603. *Alkaloid Biosynthesis. Part VIII.*[1] *Use of Optically Active Precursors for Investigations on the Biosynthesis of Morphine Alkaloids*

By A. R. Battersby, D. M. Foulkes, and (in part) R. Binks

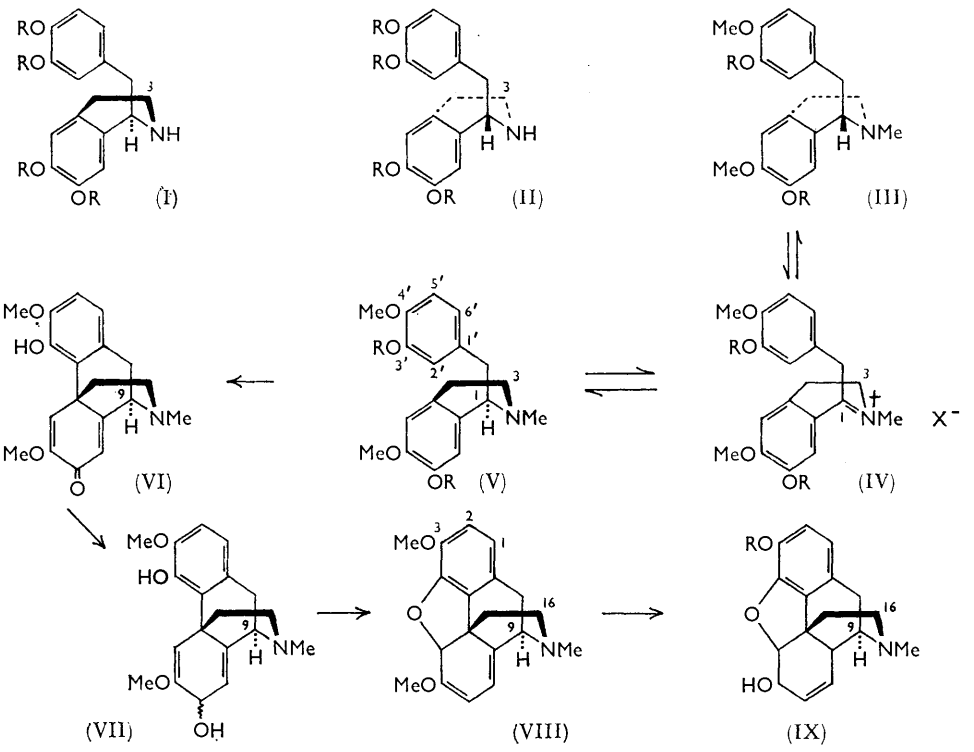
The quadruply-labelled (+)-, and (−)-forms of reticuline have been synthesised, and their incorporations into thebaine, codeine, and morphine in the opium poppy have been studied. The results prove (−)-reticuline (V; R = H) to be the direct precursor of these alkaloids and that it is in oxidation–reduction equilibrium with 1,2-dehydroreticuline and with (+)-reticuline. Labelled 1,2-dehydroreticuline (IV; R = H) has been synthesised and incorporated in high yield into morphine. It is suggested that 1,2-dehydroreticuline stands at a branching point on the biosynthetic pathway. Incorporation experiments with labelled (+)- and (−)-norlaudanosolines (I; R = H) and (II; R = H) have given unexpected results, which are discussed. The present work illustrates how detailed information can be gained by the use of resolved, multiply labelled precursors.

The three diphenolic structural isomers of reticuline have been synthesised in labelled form, and are shown not to act as precursors of morphine.

The incorporation of ^{14}C-labelled (±)-norlaudanosoline (as I; R = H) without randomisation of the label into thebaine (VIII), codeine (IX; R = Me), and morphine (IX;

[1] Part VII, D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson, and H. Ramuz, 1965, 2423; for preliminary Papers see D. H. R. Barton, G. W. Kirby, W. Steglich, and G. M. Thomas, *Proc. Chem. Soc.*, 1963, 203, and A. R. Battersby, R. Binks, D. M. Foulkes, R. J. Francis, and H. Ramuz, *ibid.*, 1963, 203.

R = H) proved [2,3] that oxidative cyclisation of a 1-benzyltetrahydroisoquinoline precursor occurs in *Papaver somniferum* plants to yield morphine, as had been proposed [4,5] by



Robinson and Barton. Good reasons were advanced [5–7] to support the view that methylation of the norlaudanosoline system occurs in the poppy to afford reticuline [8] (as V; R = H), and that this is the species which undergoes phenol coupling. Recent work [1,3] has proved this to be so by demonstrating the high incorporation of labelled (±)-reticuline into the morphine alkaloids. It has been further shown [1] that reticuline is converted into the dienone salutaridine (VI) and thence into salutaridinol (VII) which has the phenolic hydroxyl and the allylic alcohol function correctly placed [6,9,10] for closure of the fifth ring to yield thebaine (VIII).

The labelled 1-benzylisoquinolines used in the foregoing work were all racemic. However, it would be expected that the enzyme system which carries out the oxidative coupling step would be stereospecific and that only one of the two optical isomers should act as a direct substrate. The experiments can therefore be further refined by using resolved multiply labelled precursors carrying tritium at the asymmetric centre. In order to simplify the following account, our recent work will be described first, followed by the

[2] A. R. Battersby and R. Binks, *Proc. Chem. Soc.*, 1960, 360.
[3] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J.*, 1964, 3600.
[4] J. M. Gulland and R. Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, 1925, **69**, 79; R. Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, and refs. therein.
[5] D. H. R. Barton and T. Cohen, "Festschrift Arthur Stoll," Birkhauser, Basle, 1957, p. 117.
[6] A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189.
[7] D. H. R. Barton, Hugo Muller Lecture, *Proc. Chem. Soc.*, 1963, 293.
[8] K. W. Gopinath, T. R. Govindachari, B. P. Pai, and N. Viswanathan, *Chem. Ber.*, 1959, **92**, 776.
[9] D. Ginsburg, "The Opium Alkaloids," Interscience, New York, 1962, p. 91; cf. K. W. Bentley, *Experientia*, 1956, **12**, 251.
[10] G. Stork, "The Alkaloids," ed. R. H. F. Manske, Academic Press, New York, 1960, Vol. VI, p. 223.

early experiments with (+)-, and (−)-norlaudanosolines (I; R = H) and (II; R = H), respectively.

(+)-Reticuline (III; R = H) isolated from *Anona reticulata*[8] has the illustrated absolute configuration since di-*O*-methylation affords[8] (+)-laudanosine (III; R = Me) which has been correlated chemically with the natural amino-acids.[11] The absolute configuration of the morphine alkaloids (VIII) and (IX) is firmly established by interlocking chemical[12] and *X*-ray[13] evidence. It follows that (−)-reticuline (V; R = H) should be the precursor of thebaine (VIII), codeine (IX; R = Me), and morphine (IX; R = H).

A good route to optically active reticulines was devised by fractional crystallisation of the *OO*-dibenzoyltartaric acid salt[14] of *OO*-dibenzylreticuline (as V; R = CH_2Ph); this base is the penultimate intermediate in the various syntheses[15] of (±)-reticuline. The (+)-, and (−)-*OO*-dibenzylreticulines (III; R = CH_2Ph) and (V; R = CH_2Ph), respectively, were debenzylated with hot hydrochloric acid, and (+)-reticuline (III; R = H) was methylated with diazomethane to yield optically pure (+)-laudanosine (III; R = Me). This establishes the completeness of the resolution and confirms the absolute configuration (III; R = H) assigned[8] to natural (+)-reticuline.

TABLE 1

Tracer experiments on *Papaver somniferum* Noordster

Precursor[a]	No. of plants	Year	Wt. of alkaloid (mg.)	% Incorporation[b]
0·062 mc (−)-Reticuline (V; R = H)	9	1963	Morphine, 238	5·9
			Codeine, 38	1·1
			Thebaine, 92	0·54
0·062 mc (+)-Reticuline (III; R = H)	9	1963	Morphine, 273	5·6
			Codeine, 43	0·97
			Thebaine, 73	0·61
0·062 mc (−)-Reticuline (V; R = H)	10	1964	Morphine, 330	2·3
0·062 mc (+)-Reticuline (III; R = H)	10	1964	Morphine, 330	2·6
0·05 mc (±)-Protosinomenine (XV)	8	1963	Morphine, 274	0·00
0·086 mc (±)-Orientaline (XVI)	4	1963	Morphine, 136	<0·02
0·15 mc (±)-Base (XVII)	6	1963	Morphine, 203	<0·04
0·27 mc (+)-Norlaudanosoline (I; R = H)	10	1961	Morphine, 580	0·34
0·27 mc (−)-Norlaudanosoline (II; R = H)	10	1961	Morphine, 377	4·0
0·016 mc (+)-Norlaudanosoline (I; R = H)	3	1962	Morphine, 154	0·45
0·039 mc (−)-Norlaudanosoline (II; R = H) ...	3	1962	Morphine, 244	6·7
0·052 mc 1,2-Dehydroreticuline (IV; R = H) ...	9	1964	Morphine, 303	10·5

[a] The total activities recorded refer only to ^{14}C for multiply labelled precursors. [b] For experiments based upon precursors labelled both with ^{3}H and ^{14}C, the incorporation is calculated with respect to ^{14}C only.

Quadruply labelled (±)-*OO*-dibenzylreticuline (as V; R = CH_2Ph) was prepared, in collaboration with Dr. J. Staunton and R. J. Francis, by carrying out four separate syntheses of singly labelled materials. Appropriate quantities of each were mixed to give material with ^{14}C in the skeleton at C-3 (to act as a stable internal reference), in the 4′-methoxyl group, and in the *N*-methyl group, and with ^{3}H at C-1. The synthetic routes were essentially those used for previous syntheses[3,1] of labelled 1-benzylisoquinolines, and the various modifications to allow other labels to be introduced are described in the Experimental section together with some steps where appreciable improvements have been made. Resolution of the labelled racemate was carried out as above though the small scale in the radioactive series did not allow the separation to be carried further

[11] H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, 1956, **39**, 889.
[12] J. Kalvoda, P. Buchschacher, and O. Jeger, *Helv. Chim. Acta*, 1955, **38**, 1847.
[13] G. Kartha, F. R. Ahmed, and W. H. Barnes, *Acta Cryst.*, 1962, **15**, 326.
[14] C. L. Butler and L. H. Cretcher, *J. Amer. Chem. Soc.*, 1933, **55**, 2605.
[15] M. Tomita and I. Kikkawa, *Pharm. Bull. Japan*, 1956, **4**, 230; *J. Pharm. Soc. Japan*, 1957, **77**, 195; K. W. Gopinath, T. R. Govindachari, and N. Viswanathan, *Chem. Ber.*, 1959, **92**, 1657; J. Kunimoto, *J. Pharm. Soc. Japan*, 1961, **81**, 1253; M. K. Jain, *J.*, 1962, 2203.

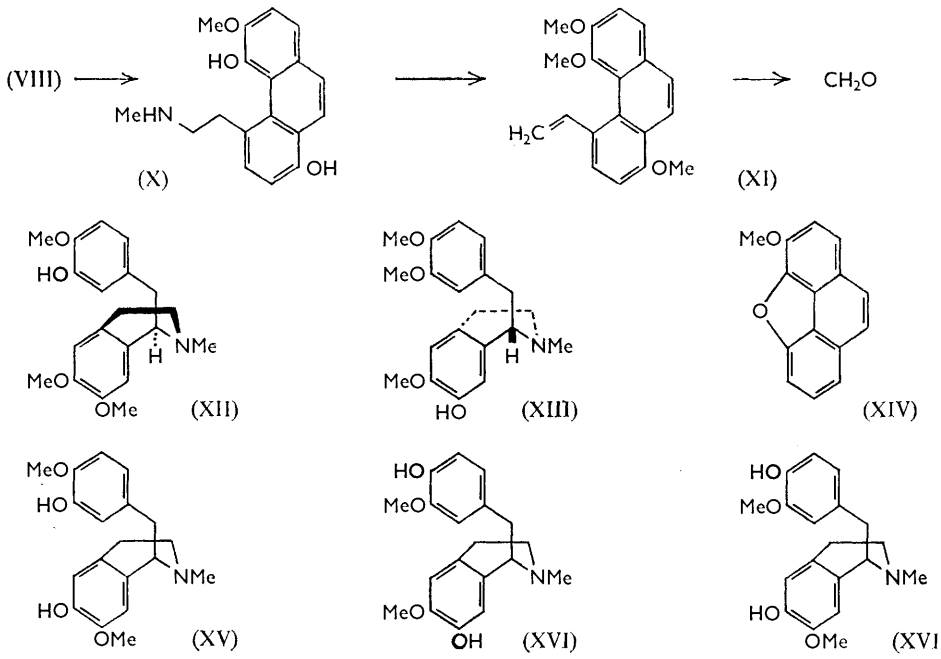
TABLE 2

Ratios of labels in (−)-reticuline and in isolated thebaine

	C-3 (^{14}C)	OMe (^{14}C)	NMe (^{14}C)	C-1 (^{3}H)
(−)-Reticuline	0·76	0·13	0·11	2·01; 100[a]
Thebaine	0·77	0·11	0·12	0·64; 32[a]

[a] Expressed as a percentage of the ratio ^{3}H : ^{14}C (at C-3) in the reticuline fed to the plants.

than *ca.* 96% optical purity; this, however, is sufficient. The two epimers were debenzylated to afford quadruply labelled (+)-reticuline (III; R = H) and (−)-reticuline (V; R = H), isolated as their hydrochlorides. These were fed by injection [16] into *P. somniferum* plants, and high incorporations from (−)-reticuline were achieved into the major opium alkaloids (Table 1). The thebaine was degraded by way of thebenine (X) to the vinylphenanthrene (XI) which was cleaved with ozone [3] to yield formaldehyde corresponding to position 16 of the original thebaine (VIII). There was no loss of ^{14}C-activity



during the conversion of thebaine into thebenine (X), as shown by counting the *NOO*-trimethyl derivative of thebenine (as its methosulphate). This taken with Zeisel *O*-demethylation of thebaine allowed the labelling at the C-3 methoxyl of thebaine to be determined (cf. ref. 1); the activity at the *N*-methyl group was obtained by Herzig–Meyer *N*-demethylation. The ^{14}C values found (Table 2) show very close agreement with the levels proved to be present in (−)-reticuline by degradation of this precursor; the degradation was carried out in collaboration with R. J. Francis.

It is apparent that (−)-reticuline (V; R = H) is incorporated into thebaine without significant cleavage of the ^{14}C-labelled parts of the molecule (cf. ref. 1). However, all the hydrophenanthrene alkaloids show a considerable loss of tritium (Table 3), and it is an increasing one in the sequence morphine, codeine, thebaine. Further discussion of these results is best deferred until those from the experiments with quadruply labelled (+)-reticuline (III; R = H) have been considered; this base has the configuration opposite to

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

TABLE 3

Tritium content of opium alkaloids

Alkaloid	Precursor (−)-Reticuline (V; R = H) Retention of ^3H (%)	(+)-Reticuline (III; R = H) Retention of ^3H (%)
Morphine	58	18
Codeine	39	17
Thebaine	32	13

that of the morphine alkaloids. (+)-Reticuline was incorporated (Table 1) almost as efficiently with respect to ^{14}C as the (−)-epimers had been. However, most of the tritium was lost from the (+)-isomer (Table 3). These results are interpreted as showing that (−)- and (+)-reticuline are undergoing interconversion in the plant by oxidation and reduction, presumably *via* the 1,2-dehydro-derivative (IV; R = H). (+)-Reticuline (III; R = H) is incorporated into thebaine (VIII), and so into codeine and morphine, by way of the intermediate (IV; R = H) with consequent loss of tritium. A small retention of tritium (up to 4%) is to be expected from the *ca.* 4% of (−)-reticuline remaining in the (+)-isomer. The possibility must also be borne in mind that transfer of tritium occurs from the oxidation–reduction enzyme system to 1,2-dehydroreticuline (IV; R = H) with the formation of a small amount of (−)-[1-^{3}H]reticuline. This may be the explanation of the somewhat higher retention observed (Table 3). A short-term feeding experiment (3 days) with the same quadruply labelled (+)- and (−)-reticuline to very young poppy capsules in the following season confirmed the foregoing results (Table 1). Here, a greater loss of tritium occurred from each isomer; 68% loss from (−)-reticuline (V; R = H) and 99% loss from (+)-reticuline (III; R = H). The combined evidence establishes the presence of a highly active oxidation–reduction system in the poppy. It further suggests that 1,2-dehydroreticuline (IV; R = H) could lie at a branching point in the biosynthetic pathway, on the one hand giving (−)-reticuline and thence on to the morphine alkaloids and laudanidine (XII), and on the other yielding (+)-reticuline, the probable precursor of codamine (XIII) and laudanosine (III; R = Me). On this basis, much more material passes along the (−)-reticuline branch, as shown by the quantities of the various alkaloids present in the opium poppy, morphine and its relatives being by far the major alkaloids.

We return now to the progressive fall in tritium retention in the order morphine, codeine, thebaine from the experiment with (−)-reticuline (Table 3). Since thebaine (VIII) is the precursor of codeine (IX; R = Me) and codeine is the precursor of morphine [17,18] (IX; R = H), the thebaine isolated from the plants is mainly drawn from (−)-reticuline which has undergone oxidation–reduction in the plant for the longest period and thus shows the greatest loss of tritium. Codeine represents (−)-reticuline of intermediate " age " in the plant and morphine mainly that which has been present for the shortest time (smallest loss of tritium). Our interpretation assumes that tritium at C-9 of the bases (VI) ⟶ (IX) inclusive is not susceptible to loss by similar oxidation and reduction; this is reasonable since a C-9, N= double bond would violate Bredt's rule.

To test 1,2-dehydroreticuline (IV; R = H) as a precursor of the opium alkaloids, the *OO*-dibenzyl[3-^{14}C]-3,4-dihydroisoquinoline iodide [8] (IV; R = CH_2Ph, X = I) was converted into the chloride by treatment with silver chloride, and the benzyl groups were cleaved to afford 1,2-dehydroreticuline chloride (IV; R = H, X = Cl). The ultraviolet spectrum of this product was closely similar to that of the starting material. When the [3-^{14}C]dehydroreticuline chloride was injected as an aqueous solution into poppy plants, there resulted the highest incorporation yet achieved (10·5%) from a 1-benzylisoquinoline precursor into morphine (Table 1). The morphine was degraded [1] to methylmorphenol (XIV) with elimination of the C-15,C-16 bridge. This phenanthrene was essentially radioinactive, proving that the label was entirely confined to the bridge and presumably

[17] H. Rapoport, F. R. Stermitz, and D. R. Baker, *J. Amer. Chem. Soc.*, 1960, **82**, 2765; F. R. Stermitz and H. Rapoport, *ibid.*, 1961, **83**, 4045.

[18] A. R. Battersby and B. J. T. Harper, *Tetrahedron Letters*, 1960, No. 27, 21.

located at C-16. The importance of 1,2-dehydroreticuline for morphine biosynthesis is clear, and the suggestion above of a dividing pathway will be tested by examining the incorporation of activity from the precursor (IV; R = H, X = Cl) into laudanosine (III; R = Me) and codamine (XIII).

Reticuline (V; R = H) has its two phenolic groups in the positions required [5] for oxidative coupling to salutaridine (VI), and it was of interest to test the three structural isomers of this diphenolic system as precursors of morphine; they are the bases (XV), (XVI), and (XVII). All three have been synthesised previously,[19,20] and our synthetic routes were essentially the same. Modifications were made to permit the introduction of labels, and these are described in the Experimental section. In this way were prepared (±)-[1-^{3}H,3-^{14}C] protosinomenine (XV), (±)-[1-^{3}H]orientaline * (XVI), and the (±)-[1-^{3}H]-benzylisoquinoline (XVII). These were fed separately under the usual conditions,[3,16] and the incorporations of activity into morphine (Table 1) ranged from negligible to zero. When this clear structural specificity is considered with earlier work [1,3] and with the proof above that the biosynthesis of morphine shows the predicted absolute stereochemical relation between precursor and product, there can be no doubt that the true precursor of the morphine alkaloids is (−)-reticuline (V; R = H).

The recent isolation [22] of (±)-reticuline from opium gives valuable proof that reticuline is a natural poppy alkaloid, and it will be of great interest to determine whether (−)-reticuline predominates in the plant. This study is in progress (Dr. G. W. Evans) in collaboration with Professor H. Rapoport and his colleagues.

The experiments with optically active norlaudanosolines (I; R = H) and (II; R = H), chronologically earlier, can now be described. These started with (+)-[3-^{14}C]tetrahydropapaverine (I; R = Me) and the (−)-isomer (II; R = Me) which are of firmly established absolute configuration; [11] our syntheses and resolutions of these labelled materials were described in Part IV.[3] In order to study the demethylation step, radioinactive (−)-tetrahydropapaverine (II; R = Me) was heated with concentrated hydrochloric acid and the resultant (−)-norlaudanosoline hydrochloride was *N*-benzoylated. Methylation of the crude *N*-benzoyl derivative (diazomethane) afforded (+)-*N*-benzoyltetrahydropapaverine, identical in properties and rotation with a specimen prepared by direct benzoylation of (−)-tetrahydropapaverine; thus, racemisation is excluded. (−)-*N*-Benzoyltetrahydropapaverine was also prepared, and these substances (and derivatives) have been of value in recent work on optical rotatory dispersion in the 1-benzyltetrahydroisoquinoline series.[23]

The labelled tetrahydropapaverines were demethylated as above to yield (+)-[3-^{14}C]-norlaudanosoline (I; R = H) hydrochloride and (−)-[3-^{14}C]norlaudanosoline (II; R = H) hydrochloride. It is the former epimer which corresponds in absolute configuration to morphine (IX; R = H) yet this isomer was incorporated into morphine to a much lower extent (Table 1) than was the (−)-isomer. Confirmatory experiments were carried out in the following season (Table 1) with essentially the same outcome. These results, surprising at the time, can now be rationalised if 1,2-dehydroreticuline (IV; R = H) is an obligatory intermediate between (−)-norlaudanosoline (II; R = H) and (−)-reticuline (VI; R = H). An experimental test based upon doubly labelled norlaudanosoline and its derivatives is in progress.

The results reported in the present Paper show the role of 1,2-dehydroreticuline in the poppy plant and establish (−)-reticuline as the direct precursor of the morphine alkaloids.

* It is convenient to have a trivial name for this isomer because of its importance [21] for the biosynthesis of the aporphine alkaloid isothebaine, found in *Papaver orientale* (hence orientaline).

[19] R. Robinson and S. Sugasawa, *J.*, 1931, 3163; 1933, 280.
[20] J. Kunimoto, *J. Pharm. Soc. Japan*, 1961, **81**, 1253.
[21] A. R. Battersby and T. H. Brown, *Proc. Chem. Soc.*, 1964, 85, and unpublished work.
[22] E. Brochmann-Hanssen and T. Furuya, *J. Pharm. Sci.*, 1964, **53**, 575.
[23] A. R. Battersby, I. R. C. Bick, W. Klyne, J. P. Jennings, P. M. Scopes, and M. J. Vernengo, *J.*, 1965, 2239.

The work illustrates the power of this approach based upon optically active, multiply labelled precursors for the study of biosynthesis.

Experimental

Cultivation of the plants and administration of the labelled precursors have been described in Part II.[16] The methods used for proof of purity of the labelled precursors and of the isolated alkaloids were described in Part III [24] together with the procedure for calculating incorporations. Melting points were determined on a Kofler hot-stage and optical rotations on a Bendix Ericsson ETL–NPL Automatic Polarimeter.

Radioactive Assay.—Activities were measured by liquid scintillation counting on a Packard " Tri-Carb " Scintillation Spectrometer (model 3003) with toluene solutions of the scintillators. Efficiencies were determined by internal standardisation with [1-^{14}C]hexadecane and [1,2-$^{3}H_2$]-hexadecane, and values in the ranges 70—75% and 25—30% for ^{14}C and ^{3}H, respectively, were obtained. This procedure allowed each isotope to be determined with an accuracy of ±5% with widely differing ratios of ^{3}H : ^{14}C; all determinations were made at least in duplicate. For the degradative work described later, the results are expressed as disintegrations (dis.) per 100 sec. per mmole. These figures are converted into relative molar activities to provide the results recorded in Table 2.

Isolation of Alkaloids.—Whole poppy plants were macerated with methanol in a Waring blender, and the mixture was then percolated in a glass column with methanol until the percolate was alkaloid free (Mayer's reagent). Evaporation of the methanolic solution left a gum which was partitioned between ethyl acetate and an excess of 2N-hydrochloric acid. The ethyl acetate layer was extracted thrice with more acid and finally washed thrice with water. After the combined acidic solution had been extracted thrice with ether, it was adjusted to pH 8·7 (sodium hydrogen carbonate) and extracted with five portions of chloroform; evaporation of the chloroform left the alkaloids as a gum. This was dissolved in chloroform–propan-2-ol (4 : 1 v/v) and the solution was extracted with N-sodium hydroxide. The organic phase and washings (below) were dried and evaporated, to leave the non-phenolic alkaloids. After back-washing the aqueous alkaline phase with chloroform–propan-2-ol, it was adjusted to pH 8·7 and again extracted with chloroform–propan-2-ol, to yield the phenolic alkaloids. Morphine, codeine, and thebaine were then isolated in pure state from these fractions as described in Part IV.[3]

(+)- and (−)-OO-*Dibenzylreticuline* (III; R = CH_2Ph) and (V; R = CH_2Ph).—A solution of (±)-*OO*-dibenzylreticuline (2·05 g.) in methanol (10 ml.) was treated with (−)-*OO*-dibenzoyltartaric acid [14] (1·84 g.), and when a homogeneous solution had been obtained ether (10 ml.) was added. The salt which slowly separated was collected and recrystallised four times from 1 : 1 ether–methanol, to afford (+)-OO-*dibenzylreticuline* (−)-OO-*dibenzoyltartrate* (1·52 g.), m. p. 130—132°, $[\alpha]_D$ +10·1° (*c* 1·0 in $CHCl_3$) (Found: C, 70·2; H, 5·7; N, 1·9. $C_{51}H_{50}NO_{12}$ requires C, 70·2; H, 5·7; N, 1·6%).

The base was recovered from this salt into ether as usual and recrystallised from aqueous ethanol, to give (+)-*OO*-dibenzylreticuline (0·79 g.), m. p. 90—91°, $[\alpha]_D$ +44° (*c* 1·0 in $CHCl_3$).

The mother-liquors from the above resolution were worked for base and this was treated in methanol–ether as before with (+)-*OO*-dibenzoyltartaric acid (1·14 g.). In this way was obtained (−)-*OO*-dibenzylreticuline (0·77 g.), m. p. 90—93°, $[\alpha]_D$ −42° (*c* 1·0 in $CHCl_3$).

Debenzylation of each isomer with hot concentrated hydrochloric acid [15] afforded (+)- and (−)-reticuline hydrochloride, $[\alpha]_D$ +73·1 and −75°, respectively (*c* 1·0 in water). These materials were identified by comparison with (±)-reticuline (infrared and thin-layer chromatography).

The base was recovered from (+)-reticuline hydrochloride (90 mg.) and methylated in methanol (10 ml.) with ethereal diazomethane (50 ml.; 3%) for 3 days. Chromatography of the resultant bases on neutral alumina (activity I) with 1 : 1 benzene–chloroform afforded (+)-laudanosine, m. p. 88—90° [from ether–light petroleum (b. p. 60—80°)], $[\alpha]_D$ +54·1° (*c* 1·0 in $CHCl_3$) (lit.,[25] m. p. 89—90°, $[\alpha]_D$ +52—53·5°). The product gave an infrared spectrum identical with that of (±)-laudanosine.

[24] A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J.*, 1964, 1595.

[25] B. Frydman, R. Bendisch, and V. Deulofeu, *Tetrahedron*, 1958, **4**, 342.

Quadruply Labelled (+)- *and* (−)-*Reticuline* (III; R = H) *and* (V; R = H).—Sodium borotritiide (0·88 mg.; 2·38 mc) was added to a solution of 1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline methiodide [15] (25 mg.) in dimethyl sulphoxide (0·2 ml.). After the solution had been kept at room temperature under nitrogen for 86 hr., sodium borohydride (10 mg.) was added to it, followed, after 0·5 hr., by water (3 ml.). Extraction with 4 : 1 ether–chloroform afforded (±)-[1-^{3}H]-*OO*-dibenzylreticuline * (23 mg.; 0·34 mc). Further preparations in this way afforded more material for combination with the products below.

(±)-[3-^{14}C]-*OO*-Dibenzylreticuline (290 mg.; 1·0 mc) was synthesised as previously,[3,1] The preparation [1] of (±)-[N-*methyl*-^{14}C]-*OO*-dibenzylreticuline (0·1 g.; 0·1 mc) was carried out by Dr. J. Staunton [26] and that of the (±)-[4′-O-*methyl*-^{14}C]-base [1] (166 mg.; 0·1 mc) by R. J. Francis.[26] Appropriate quantities of these four specimens were combined, and the product (total 454 mg.) was resolved as above. Debenzylation of the resultant epimers afforded quadruply labelled (+)-reticuline hydrochloride (127 mg.; 0·26 mc ^{14}C and 0·4 mc ^{3}H), $[\alpha]_D^{20}$ +69·4° (*c* 1·5 in water), and the corresponding (−)-salt (121 mg. 0·25 mc ^{14}C and 0·39 mc ^{3}H), $[\alpha]_D^{20}$ −70·1° (*c* 1·5 in water).

[3-^{14}C]-1,2-*Dehydroreticuline Chloride* (IV; R = H, X = Cl).—Freshly prepared silver chloride (0·5 g.) was stirred for 1 hr. with a solution of 1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline methiodide (0·1 g.) in 1 : 1 aqueous methanol (10 ml.). The solids were filtered off, and the solution was evaporated to a gum which crystallised from acetone–ether to yield OO-*dibenzyl*-1,2-*dehydroreticuline chloride* (80 mg.), m. p. 118—121° (Found: C, 72·8; H, 6·6. $C_{33}H_{34}ClNO_4$ requires C, 72·9; H, 6·3%), $\lambda_{max.}$ 250, 317 mμ in 1 : 1 aqueous ethanol. Acid catalysed debenzylation [15] of this product (50 mg.) afforded the unstable 1,2-dehydroreticuline chloride (29 mg.), m. p. 190—200° (decomp.), $\lambda_{max.}$ 250, 323 mμ, which was very hygroscopic and not susceptible to analysis. It was shown to be homogeneous by thin-layer chromatography.

In the radioactive series, the [3-^{14}C]-methiodide (50 mg.; 0·05 mc) gave [3-^{14}C]-1,2-dehydroreticuline chloride (28·4 mg.; 0·05 mc) which was stored at −196° until it was administered to the plants a few hours later.

(±)-[1-^{3}H]*Orientaline* (XVI).—The synthesis of this base followed that in the radioinactive series [20] save that the acid chloride preparation was improved.

3-Methoxy-4-benzyloxyphenylacetic acid (0·25 g.) in hot anhydrous benzene (20 ml.) was treated with freshly purified thionyl chloride (3 ml.). After the solution had been kept without further heating for 1 hr., it was evaporated to leave crystalline acid chloride suitable for direct use in amide formation. This method was used for all preparations of acid chlorides.

A solution of 1-(3-methoxy-4-benzyloxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline methiodide [20] (62·4 mg.) was reduced with sodium borotritiide in dimethyl sulphoxide as above to yield (±)-[1-^{3}H]-*OO*-dibenzylorientaline (36·2 mg.; 0·25 mc), m. p. 98—100° (lit.,[20] m. p. 91—93°) (Found: C, 77·9; H, 6·9; N, 2·8. Calc. for $C_{33}H_{35}NO_4$: C, 77·7; H, 6·9; N, 2·75%). This was debenzylated as for reticuline, above, to yield orientaline hydrochloride (20 mg.; 0·17 mc).

(±)-[1-^{3}H,3-^{14}C]*Protosinomenine* (XV).—[3-^{14}C]-*OO*-Dibenzylprotosinomenine was prepared [15,19,20] from [1-^{14}C]-3-benzyloxy-4-methoxyphenethylamine (0·1 g.; 0·83 mc) which was synthesised precisely as for [1-^{14}C]-3-methoxy-4-benzyloxyphenethylamine.[3] 1-(3-Benzyl-4-methoxy)-6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline methiodide [20] (199 mg.) was reduced as above with sodium borotritiide, to yield (±)-[1-^{3}H]-*OO*-dibenzylprotosinomenine (51·5 mg.; 0·43 mc) which was isolated as the *picrolonate*, m. p. 180° (decomp.) (from methanol) (Found: C, 66·8; H, 5·6; N, 9·0. $C_{43}H_{43}N_5O_9$ requires C, 66·7; H, 5·6; N, 9·1%). The base was recovered from the picrolonate by running a solution in chloroform through a short column of alumina. Combination of the singly labelled species and debenzylation as above gave (±)-[1-^{3}H,3-^{14}C]protosinomenine hydrochloride (51 mg.; 0·09 mc ^{14}C, 0·4 mc ^{3}H).

(±)-[1-^{3}H]-1-(3-*Methoxy*-4-*hydroxybenzyl*)-2-*methyl*-6-*hydroxy*-7-*methoxy*-1,2,3,4-*tetrahydroisoquinoline* (XVII).—The corresponding 3,4-dihydroisoquinoline methiodide (62 mg.), prepared essentially as previously,[15,20] was reduced as above with sodium borotritiide to afford the

* [*Added in Proof, March 19th*, 1965.—Repetition of this reduction with sodium borodeuteride (98% isotropic purity) in place of borotritiide gave (±)-*OO*-dibenzylreticuline which was shown by mass spectrometry to carry deuterium solely at position 1.]

[26] A. R. Battersby, R. J. Francis, and J. Staunton, forthcoming Paper.

OO-dibenzyl base (XVII, dibenzyl ether) (51 mg.; 0·25 mc). This was characterised as the *picrolonate*, m. p. 185° (decomp.) (from methanol) (Found: C, 66·7; H, 5·7; N, 9·4. $C_{43}H_{43}N_5O_9$ requires C, 66·7; H, 5·6; N, 9·1%). Debenzylation of this base gave the phenolic base (XVII) as its hydrochloride (39 mg.; 0·15 mc).

Degradation of Thebaine from (−)-*Reticuline Feeding.*—The thebaine isolated from the plants was diluted with inactive alkaloid and purified to a constant activity of $5·12 \times 10^6$ dis./100 sec./mmole (^{14}C) and $2·5 \times 10^6$ dis./100 sec./mmole (^3H).

Part (50 mg.) was demethylated by Zeisel's method, to yield triethylamine methiodide (71 mg.; $5·83 \times 10^5$ dis./100 sec./mmole ^{14}C) and by the Herzig–Meyer *N*-methyl procedure to give the same salt (22 mg.; $6·35 \times 10^5$ dis./100 sec./mmole ^{14}C).

A further portion (150 mg.) was dissolved in 15% (w/v) aqueous hydrochloric acid (1·5 ml.) and methanol (0·3 ml.), and the solution was heated under reflux for 2 min. and cooled in ice. The liquid was drawn off the precipitated gum, and a solution of the gum in methanol (0·6 ml.) and water (0·2 ml.) was treated, whilst being stirred by a fast stream of nitrogen, with 30% (w/v) sodium hydroxide solution until the precipitate which first formed just redissolved. Dimethyl sulphate was added dropwise until the mixture had pH 5, when the steps of alkali addition followed by dimethyl sulphate were repeated. The crystals which separated (2 days at 0°) were collected and recrystallised from ethanol–ether to yield *NOO*-trimethylthebenine methosulphate [3] (139 mg.; $5·09 \times 10^6$, ^{14}C, and $8·15 \times 10^5$, ^3H, dis./100 sec./mmole), m. p. 233° (decomp.). Hofmann degradation of this salt [3] afforded the phenanthrene (XI; 83 mg.; $4·61 \times 10^6$ dis./100 sec./mmole ^{14}C) which was divided into two equal parts. One was cleaved by ozonolysis [3] to formaldehyde dimethone (18 mg.; $4·01 \times 10^6$ dis./100 sec./mmole ^{14}C) and the other was oxidised with potassium permanganate to yield 3,4,8-trimethoxyphenanthrene-5-aldehyde [27] (17 mg.; $5·6 \times 10^5$ dis./100 sec./mmole ^{14}C).

Degradation of Morphine from 1,2-*Dehydroreticuline Feeding.*—This was carried out as previously [1] and the starting material and products had the following activities (dis./100 sec./mmole). Morphine $4·83 \times 10^5$, codeine methiodide $4·66 \times 10^5$, α-codeimethine $4·44 \times 10^5$, α-codeimethine methiodide $4·42 \times 10^5$, methylmorphenol $2·69 \times 10^3$.

(−)- and (+)-N-*Benzoyl*-1,2,3,4-*tetrahydropapaverine.*—A solution of (+)-1,2,3,4-tetrahydropapaverine [11] (0·1 g.) in acetone 0·5 ml.) was shaken with benzoyl chloride (0·5 ml.) and 10% aqueous potassium hydroxide (5 ml.) until the reaction was complete. Dilution with water gave a solid which was recrystallised from aqueous ethanol to yield (−)-N-*benzoyltetrahydropapaverine* (95 mg.), m. p. 160°, $[\alpha]_D$ −62·6° (*c* 1·1 in EtOH) (Found: C, 72·6; H, 6·3. $C_{27}H_{29}NO_5$ requires C, 72·4; H, 6·5%).

The (+)-*isomer* was prepared similarly, m. p. 160°, $[\alpha]_D$ +63·4° (*c* 1·0 in EtOH) (Found: C, 72·3; H, 6·5%).

(+)- and (−)-*Norlaudanosoline Hydrochloride* (I; R = H) and (II; R = H).—(+)-Tetrahydropapaverine (0·5 g.) was demethylated [3,28] to yield (+)-norlaudanosoline hydrochloride (320 mg.), m. p. 282° (decomp.), $[\alpha]_D$ +17·1° (*c* 1·0 in 1 : 1 by vol. aqueous acetone).

The (−)-isomer was prepared in the same way, m. p. 280° (decomp.), $[\alpha]_D$ −16·5° (*c* 1·0 in 1 1 1 by vol. aqueous acetone). Both samples were identified by comparison with authentic (±)-norlaudanosoline hydrochloride.[3,28]

Methylation of (−)-*Norlaudanosoline.*—A solution of (−)-norlaudanosoline hydrochloride (147 mg.), benzoic anhydride (110 mg.), and sodium benzoate (64 mg.) in dry dimethyl sulphoxide (0·5 ml.) was heated at 90° under nitrogen for 1 hr. The reaction mixture was diluted with methanol (10 ml.), mixed with a 5% solution of diazomethane in ether (20 ml.), and kept at room temperature for 3 days. The excess of reagent was destroyed with acetic acid and the solution was evaporated to dryness to leave a gum. This was worked for neutral material (179 mg.), as usual, which was fractionated on neutral alumina (activity I) in 3 : 1 (by vol.) chloroform–benzene. Recrystallisation of the fast-running fraction from aqueous ethanol gave (+)-*N*-benzoyl-1,2,3,4-tetrahydropapaverine (21 mg.), m. p. 160° $[\alpha]_D$ +64·2° (*c* 1·0 in EtOH); infrared spectrum identical with that of above product (Found: C, 72·3; H, 6·5%).

(+)- and (−)-[3-^{14}C]*Norlaudanosoline Hydrochloride.*—A solution of (+)-[3-^{14}C]tetrahydropapaverine [3] (50·3 mg.) in AnalaR concentrated hydrochloric acid (0·55 ml.) was heated in an evacuated sealed tube at 160—165° for 50 min. (+)-[3-^{14}C]Norlaudanosoline hydrochloride (48·5 mg.) separated from the cold solution; $[\alpha]_D$ shown to be positive in water.

[27] J. M. Gulland and C. J. Virden, *J.*, 1928, 921.
[28] F. L. Pyman, *J.*, 1909, **95**, 1619.

Similarly, (−)-[3-^{14}C]norlaudanosoline hydrochloride (51·1 mg.) was prepared from the (+)-tetrahydropapaverine (62·5 mg.); $[\alpha]_D$ shown to be negative in water.

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