Biosynthesis of Unnatural Morphine Derivatives in *Papaver somniferum*

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Demethylation of various codeine derivatives to give the corresponding, unnatural derivatives of morphine has been studied in *Papaver somniferum*. The efficiencies of the unnatural processes have been compared with that of the natural, codeine into morphine, conversion by feeding mixtures of [2-^{3}H]-labelled codeine derivatives with [N-*methyl*-^{14}C]codeine. Demethylation of dihydrocodeine (efficiency relative to codeine into morphine conversion, 47%), isocodeine (15%), codeine methyl ether (69%), dihydrodeoxycodeine (108%), and 1-bromocodeine (5·8%), to give the corresponding morphines, was observed.

BIOSYNTHETIC studies, especially with intact organisms, rely heavily on experiments with radiolabelled precursors. Incorporation of a labelled compound, without prior fragmentation and reassembly, into a natural product often provides the first indication that the compound is a natural precursor. Occasionally, a labelled compound, closely related structurally to an established precursor, is fed to a living organism. Lack † of incorporation into a natural product then adds weight to the positive results obtained with the correct precursor. We have examined a third possibility, namely the biosynthetic conversion of a structurally modified precursor into the correspondingly modified, unnatural end product. Systematic modification of the precursor's structure can reveal the specificity of the enzyme or enzymes involved in the transformation although these enzymes may not, for technical reasons, be accessible for study in the free state.

Few examples are available of the transformation in higher plants of an unnatural precursor into an unnatural end product.[2] Reuppel and Rapoport have recently reported [3] the conversion, in *Nicotiana glutinosa*, of methylated analogues of 1-methyl-1-pyrrolinium chloride, a natural precursor of nicotine [3-(1-methylpyrrolidin-2-yl)pyridine], into the correspondingly methylated nicotines. The incorporation of 1,3-dimethyl-1-pyrrolinium chloride into 3′-methylnicotine was especially efficient (6·5—13·8%). Similarly, in *Nicotiana tabacum*, 5-fluoronicotinic acid was converted (0·15%) into 5-fluoronicotine.[2] In neither of these studies was a direct comparison made of the efficiencies for the natural and unnatural processes. We selected the demethylation [4] of codeine (1; R^1 = Me, R^2 = OH, R^3 = H) to give morphine (1; R^1 = R^3 = H, R^2 = OH) in *Papaver somniferum* for systematic study. Both the plant and its alkaloids were readily available and the biosynthesis of morphine is specially well understood.[5]

Mixtures of various derivatives [(C′) in the Scheme] of [2-^{3}H]codeine with [N-*methyl*-^{14}C]codeine (C) were fed separately to mature *P. somniferum* plants. After a suitable period of metabolism the total alkaloids were isolated and diluted with inactive samples of codeine (C), morphine (M), the modified codeine (C′), and the correspondingly modified morphine (M′). These four alkaloids were separated, rigorously purified, and counted for ^{3}H and ^{14}C. Comparison of the incorporations of ^{3}H

† Conversion of an unnatural precursor into a natural product may, however, occasionally be observed.[1]

[1] *E.g.*, T. J. Gilbertson and E. Leete, *J. Amer. Chem. Soc.*, 1967, **89**, 7085.

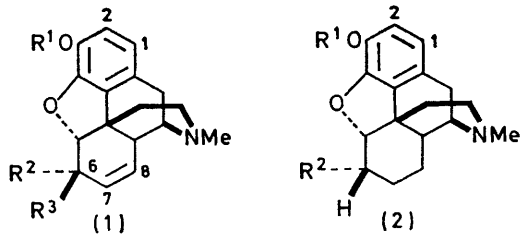
[2] E. Leete, G. B. Bodem, and M. F. Manuel, *Phytochemistry*, 1971, **10**, 2687, and reference cited therein.

[3] M. L. Rueppel and H. Rapoport, *J. Amer. Chem. Soc.*, 1970, **92**, 5528; 1971, **93**, 7021.

[4] A. R. Battersby and B. J. T. Harper, *Tetrahedron Letters*, 1960, 21; F. R. Stermitz and H. Rapoport, *J. Amer. Chem. Soc.*, 1961, **83**, 4045.

[5] G. W. Kirby, *Science*, 1967, **155**, 170.

into (M′) and ^{14}C into (M) afforded a measure of the relative efficiencies for the unnatural and natural



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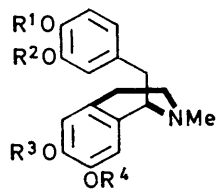
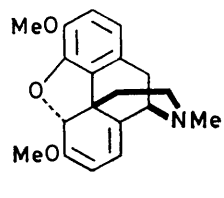


(3a) $R^1 = R^3 = Me, R^2 = R^4 = H$
(3b) $R^1 = R^4 = Me, R^2 = R^3 = H$
(3c) $R^1 = R^4 = H, R^2 = R^3 = Me$
(3d) $R^1 = R^3 = H, R^2 = R^4 = Me$



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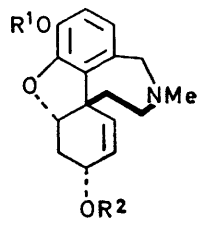

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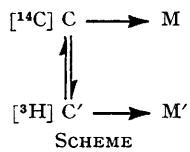
conversions under exactly the same conditions. Further, interconversions of the type, (C) ⇌ (C′), when



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they occurred, were apparent from the transfer of ^3H into (C) and (M) and of ^{14}C into (C′) and (M′).

Labelling of Precursors.—[2-^{3}H]Morphine was prepared by heating morphine (1; $R^1 = R^3 = H$, $R^2 = OH$) in dimethylformamide containing tritiated water.[6] Methylation [7] with phenyltrimethylammonium ethoxide gave [2-^{3}H]codeine together with a by-product (*ca.* 7%) identified as [2-^{3}H]codeine methyl ether. This labelled ether was used for biosynthetic experiments. Other derivatives of [2-^{3}H]codeine were prepared by published methods or variants thereof (see Experimental) as were the various inactive alkaloids required for dilution of metabolite mixtures. During this work the n.m.r. spectrum of 3-*O*-methoxymethylmorphine (1; R^1 = MeO·CH_2, R^2 = OH, R^3 = H) an intermediate in the synthesis [8] of 6-*O*-methylmorphine (1; $R^1 = R^3 = H$, R^2 = MeO), was examined. The methylene protons of the methoxymethyl group gave an AB quartet, τ ($CDCl_3$) 4·52 and 5·00 (*J* 6·0 Hz). The methylene protons are, of course, diastereotopic and should therefore give separate pairs of signals,[9] but the chemical shift difference appears unusually large for a methylene group four atoms removed from the nearest chiral centre. Curiously, the corresponding methylene signals of the derived *N*-oxide [τ (D_2O) 4·80], which contains an additional chiral centre, and of 3-*O*-benzylmorphine (1; R^1 = $PhCH_2$, R^2 = OH, R^3 = H) [τ ($CDCl_3$) 4·90] were singlets.

Isolation and Purification of Alkaloids.—The total alkaloid mixture from each feeding experiment was diluted with the appropriate inactive alkaloids (see above) and separated by anion exchange chromatography into phenolic and non-phenolic fractions. Control experiments with mixtures of labelled codeine and morphine showed high recovery (>90%) of each component and low contamination of codeine by morphine (<0·2%) and morphine by codeine (<0·02%). The phenolic and non-phenolic fractions were each separated by preparative thin-layer chromatography (t.l.c.) into the two major components. Repeated chromatography was used, when necessary, to achieve further purification. Generally, each radioactive component was mixed with an inactive specimen of its major companion and the mixture again separated by t.l.c. This 'washing' procedure ensured high radiochemical purity even for metabolites poorly separated chromatographically from possible contaminants. The purified alkaloids were crystallised to constant specific activity and converted into suitable derivatives as a final check on radiochemical purity.

Discussion of Results.—Battersby *et al.*[10] showed that the conversion of [2-^{3}H]codeine into [2-^{3}H]morphine in *P. somniferum* occurs without detectable loss or 'scrambling' of the tritium label. Further, the incorporation of [N-*methyl*-^{14}C]-labelled precursors into morphine is known [11-13] to take place without significant de-*N*-methylation. Nevertheless, a control experiment using [N-*methyl*-^{14}C,2-^{3}H]codeine was carried out (experiment 1 in Table) to test the suitability of our labelling pattern. The ^3H : ^{14}C ratio of the precursor was set at 5·32 and the observed ratios for the biosynthetic morphine and recovered codeine were, respectively, 5·57 and 5·70. This increase in the ^3H : ^{14}C ratios, possibly resulting from partial de-*N*-methylation in the plant, was considered too small to seriously affect interpretation of the experiments with codeine analogues.

Our findings (see Table) can now be discussed in detail. In all experiments with 'doubly-labelled' precursors the tritiated codeine analogue (typically 10 mg, 40 μCi) was mixed with a much smaller weight (typically 0·1 mg,

[6] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
[7] W. Rodionow, *Bull. Soc. chim. France*, 1926, **39**, (4), 305.
[8] C. Mannich, *Arch. Pharm.*, 1916, **254**, 349.
[9] M. van Gorkon and G. E. Hall, *Quart. Rev.*, 1968, **22**, 14.
[10] A. R. Battersby, J. A. Martin, and E. Brochmann-Hanssen, *J. Chem. Soc.* (*C*), 1967, 1785.
[11] D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson, and H. Ramuz, *J. Chem. Soc.*, 1965, 2423.
[12] A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.
[13] A. R. Battersby, D. M. Foulkes, M. Hirst, G. V. Parry, and J. Staunton, *J. Chem. Soc.* (*C*), 1968, 210.

10 μCi) of [N-*methyl*-^{14}C]codeine in order to allow, qualitatively, for the unavoidable dilution of [^{14}C]-codeine by endogenous alkaloid and thus provide a more equitable comparison of the metabolic fates of the unnatural and natural precursor. [2-^{3}H]Dihydrocodeine (2; R^1 = Me, R^2 = OH, ^{3}H for H at position 2) was converted (experiment 2 in Table) into [2-^{3}H]dihydromorphine with an efficiency (0·59% incorporation *) almost half that of the concurrent, natural conversion (1·26%) of [^{14}C]codeine into [^{14}C]morphine. The location of tritium in the biosynthetic [2-^{3}H]dihydromorphine was determined by base-catalysed exchange [6,10] in aqueous methanolic potassium carbonate. Under these conditions tritium should be removed only from the position *ortho* to the phenolic hydroxy-group: in fact 96% of the tritium was so lost, showing that metabolism of the precursor had occurred without significant 'scrambling' of the label. No cross-conversions (C ⇌ C′ in Scheme) were observed but the conversion (C′ → C) of [2-^{3}H]dihydrocodeine into [2-^{3}H]codeine would not easily have been detected since small amounts of tritium activity in the isolated codeine would have been obscured by the high ^{14}C activity. To clarify this point, [2-^{3}H]dihydrocodeine was fed alone to *P. somniferum*. Again, good conversion (0·51%, experiment 3) into dihydromorphine was achieved. Apparent incorporations of tritium into codeine (0·11%) and morphine (0·02%) were also observed but seemed sufficiently small to be attributable to contamination of the [2-^{3}H]dihydrocodeine precursor by [2-^{3}H]codeine, its synthetic progenitor. Radiodilution analysis of the precursor using inactive codeine showed, indeed, 0·74% contamination by [^{3}H]codeine. The apparent conversion of dihydrocodeine into codeine might, therefore, merely represent a 15% recovery of the labelled codeine inadvertently fed to the plant since recoveries of this order are common (experiments 2, 4, and 6). Thus, dehydrogenation of dihydrocodeine, if it occurred at all, was below the detection limit of this experiment and was inefficient (<0·1% incorporation).

Isocodeine (1; R^1 = Me, R^2 = H, R^3 = OH) was selected to test the response of the plant's demethylating system to a change in stereochemistry of the 6-hydroxy-function. The usual mixture of [N-*methyl*-^{14}C]codeine and [2-^{3}H]isocodeine was fed to *P. somniferum* (experiment 4). High incorporation (9·1%) of codeine into morphine was observed together with substantial, though smaller (1·38%), incorporation of isocodeine into isomorphine. Base-catalysed exchange of the [2-^{3}H]isomorphine caused, as expected, loss of most (94·4%) of the tritium. A separate feeding of [2-^{3}H]isocodeine gave [2-^{3}H]isomorphine (0·27%) and no interconversions of the type C ⇌ C′ or M ⇌ M′ were detected in either experiment.

Incorporations (%) of [N-*methyl*-^{14}C]codeine (C) and [2-^{3}H]codeine derivatives (C′) into morphine (M) and morphine derivatives (M′) in *P. somniferum*

Experiment	Precursor	C	C′	M	M′	M′/M
1.	[^{14}C]Codeine	2·69		0·99		
	[^{3}H]Codeine	2·88		1·04		
2.	[^{14}C]Codeine	19·4		1·26		0·47
	[^{3}H]Dihydrocodeine		31·8		0·59	
3.	[^{3}H]Dihydrocodeine	(0·11) *	39	(0·02) *	0·51	
4.	[^{14}C]Codeine	16·2	<0·01	9·1	<0·05	0·15
	[^{3}H]Isocodeine	<0·003	32	<0·01	1·38	
5.	[^{3}H]Isocodeine	<0·008	9·4	<0·02	0·27	
6.	[^{14}C]Codeine	14·6	0·182	0·90	<0·012	0·69
	[^{3}H]Codeine methyl ether	0·49	18·4	0·08	0·62	
7.	[^{3}H]Codeine methyl ether	0·88	16·4	0·048	1·02	
8.	[^{14}C]Codeine	38·4	<0·08	8·55	<0·07	1·08
	[^{3}H]Dihydrodeoxycodeine		30·6		9·27	
9.	[^{14}C]Codeine	61	<0·03	2·57	<0·04	0·058
	1-Bromo[^{3}H]codeine	<0·02	35·2	<0·02	0·15	

* Not significant conversions: see text.

The metabolism of codeine methyl ether (1; R^1 = Me, R^2 = MeO, R^3 = H) in *P. somniferum* was studied by Blaschke *et al.*[14] They recorded incorporation into codeine (4·7%) and morphine (3%) whereas, in a parallel experiment, the corresponding incorporations for codeinone, the probable immediate progenitor of codeine on the biosynthetic pathway, were 14·5 and 2·8%. Our studies show that biological cleavage of both the 6-methoxy- (process C′ → C) and 3-methoxy- (C′ → M′) groups occurs readily (experiment 6). Incorporation (0·62%) or [2-^{3}H]codeine methyl ether into [2-^{3}H]-morphine methyl ether was observed, the product losing 96·7% of its tritium by subsequent exchange. The concurrent incorporation of [^{14}C]codeine into [^{14}C]morphine was 0·90%. Substantial demethylation of codeine methyl ether in the alternative sense, to yield codeine (0·49%), also took place and a little tritium activity (0·08%) passed on, as expected, into morphine. Radiodilution analysis of the [2-^{3}H]codeine methyl ether precursor revealed only 0·006% contamination by codeine: the observed demethylation of this precursor

* Incorporation is, as usual, defined as the ratio of the total activity of the product to that of the precursor, expressed as a percentage.

[14] G. Blaschke, H. I. Parker, and H. Rapoport, *J. Amer. Chem. Soc.*, 1967, **89**, 1540.

to give codeine, was therefore real and confirms earlier findings. For the first time, a transformation of the type C ⟶ C′ was detected. Codeine methyl ether, isolated from plants fed with the mixture of [2-^{3}H]-codeine methyl ether and [N-*methyl*-^{14}C]codeine, contained tritium (18·4% recovery) and ^{14}C (0·182% incorporation). Careful radio-assay of the alkaloid during purification showed that the ^{14}C activity, though small, was significant. Insufficient material was available for degradation but it appeared clear that 6-*O*-demethylation of the precursor was reversible in *P. somniferum*. This result implies that codeine methyl ether is a natural constituent of mature poppy plants and supports an earlier claim to this effect.[15] The normal concentration of codeine methyl ether in the plants may however be very small since, in our experiments, the methyl ether present in the precursor mixture would serve to trap [^{14}C]-labelled material formed, reversibly, from [^{14}C]codeine. Whatever the status of codeine methyl ether as a natural product, it is metabolised most efficiently in *P. somniferum*. The total conversion, 1·19% (0·49 + 0·08 + 0·62), into other alkaloids was similar to that observed concurrently for codeine, 1·08% (0·18 + 0·90). The corresponding recoveries of these two precursors, 18·4 and 14·6%, also did not differ substantially. When [2-^{3}H]codeine methyl ether was fed alone to poppies (experiment 7), conversion into morphine methyl ether (1·02%), codeine (0·88%), and morphine (0·048%) was observed: 16·4% of the labelled precursor was recovered.

The most striking example of an efficient, unnatural demethylation was provided (experiment 8) by dihydrodeoxycodeine (2; R^1 = Me, R^2 = H). [2-^{3}H]-Labelled material, mixed as usual with [N-*methyl*-^{14}C]codeine, was incorporated more efficiently (9·27%) into dihydrodeoxymorphine (2; $R^1 = R^2$ = H) than was codeine into morphine (8·55%). Tritium was located at position 2 in the unnatural metabolite by exchange (95·9% loss of activity). In contrast, introduction of bromine into the aromatic ring of codeine substantially inhibited demethylation (experiment 9). Only 0·15% incorporation of 1-bromo[2-^{3}H]codeine into 1-bromo[2-^{3}H]-morphine occurred although demethylation of codeine proceeded with reasonable efficiency (2·57%).

The conversion of codeine into morphine varied in efficiency over a wide range (0·90—9·1%) throughout the series of feeding experiments, which were, however, carried out as far as possible under similar conditions. Doubtless, this was due in part to variations in the vigour of plants and weather conditions at the time of feeding. The presence of unnatural compounds in the precursor mixtures may also have affected the efficiency of the natural process but the tabulated results provide no convincing evidence for this. For example, in experiment 8 (see Table) extensive demethylation of codeine (8·55%) was observed in the presence of a derivative which was itself very efficiently (9·27%) demethylated, whereas in experiment 4 even greater demethylation of codeine (9·1%) was associated with much less (1·38%) demethylation of the unnatural precursor. The relative efficiencies for unnatural and natural processes (M′%/M% in the Table), obtained from the mixed-precursor feeding experiments, are intrinsically free from the effects of botanical and meteorological variations and do, we believe, provide a reliable indication of the specificity of the demethylating system of *P. somniferum*. The results of a preliminary feeding of [N-*methyl*-^{14}C]codeine and [2-^{3}H]codeine methyl ether illustrate this point. Concurrent conversions of codeine into morphine (0·67%), and of codeine methyl ether into codeine (0·35%), morphine (*ca.* 0·01%), and morphine methyl ether (0·46%) were observed. All these values were lower than those from the later experiment (experiment 6 in Table) but the relative efficiencies of the processes C′ ⟶ M′ and C ⟶ M were the same (0·69) on both occasions. Further, the efficiency ratios (C′ ⟶ C) : (C ⟶ M) were 0·52 and 0·54 in the preliminary and tabulated experiments. This agreement between results obtained one year apart by different workers with plants grown in different locations is reassuring.

The most surprising feature of our results was the ease with which unnatural codeine derivatives, especially dihydrodeoxycodeine (2; R^1 = Me, R^2 = H), codeine methyl ether (1; R^1 = Me, R^2 = MeO, R^3 = H), and dihydrocodeine (2; R^1 = Me, R^2 = OH) were converted biologically into their morphine analogues. Clearly neither the hydroxy-group nor 7,8-double bond of codeine (1; R^2 = Me, R^2 = OH, R^3 = H) is important for binding to the demethylating enzyme (see below). Isocodeine (1; R^1 = Me, R^2 = H, R^3 = OH) was less efficiently demethylated suggesting, perhaps, that a hydroxy-group *cis* to the ethanamine bridge may actually hinder approach to the enzyme. Only for 1-bromocodeine was serious inhibition of demethylation observed and here, of course, the substituent could exert electronic and steric effects close to the site of enzymic attack.

Experiments of this type, with intact organisms, cannot distinguish between the operation of one enzyme, or enzyme system, of limited substrate specificity and an array of enzymes of widely varying specificity. It might be argued that demethylation of codeine is controlled by a single, substrate-specific enzyme, while demethylation of codeine derivatives results from attack by other, non-specific enzymes present in the plant. This seems unlikely because of the very similar demethylation efficiencies observed for codeine and several derivatives. Also it is known that cleavage of phenolic methyl ethers is by no means indiscriminate in *P. somniferum*. Reticuline (3a), a key intermediate in morphine biosynthesis, labelled with ^{14}C in the methoxy-groups was incorporated into thebaine (4) without loss of methoxy-activity.[11] Further, Battersby *et al.* showed [12] that none of the three structural isomers (3b), (3c), and (3d) was a precursor of the morphine alkaloids. Double

[15] E. Brochmann-Hanssen and B. Nielsen, *J. Pharm. Sci.*, 1965, **54**, 1393.

demethylation of the ethers (3) would have given landanosoline (3; $R^1 = R^2 = R^3 = R^4 = H$), a known [13] precursor of morphine, and non-selective mono-demethylation of (3a), (3b), or (3c) would, presumably, have produced a triphenolic precursor of reticuline. Thus, contrary to observation, conversion of labelled reticuline into thebaine would have involved substantial loss of methoxy-activity and incorporation of the isomers (3b), (3c), and (3d) into morphine would have occurred.

The non-specific character of methyl ether cleavage in *P. somniferum* has certain implications. Thebaine, the progenitor of codeine and morphine, exists in substantial amounts in the opium poppy. The corresponding phenol, oripavine, previously isolated only from *P. orientale* and *P. bracteatum*, might therefore be expected to occur, at least to some extent, in *P. somniferum*. Similarly, the phenolic analogues of the transient intermediate codeinone and the minor alkaloid neopine may also be naturally-occurring. The daffodil alkaloid, galanthamine (5; $R^1 = Me$, $R^2 = H$) is converted [16] in *Chlidanthus fragrans* into the phenol, chlidanthine (5; $R^1 = H$, $R^2 = Me$). It would be interesting to discover if demethylation of galanthamine can take place in *P. somniferum* and, more generally, to investigate whether biosynthetic processes occurring naturally in one organism may be effected in other, more accessible organisms, with useful efficiency.

EXPERIMENTAL

Counting Methods.—^3H and ^{14}C Activities were measured using a Beckman CPM-100 liquid scintillation spectrometer calibrated with [1,2-$^3H_2$]- and [1-^{14}C]-hexadecane (Radiochemical Centre, Amersham).

Preparation of Labelled Precursors.—Anhydrous morphine (0·48 g) was heated under nitrogen in dry dimethylformamide (2 ml) containing tritiated water (0·5 ml) (3·6 mCi mmol^{-1}) at 100 °C for 100 h to give [6] [2-^3H]morphine (0·41 g) (1·4 mCi mmol^{-1}). [2-^3H]Codeine was prepared from the labelled morphine by treatment [7] with *NNN*-trimethylanilinium ethoxide in hot toluene. A by-product (*ca.* 7%) was isolated, by chromatography on grade III alumina using chloroform for elution, and identified as [2-^3H]codeine methyl ether. Catalytic (10% palladium–carbon) hydrogenation of [2-^3H]codeine in methanol gave [2-^3H]dihydrocodeine. [2-^3H]Isocodeine was prepared [17] from [2-^3H]-codeine *via* the corresponding toluene-*p*-sulphonate. Successive reduction of [2-^3H]codeine toluene-*p*-sulphonate with lithium aluminium hydride and platinum oxide–hydrogen gave [18] [2-^3H]dihydrodeoxycodeine. Bromination [10] of [2-^3H]codeine acetate and hydrolysis of the product gave 1-bromo[2-^3H]codeine. [N-*Methyl*-^{14}C]-Codeine was bought from the Radiochemical Centre, Amersham.

Preparation of Inactive Morphine and Codeine Derivatives.—Catalytic (10% palladium–carbon) hydrogenation of morphine in ethanol gave dihydromorphine. Isomorphine was prepared [19] by the hydrolysis of 3-*O*-acetylmorphine toluene-*p*-sulphonate in aqueous acetic acid and purified *via* the 3-*O*-acetyl derivative,[20] which was chromatographed on grade V alumina and eluted with chloroform. Methylation of 3-*O*-methoxymethylmorphine *N*-oxide followed by hydrolysis and reduction gave 6-*O*-methylmorphine.[8] Cleavage of dihydrodeoxycodeine with pyridine hydrochloride gave dihydrodeoxymorphine.[18] 1-Bromomorphine was prepared by treating diacetylmorphine with bromine [10] (1 mol) in acetic acid–acetic anhydride (16 : 1) at room temperature for 45 h and then removing the acetyl groups with methanolic sodium hydroxide. The product, m.p. >250 °C, was characterised by conversion, with diazomethane in ether–methanol, into 1-bromocodeine (see before). Inactive codeine derivatives were prepared by the methods used for the labelled materials with the exception of codeine methyl ether which was obtained [8] from the pyrolysis of codeine methyl ether methochloride.

Feeding of Precursors.—Aqueous solutions of precursors, pH *ca.* 7, were injected into the ripening seed capsules of *Papaver somniferum* (Halle variety) plants 5–7 days after petal fall. Water was injected from time to time thereafter and the plants were harvested 7—8 days after injection of the precursors.

Isolation of Phenolic and Non-phenolic Alkaloids.—The total plant material was cut into small pieces and stored in ethanol for *ca.* 2 weeks. The ethanolic suspension was homogenised, and filtered through Celite. The filtrate was evaporated to give a green residue which was dissolved in 0·5N-hydrochloric acid and washed with ether. The alkaloids were liberated from the aqueous solution with an excess of sodium hydrogen carbonate then extracted with chloroform–propan-2-ol (9 : 1). An anion exchange column (De-Acidite FF resin, Permutit Co. Ltd., SRA 71, 100—200 mesh) was prepared in the hydroxide form by repeated cycling through the chloride and hydroxide forms and finally washing with water–methanol (1 : 1). The total alkaloids were dissolved in hydrochloric acid and the solution was quickly adjusted to pH 12 and run onto the column. Elution with water–methanol (1 : 1) and evaporation of the eluate gave the non-phenolic alkaloids. Elution with 0·5N-hydrochloric acid gave the phenolic alkaloids. The acidic eluate was neutralised with sodium hydrogen carbonate and evaporated. The residue was dissolved in water and the alkaloids extracted with chloroform–propan-2-ol (9 : 1).

Thin-layer Chromatographic Systems.—A slurry of Merck GF_{254} alumina (45 g) in water (68 ml) was spread in a thin (0·25 mm) layer over 20 plates (20 × 5 cm). The plates were activated at 140 °C for 2 h. Merck PF_{254} alumina (90 g) in water (120 ml) was used for thick (0·5 mm) plates (1 m × 20 cm). These were allowed to dry at room temperature for 12 h before activation at 120° for 2 h. Silver nitrate–alumina plates were prepared in the same way except that silver nitrate (1 g per 10 g alumina) was dissolved in the water used for slurrying. Basic silica plates were prepared with Merck GF_{254} silica (50 g) in 0·1N-sodium hydroxide (90 ml) and activated at 140 °C for 2 h.

Separation of Alkaloid Mixtures.—Basic silica plates (prepared as before) developed in methanol were used to

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separate mixtures of dihydrocodeine and codeine, dihydromorphine and morphine, 1-bromocodeine and codeine, and 1-bromomorphine and morphine. Silver nitrate–alumina plates were occasionally used to separate dihydrocodeine from codeine. Alumina plates developed in chloroform separated codeine methyl ether from codeine and dihydrodeoxycodeine from codeine. Morphine methyl ether and morphine were separated on alumina plates developed with n-butanol–di-n-butyl ether–acetic acid (4 : 5 : 1). Isocodeine and codeine were separated on grade III neutral alumina columns by elution with chloroform. Separation of dihydrodeoxymorphine and morphine was similarly effected using grade V neutral alumina. Mixtures of isomorphine and morphine were treated with acetic anhydride in aqueous sodium hydrogen carbonate to give the corresponding 3-*O*-acetyl derivatives [20] which were separated on grade V neutral alumina columns by elution with chloroform.

Purification of Labelled Alkaloids.—Chromatographically pure compounds were crystallised to constant specific activity. As a check on radiochemical purity suitable derivatives were prepared and crystallised: in no case was a significant drop in molar specific activity observed. The following derivatives were used: the picrates of codeine, codeine methyl ether, morphine methyl ether, dihydrodeoxycodeine, and dihydrodeoxymorphine (all crystallised from ethanol); diacetylmorphine (from ethyl acetate); diacetyldihydromorphine (from ethyl acetate); diacetyl-1-bromomorphine (from methanol); and acetyl-1-bromocodeine (from ethanol). Isomorphine was converted (diazomethane in ether–methanol) into isocodeine (crystallised from ethyl acetate).

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