36), 137 (65), and 91 (100).

7-*Benzyloxy*-3,4-*dihydro*-1-(3-*hydroxy*-4-*methoxybenzyl*)-6-*methoxyisoquinoline Hydrochloride*.—The foregoing amide was cyclised as for the synthesis of the 6,7-methylenedioxy-3,4-dihydroisoquinoline above to yield the crude *dihydroisoquinoline hydrochloride*, which on trituration with 95% ethanol (14 ml g^{-1} of original amide) gave almost pure salt (yield >90%), m.p. 224—229° (from methanol) (Found: C, 68·5; H, 6·25; N, 3·2. $C_{25}H_{26}ClNO_4$ requires C, 68·3; H, 5·9; N, 3·2%); $\nu_{max.}$ (Nujol) 1655 cm^{-1}; $\lambda_{max.}$ 357, 306, 287, and 251sh nm, shifting to 306, 281, and 230 nm with alkali; τ ($CDCl_3$–D_2O) 2·40 and 2·91 (each 1H, s), 2·64 (5H, s), 3·1—3·4 (3H, m, aryl H), 4·91 (2H, s, OCH_2), 5·69 (2H, s, CH_2–C=N^+), *ca.* 6·2 (2H, m, CH_2), 6·28 (6H, s, MeO), and 7·0 (2H, t, CH_2); *m/e* 403 (M^+, 81%), 313 (25), and 91 (100).

(RS)-[8-^{14}C]*Isocorypalmine* (6).—The above imine hydrochloride (79 mg) was reduced in methanol (10 ml) with sodium borohydride (14 mg), and when there was no absorption above 300 nm the solution was acidified to pH 3 with hydrochloric acid; $\lambda_{max.}$ 283 and 232sh nm shifting to 291 and 234sh with alkali. Water (10 ml) was added, the pH was adjusted to 9 with sodium carbonate, and ring closure with [^{14}C]formaldehyde was carried out as described for the preparation of (*RS*)-nandinine. The resultant mixture of (*RS*)-*O*-benzylscoulerine and (*RS*)-*O*-benzylcoreximine was extracted with chloroform and treated in methanol with an excess of ethereal diazomethane. When methylation was complete (no spots on t.l.c. which stained pink with iodine) the mixture of (*RS*)-*O*-benzylisocorypalmine and its isomer was acidified with concentrated hydrochloric acid (0·5 ml) and hydrogenated over 10% palladised charcoal (20 mg) until uptake ceased. The filtered solution was evaporated

[23] E. Späth and N. Lang, *Monatsh.*, 1921, **42**, 273.

and the residue was partitioned between chloroform and aqueous sodium hydrogen carbonate; the material in the chloroform was fractionated by p.l.c. in ether. The two bases were converted into their hydrochlorides and the salts were recrystallised from methanol–ether to give (*RS*)-[8-^{14}C]isocorypalmine hydrochloride (8·4 mg; 2·44 × 10^8 disint. per 100 s per mg; 12·3%) and its isomer (12·7 mg; 2·43 × 10^8 disint. per 100 s per mg; 18·6%). The labelled *isocorypalmine base* had m.p. 175—185° (decomp.) (Found: M^+, 341·1618. $C_{20}H_{23}O_4$ requires M, 341·1621); *m/e* 341 (M^+, 64%), 340 (41), 176 (20), 170·5 (2·5), 164 (95), and 149 (100). *Pseudoisocorypalmine base* had m.p. 180—190° (decomp.) (Found: M^+, 341·1619); $\nu_{max.}$ (both alkaloids) 3500 and 2780—2760 cm^{-1} (Bohlmann bands).

Correlation of (RS)-*Isocorypalmine* (6) *with Tetrahydropalmatine* [*Methyl Ether of* (6)].—The mixture of *O*-benzylisocorypalmine and *O*-benzylpseudoisocorypalmine prepared as above was separated by p.l.c. on silica with ether. The former showed *m/e* 431 (M^+, 67%), 340 (27), 266 (14), 215·5 (0·5), 164 (100), and 149 (62), and the latter *m/e* 431 (M^+, 46%), 340 (11), 266 (16), 215·5 (0·2), 164 (100), and 149 (9). Each base was separately debenzylated as before, the free phenols were methylated, and the products were compared with authentic (*RS*)-tetrahydropalmatine. The synthetic and authentic samples of (*RS*)-tetrahydropalmatine were identical (full spectroscopic and chromatographic comparison); on t.l.c. both stained orange-yellow with iodine (R_F 0·5 in 1 : 19 propan-2-ol–ethyl acetate) whereas the isomer gave a yellow colour (R_F 0·3). Tetrahydropalmatine showed *m/e* 355 (M^+, 75%), 190 (31), 177·5 (2·2), 164 (100), and 149 (58); pseudotetrahydropalmatine showed *m/e* 355 (M^+, 45%), 190 (12), 177·5 (1·7), 164 (100), and 149 (6). These fragmentations are in agreement with the assigned structures.[24]

Experiments with Papaver somniferum *and Isolation of Alkaloids.*—*P. somniferum* plants (variety Schlandstedt) were grown in pots in the open and transferred at flowering time into a greenhouse. The solutions of precursors were injected into the capsules at flowering (0·3—0·5 ml per head) and just the capsules which had been ' fed ' plus *ca.* 3 in of stem were harvested after 5 days. Test runs with labelled precursors showed that all the radioactivity was confined to the caspule.

The extraction was performed as in the preceding paper [1] for *C. majus* with addition of carrier materials and gave extracts A—C as before. Narcotine and (*RS*)-canadine from extract A were separated by p.l.c. on silica with ether (R_F 0·4 and 0·6, respectively); m.p.s 175° (from methanol) and 169—172° (from chloroform–methanol), respectively

Extract B contained protopine, dihydroprotopine, and allocryptopine, of which the last was purified by p.l.c. on silica in 1 : 1 : 1 chloroform–methanol–ethyl acetate. The former two were then resolved by p.l.c. on silica with ether (dihydroprotopine, R_F 0·4; protopine, R_F 0·1). Protopine had m.p. 209—210° (from chloroform–methanol); allocryptopine had m.p. 157—159° (from methanol); dihydroprotopine had m.p. 151—153° (from methanol–water or chloroform–hexane).

Extract C was handled as for *C. majus*.[1] The berberine isolated from experiment 5, Table 4, was heavily diluted with radioinactive (*RS*)-canadine and was then reisolated and purified before reduction to (*RS*)-canadine for crystallisation to constant activity.

We thank Dr. C. Fuganti and Dr. M. Hirst for the indicated experiments, Mr. J. K. Hulme (Ness Botanic Gardents, Wirral) and Mr. J. Symonds (University Botanic Garden, Cambridge) for cultivation of the plants, and the University of Liverpool for a scholarship (to R. S.). We also thank the S.R.C. for studentships (to R. J. F. and H. R. W.) and for financial support.

[4/2572 *Received*, 9*th December*, 1974]

[24] C.-Y. Chen and D. B. Maclean, *Canad. J. Chem.*, 1968, **46**, 2501.