Opium Alkaloids. Part XVI.† The Biosynthesis of 1-Benzylisoquinolines in *Papaver somniferum*. Preferred and Secondary Pathways; Stereochemical Aspects

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(−)-Tetrahydropapaverine (VI), derived from (−)-nor-reticuline (II) and (−)-nororientaline (III), is the principal immediate precursor of papaverine (VIII) in *Papaver somniferum*. The trimethyl ethers of papaveroline (XIa) and b) are incorporated less effectively into papaverine, the dimethyl ether (XIc) not at all. Norprotosinomenine (IV) and noriso-orientaline(V) can also be biotransformed into papaverine in the plant, but this occurs by an aberrant route which does not involve tetrahydropapaverine as an intermediate.

The stereoselectivity of various *O*- and *N*-methylations and of dehydrogenation of tetrahydropapaverine is discussed. ' Unnatural ' (−)-laudanosine was biosynthesized from ' unnatural ' (+)-tetrahydropapaverine, but could not be obtained from ' unnatural ' (+)-nor-reticuline. Enzymic racemization of benzyltetrahydroisoquinolines, which is a prerequisite for the biosynthesis of morphine alkaloids, appears to be limited to reticuline (Xa). *N*-Methylations may occur at several stages along the biosynthetic routes, and there is evidence that enzymatic *N*-demethylations may take place to an appreciable extent.

PAPAVERINE (VIII) had been shown to be biosynthesized in the opium poppy from tyrosine [1,2] *via* 3,4-dihydroxyphenylalanine (DOPA) and norlaudanosoline [3] (I) as proposed originally by Winterstein and Trier [4] in 1910. In an earlier paper [5] we reported that nor-reticuline (II) was an efficient precursor of papaverine, and the question arose as to the sequence of *O*-methylation and the stage at which aromatization of ring B takes place.

O-Methylation of norlaudanosoline by catechol *O*-methyltransferase (COMT) may conceivably produce four isomeric dimethyl ethers, nor-reticuline (II), nor-orientaline (III), norprotosinomenine (IV), and the base (V), which in the following will be referred to as noriso-orientaline. Although these bases have not been isolated from opium or the opium poppy, two *N*-methyl

† Part XV, *J. Pharm. Sci.*, in the press.

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[2] A. R. Battersby, R. Binks, and B. J. T. Harper, *J. Chem. Soc.*, 1962, 3534.

[3] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.

[4] E. Winterstein and G. Trier, ' Die Alkaloide,' Bornträger, Berlin, 1910, p. 307.

[5] E. Brochmann-Hanssen, C.-C. Fu, A. Y. Leung, and G. Zanati, *J. Pharm. Sci.*, 1971, **60**, 1672.

derivatives are known to be present, namely reticuline [6-8] (Xa) and orientaline [9] (Xb). Complete *O*-methylation of the four dimethyl ethers would give tetrahydropapaverine (VI), which was detected in the opium poppy by isotope dilution.[5] Dehydrogenation * to papaverine might then follow.

Alternatively, dehydrogenation might occur at the dimethyl or trimethyl ether stage. Two papaveroline trimethyl ethers are known to exist in the opium poppy, namely palaudine [10] (XIa) and pacodine [5] (XIb).

It is generally believed, and has been demonstrated experimentally in several instances,[11-14] that biosynthetic methylations and demethylations do not occur at L(*S*)-configuration. Two benzyltetrahydroisoquinolines occur in both enantiomeric forms in *Papaver somniferum*, namely reticuline [7,8] and laudanine [(±)-laudanidine] (Xc). Battersby *et al.*[15] demonstrated that reticuline undergoes racemization in the opium poppy *via* a reversible oxidation–reduction system, a process essential for the formation of morphine alkaloids which are derived from (—)-D(*R*)-reticuline. It is not known to what extent other alkaloids are being racemized in this plant.

The present study was undertaken to determine the sequence and stereoselectivity of enzymic methylations and dehydrogenation in the biosynthesis of papaverine,

TABLE 1

Tracer experiments on *Papaver somniferum*

Precursor	Incorporation of radioactivity (%) into: Papaverine	Laudanosine [a]	Tetrahydropapaverine [a]	Palaudine [a]	Isopacodine [a]	Morphine
(±)-Tetrahydropapaverine (VI) [b]	17·6	2·0				
(±)-Norlaudanine (Xe) [b]	5·3	0·30	1·2	15·6		0·007
(±)-Norprotosinomenine (IV) [b]	1·7	0·02	0·05		12·2	
(±)-Noriso-orientaline (V) [b]	1·2	0·007	0·02			
(±)-Nororientaline (III) [c]	1·7	0·27	0·13			<0·004
Palaudine (XIa) [d]	0·17					
Pacodine (XIb) [c]	0·17					
Papaveroline 4′,6-dimethyl ether (XIc) [c]	<0·0001					
Papaveroline 4′,6-dimethyl ether (XIc) [d]	0·000					
(±)-Orientaline (Xb) [e]		0·06				
(+)-Laudanosoline (Xg) [b]	1·0	0·18				2·0
(—)-Laudanosoline (Xg) [b]	0·07	0·07				0·43
(±)-Laudanosoline (Xg) [e]		0·14				5·0 (into thebaine)

[a] Isolated by carrier dilution. [b] [3-^{14}C]. [c] [^{3}H]. [d] [1-^{14}C]. [e] [N-*methyl*-^{14}C].

TABLE 2

Tracer experiments on *Papaver somniferum* with [^{3}H,^{14}C]precursors having ^{3}H at the asymmetric centre

Precursor	^{3}H : ^{14}C	^{14}C Incorporation (%) and ^{3}H retention (%) in: Papaverine ^{14}C inc.	Papaverine ^{3}H ret.	Laudanosine [a] ^{14}C inc.	Laudanosine [a] ^{3}H ret.	Tetrahydropapaverine [a] ^{14}C inc.	Tetrahydropapaverine [a] ^{3}H ret.	Morphine ^{14}C inc.	Morphine ^{3}H ret.
(—)-Nor-reticuline (II) [b]	9·04	7·3	0·0	3·8	0·4	1·3	95·0	2·8	0·5
(+)-Nor-reticuline (II) [b]	9·06	0·14	0·0	0·01	26·5			0·7	96·2
(—)-Tetrahydropapaverine (VI) [b]	9·05	12·4	0·0	31·9	100				
(+)-Tetrahydropapaverine (VI) [b]	9·08	0·1	0·0	20·9	98·1				
(±)-Laudanine (Xc) [c]	0·94	0·7	0·0	2·0	100				
(±)-Orientaline (Xb) [c]	1·20	0·08	0·0	0·02	100				

[a] Isolated by carrier dilution. [b] [1-^{3}H, 3-^{14}C, 6-O-*methyl*-^{14}C]. [c] [1-^{3}H, 3-^{14}C].

random, but according to a definite pattern which may well determine the direction of alkaloid biosynthesis in a given plant. Furthermore, these reactions show considerable stereoselectivity. There is evidence that norlaudanosoline produced from DOPA in the opium poppy is the laevorotatory enantiomer [15] which has the and also to explore the extent to which the integrity of the chiral centre at C-1 is being maintained in the biosynthetic process.

The results of the tracer experiments with labelled presumptive precursors are given in Tables 1 and 2. A

* The term dehydrogenation is used to denote aromatization of ring B; we recognize that the mechanism, which is unknown at present, might involve several enzymic reactions.

[6] E. Brochmann-Hanssen and T. Furuya, *J. Pharm. Sci.*, 1964, **53**, 575; *Planta Med.*, 1964, **12**, 328.

[7] E. Brochmann-Hanssen and B. Nielsen, *Tetrahedron Letters*, 1965, 1271.

[8] A. R. Battersby, G. W. Evans, R. O. Martin, M. E. Warren, jun., and H. Rapoport, *Tetrahedron Letters*, 1965, 1275.

[9] E. Brochmann-Hanssen, C. H. Chen, H.-C. Chiang, C.-C. Fu, and H. Nemoto, *J. Pharm. Sci.*, 1973, **62**, 1291.

[10] E. Brochmann-Hanssen and K. Hirai, *J. Pharm. Sci.*, 1968, **57**, 940.

[11] H. Rapoport, F. R. Stermitz, and D. R. Baker, *J. Amer. Chem. Soc.*, 1960, **82**, 2765.

[12] F. R. Stermitz and H. Rapoport, *Nature*, 1961, **189**, 310; *J. Amer. Chem. Soc.*, 1961, **83**, 4045.

[13] 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.

[14] A. G. Paul, *Lloydia*, 1973, **36**, 36.

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

selection of isolated radioactive alkaloids were subjected to controlled degradation for determination of the position of the label. The results are recorded in Table 3.

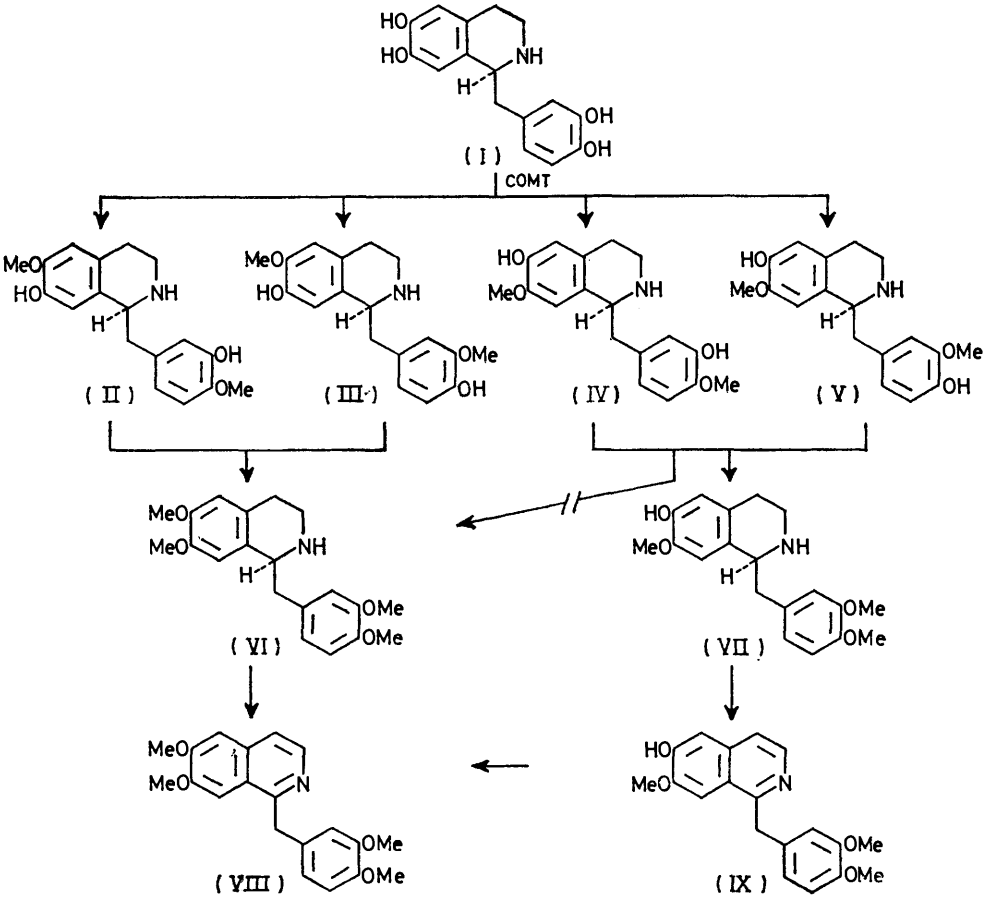
TABLE 3
Controlled degradation of radioactive alkaloids

Precursor fed	Alkaloid degraded		Rel. activity in isolated fragment (%)
(±)-Norprotosinomenine [a]	Papaverine		99·5
(±)-Noriso-orientaline [a]	Papaverine		100·1
(±)-Norlaudanine [a]	Palaudine		99·0
(±)-Laudanine [b]	Laudanosine	[1-^{3}H] [d]	97·4
		[3-^{14}C]	97·0
(−)-Tetrahydropapaverine [c]	Laudanosine	[1-^{3}H] [d]	98·3
		[3-^{14}C]	99·0

[a] [3-^{14}C]. [b] [1-^{3}H, 3-^{14}C]. [c] [1-^{3}H, 3-^{14}C, 6-O-*methyl*-^{14}C]. [d] Determined indirectly from the difference between the specific ^{3}H activities of *trans*-laudanosine methine and 4,5-dimethoxy-2-vinylbenzoic acid.[1]

[2′,6′,8-^{3}H]Papaveroline 4′,6-dimethyl ether (XIc) was not incorporated into papaverine. Because of the possibility of loss of ^{3}H by isotope exchange, the experiment was repeated the following year with the ^{14}C-labelled compound. The result was the same and leads to the conclusion that dehydrogenation does not take place at the nor-reticuline stage. At the same time, the relatively low incorporations (0·17%) of palaudine and pacodine indicated that these substances are not important intermediates in the biosynthesis of papaverine. Tetrahydropapaverine, on the other hand, gave excellent incorporation into papaverine, in one experiment as high as 17·6%, and should be considered the principal immediate precursor of papaverine.

All four isomeric dimethyl ethers of norlaudanosoline were incorporated into papaverine without randomization of the radioactive labels. However, they differed in one important respect. While nor-reticuline and nororientaline were incorporated into tetrahydropapaverine and laudanosine (Xd), norprotosinomenine and noriso-orientaline did not proceed along this route. The C-rings of norprotosinomenine and noriso-orientaline correspond to those of nor-reticuline and nororientaline, respectively, and it would seem reasonable to assume that the phenolic hydroxy-functions of the C-rings can be methylated in the normal manner to give norlaudanosoline 3′,4′,7-trimethyl ether (' norisocodamine ') (VII).

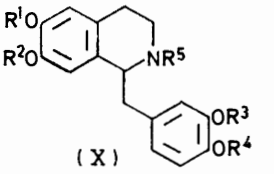


SCHEME 1 Potential pathways for biosynthesis of papaverine based on tracer experiments

The A-ring differs in its methylation pattern from that found in all known opium alkaloids. It appears that the opium poppy is not capable of methylating the hydroxy-group in the 6-position once the 7-OH is

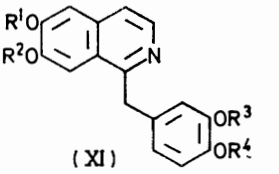
methylated, *i.e.* 6-*O*-methylation must precede 7-*O*-methylation. This is consistent with the sequence of *O*-methylation of phenethylamine and tetrahydroisoquinolines in the biosynthesis of peyote alkaloids.[14] It is also analogous to the biotransformation by COMT in mammalian systems of catecholamines and tetrahydroisoquinolines containing the catechol function.[16-18] After the C-ring is fully methylated, the resulting trimethyl ether can be dehydrogenated to the corresponding aromatic benzylisoquinoline (IX) [papaveroline 3',4',7-trimethyl ether (' isopacodine ')], which may then be methylated to give papaverine (Scheme 1). This conclusion is based on the following observations. Norprotosinomenine and noriso-orientaline were not incorporated into tetrahydropapaverine and laudanosine to a significant degree, but gave good incorporation into papaverine. Labelled norprotosinomenine was found to produce radioactive ' isopacodine ' (12·2% incorp.). Furthermore, the high incorporation of norlaudanine (Xe) into palaudine (15·6%) confirms that the plant does not require complete methylation of all four phenolic hydroxy-groups before dehydrogenation can take place. However, unless their presence in the opium poppy can be demonstrated by isolation or by isotope dilution, norprotosinomenine, noriso-orientaline, norisocodamine, and isopacodine should not be considered genuine opium alkaloids. When administered to the plant, they enter the pathways of secondary metabolism and are modified to the extent that their structures permit interaction with the active sites of available enzyme systems.

N-Methylation of tetrahydropapaverine shows only a low order of stereoselectivity. Both isomers gave good incorporation into laudanosine with little or no loss of ^3H from the asymmetric centre. Consequently, administration of the ' unnatural ' precursor (+)-(*R*)-tetrahydropapaverine had produced the ' unnatural ' alkaloid (−)-(*R*)-laudanosine. As expected, incorporation of (−)-(*S*)-nor-reticuline into morphine resulted in complete loss of ^3H. It was surprising, however, that the ' unnatural ' (+)-(*R*)-nor-reticuline—although a much less efficient precursor of morphine than its laevorotatory isomer—gave morphine with almost complete retention of ^3H. Battersby *et al.*[15] had shown that (−)-(*R*)-reticuline gave morphine with only about 50% retention of ^3H at the asymmetric centre because of the efficiency and reversibility of the racemization process. If *N*-methylation of nor-reticuline were non-stereoselective—as it appears to be for tetrahydropapaverine—the product of *N*-methylation of (+)-nor-reticuline would be (−)-reticuline, which would be expected to give good incorporation into morphine and about 50% loss of ^3H. The low incorporation observed, together with only about 4% ^3H loss, may be interpreted to mean that *N*-methylation of (+)-nor-reticuline occurs to only a very small extent, if at all. Instead, (+)-nor-reticuline, which is not racemized, undergoes oxidative phenol coupling to *N*-norsalutaridine (XIIa), followed by *N*-methylation and conversion of salutaridine (XIIb) into morphine in the usual way.[13,19] Stereospecific *N*-methylation of (−)-nor-reticuline is also supported by the fact that (+)-nor-reticuline was not incorporated into laudanosine.



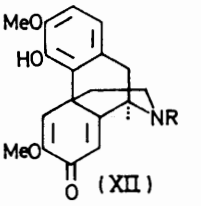
(X)

a; $R^1 = R^4 = R^5 = Me$, $R^2 = R^3 = H$
b; $R^1 = R^3 = R^5 = Me$, $R^2 = R^4 = H$
c; $R^1 = R^2 = R^4 = R^5 = Me$, $R^3 = H$
d; $R^1 = R^2 = R^3 = R^4 = R^5 = Me$
e; $R^1 = R^2 = R^4 = Me$, $R^3 = R^5 = H$
f; $R^1 = R^3 = R^4 = R^5 = Me$, $R^2 = H$
g; $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = Me$
h; $R^1 = R^3 = R^4 = Me$, $R^2 = R^5 = H$



(XI)

a; $R^1 = R^2 = R^4 = Me$, $R^3 = H$
b; $R^1 = R^3 = R^4 = Me$, $R^2 = H$
c; $R^1 = R^4 = Me$, $R^2 = R^3 = H$



(XII)

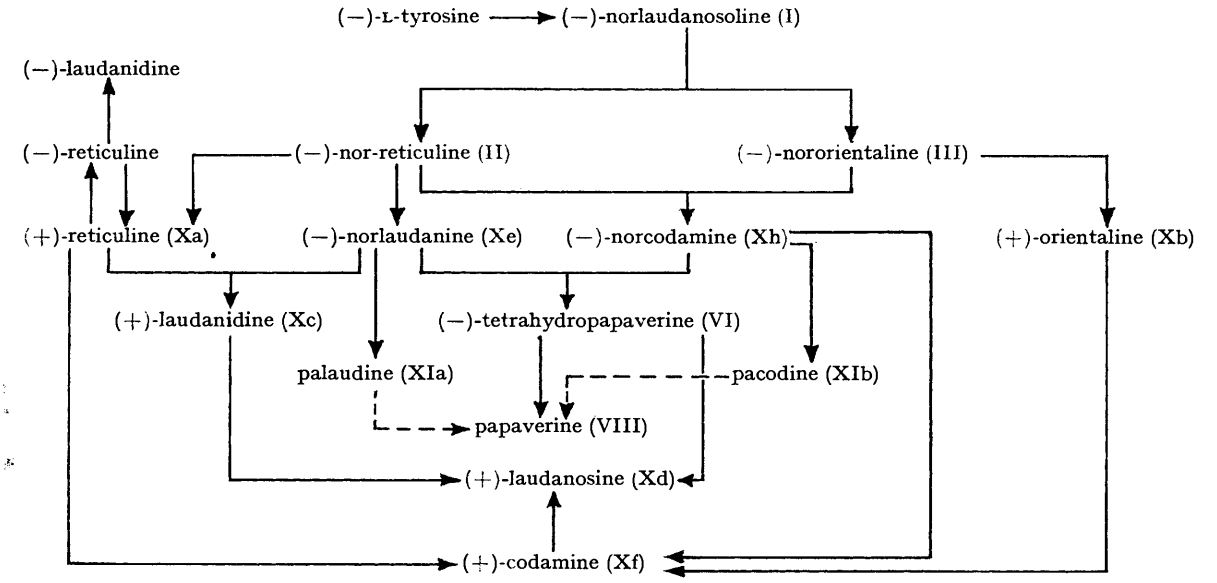
a; R = H
b; R = Me

During the biosynthesis of laudanosine from (−)-nor-reticuline the ^3H label was completely lost. Since it is evident from other tracer experiments that laudanosine is not racemized in the opium poppy, *e.g.* tetrahydropapaverine and laudanine feedings (Table 2), and since (−)-nor-reticuline produced tetrahydropapaverine without significant loss of ^3H, the total loss of ^3H from laudanosine must mean that *N*-methylation to reticuline—and racemization of reticuline—preceded *O*-methylation. This suggests that the pathway to laudanosine is from (+)-reticuline *via* (+)-laudanidine and codamine (Xf) as proposed earlier.[5] On the other hand, tracer experiments with tetrahydropapaverine make it evident that laudanosine can also be biosynthesized from this precursor. *N*-Methylation appears to be one of the principal mechanisms available to the plant for disposition and ' detoxification.' Normally, the pool sizes of the secondary heterocyclic amines are very small, and no benzyltetrahydroisoquinoline lacking the *N*-methyl group has been isolated from opium or the opium poppy. When the pools of secondary benzyltetrahydroisoquinoline alkaloids are increased in an

[16] J. Axelrod, J. K. Inscoe, S. Senohu, and B. Witkop, *Biochem. Biophys. Acta*, 1958, **27**, 210.
[17] I. J. Kopin, J. Axelrod, and E. Gordon, *J. Biol. Chem.*, 1961, **236**, 2109.
[18] M. J. Walsh, *Ann. New York Acad. Sci.*, 1973, **215**, 98.
[19] D. H. R. Barton, D. S. Bhakuni, R. James, and G. W. Kirby, *J. Chem. Soc.* (*C*), 1967, 128.

artificial manner during tracer experiments, *N*-methylation may be the simplest means of disposing of the excess. This may explain why, in general, secondary benzyltetrahydroquinolines show more efficient incorporation into laudanosine than do the tertiary analogues. It therefore seems reasonable to assume that laudanosine may be biosynthesized by several routes depending on the sequence of *O*- and *N*-methylation of norlaudanosoline.

The stereoselectivity of *O*-methylation depends on the position of the hydroxy-group. Methylation of the 7-OH group shows little, if any, stereoselectivity. Both isomers of reticuline are readily methylated at the 7-position to give (+)- and (−)-laudanidine. The enantiomeric laudanidines are probably not produced by racemization in the plant, since doubly labelled *N*-demethylation in the plant. The relatively high incorporations of laudanine and (+)-laudanosoline (Xg) into papaverine tend to suggest that enzymic *N*-demethylation may be at least partly responsible. Miller, Jolles, and Rapoport [20] showed that morphine was *N*-demethylated to the extent of 2—2·5% when exposed to strong acid, heat, and air. At the same time, they also established normorphine as a metabolite of morphine in the opium poppy. Several other alkaloids have been shown to undergo *N*-demethylation in plants [21-24] and it is possible, as was suggested by Miller *et al.*, that alkaloids may play an important biochemical function in plants as specific methylating agents, ' the relative stabilities of the successive methyl groups affording the plant a potentially sensitive control of methylation processes.'



SCHEME 2 Pathways for biosynthesis of benzylisoquinoline alkaloids in *Papaver somniferum*; broken lines designate minor pathways

laudanine containing ^3H at the asymmetric centre gave laudanosine with complete retention of ^3H. Methylation of the 3'-OH group is very stereoselective, and only the dextrorotatory enantiomers of codamine and laudanosine are present in the plant.

Aromatization of ring B is also a stereoselective process, and only the (−)-(*S*)-isomers of tetrahydropapaverine and nor-reticuline could serve as precursors of papaverine.* It was surprising, however, although it agrees with an earlier observation,[5] that *N*-methyl benzyltetrahydroisoquinolines were *N*-demethylated to a significant degree and converted into papaverine. It still remains to be established unequivocally whether partial *N*-demethylation took place during the syntheses of the precursors because of treatment with strong acid at elevated temperatures, or was the result of enzymatic

Racemization of reticuline, which is a necessary step for the biosynthesis of morphine alkaloids, appears to be very substrate-specific. There is no evidence that even closely related benzyltetrahydroisoquinolines are racemized in the opium poppy. The picture is not entirely clear for laudanosoline, which may or may not be a genuine opium alkaloid. Both isomers were incorporated into morphine and laudanosine albeit not to the same extent. The experiment will be repeated with doubly labelled laudanosoline containing ^3H at position 1.

Based on the tracer experiments recorded in Tables 1 and 2, the biosynthetic pathways leading from L-tyrosine to the various benzylisoquinolines in *Papaver somniferum* may be summarized as shown in Scheme 2.

* The incorporations of the (+)-isomers recorded in Table 2 were probably due to incomplete resolution of the enantiomers.

[20] R. J. Miller, C. Jolles, and H. Rapoport, *Phytochemistry*, 1973, **12**, 597.

[21] J. N. Cassady, C. I. Abou-Chaar, and H. G. Floss, *Lloydia*, 1973, **36**, 390.

[22] R. F. Dawson, *J. Amer. Chem. Soc.*, 1945, **67**, 503; 1951, **73**, 4218.

[23] E. Leete and V. M. Bell, *J. Amer. Chem. Soc.*, 1959, **81**, 4358.

[24] A. Romeike, *Naturwiss.*, 1962, **49**, 426.

EXPERIMENTAL

The methods used for cultivation of the plants, administration of labelled precursors, extraction, separation, purification, and controlled degradation of alkaloids, and determination of radioactivity of singly labelled compounds have been described.[1,5,25] Simultaneous determination of ^{3}H and ^{14}C activities in intact molecules was carried out by double-label counting in a Searle Analytic, Mark III liquid scintillation spectrometer. *Papaver somniferum*, Noordster variety, was used throughout. About 1 mg of labelled precursor was administered to each capsule (3—5 capsules per plant). The specific ^{14}C activity of most precursors was *ca.* 1 mCi mmol^{-1}; the specific ^{3}H activities of the compounds which were labelled by isotope exchange ranged from *ca.* 2·5 to *ca.* 7·5 mCi mmol^{-1}.

1-Benzyl-isoquinolines and -tetrahydroisoquinolines were synthesized by standard methods.[3,5,10,13,15,25] The radioactive purity of labelled potential precursors was determined by radioactivity scanning with a Berthold radio scanner (Varian Aerograph Co.) of thin-layer chromatograms prepared with at least two solvent systems. Multiply labelled compounds were prepared by mixing the singly labelled compounds in a known proportion and crystallizing the mixture. The calculated ^{3}H : ^{14}C ratio was compared with that determined by actual double-label counting, the latter being used for calculation of incorporation and ^{3}H retention in the isolated alkaloids.

M.p.s were determined with a Kofler hot-stage apparatus, and optical rotations with a Bendix automatic polarimeter.

Tritiation of Phenolic Benzylisoquinolines. Nororientaline (III), papaveroline 3′,4′,6-trimethyl ether (pacodine) (XIb), and papaveroline 4′,6-dimethyl ether (XIc) were tritiated by base-catalysed isotope exchange with tritiated water.[26,27] This introduced the ^{3}H label into unsubstituted positions *ortho* and *para* to the phenolic hydroxy-groups. The tritiated products were purified by column chromatography on silica gel and by crystallization.

Optically Active, Labelled Nor-reticulines (II).—(±)-[1-^{3}H,3-^{14}C,6-O-*methyl*-^{14}C]-*OO*-Dibenzylnor-reticuline hydrochloride[5] (1·173 g, 2·21 mmol; 1-^{3}H : 3-^{14}C : 6-*O*-$^{14}CH_3$ 5·5 : 0·491 : 0·116) was converted into the free base and dissolved in methanol (5 ml) together with (−)-*OO*-dibenzoyltartaric acid (914 mg, 2·43 mmol). Ether (10 ml) was added and the solution set aside to crystallize at 0—5°. The salt was recrystallized 3 times from methanol–ether to give (+)-[1-^{3}H,3-^{14}C,6-O-*methyl*-^{14}C]-*OO*-dibenzylnor-reticuline (−)-*OO*-dibenzoyltartrate, m.p. 159—160° (lit.,[28] 150—160°), $[\alpha]_D^{22}$ −44° (*c* 1·0 in $CHCl_3$).

The *OO*-dibenzoyltartrate was converted into the free base (a colourless oil), which was debenzylated with hot methanolic hydrochloric acid;[28] the (+)-nor-reticuline was purified as the hydrochloride, $[\alpha]_D^{23}$ +13·2° (*c* 0·375 in MeOH) (lit.,[28] +13·3° in MeOH); specific activities ^{3}H 0·988, ^{14}C 0·109 mCi mmol^{-1}, ^{3}H : ^{14}C 9·06 : 1.

The absolute configuration of (+)-nor-reticuline obtained in this way was established as D(*R*) by means of non-radioactive nor-reticuline resolved as above. (+)-*OO*-Dibenzylnor-reticuline (−)-*OO*-dibenzoyltartrate, $[\alpha]_D^{23}$ −40·9 (*c* 1·0 in $CHCl_3$), was converted into the free base and *N*-methylated with formaldehyde and sodium borohydride[29] to give (−)-*OO*-dibenzylreticuline, m.p. 90·5—92°, $[\alpha]_D^{23}$ −42·2° (*c* 1·0 in $CHCl_3$) (lit.,[15] m.p. 90—93°, $[\alpha]_D^{23}$ −42·0° in $CHCl_3$). Debenzylation with hot alcoholic hydrochloric acid and purification of the hydrochloride afforded (−)-reticuline hydrochloride, $[\alpha]_D^{23}$ −73·5° (*c* 0·8 in H_2O) (lit.,[15] −75° in H_2O), which has been shown to have the *R*-configuration.

The mother liquors from the resolution of (+)-[1-^{3}H,3-^{14}C,6-O-*methyl*-^{14}C]-*OO*-dibenzylnor-reticuline (−)-*OO*-dibenzoyltartrate were combined; the material therein was converted into the base and mixed with (+)-*OO*-dibenzoyltartaric acid.[30] Resolution was carried out as before to afford (−)-*OO*-dibenzylnor-reticuline (+)-*OO*-dibenzoyltartrate, m.p. 159—160°, $[\alpha]_D^{22}$ +43·6° (*c* 1·0 in $CHCl_3$). The base, recovered from the salt, was debenzylated with hot alcoholic hydrochloric acid and purified as the hydrochloride, $[\alpha]_D^{22}$ −13·0° (*c* 0·426 in MeOH) (lit.,[28] $[\alpha]_D$ −12·9° in MeOH); specific activities ^{3}H 0·994, ^{14}C 0·110 mCi mmol^{-1}, ^{3}H : ^{14}C 9·04 : 1.

Optically Active, Labelled Tetrahydropapaverines (VI).—(−)-[1-^{3}H,3-^{14}C,6-O-*methyl*-^{14}C]nor-reticuline (54 mg) was methylated in methanol (25 ml) with ethereal diazomethane for 3 days. The solvent was removed and the residue purified by liquid–liquid extraction to remove unchanged phenols, then by chromatography on silica gel, and crystallized as the hydrochloride from methanol–ethyl acetate; yield 10·7 mg; m.p. 166—168° (lit.,[31] 167°); specific activities ^{3}H 0·996, ^{14}C 0·110 mCi mmol^{-1}, ^{3}H : ^{14}C 9·05 : 1.

(+)-[1-^{3}H,3-^{14}C,6,O-*methyl*-^{14}C]Nor-reticuline (50·6 mg) was methylated with diazomethane and purified as described for the (−)-isomer; yield 13·8 mg; m.p. 166—168°; specific activities ^{3}H 0·990, ^{14}C 0·109 mCi mmol^{-1}, ^{3}H : ^{14}C 9·08 : 1.

(+)- and (−)-Tetrahydropapaverine prepared in this way by methylation of the corresponding isomers of nor-reticuline were identical with the products obtained by direct resolution of tetrahydropapaverine as described below.

Optically Active [3-^{14}C]*Laudanosoline* (Xg).—(±)-[3-^{14}C]-Tetrahydropapaverine (481 mg) and *N*-acetyl-L-leucine (243 mg, 1 equiv.) were dissolved in methanol (4 ml) and ether (6 ml) and the product was allowed to crystallize at 0—5°. Three recrystallizations from methanol–ether gave (−)-[3-^{14}C]tetrahydropapaverine *N*-acetyl-L-leucinate (112 mg), $[\alpha]_D^{23}$ +14·8° (*c* 1·8 in EtOH) (lit.,[31] +14° in EtOH). The base was recovered as an oil which was induced to crystallize from ether–petroleum; m.p. 96—98°, $[\alpha]_D^{23}$ −28·3° (*c* 3·52 in $CHCl_3$) {lit.,[3] m.p. 97·5—98·5°; lit.,[31] $[\alpha]_D$ −21° (as an oil in $CHCl_3$)}. (−)-[3-^{14}C]Tetrahydropapaverine was *N*-methylated with formaldehyde and sodium borohydride[29] to give (+)-[3-^{14}C]laudanosine, which was purified by chromatography on silica gel with chloroform–methanol (20 : 1), and crystallized from ether–petroleum; m.p. 88—90°, $[\alpha]_D^{23}$ +104° (*c* 0·63 in EtOH) (lit.,[7] m.p. 89·5°, $[\alpha]_D$ +105·3° in EtOH); specific activity 0·765 mCi mmol^{-1}.

(+)-[3-^{14}C]Laudanosine (50 mg) was *O*-demethylated

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with hydrochloric acid (0·5 ml) in a sealed tube at 165° for 90 min.[3] Water was removed azeotropically with absolute ethanol and benzene, and the residue was crystallized from methanol and ether to yield (+)-[3-^{14}C]laudanosoline hydrochloride (23 mg), $[\alpha]_D^{23}$ *ca.* +50° (*c* 0·5 in H_2O) (lit.,[32] +48° ± 3° for the hydrobromide in water).

The mother liquors from the crystallization of (−)-[3-^{14}C]tetrahydropapaverine *N*-acetyl-L-leucinate were combined, and the base was isolated in the usual way and treated with *N*-acetyl-D-leucine [33] to give (+)-[3-^{14}C]tetrahydropapaverine *N*-acetyl-D-leucinate, $[\alpha]_D^{23}$ −13·8° (*c* 1·2 in EtOH). The base was recovered from the salt and crystallized from ether–petroleum; m.p. 96—98°, $[\alpha]_D^{23}$ +27·8° (*c* 1·8 in $CHCl_3$) (lit.,[3] $[\alpha]_D$ +26° ± 8° in $CHCl_3$). (+)-[3-^{14}C]Tetrahydropapaverine was *N*-methylated to afford (−)-[3-^{14}C]laudanosine, which was purified by column chromatography, first on silica gel, then on neutral alumina, and crystallized from ether–petroleum; m.p. 87—88°, $[\alpha]_D^{23}$ −102° (*c* 0·5 in EtOH); specific activity 0·767 mCi mmol^{-1}.

(−)-[3-^{14}C]Laudanosine was *O*-demethylated with hydrochloric acid in a sealed tube and purified as the hydrochloride. The product was laevorotatory in water.

(±)-[N-*methyl*-^{14}C]Laudanosoline was prepared by *O*-demethylation of [N-*methyl*-^{14}C]laudanine [5] as above; specific activity 1·53 mCi mmol^{-1}.

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