Alkaloid Biosynthesis. Part XII.[1] The Biosynthesis of Narcotine

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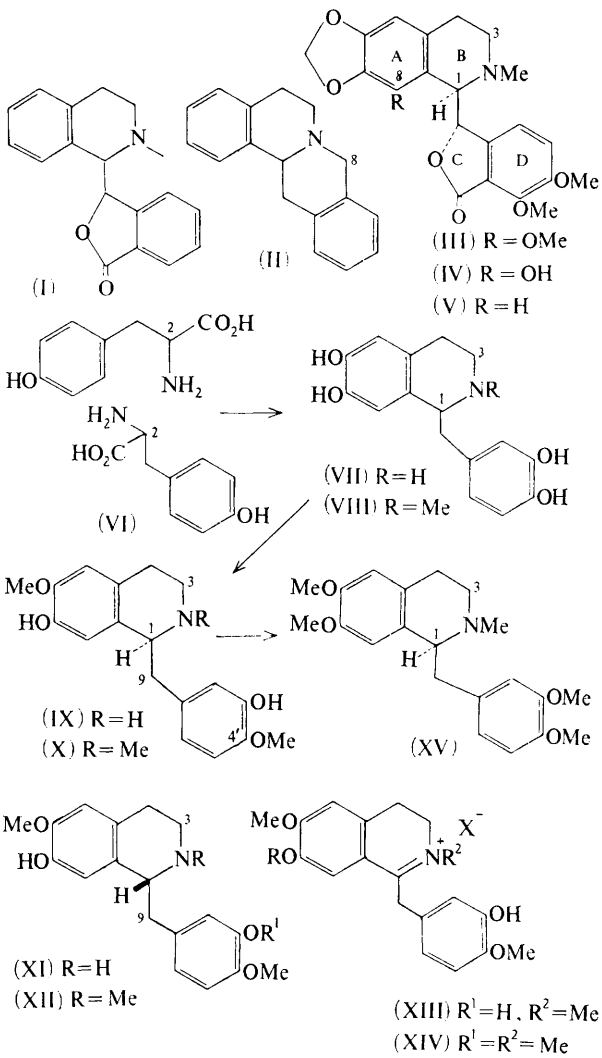
Schemes for the degradation of narcotine (III) are devised which allow unambiguous isolation of labelled atoms from the alkaloid isolated from *Papaver somniferum* plants fed with a variety of precursors, both singly and multiply-labelled. It is shown in this way that the biosynthesis involves combination of two Ar–C–C units, derivable in the living plant from tyrosine, to generate norlaudanosoline (VII). *O*-, and *N*-Methylation from the *S*-methyl group of methionine then yields reticuline [as (X)], the (+)-isomer of which (X) undergoes oxidative ring-closure to yield (−)-scoulerine (XLII). Further oxidative attack at positions 1, 8, and 13 are then involved in the final steps leading to narcotine. The results from ^{3}H,^{14}C-labelling experiments are in agreement with stereospecific attack at position 13.

The synthesis and resolution of labelled 1-benzylisoquinolines and scoulerine are described.

NARCOTINE (III) belongs to the phthalideisoquinoline alkaloids [2,3] which are based upon the skeleton (I). The various members can differ in oxygenation pattern, in degree of *O*-methylation (or *OO*-methylenation), and in stereochemistry. The occurrence of narcotine in opium, which has long been readily available and of pharmacological interest, led to its isolation as one of the earliest known pure alkaloids.[4] It was considered of little value in medicine but the demand for narcotine has been increased by the discovery of its antitussive activity.[5] The structure of narcotine has been firmly established by degradation and by synthesis [2,3] and recent studies [6] have defined the illustrated absolute stereochemistry.

Nothing was known at the outset of our work concerning the biosynthesis of phthalideisoquinolines and our early experiments were guided by Robinson's proposal [7] that the system (I) probably arises in Nature by oxidative modification of the tetrahydroprotoberberine skeleton (II). In addition, we were able to use knowledge gained from tracer experiments on many other isoquinoline alkaloids.[8] The experimental study to be described falls into three parts (*a*) origin of the carbon skeleton and attached groups, (*b*) 1-benzylisoquinoline intermediates, and (*c*) studies with tetrahydroprotoberberines. Preliminary accounts of some of the results have been published.[9–11] Other investigators have carried out parallel work relating to section (*a*) and their results will be considered at the appropriate points.

Our experiments were carried out entirely with the Schlendstedt variety of *Papaver somniferum* which though not a rich source, affords workable amounts of narcotine. The various labelled materials, usually as the hydrochlorides, were administered to the plants by injection of aqueous solutions at pH 5·5—7·0 into the



[1] Part XI, A. R. Battersby, D. M. Foulkes, M. Hirst, G. V. Parry, and J. Staunton, *J. Chem. Soc.*, 1968, 210.

[2] J. Stanek and R. H. F. Manske, 'The Alkaloids,' ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1954, vol. IV, p. 167.

[3] H.-G. Boit, 'Ergebnisse der Alkaloid-Chemie bis 1960,' Akademie-Verlag, Berlin, 1961, p. 354.

[4] M. Derosne, *Ann. Chim.*, 1803, [1] **45**, 257; M. Robiquet, *Ann. Chim.*, 1817, [2] **5**, 275.

[5] *E.g.* M. S. Segal, M. M. Goldstein, and E. O. Attinger, *Diseases Chest*, 1957, **32**, 305.

[6] A. R. Battersby and H. Spencer, *Tetrahedron Letters*, 1964, 11; *J. Chem. Soc.*, 1965, 1087; M. Ohta, H. Tani, S. Morosumi, S. Kodaira, and S. Kuriyama, *Tetrahedron Letters*, 1963, 1857; S. Safe and R. Y. Moir, *Canad. J. Chem.*, 1964, **42**, 160; K. Blaha, J. Hrbek, J. Kovař, L. Pijewska, and F. Šantavý, *Coll. Czech. Chem. Comm.*, 1964, **29**, 2328.

[7] R. Robinson, 'The Structural Relations of Natural Products,' Clarendon Press, Oxford, 1955.

[8] A. R. Battersby, *Quart. Rev.*, 1961, **15**, 259.

[9] A. R. Battersby and D. J. McCaldin, *Proc. Chem. Soc.*, 1962, 365.

[10] A. R. Battersby and M. Hirst, *Tetrahedron Letters*, 1965, 669.

[11] A. R. Battersby, R. J. Francis, M. Hirst, R. Southgate, and J. Staunton, *Chem. Comm.*, 1967, 602.

young seed capsules at petal fall.[12] Extraction and purification of the alkaloids was carried out largely by the methods described earlier [13,14] but an improved procedure was developed during the later phases of the present work.

Origin of the Carbon Skeleton.—The clear structural relation of narcotine to 1-benzylisoquinoline alkaloids [7] led us to expect that the carbon skeleton, apart from the lactonic carbonyl group, would originate from two aromatic C_6–C_2 units derivable in the plant from tyrosine [15] (VI). When (±)-[2-^{14}C]tyrosine was fed to *P. somniferum* plants, radioactive narcotine was isolated (Expt. 1, Table 1) and this material of proven radiochemical purity was degraded by the following sequence. The exploratory work was carried out with radioinactive materials. Some of the steps had been described previously in old papers and much experimental work was often necessary to define satisfactory conditions; details are reported in these cases.

TABLE 1

Tracer experiments on *Papaver somniferum* Schlandstedt

Expt. No.	Precursor [a]	No. of plants	Year	Wt. of Narcotine (mg.)	Incorpn.[b] (%)	^{3}H/^{14}C ratio in precursor	^{3}H/^{14}C ratio in narcotine
1	0·2 mc (±)-[2-^{14}C]Tyrosine	20	1960	92	0·22		
2	0·5 mc Sodium ^{14}C-formate	10	1960	60	0·05		
3	0·2 mc L-[*methyl*-^{14}C]Methionine	20	1962	87	0·04		
4	0·2 mc (±)-[1-^{14}C]Norlaudanosoline (VII)	20	1960	65	0·06		
5	0·045 mc (±)-[1-^{3}H,3-^{14}C]Norlaudanosoline (VII)	6	1963	7	0·012	0·38	0·36
6	0·05 mc (±)-[1-^{3}H,3-^{14}C,N-*methyl*-^{14}C]Laudanosoline (VIII)	10	1963	16	0·022	0·36	0·20
7	0·05 mc (+)-[1-^{3}H,3-^{14}C,N-*methyl*-^{14}C,-4′-O-*methyl*-^{14}C]-Reticuline (X)	9	1963	27	0·042	1·47	0·35
8	0·05 mc (−)-[1-^{3}H,3-^{14}C,N-*methyl*-^{14}C,-4′-O-*methyl*-^{14}C]-Reticuline (XII)	9	1963	24	0·032	1·47	0·66
9	0·03 mc (+)-[3-^{14}C,9-^{3}H]Reticuline (X)	8	1964	21	0·088	12·0	6·5
10	0·03 mc (−)-[3-^{14}C,9-^{3}H]Reticuline (XII)	8	1964	22	0·032	12·0	5·4
11	0·034 mc (−)-[6-^{14}C,14-^{3}H]Scoulerine (XLII)	5	1965	35	2·3	5·07	4·42
12	0·032 mc (+)-[6-^{14}C,14-^{3}H]Scoulerine (XLIII)	5	1965	40	0·02	5·07	

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

Oxidative cleavage of narcotine with hot nitric acid [16] afforded opianic acid (XXII) and cotarnine (XVI) in good yield. The latter reacted with methyl iodide [17] and the resultant methiodide (XVII) was converted first into the oximino-iodide (XVIII) and then by ion-exchange into the corresponding chloride (XIX). This oxime was readily dehydrated with hot acetic anhydride to generate the required benzonitrile residue and, in addition, Hofmann elimination occurred giving (XXI). Enhancement of the acidity of the β-hydrogen [see (XVIII)] by the oximino- or nitrile functions probably accounts for the unusual ease of this elimination; the product was identical with a sample prepared by a different route.[18] It is known that *o*-disubstituted benzonitriles are difficult to hydrolyse and hot 100% phosphoric acid has been used with success.[19] Such conditions would be expected in our case (XXI) to cause decarboxylation of the resultant acid and this occurred. The evolved carbon dioxide was trapped as barium carbonate (68% yield) for radioactive assay. Because of the vigorous nature of the last step, it was necessary to ensure that carbon dioxide was not being formed by non-specific oxidative processes. Accordingly, the alcohol [17] (XXV) was treated under the same conditions; no carbon dioxide resulted. A satisfactory degradative route for the isolation of C-1 from narcotine was thus available.

The sequence for the isolation of C-3 also depended upon the aldehyde (XVII) which was converted [17,18] by Hofmann degradation into the styrene (XX). Hydroxylation of this product with osmium tetroxide and cleavage of the diol (XXIII or tautomers) with periodate [17] gave the phthalaldehyde (XXVI) and formaldehyde, corresponding to C-3 of narcotine, isolated as the dimethone.

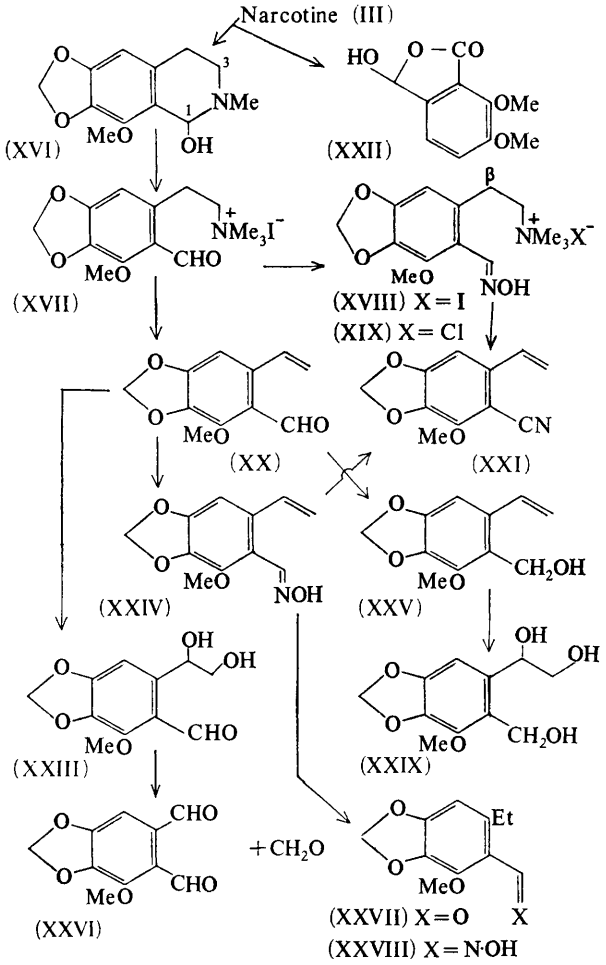
TABLE 2

Degradation of Narcotine: Cotarnine route

Narcotine and degradation products	Relative activities Expt. No. 1	Expt. No. 2	Expt. No. 4	Expt. No. 11
Narcotine	1·00	1·00	1·00	1·00
Opianic acid (XXII)	<0·02	0·48	0·00	
CO_2 from opianic acid (XXII)		0·16		
Cotarnine (XVI)				0·96
Quaternary aldehyde (XVII)	0·97		0·99	
Quaternary oxime (XVIII)		0·49	0·99	
Nitrile (XXI)	0·99		0·97	
CO_2 from nitrile (XXI)	0·51		0·94	
The phthalaldehyde (XXVI)	0·44			
Formaldehyde dimethone from (XXIII)	0·51			
The aldehyde (XXVII)				0·96
p-Bromophenacyl acetate				0·98
N-Methylphthalimide				0·97

[12] A. R. Battersby, R. Binks, and B. J. T. Harper, *J. Chem. Soc.*, 1962, 3534.
[13] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.
[14] A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.
[15] A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189.
[16] T. Anderson, *Annalen*, 1853, **86**, 179.
[17] J. Blair and G. T. Newbold, *J. Chem. Soc.*, 1954, 1836.
[18] W. Roser, *Annalen*, 1888, **249**, 156 and refs. therein.
[19] G. Berger and S. C. J. Olivier, *Rec. Trav. chim.*, 1927, **46**, 600.

Application of these two schemes to the radioactive narcotine isolated from plants fed with (±)-[2-^{14}C]-tyrosine (Expt. 1, Table 1) gave the results collected in Table 2. These established that the original narcotine



was labelled specifically and equally, within experimental error, at C-1 and C-3. The benzylisoquinoline system of narcotine is thus derived biologically from two Ar–C–C units * which can arise from tyrosine in the plant. Parallel studies by Kleinschmidt and Mothes [20] on narcotoline (IV) and by Gear and Spenser [21] on hydrastine (V) afforded similar results from experiments in which, respectively, generally labelled tyrosine and (±)-[2-^{14}C]tyrosine were fed to the appropriate plants.

Several substances were prepared during the exploratory degradations of narcotine which were found unsuitable for use in the radioactive series. Thus, mild hydrogenation of the oxime (XXIV) afforded the corresponding dihydro-derivative (XXVIII). The alcohol (XXV), prepared [17] by borohydride reduction of (XX), was hydroxylated with osmium tetroxide to afford a low yield of the triol (XXIX).

Attention turned next to the origin of the lactonic carbonyl group. If the tetrahydroprotoberberine system [*e.g.* (II)] is an intermediate, then the carbonyl group of narcotine corresponds to the so-called 'berberine-bridge' [C-8 in (II)] and this was considered [7] to enter the biosynthetic process as a one-carbon unit. Accordingly, both ^{14}C-formate and [methyl-^{14}C]methionine were studied as sources of the carbonyl group in narcotine. Radioactive alkaloid was isolated from both experiments (No. 2 and 3, Table 1) and the formate-derived narcotine was degraded to opianic acid (XXII). This underwent decarboxylation when heated with quinoline and copper chromite and the radioactive carbon dioxide was collected as barium carbonate. Its relative activity of 0·13 was subsequently found to be a minimum value since small amounts of carbon dioxide were produced under the conditions used from closely similar substances having no carbonyl function; correction for this led to a relative activity value of 0·16 (see Table 2). This result proved that the carbonyl group of narcotine is derived from a one-carbon source and further refinement was unnecessary because more extensive data became available from the incorporation of methionine. The resultant radioactive narcotine was degraded by a different method based upon the following work.

Phenylmagnesium bromide reacts with narcotine to form the hemiacetal [gross structure as (XXX)] and the properties of our product compared favourably with those recorded.[22] However, the m.p. changed as the substance was recrystallised and t.l.c. revealed that two readily interconvertible materials are present. Preparations highly enriched in one or the other, isolated from thick-layer plates, reverted to the same mixture of the two by being kept in solution. These results are best explained by the presence of two stereoisomers (XXX) and (XXXI) which interconvert *via* the ketone (XXXII). Recrystallisation of the mixture from methanol gave mainly one isomer, m.p. 109°. This was oxidised with permanganate to yield 2-benzoyl-3,4-dimethoxybenzoic acid (XXXIV) and benzoic acid. The former product increases the value of this sequence by allowing assay of radioactivity carried by ring C and groups attached thereto in the original narcotine (III). The acid (XXXIV) had been obtained previously [23] and our product was identical with that kindly provided by Professor E. Bergmann. A further one-carbon unit was examined by hydrolysis of narcotine with sulphuric acid which cleaved the methylenedioxy-group; the liberated formaldehyde was trapped as its dimethone.

When these procedures were applied to the narcotine isolated from the methionine feeding (Expt. 3, Table 1),

* One of the residues which appears in narcotine as a C_6–C_2 unit may, at the time of combination with the other, be a C_6–C_3 unit, *e.g.*, 3,4-dihydroxyphenylpyruvic acid, with loss of one carbon in a subsequent decarboxylation step.

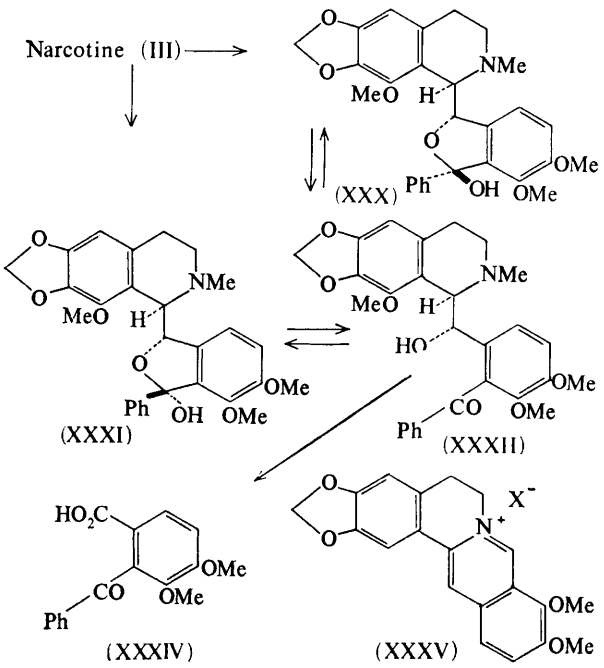
[20] G. Kleinschmidt and K. Mothes, *Z. Naturforsch.*, 1959, **14***b*, 52.

[21] J. R. Gear and I. D. Spenser, *Canad. J. Chem.*, 1963, **41**, 783.

[22] M.-M. Janot and H. Pourrat, *Bull. Soc. chim. France*, 1955, 823.

[23] Ch. Weizmann and E. Bergmann, *J. Chem. Soc.*, 1936, 567.

the values collected in Table 3 were obtained. These prove that the carbonyl and methylenedioxy-groups of narcotine are derived from the *S*-methyl group of



methionine and both carry, within experimental error, one sixth of the total activity. In addition, the activity of the acid (XXXIV) is in agreement with equal labelling of the three one-carbon units attached to rings A and B (OCH_2O, OMe, and NMe) and the three attached to ring C (2 × OMe, CO). The results reported by Gupta and Spenser [24] on the incorporation of labelled methionine into hydrastine are in full agreement.

Two related studies yielded important information during the later stages of the foregoing work. Barton and his co-workers [25] proved that a methylenedioxy-group is formed by ring-closure of the *o*-methoxyphenol system and clearly incorporation of *S*-methyl from methionine into the OCH_2O group of narcotine (III) is in accord with such a biosynthesis. Further, it was shown by the Imperial College group [26] and by our own [27] that the 'berberine-bridge' of berberine (XXXV) is built by oxidative cyclisation of an *N*-methyl group. This, taken with our findings that the carbonyl group of narcotine is derived from the *S*-methyl of methionine, indicated that the biosynthetic pathway to narcotine could run thus: two Ar–C–C units combine to yield a 1-benzyltetrahydroisoquinoline followed by suitable *O*-, and *N*-methylation, ring-closure of the *N*-methyl group to form a tetrahydroprotoberberine system [*e.g.* (XLII)] and finally several oxidative steps to complete the formation of narcotine. Obviously, this is only a vague outline and the experiments to be described in the sequel were designed to identify the intermediates and to determine the sequence.

Benzylisoquinoline Intermediates.—The majority of phthalideisoquinoline alkaloids do not carry oxygen at position 8 and therefore it seemed probable that the hydroxy-group at this site in narcotoline (IV) and the corresponding methoxy-group of narcotine (III) are introduced by a late oxidative step. Accordingly, (±)-[1-^{14}C]norlaudanosoline [as (VII)] was fed to the plants. Radioactive narcotine was isolated (Expt. 4, Table 1) and degradation by the cotarnine route already described showed the alkaloid to be labelled solely at position 1 (Table 2). Biological derivation of narcotine from a 1-benzylisoquinoline system is thus established. *N*-, and *O*-Methylation must occur at some stage(s) and bearing in mind the importance of reticuline [as (X)] for the biosynthesis of many benzylisoquinoline alkaloids,[28] it was possible that narcotine is also derived from this precursor. Both (+)-reticuline (X) and (−)-reticuline (XII) were fed in multiply-labelled form (Expt. 7 and 8, Table 1); the synthesis of these precursors has been published.[14] Doubly-labelled (±)-laudanosoline (VIII) was included in the feeding programme (Expt. 6, Table 1) to gain evidence about the sequence of methylation steps. Table 1 shows that all these precursors were incorporated into narcotine and the two alkaloid samples derived from the enantiomeric reticulines were degraded by the phenylhemiacetal route. The results collected in Table 3 prove that (*a*) reticuline is specifically in-

TABLE 3

Degradation of Narcotine: hemiacetal route

	Expt. No. 3		Expt. No. 7		Expt. No. 8	
Narcotine and degradation products	Found	Expected [a]	Found	Expected	Found	Expected [b]
Narcotine	1·00		1·00		1·00	
The hemiacetal (as XXX)	1·00	1·00	0·99	1·00	1·01	1·00
2-Benzoylveratric acid (XXXIV)	0·50	0·50	0·25	0·24	0·25	0·24
Benzoic acid	0·16	0·17	0·11	0·11	0·12	0·11
Formaldehyde dimethone—from OCH_2O	0·17	0·17				

[a] Expected values if all residues derived from methionine are equally labelled. [b] The ^{14}C-activity of the precurosr [14] was divided thus: C-3, 0·76; 4-OMe, 0·13; NMe, 0·11.

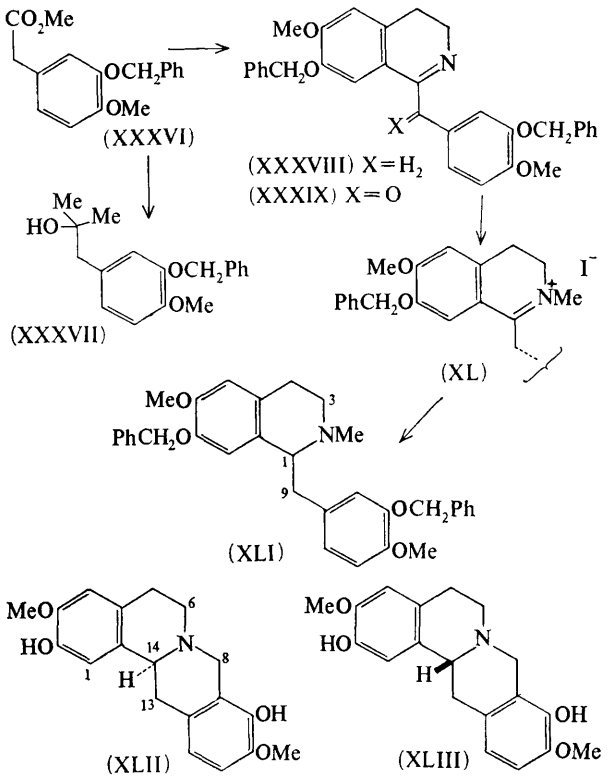
[24] R. N. Gupta and I. D. Spenser, *Canad. J. Chem.*, 1965, **43**, 133.
[25] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J. Chem. Soc.*, 1963, 4545.
[26] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *Proc. Chem. Soc.*, 1963, 267.
[27] A. R. Battersby, R. J. Francis, M. Hirst, and J. Staunton, *Proc. Chem. Soc.*, 1963, 268.
[28] *E.g.*, A. R. Battersby, 'Organic Substances of Natural Origin, Vol. I, Oxidative Coupling of Phenols,' ed. A. R. Battersby and W. I. Taylor, Marcel Dekker, New York, 1967, p. 119.

corporated into narcotine, (*b*) *N*-methylation can precede *O*-methylation, (*c*) the 4′-*O*-methyl group is retained throughout the biosynthetic process, and (*d*) the lactone carbonyl group is derived from the *N*-methyl group of reticuline by a process not involving significant fission of the *N*-methyl bond before the bond to ring D of narcotine has been established. The formation of scoulerine [as (XLII)] as the next step in the biosynthesis after reticuline would be in accord with the foregoing results and this idea is examined in the sequel. First, however, the stereochemical aspects must be considered.

The absolute stereochemistry of narcotine [6] (III) corresponds to that of (+)-reticuline (X) and this enantiomer is incorporated to a somewhat higher extent into narcotine than is the (−)-enantiomer (Expt. 7 and 8, Table 1) but the difference is not large. A greater selectivity was observed in the following season when (+)-reticuline was used *ca.* three times more efficiently than (−)-reticuline (Expt. 9 and 10, Table 1). The ' wrong ' isomer is presumably incorporated as a result of oxidation–reduction *via* 1,2-dehydroreticuline (XIII) and this process is known to be important in the opium poppy.[14] In keeping with this, the $^3H : ^{14}C$ ratio found for the narcotine resulting from (+)-reticuline (Expt. 7) corresponded to loss of 76% of the original ^3H from position-1. Similarly, the change of $^3H : ^{14}C$ ratio in Expt. 6 indicates a loss of 45% of ^3H from the same position of (±)-laudanosoline (VIII). The result found for (±)-[1-^{3}H,3-^{14}C]norlaudanosoline (Expt. 5) may be a further case of the phenomenon now discussed in relation to the $^3H : ^{14}C$ ratio found for narcotine derived from (−)-reticuline, the ' wrong ' enantiomer (Expt. 8). Comparison of Expts. 7 and 8 shows that the $^3H : ^{14}C$ ratio observed for narcotine is higher from Expt. 8 than from 7 when, in the absence of complicating factors, the reverse would have been expected. In contrast, the morphine from Expt. 7 and 8 had previously been isolated and the $^3H : ^{14}C$ ratios found for this alkaloid [1] were, respectively, 0·12 and 0·91; (−)-reticuline corresponds in absolute configuration to morphine and so these values, including the small retention of ^3H from the ' wrong ' isomer are readily explained.[14] The results also agree well with earlier findings.[14]

The correct explanation of the ^3H results from Expt. 7 and 8 for narcotine cannot be selected on the present data, though there are several possibilities *e.g.* preferential removal of ^{14}C-labelled species along other pathways so leading to an apparent ' rise ' in $^3H : ^{14}C$ ratio. This phenomenon has not been examined further because clear-cut results were obtained in later work with scoulerine as the precursor. However, the present findings indicate caution in interpretation of results from $^3H : ^{14}C$ doubly-labelled materials if the incorporation is low, the precursor is also involved in other much more active pathways and, probably most important, the ^3H label is at a site which may readily be attacked in the living system.

Of the precursors used in the foregoing work, only laudanosoline for Expt. 6 has not been described in earlier Parts. [3-^{14}C]-3,4-Dihydropapaverine [13] was converted into the methiodide (XIV; X = I) and reduced with borohydride to afford (±)-[3-^{14}C]laudanosine [as (XV)]. Repetition of the last step with radioinactive methiodide but with borotritiide produced (±)-[1-^{3}H]-laudanosine; the specificity of labelling by this method has been established.[14] Labelling at the *N*-methyl group involved reaction of 3,4-dihydropapaverine with ^{14}C-methyl iodide and reduction of the product with borohydride. Appropriate quantities of the three products were mixed and the triply-labelled material was then demethylated with hot hydrochloric acid to give (±)-[1-^{3}H,3-^{14}C,*N*-methyl-^{14}C]laudanosoline (VIII) as the hydrochloride. The labelling ratios calculated from the amounts and activities of the three components used were confirmed by *O*-methylation of a small amount of the active laudanosoline which was then diluted with radioinactive (±)-laudanosine [as (XV)]. Rigorous purification and counting confirmed the $^3H : ^{14}C$ ratio



and Hertzig-Meyer demethylation to methyl iodide (assayed as methyltriethylammonium iodide) gave an independent check on the ratio of the two ^{14}C-labels.

Tetrahydroprotoberberine Intermediates.—The conviction that scoulerine [as (XLII)] is an important intermediate for the biosynthesis of the many alkaloids structurally related to the tetrahydroprotoberberine skeleton (II) had already led us to devise flexible syntheses [29] of (+)-, and (−)-scoulerine *via* the optically

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

active forms of nor-reticuline (IX) and (XI). By using 4-benzyloxy-3-methoxyphen[1-^{14}C]ethylamine [13] in this synthesis, the *OO*-dibenzyl ether of (±)-[3-^{14}C]nor-reticuline [as (IX)] was prepared; the corresponding (±)-[1-^{3}H]-labelled species was available by standard methods. Appropriate quantities of these products were mixed and resolution followed by *O*-debenzylation afforded doubly-labelled (−)-nor-reticuline (IX) and (+)-nor-reticuline (XI). These were then converted as earlier [29] into (−)-scoulerine (XLII) and (+)-scoulerine (XLIII), respectively, for Expt. 11 and 12 (Table 1). The results show that (−)-scoulerine which corresponds to narcotine in absolute configuration is over one hundred times more effective than the (+)-form. There was only slight loss of ^{3}H from the asymmetric centre of (−)-scoulerine (Table 1) and these combined results demonstrate that interconversion of (−)-, and (+)-scoulerine by oxidation–reduction is not of importance in the Schlanstedt poppy plants over the period of our experiments.

Degradation of narcotine from Expt. 11 (Table 1) followed the route outlined earlier to the quaternary salt (XVII) which readily undergoes Hofmann elimination to generate the aldehyde [17,18] (XX). Mild hydrogenation then afforded the dihydro-derivative (XXVII) which was subjected to Kuhn-Roth oxidation and the resultant acetic and propionic acids were separated as their *p*-bromophenacyl esters. The acetic acid recovered by hydrolysis of the acetate ester was further degraded by the Schmidt reaction to methylamine, isolated as *N*-methylphthalimide. The activities of the degradation products (Table 2) prove 97% of the original activity of narcotine to be located at C-3 [see (III)] thus establishing the protoberberine ⟶ phthalideisoquinoline conversion. It is of interest that scoulerine has recently been isolated from opium.[30]

The stages beyond scoulerine (XLII) must involve oxidation at positions 1, 8, and 13 among others. The last has been examined by synthesising (+)-, and (−)-[3-^{14}C,9-^{3}H]reticuline (X) and (XII). Initially it was planned to synthesise the [9-^{3}H]-labelled material from the ester (XXXVI). Ready exchange of the methylene protons of this substance occurred when it was heated in anhydrous tetrahydrofuran with tritiated water and an excess of magnesium methoxide. The radioactive product was converted by reaction with methylmagnesium iodide into the alcohol (XXXVII) as the first step of a degradative scheme but later findings made further work along these lines unnecessary. By heating the ester with 4-benzyloxy-3-methoxyphenethylamine, it was converted into the amide which was taken through the standard stages (XXXVIII) ⟶ (XL) ⟶ (XLI). Serious losses of tritium occurred at each of these steps save the last and the final product had a molar activity only 3·5% of that of the ester (XXXVI). The losses of ^{3}H in the ring-closure reaction and during *N*-methylation (carried out on free base) suggested that the required labelling might conveniently be achieved at the imine stage (XXXVIII). In practice, when a benzene solution of the imine was shaken with tritiated water, highly active base was formed. Specificity of labelling at C-9 was demonstrated by oxidation of the imine (XXXVIII) to the ketone (XXXIX) with manganese dioxide; this method of oxidation is more convenient than earlier procedures.[31] Less than 1% of the ^{3}H-activity remained in the ketone. The synthesis was completed from the ^{3}H-imine as before to yield (±)-*OO*-dibenzyl-[9-^{3}H]reticuline. This was mixed in suitable amounts with (±)-*OO*-dibenzyl-[3-^{14}C]reticuline [13] before being resolved by an improved method (Experimental section). Debenzylation of the (+)-, and (−)-products then afforded the precursors for Expt. 9 and 10 (Table 1).

The efficiency of resolution of *OO*-dibenzylreticuline was determined in the following way. Methylation of a small quantity of the radioactive (+)-reticuline (X) with diazomethane afforded crude (+)-laudanosine (XV). This was greatly diluted (>200 fold) with racemic, radioinactive laudanosine. By this means, the two enantiomers in racemic laudanosine became labelled to an extent corresponding to the degree of resolution of the original (+)-reticuline. Resolution of the (±)-laudanosine using salts with (+)-, and (−)-*OO*-di-*p*-toluoyltartaric acid (a considerable improvement for small scale work over older methods [32]) gave products which were crystallised to constant radioactivity. The degree of resolution of (+)-[3-^{14}C,9-^{3}H]reticuline was shown in this way to be 98% and that of the (−)-enantiomer to be 96%.

Table 1 (Expt. 9) shows that (+)-reticuline (X) is incorporated more efficiently into narcotine than is the (−)-isomer (Expt. 10) and retention of ^{3}H at C-9 is 50%, within experimental error. Loss of half the ^{3}H from this position is the expected value for a *stereospecific* oxidative step which does not involve an isotope effect. This stage is being examined further by the preparation of scoulerine [as (XLII)] labelled at C-13 with ^{3}H in known absolute configuration.

The foregoing results in sum clearly define the major part of the biosynthetic pathway to narcotine. Generation of the 1-benzylisoquinoline system (VII) from two Ar–C–C units (see footnote on page 2165) is followed by *N*-, and *O*-methylation from methionine to afford reticuline. Oxidative ring-closure of the *N*-methyl group of (+)-reticuline (X) then generates (−)-scoulerine (XLII) which by further oxidative modification is converted into narcotine (III). It is probable that the oxidative attack at C-13 of (−)-scoulerine is stereospecific.

EXPERIMENTAL

General Directions.—For cultivation of the poppy plants and administration of the labelled precursors, see Part II.[12]

[30] E. Brochmann-Hanssen and B. Nielsen, *Tetrahedron Letters*, 1966, 2261.

[31] J. S. Buck, R. D. Haworth, and W. H. Perkin, *J. Chem. Soc.*, 1924, **125**, 2176.

[32] A. Pictet and B. Athanasescu, *Ber.*, 1900, **33**, 2346.

The methods used for proof of purity of the labelled precursors and of the isolated alkaloids were described in Part III [33] together with the procedure for calculating incorporations. For radio-active assay, see Part VIII.[14] The proportions for mixed solvents are by volume unless stated otherwise.

Isolation of Narcotine.—(a) *By countercurrent distribution.* The acidic aqueous solution containing the total alkaloids from plants extracted with methanol [14] was adjusted to >pH 10 with potassium hydroxide and extracted with 4 : 1 chloroform–propan-2-ol. After the extracts had been washed with water, they were dried and evaporated to afford the non-phenolic alkaloids (usually 250—300 mg. from 10 plants). These were partitioned between the layers of the system made by equilibration of ethyl acetate (200 ml.), 0·2N-sodium acetate (20 ml.), and 0·2N-acetic acid (180 ml.). The weak bases (narcotine and papaverine) were isolated from the organic layer and the stronger bases (codeine and thebaine) from the aqueous layer. The former (usually 70—80 mg.) were fractionated by countercurrent distribution (10 ml. phases) in the system formed by ethyl acetate (1200 ml.) with 0·2N-acetic acid (1130 ml.) and 0·2N-sodium acetate (70 ml.). After 98 transfers, narcotine was isolated as usual from tubes 65—85. This was diluted with radioinactive narcotine and crystallised to constant activity.

(b) *By thick-layer chromatography.* The solution of total alkaloids in hydrochloric acid used at the outset of (a) above (total vol. *ca.* 500 ml.) was extracted twice with ether and then with chloroform (6 × 150 ml.). The residue left by evaporation of the chloroform was dissolved in the minimum volume of methanol, and the solution was diluted with water (50 ml.), basified with sodium hydrogen carbonate, and extracted thrice with chloroform. This afforded the crude narcotine–papaverine mixture which was separated by thick-layer chromatography on Merck silica GF 254 with 10% methanol in benzene. The pure narcotine, crystallised from methanol, had m.p. 176—177°.

Oxidative Cleavage of Narcotine.—Narcotine (368 mg.) was added portionwise during ½ hr. to a mixture (4 ml.) composed of water (160 ml.) and concentrated nitric acid (47 ml.) at 50°. After 1 hr. further, the cooled suspension was filtered, the filtrate was made alkaline (>pH 12) with potassium hydroxide, and the crystals (210 mg.) were collected and washed with water. This product was suitable for use in the next stage but could be recrystallised from benzene to afford pure cotarnine, m.p. 130—132°. Acidification of the aqueous alkaline filtrate and extraction with ether afforded opianic acid (156 mg.), m.p. 150·5—151·5° after recrystallisation from water.

Methylation of Cotarnine (cf. ref. 17).—A solution of cotarnine (197 mg.) in dioxan (5·5 ml.) and 10% aqueous potassium carbonate (3 ml.) was mixed with methyl iodide (0·75 ml.) and heated under reflux for 2 hr. Evaporation of the solution to dryness and crystallisation of the residue from water yielded the methiodide (XVII) (289 mg.), m.p. 217—220° (decomp.) (lit.,[17] m.p. 217—218°).

The Oxime (XVIII).—The foregoing methiodide (289 mg.) in ethanol (10 ml.) and water (3 ml.) containing hydroxylamine hydrochloride (210 mg.) and anhydrous sodium acetate (200 mg.) was heated under reflux for 2·5 hr. and then evaporated. Methanol (10 ml.) was added to the residue and then distilled off; the *product* (282 mg.) had m.p. 223—223·5° (from 10% aqueous potassium iodide) (Found: C, 42·7; H, 5·2; N, 6·9. $C_{14}H_{21}N_2O_4I$ requires C, 42·6; H, 5·3; N, 6·9%).

Preparation and Hydrolysis of the Nitrile (XXI).—A solution of the foregoing oxime (274 mg.) in 50% aqueous methanol was passed through a column of Amberlite IRA-400 resin (chloride phase) and eluted with the same solvent (100 ml.). Evaporation of the percolate gave the oxime chloride (XIX) (166 mg.) (from methanol–ethyl acetate). Treatment of this product (63 mg.) in water with potassium iodide afforded the original oxime iodide (75 mg., 93%), m.p. 217—220° (decomp.). The oxime chloride (157 mg.) was heated under reflux (bath 170°) with acetic anhydride (15 ml.) for 4 hr., the anhydride was then evaporated, and a solution of the residue in chloroform : ether (1 : 3) (100 ml.) was washed with water (4 × 3 ml.). Evaporation of the organic solution left the nitrile (XXI) (67 mg.), m.p. and mixed m.p. with material prepared by dehydration of the oxime [18] (XXIV) 168—170° after recrystallisation from aqueous acetone.

Phosphoric oxide (2·6 g.) was mixed with 90% orthophosphoric acid (10 g.) in suitable equipment for decarboxylation reactions [21] and the nitrile (XXI) (80 mg.) was added. The mixture was heated in carbon dioxide-free nitrogen at 240° (bath) for 1 hr. and the carbon dioxide evolved was collected and assayed for radioactivity as earlier.[34]

Degradation of Cotarnine to Isolate C-3.—This sequence mainly followed earlier work.[17,18] Cotarnine (77 mg.) yielded the aldehyde (XX) (50 mg.) which in benzene (3 ml.) and pyridine (0·05 ml.) was treated with a solution of osmium tetroxide (70 mg.) in benzene (10 ml.). The complex was collected after 24 hr. and heated under reflux for 1 hr. with ethanol (5 ml.), water (3 ml.), and sodium sulphite (1 g.). After filtration and extraction of the solid with hot ethanol, the total aqueous ethanolic solution was evaporated to dryness and the residue in water (5 ml.) was treated with sodium metaperiodate (80 mg.) and then a further portion (83 mg.) after 20 min. The solution was kept at 20° for 15 hr. and then was added dropwise to a saturated aqueous solution of arsenious oxide (50 ml.). The filtered solution was extracted thrice with ether which yielded the phthalaldehyde (XXVI) (32 mg.), m.p. and mixed m.p. 179—180° from light petroleum (b.p. 60—80°) (lit.,[17] m.p. 179°). Dimedone (0·1 g.) was added to the aqueous solution which had previously been adjusted to pH 9 with potassium carbonate and when dissolution was complete, adjustment to pH 6 was made with hydrochloric acid. After 2 days, the formaldehyde dimethone was collected and recrystallised from aqueous ethanol (31 mg.), m.p. 193—194°.

6-Ethyl-2-methoxy-3,4-methylenedioxybenzaldoxime (XXVIII).—A solution of the corresponding styrene (XXIV) (194 mg.) in ethanol (20 ml.) was shaken at 20°/760 mm. with hydrogen and platinic oxide (45 mg.). Uptake ceased after 30 min. and the filtered solution was evaporated. Recrystallisation of the residue from aqueous ethanol and ether–light petroleum (b.p. 60—80°) gave the *6-ethylbenzaldoxime* (41 mg.), m.p. 110—111° (Found: C, 59·3; H, 6·0; N, 6·4. $C_{11}H_{13}NO_4$ requires C, 59·2; H, 5·9; N, 6·3%).

The Triol (XXIX).—Osmium tetroxide (0·2 g.) was added at 20° to a solution of the alcohol [17] (XXV) (170 mg.)

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

[34] A. R. Battersby and B. J. T. Harper, *J. Chem. Soc.*, 1962, 3526.

in ether (50 ml.) containing pyridine (0·5 ml.). After 2 days, the solid was collected and heated under reflux for 1 hr. with ethanol (10 ml.), water (10 ml.), and sodium sulphite (3 g.). The work up was as above save that chloroform was the extracting solvent. This afforded the *triol* (XXIX) (33 mg.), m.p. 123·5—124·5° from chloroform–light petroleum (b.p. 60—80°) (Found: C, 54·5; H, 5·8. $C_{11}H_{14}O_6$ requires C, 54·2; H, 5·6%).

Decarboxylation of Opianic Acid (XXII).—This material (75 mg.) was heated with quinoline (5 ml.) and copper chromite (0·5 g.) as in Part XI[1] to yield barium carbonate (65 mg.) as an infinitely thick disc for counting.

Degradation of Narcotine via *the Phenylhemiacetal* [(XXX) and (XXXI)].—Narcotine (157 mg.) in anhydrous benzene (20 ml.) was added at −5° to a solution of phenylmagnesium bromide [from bromobenzene (4·8 g.) and magnesium (0·8 g.)] following Janot and Pourat's method.[22] The product crystallised from benzene–light petroleum (b.p. 40—60°) and had m.p. 130—131° after much sintering at 80° (lit.,[22] m.p. 132°). This crude product showed $[\alpha]_D^{20}$ −374° (in benzene) and after chromatography on alumina (activity III) in benzene, which removed diphenyl, the m.p. changed to 113—113·5° and $[\alpha]_D^{20}$ −206°. The best material was isolated by chromatography of the crude product on alumina (activity I) in benzene–light petroleum (b.p. 40—60°) (1 : 4) which removed diphenyl. Chloroform–benzene (1 : 5) eluted the hemiacetal which crystallised from aqueous methanol. This was rechromatographed on alumina (activity III) in benzene and the appropriate fractions yielded the pure product (134 mg.), m.p. 109—110° from aqueous methanol (Found: C, 66·9; H, 6·0; O, 23·7. $C_{28}H_{29}NO_7,CH_3OH$ requires C, 66·5; H, 6·0; O, 23·1%).

The hemiacetal (99 mg.) in acetic acid (2 ml.) and water (10 ml.) at 70—75° was treated during 1 hr. with potassium permanganate (1 g.) in water (100 ml.). After the mixture had been kept at 75° for 6 hr., sodium metabisulphite was added to give a clear solution which was extracted continuously with ether for 36 hr. Evaporation of the ether left a wet acetic acid solution which was kept at 12 mm. over sodium hydroxide pellets and phosphoric oxide. The benzene-soluble fraction from the crystalline residue (51 mg.) was chromatographed on silica (3 g.) in ether–light petroleum (1 : 9) to yield benzoic acid (11·2 mg.), further purified by sublimation twice at 105°, m.p. 120—121°. Further elution of the column with ether–light petroleum (1 : 1) yielded 2-benzoyl-3,4-dimethoxybenzoic acid (10·7 mg.), m.p. and mixed m.p. with authentic material 195—196° after recrystallisation from aqueous methanol (Found: C, 67·2; H, 5·0. Calc. for $C_{16}H_{14}O_5$: C, 67·1; H, 4·9%).

Hydrolysis of Narcotine.—A solution of narcotine (22·6 mg.) in 25% aqueous sulphuric acid (50 ml.) was heated at 150—155° in a stream of nitrogen and water was added continuously to maintain a constant volume. The distillate (150 ml.) was mixed with a solution of dimedone (106 mg.) in water (25 ml.) and, after 24 hr., the dimethone (4·3 mg.) was collected and recrystallised from aqueous methanol (2·6 mg.), m.p. 193—194°.

(±)-[1-^{3}H,3-^{14}C,N-methyl-^{14}C]*Laudanosoline* (VIII).—[3-^{14}C]-3,4-Dihydropapaverine[13] (90 mg.) was heated under reflux for 2 hr. under nitrogen and in the dark with ethyl acetate (22 ml.), ethanol (2·5 ml.), methyl iodide (5 ml.), and sodium hydrogen carbonate (0·34 g.). The solvents were then evaporated and the residue in methanol (10 ml.) was treated with sodium borohydride (0·2 g.). After 5 hr., the solvent was evaporated and the residue was dissolved in dilute hydrochloric acid, the solution basified and then extracted with chloroform–ether (1 : 4 v/v) to give a gum. This was chromatographed on alumina (activity I) initially in 1 : 4 and later 2 : 3 chloroform–benzene to give the laudanosine fraction (62 mg.) which was converted into the picrate and this recrystallised from methanol, m.p. 175—176°. Recovery of the base by passing a chloroform solution of it over alumina gave (±)-[3-^{14}C]-laudanosine (43·1 mg., 0·165 mc), m.p. 113—114°.

3,4-Dihydropapaverine methiodide[35] (48 mg.) in dimethyl sulphoxide (0·4 ml.) was treated with sodium borotriti-ide (1·14 mg., 3·0 mc). After being kept at 20° for 3 days, the solution was diluted with methanol (2 ml.) and sodium borohydride (100 mg.) was added. Water (15 ml.) was added after 2 hr. and extraction with chloroform–ether (1 : 3) gave a gum which was purified by chromatography as above. The laudanosine fraction (27 mg.) was diluted with radioinactive laudanosine (20·4 mg.), further purified *via* the picrate, and recovered therefrom as earlier, to give (±)-[1-^{3}H]laudanosine (24·3 mg., 0·087 mc), m.p. 113—114°.

^{14}C-Methyl iodide (12·6 mg., 1 mc) was transferred by a vacuum line into a tube containing 3,4-dihydropapaverine (99 mg.) in ethyl acetate (7 ml.) which was then sealed and kept at 50° for 2 days. The contents of the tube were then mixed with ethyl acetate (10 ml.) and methyl iodide (10 ml.) and heated under reflux for 1 hr. The residue left by evaporation was dissolved in methanol (10 ml.) and treated with sodium borohydride (0·2 g.). Work up and purification as above gave (±)-[N-*methyl*-^{14}C]laudanosine (41 mg., 0·092 mc), m.p. 113—114°.

The following quantities of the three (±)laudanosines with the indicated labels were mixed: [1-^{3}H], 23·6 mg., 0·087 mc; [3-^{14}C], 36·5 mg., 0·14 mc; [N-*methyl*-^{14}C], 41·4 mg., 0·092 mc. A solution of the resultant triply-labelled material in concentrated hydrochloric acid (2 ml.) was heated in an evacuated sealed tube at 160° for 1 hr., and from the cooled solution separated (±)-[1-^{3}H,3-^{14}C,N-*methyl*-^{14}C]laudanosoline hydrochloride (67·7 mg.), 0·16 mc ^{14}C, 0·061 mc ^{3}H, m.p. 239—242° (lit.,[36] 240—242°).

The various activities reported above were determined by extensive dilution of standard solutions and appreciable errors can arise. Independent determinations were made as follows. [With Dr. R. J. Francis]. The triply-labelled laudanosoline hydrochloride (*ca.* 1 mg.) was diluted with radioinactive material (45 mg.) and treated in methanol (15 ml.) with an excess of ethereal diazomethane for 3 days. The residue left on evaporation was mixed with (±)-laudanosine (29 mg.), the mixture was rigorously purified as above, and finally crystallised to constant activity, m.p. 113—114°. Assay for ^{3}H and ^{14}C then yielded the value in Table 1.

A solution of the pure laudanosine (52 mg.) in phenol (0·4 g.) was heated to 200° over 1 hr. with hydriodic acid (2 ml., *d* 1·7), chloroauric acid solution (4 drops, 2% w/v) and ammonium iodide. The liberated methyl iodide was swept in nitrogen into a trap containing ethanolic triethylamine (5 ml.; 5% v/v) at −78°. The trap was then replaced by a similar one and the foregoing process repeated. After again replacing the trap, the temperature of the reaction mixture was raised to 340° during 1 hr. and then

[35] Z. Kitasato and R. Robinson, *J. Chem. Soc.*, 1932, 785.
[36] R. Robinson and S. Sugasawa, *J. Chem. Soc.*, 1932, 789.

maintained at this temperature for 0·5 hr. The contents of the third trap were kept at 20° for 16 hr., evaporated to dryness, and the residue crystallised to constant activity from ethanol–ether. The methyltriethylammonium iodide (27·7 mg.) had m.p. 296° (decomp.) and contained, on a molar basis, 32% of the original ^{14}C-activity.

(+)-, *and* (−)-[6-^{14}C,14-^{3}H]*Scoulerine* (XLIII) *and* (XLII).— 3-Methoxy-4-benzyloxyphen[1-^{14}C]ethylamine [13] (173 mg., 1·1 mc) was converted by the previously developed route [29] into (±)-*OO*-dibenzyl-[3-^{14}C]nor-reticuline (220 mg., 0·55 mc).

The base recovered as usual from 7-benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-3,4-dihydroisoquinoline hydrochloride [13] (150 mg.) was dissolved in dimethyl sulphoxide (2·5 ml.) and treated with sodium borotriti-ide (5 mg., *ca.* 100 mc). After 1 day, sodium borohydride (25 mg.) was added, the mixture was kept for 3 hr., and then diluted with water (10 ml.) and extracted with ether. The base so obtained was purified by chromatography on alumina in 1 : 1 chloroform–benzene before conversion into (±)-*OO*-dibenzyl-[1-^{3}H]nor-reticuline hydrochloride (89 mg. *ca.* 9 mc).

The ^{3}H-, and ^{14}C-labelled samples were mixed with radioinactive material to give doubly-labelled base (405 mg.) having ^{3}H : ^{14}C ratio *ca.* 5 : 1. Resolution was carried out as earlier [29] and the pure enantiomers were debenzylated with acid [29] to afford (−)-[1-^{3}H,3-^{14}C]nor-reticuline hydrochloride (105 mg.) and the corresponding (+)-isomer (107 mg.). The former was converted [29] into (−)-[6-^{14}C,14-^{3}H]-scoulerine (21 mg., 0·034 mc ^{14}C, 0·17 mc ^{3}H) and the latter into the (+)-enantiomer (29 mg., 0·032 mc ^{14}C, 0·16 mc ^{3}H); $[\alpha]_D^{25}$ −284° and +282°, respectively, (*c* 0·64 and 0·60 in methanol).

6-*Ethyl*-2-*methoxy*-3,4-*methylenedioxybenzaldehyde* (XXVII).—A solution of the aldehyde (XX) (140 mg.) in ethanol (10 ml.) was shaken at 20°/755 mm. with hydrogen and 5% palladised charcoal (15 mg.). Uptake (1·0 mol.) ceased after 1·5 hr. The catalyst was filtered off and the solution was evaporated to leave a gum which was distilled at 50° (bath)/0·1 mm. to give the *aldehyde* (XXVII) as needles (105 mg.), m.p. 43—44° (Found: C, 63·4; H, 5·7. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·8%), $\nu_{max.}$ 1678 cm.$^{-1}$.

Kuhn-Roth Oxidation and Degradation of the Acetic Acid.— The foregoing product in the radioactive series (50 mg.) was heated under reflux for 2·5 hr. with chromic acid [30 ml. of a mixture made by dissolving chromium trioxide (33·6 g.) in water (200 ml.) and concentrated sulphuric acid (40 ml.)]. Steam distillation was then carried out until 300 ml. of distillate had been collected which was adjusted to pH 11 with lithium hydroxide and evaporated to dryness. A solution of the residue in water (1 ml.) was adjusted to pH 8·5, mixed with an ethanolic solution (10 ml.) of *p*-bromophenacyl bromide (60 mg.) and heated under reflux for 1 hr. The residue obtained by evaporation was chromatographed in benzene on thick-layer plates (silica) to yield *p*-bromophenacyl acetate (40 mg.), m.p. 84—85° from light petroleum (b.p. 60—80°). This product was diluted with radioinactive *p*-bromophenacyl acetate and the product (100 mg.) was heated under reflux for 0·5 hr. with 1% sodium hydroxide in ethanol (25 ml.). Acidification with phosphoric acid followed by steam distillation gave a solution (350 ml.) of acetic acid which was adjusted to pH 11 with sodium hydroxide and evaporated. A solution of the residue in water (5 ml.) was acidified with hydrochloric acid, filtered, basified to pH 11 with sodium hydroxide, and again evaporated. The residual sodium acetate in water (1 ml.) was mixed with sodium azide (75 mg.) and the residue left on evaporation was heated with polyphosphoric acid (5 g.) at 100° for 2·5 hr. Water (40 ml.) was added to the cooled mixture, followed by 40% sodium hydroxide, with cooling, until the solution was strongly alkaline. The methylamine was steam distilled into 2N-hydrochloric acid (5 ml.), the distillate (40 ml.) was evaporated to dryness, and the residue in acetic acid (2 ml.) was heated under reflux for 1 hr. with phthalic anhydride (75 mg.) and sodium acetate (50 mg.). The residue left by evaporation was fractionated by thick-layer chromatography on silica in 5% methanol in chloroform. Crystallisation of the appropriate fraction from ethanol gave *N*-methylphthalimide (27 mg.), m.p. 133—135°. Sublimation at 80°/0·1 mm. and further recrystallisation from ethanol left the m.p. and specific activity unchanged (Found: C, 67·0; H, 4·5. Calc. for $C_9H_7NO_2$ C, 67·1; H, 4·8%).

^{3}H-*Labelling of Methyl* 3-*Benzyloxy*-4-*methoxyphenylacetate* [13] (XXXVI).—A mixture of the ester (XXXVI) (243 mg.), magnesium methoxide (204 mg.), ether saturated with tritiated water (0·5 ml.; *ca.* 60 mc) and anhydrous tetrahydrofuran (20 ml.) was heated under reflux for 2 days. 2N-Acetic acid (100 ml.) was added, the product was extracted into ether and the solution was washed four times with water. Evaporation of the ether left the tritiated ester (234 mg.; 9·2 mc).

(±)-*OO-Dibenzyl*[9-^{3}H]*reticuline* (XLI).—(a) *From the ester* (XXXVI). The foregoing ester (50·6 mg.; $4{\cdot}7 \times 10^8$ dis./sec./mmole) and 4-benzyloxy-3-methoxyphenethylamine were heated together in an evacuated sealed tube at 100—120° for 2 days. The product in chloroform was washed twice with 2N-hydrochloric acid, then with water and the solution was evaporated. Chromatography on alumina (activity I) in benzene–chloroform (2 : 1) gave the required amide [13] (94·7 mg.), m.p. 138—140° ($1{\cdot}22 \times 10^8$ dis./sec./mmole). This was diluted with radioinactive amide to give material counting $2{\cdot}8 \times 10^5$ dis./sec./mmole and part (155 mg.) was ring-closed as usual [13] to give the dihydroisoquinoline (XXXVIII), $1{\cdot}6 \times 10^5$ dis./sec./mmole, as the hydrochloride (145 mg.), m.p. 201—203°. Further characterisation was carried out on radioinactive material as the *picrate*, m.p. 138—139° (Found: C, 63·1; H, 4·8; N, 7·9. $C_{30}H_{34}N_6O_4$ requires C, 63·2; H, 4·8; N, 7·8%). *N*-Methylation [13,37] of the ^{3}H-dihydroisoquinoline hydrochloride (136 mg.) afforded the methiodide (XL), $3{\cdot}5 \times 10^4$ dis./sec./mmole (117 mg.) and this was reduced with borohydride [13,37] to (±)-*OO*-dibenzyl[9-^{3}H]-reticuline (86 mg.), m.p. 96—97°, $3{\cdot}8 \times 10^4$ dis./sec./mmole.

(b) *From the dihydroisoquinoline* (XXXVIII) *by exchange.* A solution of the dihydroisoquinoline (XXXVIII) (318 mg.) in anhydrous benzene (12 ml.) was shaken vigorously with a 1 : 4 mixture of tritiated water and methanol (0·1 ml.; 100 mc) for 18 hr. and the solvents were then evaporated. Part of the residue was diluted with radioinactive material and oxidised as below. The rest in ethyl acetate (15 ml.) was kept for 18 hr. with methyl iodide (6 ml.) and the resultant crystals (248 mg.) were treated in isopropyl alcohol (7 ml.) with sodium borohydride (25 mg.). After 2 days, the solution was acidified with hydrochloric acid, basified to pH 10 with sodium hydroxide, and extracted

[37] 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.

with benzene. The gum (211 mg.) so obtained was converted as usual into the picrolonate [37] and the base recovered from it on alumina had m.p. 98—100° (149 mg.; 4·45 mc).

(±)-OO-*Dibenzyl*-9-*oxoreticuline* (XXXIX).—A solution in benzene (20 ml.) of the foregoing low activity [9-^{3}H]dihydroisoquinoline (XXXVIII) (88 mg.), $2{\cdot}52 \times 10^6$ dis./sec./mmole, was percolated during 40 min. through a column of manganese dioxide (9 g.). The base left by evaporation of the percolate was chromatographed on alumina (activity III) in benzene and the base from appropriate fractions (56 mg.) was converted into its *picrate* (70 mg.) in methanol, m.p. 163—165° (Found: C, 62·0; H, 4·4; N, 7·3. $C_{38}H_{32}N_4O_{12}$ requires C, 61·9; H, 4·4; N, 7·6%). Recovery of the keto-base from the picrate by passing a chloroform solution of it over alumina gave the sample for assay, $1{\cdot}94 \times 10^4$ dis./sec./mmole.

2-(3-*Benzyloxy*-4-*methoxybenzyl*)*propan*-2-*ol* (XXXVII).—A solution of methyl 3-benzyloxy-4-methoxyphenylacetate (236 mg.) in ether (10 ml.) was added to methylmagnesium iodide prepared in ether (10 ml.) from magnesium (0·3 g.) and methyl iodide (1·5 g.). The mixture was heated under reflux for 0·5 hr., cooled, and treated with saturated aqueous ammonium chloride (25 ml.) before adjustment to pH 5·7 with acetic acid. Separation of the ether layer and further extraction of the aqueous solution with ether afforded the *alcohol* (XXXVII) which was recrystallised from benzene–light petroleum (b.p. 60—80°), m.p. 80—82° (201 mg.) (Found: C, 75·3; H, 7·6. $C_{18}H_{22}O_3$ requires C, 75·5; H, 7·7%).

Resolution of (±)-OO-*Dibenzyl*[3-^{14}C,9-^{3}H]*reticuline*.—A mixture of (±)-*OO*-dibenzyl[3-^{14}C]reticuline (131 mg.; 0·28 mc) and (±)-*OO*-dibenzyl[9-^{3}H]reticuline (110 mg.; 3·27 mc) in ethyl acetate (10 ml.) was treated with (−)-*OO*-dibenzoyltartaric acid (187 mg.) and warmed until dissolution was complete. The salt which separated on cooling the solution was recrystallised four times from ethyl acetate (8 ml. portions) to give (+)-*OO*-dibenzyl[3-^{14}C,9-^{3}H]-reticuline (−)-*OO*-dibenzoyltartrate, m.p. 130—132°. The base recovered from the first mother liquor in the resolution was treated with (+)-*OO*-dibenzoyltartartic acid (130 mg.) to give after three recrystallisations (−)-*OO*-dibenzyl-[3-^{14}C,9-^{3}H]-reticuline (+)-*OO*-dibenzoyltartrate, m.p. 130—131°. The bases recovered from the salts showed, respectively, m.p. 90—92°, (104 mg.), $[\alpha]_D$ +43·4° (*c*, 1·0 in $CHCl_3$) and m.p. 90—92°, (55 mg.), $[\alpha]_D$ −42·6° (*c*, 1·0 in $CHCl_3$). These were debenzylated with hydrochloric acid as usual to give (+)-[3-^{14}C,9-^{3}H]reticuline hydrochloride (70 mg.) and the (−)-enantiomer (43 mg.) for Expts. 9 and 10.

A small samples (*ca.* 1 mg.) of the (+)-[3-^{14}C,9-^{3}H]-reticuline hydrochloride in methanol (1 ml.) was treated with a vast excess of ethereal diazomethane (*ca.* 5% solution). After 3 days, radioinactive (±)-laudanosine (400 mg.) was added and the total base was chromatographed on alumina (activity I) in benzene–chloroform (4 : 1). The pure laudanosine fractions were combined and the base (330 mg.) in ethyl acetate (20 ml.) was treated with (−)-*OO*-di-*p*-toluoyltartaric acid (350 mg.). After warming to complete the dissolution, the mixture was cooled and the crystals were recrystallised from ethyl acetate–acetone to constant specific rotation and specific activity yielding (+)-*laudanosine* (−)-OO-*di*-p-*toluoyltartrate* (227 mg.), m.p. 117—118°, $[\alpha]_D^{27}$ −26·3° (in methanol) (Found: C, 64·9; H, 6·3; N, 1·8. $C_{41}H_{45}N_{12},H_2O$ requires C, 64·7; H, 6·2; N, 1·8%). The base (153 mg.) recovered from the mother liquors of the resolution was treated as before with (+)-*OO*-di-*p*-toluoyltartaric acid (155 mg.) in ethyl acetate (10 ml.) and acetone (2·5 ml.) and the salt was recrystallised as before to give (−)-*laudanosine* (+)-OO-*di*-p-*toluoyltartrate* (92 mg.), m.p. 117—118°, $[\alpha]_D^{27}$ +26·8° (in methanol) (Found: C, 64·5; H, 6·3; N, 1·7%).

(+)-, and (−)-Laudanosine were recovered from these salts and showed m.p. 109°, $[\alpha]_D$ +56·8° and −56·8° ± 2° (in $CHCl_3$).

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