Studies of Enzyme-mediated Reactions. Part V.[1] Synthesis of (13*S*)- and (13*R*)-[13-$^3H_1$]Scoulerine from Stereospecifically Labelled (*R*)- and (*S*)-[α-$^3H_1$]Benzyl Alcohols; Stereochemistry of Enzymic Reactions at Saturated Benzylic Carbon

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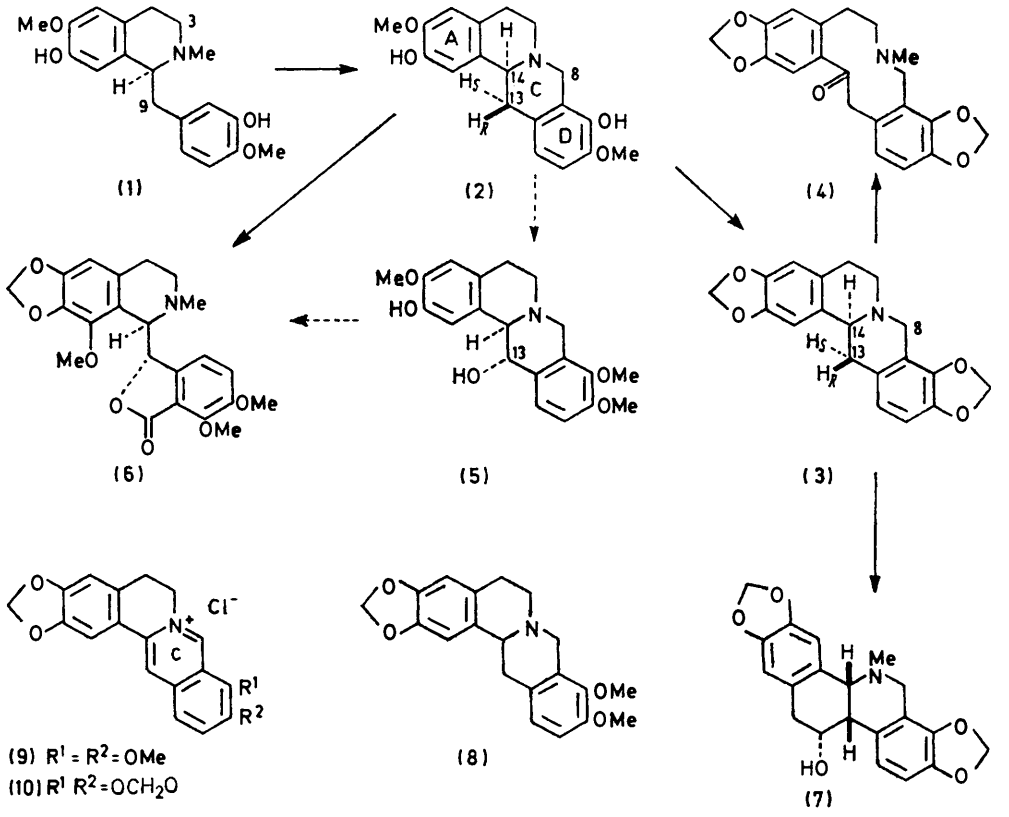
A synthesis of (14*RS*)-scoulerine [as (2)] has been developed which allows stereospecific labelling with deuterium or tritium at C-13; (14*RS*)-coreximine (45) is a valuable second product from the synthesis. The configurational purity of the labelled scoulerine was determined by degradation of a suitable intermediate, in the ^2H series to monodeuteriosuccinic acid, and in the ^3H-series to [^3H]malic acid for configurational assay by fumarase.

Incorporation experiments with (13*R*)- and (13*S*)-[13-$^3H_1$]scoulerine have proved that the biosynthesis of both narcotine (6) and chelidonine (7) involves stereospecific removal of the *pro-S* hydrogen atom from the saturated benzylic carbon, C-13 of scoulerine (2). The mechanistic implications of these findings are considered.

EXPERIMENTS with randomly labelled (+)-(1*S*)-[3-^{14}C,-9-$^3H_1$] reticuline (1) in the opium poppy, *Papaver somniferum*, showed that its incorporation into narcotine (6) occurred with retention of 50% of the tritium † relative to the ^{14}C internal standard.[2] This result is indicative of a stereospecific biochemical reaction and would agree, for example, with the formation of a 13-hydroxytetrahydroprotoberberine [*e.g.* (5)] as an intermediate. In contrast, the same precursor (1) was converted in *Chelidonium majus* plants into chelidonine (7) having a ^3H : ^{14}C ratio equivalent to 82% of that in the precursor.[3] Two explanations of the latter result are possible:



the removal of hydrogen from C-13 of the intermediate stylopine[4] (3), which corresponds to C-9 of reticuline, could be either (*a*) a non-stereospecific process or (*b*) a

† To avoid repetition, the phrase ' within experimental error ' has been omitted in all cases.

[1] Part IV, A. R. Battersby, J. Staunton, and H. R. Wiltshire, preceding paper.

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

[3] A. R. Battersby, R. J. Francis, R. Southgate, J. Staunton, and H. R. Wiltshire, *J.C.S. Perkin I*, 1975, 1147.

[4] A. R. Battersby, R. J. Francis, M. Hirst, E. A. Ruveda, and J. Staunton, *J.C.S. Perkin I*, 1975, 1140.

stereospecific one with 50% retention of ^3H in that step but obscured by a general rise in the ^3H : ^{14}C ratio (for a fuller discussion of these possibilities, see ref. 3). It was clear that syntheses of scoulerine (2) stereospecifically labelled at C-13 would allow elimination of possibility (*a*) or (*b*). Importantly, this approach would also discover the stereochemistry of the enzymic reaction at the benzylic C-13 of the tetrahydroprotoberberine skeleton as narcotine is biosynthesised and similarly for chelidonine if explanation (*b*) were the true one.

The preceding paper described preparative routes to the substituted (*R*)- and (*S*)-[α-$^3H_1$]benzyl alcohols (16) and (18) and the corresponding chlorides (17) and (19). A scheme for the conversion of these building blocks into scoulerine (2) was therefore needed which would preserve configurational purity to as great an extent as possible. Earlier experience [2] indicated that the normal route [5] to scoulerine might run into difficulties, and this occurred at the outset. Treatment of the dideuterio-chloride (15; >99% $^2H_2$) with potassium cyanide in dimethyl sulphoxide or dimethylformamide gave the corresponding nitrile which contained *ca.* 53% $^2H_0$, 38% $^2H_1$, and 9% $^2H_2$. The deuterium content did not fall further on treatment of this product with either solvent, but more loss occurred when potassium cyanide was added.

We therefore sought a route which avoids placing an electron sink adjacent to the labelled chiral centre at any stage in the synthesis. One possibility was suggested by the work of Dr. F. R. Atherton (Roche Products Limited) on the generation and use of anions from imines of the type PhCH=N–CH_2Ar; we are indebted to him for information in advance of publication. The symmetrical aza-allyl carbanion (12) was generated from the imine (10) with sodium hydride in dimethylformamide and it reacted smoothly with the chloride (14) to yield, after hydrolysis of the intermediate (20), the known amine [6] (24). The same sequence was then used with the anion (13) and the dideuterio-chloride (15) to give the imine (21), which yielded the base (25) and, importantly, there was no loss of label; this augured well for work with chiral systems. It should be noticed that the label is now at a stable location in structure (25).

Two further rings had to be built and the first step was condensation of the bases (24) and (26) with 2,2-diethoxyacetaldehyde; reduction of the resultant imine with borohydride yielded the acetals (29) and (30). Simple acetals of this type have been converted into 1,2,3,4-tetrahydroisoquinolines by treatment with acid followed by hydrogenolysis of the 4-hydroxy-derivatives first formed.[7] Attempts to achieve this conversion by treating the acetal (29) with acid under a variety of conditions gave three products. These were shown to be isopavine (48), the major product, by direct comparison [8] and the two isomeric 4-hydroxytetrahydroisoquinolines (49), mainly by mass spectrometry and n.m.r.

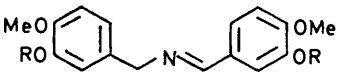
The formation of the isopavine system was avoided in in the main synthesis by constructing ring C of scoulerine (2) before ring B. Debenzylation of the amino-acetal (30) led to the diphenol (31), which reacted with formaldehyde to yield the isomeric products (34) and (37). These were difficult to separate and so they were carried together through the second, acid-catalysed ring closure. Hydrogenolysis of the resultant mixture of alcohols (40) and (44) then afforded (*RS*)-scoulerine (41) and (*RS*)-coreximine (45), which are easily separable.[5]

The (*R*)- and (*S*)-[α-$^3H_1$]benzyl chlorides (17) and (19) described in the preceding paper had configurational purity amply high enough for our purpose and the way was clear for the synthesis of (13*S*)- and (13*R*)-[13-$^3H_1$]-scoulerines (42) and (43). However, a determination was necessary of the configurational purity of some product after the carbon–carbon bond-forming step, *e.g.* (17) ⟶ (22), and the deuterio-amine (50) (Scheme) was chosen for the first degradation. This amine was prepared from the deuterio-alcohol (18; D in place of T) by the sequence already described, with thionyl chloride in ether being used for the preparation of the benzyl chloride. Reduction of the methochloride of part of the amine (50) with sodium amalgam gave the diarylethane (51) which was catalytically debenzylated. The resultant phenol (52) was identified by comparison with a sample synthesised by standard methods (see Experimental section) and it was then converted by ozone and peroxy-acid into monodeuteriosuccinic acid, shown to contain 68 ± 3% of (+)-(2*S*)-[2-$^2H_1$]succinic acid (53) by mass spectrometric and o.r.d. measurements. The remainder of the amine (50) was converted into (14*RS*)-scoulerine [as (2)] containing 68 ± 3% of the (13*R*)-[13-$^2H_1$]enantiomer [as (43) in ^3H series]. Importantly, there was no loss of deuterium in the final hydrogenolysis of 5-hydroxyscoulerine [as (40) with D at R^2] so fears of scrambling or loss of label in this catalytic step were allayed.

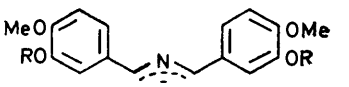
The synthetic sequence of the foregoing paragraph was precisely repeated in the ^3H series (19) ⟶ (23) ⟶ (28) to yield the (1*RS*,2*R*)-[2-$^3H_1$]amine (28), and this was converted *via* (33) ⟶ (36) ⟶ (43) by the route already described into a product containing 68 ± 3% of (13*R*,14*RS*)-[13-$^3H_1$]scoulerine (43) and 32 ± 3% of (13*S*,14*RS*)-[13-$^3H_1$]scoulerine (42). It was mixed with (14*RS*)-[8-^{14}C]scoulerine [3] as internal standard and then administered to *Chelidonium majus* and to *Papaver somniferum* plants.* Experiments A and B in Table 1 gave the first indications that a stereospecific process affecting the 13-*pro-S* hydrogen atom of the tetrahydroprotoberberine system [as (2)] is operating in the biosynthesis of narcotine (6). The ^3H retention found for chelidonine left some ambiguity and it was clear that the synthesis of both (13*R*)- and (13*S*)-[13-$^3H_1$]scoulerines

* Earlier work has shown [2,3] that (14*R*)-scoulerine [enantiomer of (2)] is essentially inert in the biosynthetic pathways of interest and so use of (14*RS*)-material is equivalent to pure (14*S*)-precursor.

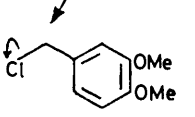
[5] A. R. Battersby, R. Southgate, J. Staunton, and M. Hirst, *J. Chem. Soc.* (*C*), 1966, 1052.
[6] A. R. Battersby and R. Binks, *J. Chem. Soc.*, 1958, 4333.
[7] J. M. Bobbitt, J. McN. Kiely, K. L. Khanna, and R. Ebermann, *J. Org. Chem.*, 1965, **30**, 2247.
[8] A. R. Battersby and D. A. Yeowell, *J. Chem. Soc.*, 1958, 1988.



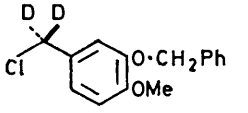
(10) R = Me
(11) R = CH_2Ph



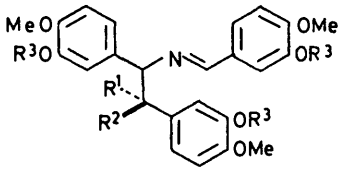
(12) R = Me
(13) R = CH_2Ph



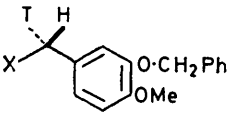
(14)



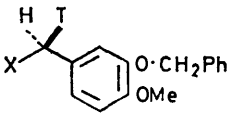
(15)



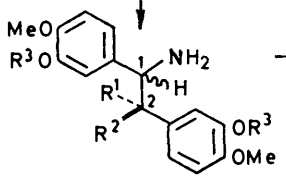
(20) $R^1 = R^2 = H, R^3 = Me$
(21) $R^1 = R^2 = D, R^3 = CH_2Ph$
(22) $R^1 = T, R^2 = H, R^3 = CH_2Ph$
(23) $R^1 = H, R^2 = T, R^3 = CH_2Ph$



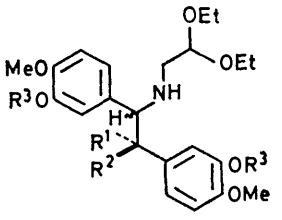
(16) X = OH
(17) X = Cl



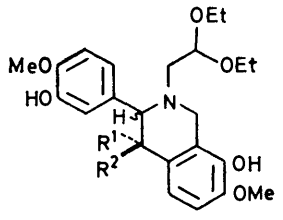
(18) X = OH
(19) X = Cl



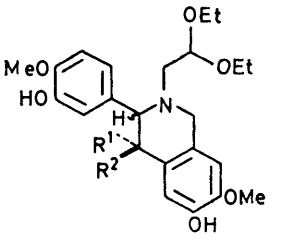
(24) $R^1 = R^2 = H, R^3 = Me$
(25) $R^1 = R^2 = D, R^3 = CH_2Ph$
(26) $R^1 = R^2 = H, R^3 = CH_2Ph$
(27) $R^1 = T, R^2 = H, R^3 = CH_2Ph$
(28) $R^1 = H, R^2 = T, R^3 = CH_2Ph$



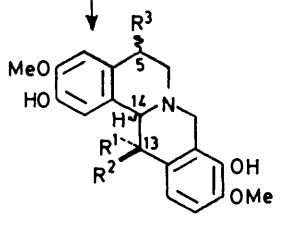
(29) $R^1 = R^2 = H, R^3 = Me$
(30) $R^1 = R^2 = H, R^3 = CH_2Ph$
(31) $R^1 = R^2 = R^3 = H$
(32) $R^1 = T, R^2 = H, R^3 = CH_2Ph$
(33) $R^1 = H, R^2 = T, R^3 = CH_2Ph$



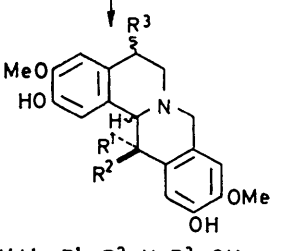
(34) $R^1 = R^2 = H$
(35) $R^1 = T, R^2 = H$
(36) $R^1 = H, R^2 = T$



(37) $R^1 = R^2 = H$
(38) $R^1 = T, R^2 = H$
(39) $R^1 = H, R^2 = T$

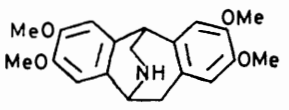


(40) $R^1 = R^2 = H, R^3 = OH$
(41) $R^1 = R^2 = R^3 = H$
(42) $R^1 = T, R^2 = R^3 = H$
(43) $R^1 = R^3 = H, R^2 = T$



(44) $R^1 = R^2 = H, R^3 = OH$
(45) $R^1 = R^2 = R^3 = H$
(46) $R^1 = T, R^2 = R^3 = H$
(47) $R^1 = R^3 = H, R^2 = T$

(2) was essential. This is a good point to emphasise strongly the importance for stereochemical work of



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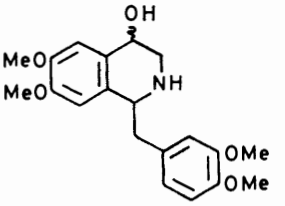
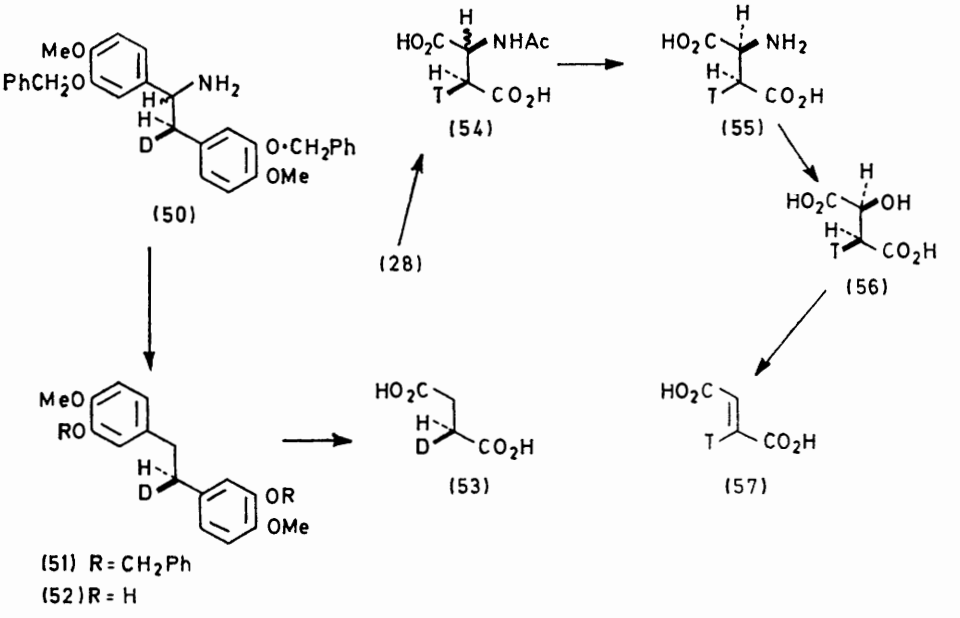


(49)

preparing *both enantiomers* by the same synthetic route from mirror-image starting materials, here the benzyl acid.[9] Therefore nitrous acid was generated separately and was blown with nitrogen into aspartic acid in water to yield virtually pure (2*S*)-[3-$^3H_1$]malic acid (56). Fumarase (malate hydro-lyase, E.C. 4.2.1.2) converted this product into fumaric acid, which was purified and counted as earlier.[9] Fumarase is at least 99·99% 3-*pro-R* specific,[10] and so the ^3H retention in the fumaric acid measures the content of (2*R*)-[2-$^3H_1$]species (28) in the original amine.

Early use of this degradative scheme was made for four samples of amine [as (27) and (28)] derived from (*S*)-[α-$^3H_1$]benzyl alcohol (18) by four different ways of generating the benzyl chloride. The results (Table 2) show the highest configurational purity for the sequence involving thionyl chloride in dioxan in agreement with a



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alcohols. This approach has the great strength of giving interlocking results from experiments with precursors labelled in a complementary way.

For the studies below we wished to work entirely in the ^3H series and here the foregoing degradation to succinic acid for determination of configuration cannot be used since the label is at tracer level. Accordingly, the amine (28) was *N*-acetylated and the amide was degraded with ozone and peroxy-acid without loss of tritium to (2*RS*)-*N*-acetylaspartic acid (54). This with hog renal acylase yielded mainly (2*S*)-aspartic acid (55) which after dilution with radioinactive (2*S*)-aspartic acid and addition of ^{14}C-generally labelled (2*S*)-aspartic acid was purified as described previously.[9] Conversion of aspartic acid into malic acid with sodium nitrite and hydrochloric acid gave varying amounts of chlorosuccinic different analytical sequence,[1] and almost complete racemisation under the last two conditions given in the Table. (*R*)- and (*S*)-[α-$^3H_1$]Benzyl alcohols (16) and (18) of high specific activity were then converted *via* (22) and (23) into the amines (27) and (28) with thionyl chloride and dioxan for the chloride preparation; the anion (13) was generated more conveniently with lithium di-isopropylamide in tetrahydrofuran. The results from the degradative studies on these amines are collected in Table 3. The main facts are clear that the amines have satisfactory configurational purities of the expected chirality. Interestingly, the sample which is mainly (1*RS*,2*R*)-[2-$^3H_1$]amine (28) was of rather lower configurational purity than the product containing mainly its enantiomer. Yet all the chemical conversions were carried out under identical conditions. One possibility

[9] P. G. Strange, J. Staunton, H. R. Wiltshire, A. R. Battersby, K. R. Hanson, and E. A. Havir, *J.C.S. Perkin I*, 1972, 2364.

[10] Reviewed by R. Bentley, 'Molecular Asymmetry in Biology,' Academic Press, New York, 1970, vol. II, p. 127.

is that liver alcohol dehydrogenase is not quantitatively stereospecific with these substances. Fuller discussion of this aspect is given in the preceding paper and it suffices to say here that by using a shorter period for enzymic reduction of the tritiated benzaldehyde, (*S*)-[α-$^3H_1$]benzyl alcohol of higher configurational purity was obtained from which the amine (28) was built as before. The composition of the amine so formed can be calculated from the data here and in ref. 1 to be 73 ± 3% of the (1*RS*,2*R*)-[2-$^3H_1$]amine (28) and 27 ± 3% of the (1*RS*,2*S*)-isomer (27)

Table 3 also shows that the malic acid from the (1*RS*,-2*S*)-amine (27) is of slightly lower ^3H : ^{14}C ratio than the aspartic acid from which it was derived. Release of tritium into the medium in the nitrous acid step has already been shown to be negligible.[9] One possible explanation was that some (2*R*)-aspartic acid was present in the supposed pure (2*S*)-material despite the extensive purification we had used. Any (2*R*)-aspartic acid would carry tritium but not ^{14}C since the added internal ^{14}C standard was (2*S*)-aspartic acid. However, dilution analysis using a large excess of unlabelled (2*R*)-aspartic acid showed that <1·2% of labelled (2*R*)-aspartic acid was present in the same sample which had been converted into (2*S*)-malic acid. We cannot at present offer an explanation of this small difference but we believe it to be real.

The two samples of labelled amine synthesised above, one largely (27) and one largely (28), were converted by the route already outlined into a sample containing 74 ± 3% (13*S*,14*RS*)-[13-$^3H_1$]scoulerine (42) and a second sample containing 73 ± 3% (13*R*,14*RS*)-[13-$^3H_1$]scoulerine (43). Each one was mixed with (14*RS*)-[8-^{14}C]scoulerine[3] as an internal standard and a small part was converted into (14*RS*)-tetrahydropalmatine {as [7; $(MeO)_2$ for CH_2O_2]} by *O*-methylation. These products, after dilution, were purified to constant ^3H : ^{14}C ratio. The rest of the labelled scoulerines were administered to *C. majus* plants[4] and to *P. somniferum* plants;[2,3] the alkaloids were isolated and separated as previously described.[2-4] Looking first at *P. somniferum*, Table 1 shows the conversion of both labelled scoulerines into the tetrahydroprotoberberine, canadine (8), with complete retention of tritium, as expected; canadine thus acts as an internal 'base-line' and the case below illustrates the value of such a substance. The ^3H retention values for incorporation of the two labelled precursors into narcotine (6) clearly show that its biosynthesis involves removal of the *pro-S* hydrogen atom from C-13 of the tetrahydroberberine skeleton. Comparing structures (2) and (6) it can be seen that hydrogen at the benzylic centre of scoulerine (C-13) is replaced by oxygen with overall retention of configuration. The few other cases which have been studied of introduction of oxygen at a saturated benzylic carbon also occur in this sense; the examples are dopamine,[11a] amphetamine,[11b] ethylbenzene,[11c] and *p*-hydroxyphenylacetonitrile.[11d]

TABLE 1

Tracer experiments in *Papaver somniferum* (P) and *Chelidonium majus* (C)

Alkaloid or derivative	(13*S*, 14*RS*)-[13-$^3H_1$, 8-^{14}C]Scoulerine [a] (42) ^3H : ^{14}C Ratio 11·8 : 1				(13*R*, 14*RS*)-[13-$^3H_1$, 8-^{14}C]Scoulerine [b] (43) ^3H : ^{14}C Ratio 1·81 : 1				
	% Incorpn. and plant [c]	^3H : ^{14}C Ratio	Retention [d] ^3H (%)	Expected % retention ^3H	Incorpn. and plant	^3H : ^{14}C Ratio	Retention [d] ^3H (%)	Adjusted [e] % retention ^3H	Expected % retention ^3H
Canadine (8)	0·05, P	11·9 : 1	101	100	0·04, P	1·83 : 1	101		100
Narcotine (6) Expt. A [f]					0·6, P	3·4 : 1	75		68 ± 3
Narcotine (6)	1·4, P	2·41 : 1	20	26 + 3	1·1, P	1·43 : 1	79		73 ± 3
Phenylnarcotine [3]		2·71 : 1	23	26 ± 3		1·53 : 1	84		73 ± 3
Berberine (9)	0·01, C	1·6 : 1	14						
Coptisine (10)	0·03, C	1·6 : 1	14		0·04, C	0·33 : 1	18	16	
Protopine (4)	0·3, C	3·2 : 1	26		0·8, C	0·2 : 1	11	10	
Stylopine (3)	0·3, C	11·8 : 1	100	100	0·3, C	2·0 : 1	110	100	100
Chelidonine (7) Expt. B [f]					0·32, C	3·9 : 1	86		68 ± 3
Chelidonine (7)	0·6, C	3·2 : 1	27	26 ± 3	0·6, C	1·5 : 1	82	75	73 ± 3
O-Acetylchelidonine		3·4 : 1	29	26 ± 3		1·5 : 1	82	75	73 ± 3

[a] Contains 74 ± 3% of (13*S*)-isomer and 26 ± 3% of (13*R*)-isomer. [b] Contains 73 ± 3% of (13*R*)-isomer and 27 ± 3% of (13*S*)-isomer. [c] See Table heading. [d] Experimental error in determination of ^3H : ^{14}C ratio is in range 2—5%; the low end of this range holds for cases of good incorporation yielding highly active materials. [e] Adjusted by taking the value for stylopine as a baseline of 100%. [f] The precursor contains 68 ± 3% of (13*R*)-isomer and 32 ± 3% of (13*S*)-isomer; its ^3H : ^{14}C ratio is 4·5 : 1.

TABLE 2

Degradation of 1,2-diaryl[2-$^3H_1$]ethylamines

Method for preparation of chloride [as (19)] from alcohol (18)	% Retention of ^3H in fumaric acid	Chirality at C-2 of original amine	
		% *R*	% *S*
Thionyl chloride–dioxan	73	73 ± 3	27 ± 3
Thionyl chloride–benzene	60	60 ± 2	40 ± 2
Thionyl chloride–ether–pyridine (1 mol. equiv.)	45	45 ± 2	55 ± 2
Triphenylphosphine–carbon tetrachloride	41	41 ± 2	59 ± 2

[11] (*a*) A. R. Battersby, P. W. Sheldrake, J. Staunton, and D. C. Williams, *J.C.S. Chem. Comm.*, 1974, 566; (*b*) K. B. Taylor, *J. Biol. Chem.*, 1974, **249**, 454; (*c*) R. E. McMahon, H. R. Sullivan, J. C. Craig, and W. E. Pereira, jun., *Arch. Biochem. Biophys.*, 1969, **132**, 574; (*d*) M. A. Rosen, K. J. F. Farnden, E. E. Conn, and K. R. Hanson, personal communication.

The work in *C. majus* (Table 1) gave values for protopine (4), berberine (9), and coptisine (10) which are in keeping with an extensive and non-stereospecific loss of hydrogen from C-13 of the tetrahydroprotoberberine skeleton. In the case of protopine, a control experiment showed that no label was lost during work-up and so the leaching of tritium presumably occurs in the plant.

Far more significant results were gained for chelidonine (7); see Table 1. The values obtained for the precursor (42) which mainly has the (13*S*)-configuration show that, as with narcotine, it is the *pro-S* hydrogen

TABLE 3

Degradation of 1,2-diaryl[2-$^3H_1$]ethylamines of high specific activity

	(2*S*)-Aspartic acid ^3H : ^{14}C ratio	(2*S*)-Malic acid [a] ^3H : ^{14}C ratio	Fumaric acid ^3H : ^{14}C ratio	Recovered (2*S*)-malic acid [a] ^3H : ^{14}C ratio	Retention ^3H (%)
(1*RS*,2*S*)Amine (27)	(6·6 ± 0·2) : 1 (6·7 ± 0·2) : 1 [b] (6·7 ± 0·2) : 1 [c]	(6·0 ± 0·2) : 1	(1·6 ± 0·1) : 1	(2·1 ± 0·1) : 1	26 ± 3
(1*RS*,2*R*)Amine (28)	(5·7 ± 0·2) : 1 (5·7 ± 0·2) : 1 [d]	(5·5 ± 0·2) : 1	(3·55 ± 0·15) : 1 (3·65 ± 0·15) : 1 [e]	(4·0 ± 0·15) : 1	65 ± 3

[a] As diphenacyl ester. [b] As dimethyl ester of α-naphthylurea. [c] As α-naphthylurea. [d] As diethyl ester of α-naphthylurea. [e] As bis-*p*-nitrobenzyl ester.

atom which is removed. The experiment with the [^{3}H]-scoulerine (43) having largely the (13*R*)-configuration is interesting in that a 10% rise in the ^3H : ^{14}C ratio has occurred through to stylopine (3). We regard this as a reflection of material being drawn off along other pathways by process(es) which involve attack at the *pro-R* hydrogen atom on C-13 of scoulerine (43). The apparent retention of 110% of the tritium in stylopine can be taken as 'base-line' and this has been used for the adjusted values also given in Table 1 for chelidonine and its derivative.

These interlocking results which are within experimental error of the expected values firmly establish a stereospecific removal of the *pro-S* hydrogen atom from C-13 of (−)-stylopine (3) generated [4] from (−)-scoulerine (2) in the biosynthesis of chelidonine (7). The evidence is in favour of a highly specific enzymic reaction. If direct removal of the two adjacent hydrogens at C-13 and C-14 occurs from (−)-stylopine* (3) to set up an enamine,[12] then it is a *cis*-dehydrogenation. Alternatively, an enamine system could be generated by hydroxylation with retention of configuration at C-13 of (−)-stylopine * (3) followed by elimination of water in an overall *cis*-manner. Finally, the stereochemical research reported here serves as a precise yardstick against which any mechanistic proposals must be measured.

EXPERIMENTAL

See ref. 4 for general directions.

3-Benzyloxy-4-methoxy[α-$^2H_2$]*benzyl Alcohol and Its Reactions*.—A solution of methyl 3-benzyloxy-4-methoxybenzoate (1·09 g) in anhydrous dioxan was heated at 70—80° C with lithium aluminium deuteride (166 mg) for 3 h. Water was then added, the suspension was evaporated to dryness, and the residue was shaken with chloroform and water. The organic layer gave the deuterio-alcohol (0·9 g), m.p. 71° [from light petroleum (b.p. 40—60°)], identified by comparison with protio-material; no signal at τ 5·53 shown by $ArCH_2OH$; *m/e* 244 (essentially 100% $^2H_2$).

This (612 mg) was treated in anhydrous ether (20 ml) with thionyl chloride (330 mg) at 20° for 2·5 h; the solvent was evaporated off and the resultant chloride crystallised from light petroleum (b.p. 40—60°); no signal at τ 5·54 shown by $ArCH_2Cl$; *m/e* 264/266 (3 : 1, M^+, essentially 100% $^2H_2$). This (132 mg) was stirred in dimethylformamide (15 ml) with potassium cyanide (56 mg) for 20 h, then treated with water (15 ml) and extracted with 1 : 1 benzene–ether to yield the arylacetonitrile, m.p. 79·5—80·5° [from light petroleum (b.p. 60—80°)]; results of mass spectrometric analysis are in the main text.

1,2-Bis-(3,4-Dimethoxyphenyl)ethylamine (24).—3,4-Dimethoxybenzylamine hydrochloride, m.p. 256°, was prepared by standard reduction of 3,4-dimethoxybenzamide with lithium aluminium hydride in tetrahydrofuran. The corresponding base was heated under partial reflux for 3 h in benzene with an equimolar quantity of 3,4-dimethoxybenzaldehyde and the solvent was evaporated off to leave the imine (10), sufficiently pure for the next step, $\nu_{max.}$ 1640 cm^{-1}.

Dimethylformamide was kept for 7 days over barium oxide and then distilled at 15 mmHg from a large excess of sodium hydride onto more sodium hydride, and the distillation was repeated. The final product was sealed in glass ampoules and opened freshly for the next stage.

A solution of the imine (10) (1·6 g) in dimethylformamide was added to a stirred slurry of sodium hydride (470 mg of 50% oil suspension) in dimethylformamide under dry oxygen-free nitrogen. After 2 h, a red solution had formed, and 3,4-dimethoxybenzyl chloride (1·1 equiv.) was added in dimethylformamide. After 16 h, the solvent was evaporated off, ether (10 ml) and 2N-hydrochloric acid (10 ml) were stirred for 12 h with the residue, and the aqueous phase was basified. Extraction with chloroform gave a basic fraction

* It is possible that (−)-stylopine has undergone some biological modification before the dehydrogenation or hydroxylation occurs; see ref. 3 for fuller discussion.

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

(1·98 g) which was fractionated on alumina and then by p.l.c. on silica to give the bisarylethylamine, m.p. of hydrochloride 208° (lit.,[6] 209°), identified by comparison with an authentic sample;[6] the base showed τ 3·0—3·5 (6H, m, aryl H), 6·18 (12H, 4 OMe), 6·3 (1H, m, CH–N), and 7·0—7·5 (4H, m, $ArCH_2$ and NH_2).

3-Benzyloxy-4-methoxybenzyl Azide.—3-Benzyloxy-4-methoxybenzyl chloride[13] (12 g) in acetone (250 ml) was heated at 50° with a solution of sodium azide (3·6 g) in water for 4 h. The acetone was evaporated off and the *azide* was extracted with methylene chloride and crystallised from hexane; m.p. 49—50° (11·4 g) (Found: C, 66·7; H, 5·8; N, 15·5. $C_{15}H_{15}N_3O_2$ requires C, 66·9; H, 5·6; N, 15·6%); $\nu_{max.}$ 2050 cm^{-1}; $\lambda_{max.}$ 282 and 232 nm; τ 2·5—2·8 (5H, m, aryl H), 3·14 (3H, s, aryl H), 4·87 (2H, s, $PhCH_2O$), 5·82 (2H, s, CH_2N_3), and 6·17 (3H, s, MeO); *m/e* 269 (M^+, 17%), 241 (3·5), 227 (3), and 91 (100).

3-Benzyloxy-4-methoxybenzylamine Hydrochloride.—The above azide (11·5 g) in anhydrous ether (40 ml) was added dropwise over 15 min to lithium aluminium hydride (2·1 g) in dry ether (80 ml). The mixture was stirred for 16 h and the excess of reductant was destroyed with saturated aqueous Rochelle salt. Benzene was added and the solid was collected and washed with benzene; the combined benzene solution was washed with water and saturated brine before being dried and evaporated. The residue in ethyl acetate was treated with ethereal hydrogen chloride to give the *amine hydrochloride* (10·2 g, 89%), m.p. 237—239° (from methanol–ether) (Found: C, 64·6; H, 6·65; N, 5·0. $C_{15}H_{18}ClNO_2$ requires C, 64·4; H, 6·4; N, 5·0%); $\lambda_{max.}$ 279 and 230 nm (ε 5000 and 12,300); free base τ 2·5—2·8 (5H, m, aryl H), 3·13 and 3·18 (3H, each s, aryl H), 4·9 (2H, s, $PhCH_2O$), 6·2 (3H, s MeO), 6·3 (2H, s, CH_2N), and 8·62 (2H, s, NH_2); *m/e* 243 (M^+, 21%) 152 (9), 108 (9), and 91 (100).

The N-*acetyl derivative* of this amine had m.p. 121° (from aqueous ethanol) (Found: C, 71·3; H, 6·6; N, 4·6. $C_{17}H_{17}NO_3$ requires C, 71·55; H, 6·7; N, 4·9%).

This amine was also made (less conveniently) by reduction with lithium aluminium hydride of *3-benzyloxy-4-methoxybenzaldehyde oxime*, m.p. 92° (from aqueous ethanol) (Found: C, 69·8; H, 5·7; N, 5·3. $C_{15}H_{15}NO_3$ requires C, 70·0; H, 5·9; N, 5·4%).

N-(3-Benzyloxy-4-methoxybenzylidene)-3-benzyloxy-4-methoxybenzylamine (11).—The base recovered from the foregoing hydrochloride (3 g) was heated in dry benzene (100 ml) with 3-benzyloxy-4-methoxybenzaldehyde (2·6 g) for 3 h under partial reflux. The solution was then concentrated to low volume and diluted with hexane to yield the *benzylidene derivative* (4·65 g, 93%), m.p. 122·5° (from benzene–hexane) (Found: C, 77·2; H, 6·2; N, 3·0. $C_{30}H_{29}NO_4$ requires C, 77·1; H, 6·3; N, 3·0%); $\nu_{max.}$ 1640 cm^{-1}; $\lambda_{max.}$ 304, 272, and 227 nm shifting to 349, 312, and 239 nm with acid; τ 1·81 (1H, s, CH=N), 2·45—3·2 (16H, m, aryl H), 4·87 and 4·90 (each 2H, s, $PhCH_2O$), 5·35 (2H, s, CH_2N), and 6·14 and 6·18 (each 3H, s, MeO); *m/e* 467 (M^+, 4%), 377 (4), 227 (13), and 91 (100).

1,2-Bis-(3-Benzyloxy-4-methoxyphenyl)ethylamine (26) *and Labelled Forms* [(25), (27), (28), *and* (50)] *with* ^2H *or* ^3H *at Position* 2.—(*a*) *Hydride method.* The foregoing benzylidene derivative (191 mg) was alkylated with the above 3-benzyloxy-4-methoxy[α-$^2H_2$]benzyl chloride (117 mg) as for the fully methoxylated analogue (24). The [2-$^2H_2$]ethylamine (25) was crystallised as the hydrochloride from methanol–ether (117 mg). N.m.r. showed no signal at τ 7·3 (see below); *m/e* 471 (M^+, 1%), and 242 (100).

(b) *Lithium di-isopropylamide method.* 3-Benzyloxy-4-methoxybenzyl alcohol in unlabelled or labelled form (310 mg) in dry highly purified dioxan (8 ml) was treated with thionyl chloride (0·015 ml), and after 15 min at 20° the solution was evaporated. The residue was transferred in anhydrous ether to a flask fitted with a right-angled male joint, the solvent was evaporated off and the chloride was kept *in vacuo* over phosphoric oxide for 10—16 h before use.

A solution of the benzylidene derivative (11) (680 mg) in dry tetrahydrofuran (25 ml) was held in a two-necked flask. The side neck carried the chloride-containing flask and the main neck was sealed with a serum-stopper. By alternately evacuating the apparatus and filling it with nitrogen, the reaction mixture was put under a slightly negative nitrogen pressure. In parallel, a solution of lithium di-isopropylamide was prepared at −42 °C (solid CO_2–acetonitrile) under nitrogen, by adding butyl-lithium (5 ml; 15% solution in hexane) to dry tetrahydrofuran (3 ml) and di-isopropylamine (2 ml) with a syringe *via* a serum-stopper. After 5 min the isopropylamide solution (1·75 ml) was added to that of the benzylidene derivative to produce a magenta colour. After a further 15 min the solution of aza-allylic carbanion was tipped onto the chloride, which dissolved as the mixture was allowed to warm to 20 °C. The solution was evaporated after 2 h and the residue was stirred for 10 min with benzene (3 ml) and water (1·5 ml) before the addition of 3N-hydrochloric acid (1·5 ml). The mixture was stirred vigorously for 16 h; ether (15 ml) and water (5 ml) were added to give a gummy precipitate which was converted into the free base by partition between aqueous sodium carbonate and ethyl acetate. The concentrated solution in ethyl acetate was warmed with oxalic acid (167 mg) to give the crystalline oxalate (425 mg, 60%); in the radioactive series, the mother liquors were retained for degradation. The corresponding *hydrochloride*, m.p. 200—204°, was identical with that obtained in (a) (Found: C, 71·4; H, 6·3; N, 2·7. $C_{30}H_{32}ClNO_4$ requires C, 71·2; H, 6·4; N, 2·8%); $\lambda_{max.}$ 280 and 230sh nm (ε 6100 and 18,000); τ 2·5—2·8 (1OH, m, aryl H), 3·1—3·2 (6H, aryl H), 4·94 and 5·0 (each 2H, s, $PhCH_2O$), 6·2 (7H, OMe and ArCHN), 7·3 (2H, $ArCH_2$), and 7·9 (2H, NH_2); *m/e* 469 (M^+, 0·4%), 452 (1), 376 (0·5), 242 (100), 200 (3·5), 152 (15), 151 (13), and 91 (58).

The stereospecifically $^3H_1$- and $^2H_1$-labelled amines (27), (28), and (50) were prepared in a strictly analogous way from the appropriate chiral benzyl alcohols[1] (16), (18), and (18; D in place of T).

2,2-Diethoxyacetaldehyde.—A suspension of (*RS*)-glyceraldehyde (4·7 g) in absolute ethanol (100 ml) was mixed with ethanolic 10% hydrogen chloride (4 ml) and stirred at 20 °C until the solid had virtually dissolved (*ca.* 2 h). The solution was neutralised at 0 °C with aqueous ammonia, ether was added to precipitate ammonium chloride, and the filtered solution was evaporated at 15°. Benzene was then added and evaporated off to remove water and the residue in more benzene was filtered and re-evaporated to give an oil homogeneous by g.l.c. (7·8 g, 91%), b.p. 83—86° at 6·5 mmHg; τ 5·5 (1H, d, *J* 6Hz, OCHO), 6·05—6·55 (7H, m, CHOH and $3CH_2$), 7·04 (2H, s, OH), and 8·76 and 8·79 (6H, dt, $2CH_3$).

The foregoing acetal (from 2·67 g of glyceraldehyde) in dry benzene (50 ml) was shaken with lead tetra-acetate (11·7 g)

[13] A. Lovecy, R. Robinson, and S. Sugasawa, *J. Chem. Soc.*, 1930, 817.

for 1 h; the suspension was filtered and the filtrate was shaken with an excess of anhydrous potassium carbonate until effervescence ceased. The filtered solution was evaporated at 15—20 °C and the residue was distilled (40—50° and 14 mmHg) to yield 2,2-*diethoxyacetaldehyde* (1·64 g, 42% overall) containing *ca.* 4% of acetic acid by g.l.c. (Found: *m/e*, 103·0760. $C_5H_{11}O_2$ requires 103·0759); $\nu_{max.}$ 1740 cm^{-1}; $\lambda_{max.}$ (hexane) 313 nm; τ 0·7 (1H, d, *J* 3 Hz, CHO), 5·62 (1H, d, *J* 3 Hz, OCHO), 6·15—6·6 (4H, dq, OCH_2), and 8·8 (6H, t, CH_3); *m/e* (no molecular ion) 104 (6%), 103 (100), 87 (24), 75 (68), and 59 (32), *m** 54·6 (103 ⟶ 75).

N-(2,2-*Diethoxyethyl*)-1,2-*bis*-(3-*benzyloxy*-4-*methoxyphenyl*)*ethylamine* (30) *and Labelled Forms* [(32) *and* (33)] *with Tritium at Position* 2.—The base (26) recovered as usual from its oxalate (477 mg) was heated with freshly prepared 2,2-diethoxyacetaldehyde (432 mg) for 5 min at 100° and the resultant oil was kept for 16 h over phosphoric oxide at 50 mmHg before being evacuated (14 mmHg) at 50—65° until of constant weight (2 h). The final residue was dissolved at 50° in methanol (6 ml) and potassium borohydride (80 mg) was added. After 2 h, the solution was added to water (10 ml) and extraction with ethyl acetate gave a gum which was chromatographed on four silica plates, developed with ether. The required zone (R_F 0·5) was scraped off and eluted with methanol; the solution was evaporated and the residue dissolved in methylene chloride. This solution was filtered and re-evaporated to give the *acetal* as a homogeneous oil (322 mg) [Found: *m/e*, 494·2331. $C_{32}H_{32}NO_4$ ($M^+ - 91$) requires 494·2301]; τ 2·5—2·8 and 3·0—3·5 (16H, m, aryl H), 4·9 and 5·1 (each 2H, s, CH_2Ph), 5·6 (1H, t, *J* 6 Hz, CHO_2), 6·15 and 6·2 (each 3H, s, MeO), 6·4 (5H, m, CHN, CH_2O), 7·25 (2H, d, *J* 6 Hz, CH_2Ar), 7·55 (2H, d, *J* 6 Hz, CH_2N), 8·4 (1H, NH), and 8·9 (3H, t, *J* 6 Hz, CH_3); *m/e* 586 (0·3%), 585 (M^+, 0·2), 584 (0·5), 540 (1·6), 494 (1·5), 453 (8·5), 359 (35), 358 (55), 313 (13), 312 (58), 268 (9), 227 (8), and 91 (100), *m** 272.

N-(2,2-Diethoxyethyl)-1,2-bis-(3,4-dimethoxyphenyl)-ethylamine [8] (29) was prepared similarly.

Study of Ring Closure of the Acetal (29).—The following describes one of the many sets of conditions studied. A solution of the acetal (29) (110 mg) in 6N-hydrochloric acid (2 ml) was kept for 1 day at 18—20 °C and was then shaken with hydrogen and palladised charcoal (20 mg) for 6 h (uptake complete). The filtered solution was basified and the product, extracted with chloroform, was separated into its three components by p.l.c. The main product was isopavine,[8] identified by full spectroscopic and chromatographic comparison, and the other two showed *m/e* 208, 190, and 151 in agreement with their being isomeric 4-hydroxytetrahydropapaverines.

2-(3-*Benzyloxy*-4-*methoxyphenyl*)-1,3-*dithian and its* 2-[2-^{2}H]*Derivative*.—A solution of 3-benzyloxy-4-methoxybenzaldehyde (5 g) and propane-1,3-dithiol (2 ml) in chloroform (100 ml) was saturated with dry hydrogen chloride. The solid which formed was dissolved by adding more chloroform (100 ml) and the solution was then washed with ice–water (2 × 100 ml), N-sodium hydroxide (2 × 100 ml), and finally water. The *dithian* from the chloroform was crystallised from methanol–chloroform (5·84 g); m.p. 168° (Found: C, 64·8; H, 6·0; S, 19·4. $C_{18}H_{20}O_2S_2$ requires C, 65·1; H, 6·1; S, 19·3%); $\lambda_{max.}$ 280 and 233 nm; τ 2·5—3·3 (8H, m, aryl H), 4·95 (2H, s, CH_2O), 4·99 (1H, s, CH), 6·14 (3H, s, MeO), 7·16 (4H, m, CH_2S), and 8·0 (2H, m, CH_2); τ (C_6D_6) 2·6—3·0 (7H, m, aryl H), 3·5 (1H, d, aryl H), 5·03 (1H, s, CH), 5·29 (2H, s, CH_2O), 6·69 (3H, s, MeO), 7·6 (4H, m, CH_2S), and 8·5 (2H, m, CH_2); *m/e* 332 (M^+, 27%), 258 (4), 241 (6), and 91 (100).

The dithian (2·27 g) in purified dry dimethylformamide (as above for alkylation reaction) was stirred at 60° with sodium hydride (2 mol. equiv.) under oxygen-free nitrogen for 4 h. Deuterium oxide (1 ml) was then added, and after the solution had been stirred for 20 h it was diluted with water (500 ml). The [2-^{2}H]*dithian* was extracted with chloroform and crystallised as above (2·1 g), m.p. 168°. The percentage deuteriation as determined by mass spectrometry was 89%.

3-*Benzyloxy*-4-*methoxybenz*[^{2}H]*aldehyde*.—The [2-^{2}H]dithian (2·19 g) in 1 : 9 water–methanol (150 ml) was heated under reflux for 4·5 h with mercury(II) chloride (3·8 g) and mercury(II) oxide (1·54 g). The filtered solution was evaporated and the residue was partitioned between chloroform and water, the organic layer being washed twice with a saturated solution of ammonium acetate and then with water. Evaporation, and recrystallisation of the residue from hexane gave the [^{2}H]aldehyde (1·34 g), m.p. 61—62° (lit.,[14] 62—63°); $\nu_{max.}$ 2100, 2050, and 1670 cm^{-1}; $\lambda_{max.}$ 307, 275, and 232 nm; τ 2·5—2·7 (7H, m, aryl H), 3·02 (1H, d, aryl H), 4·85 (1·8H, s, CH_2O), 4·11 (3H, s, MeO), only small CHO signal at τ 0·16; *m/e* 243 (M^+, 7%), 153 (1), 151 (1), and 91 (100).

In later work, the preparation and deuteriation of this dithian were carried out more conveniently by the methods of E. McDonald and E. J. Corey, to whom we are grateful for details in advance of publication. Cleavage was performed by the *N*-chlorosuccinimide method.[14]

(1R)-1,2-*Bis*-(3-*Hydroxy*-4-*methoxyphenyl*)[1-$^2H_1$]*ethane* (52).—The foregoing [^{2}H]aldehyde was converted [1] into the corresponding (*S*)-[α-$^2H_1$]benzyl alcohol, which yielded the (*S*)-[α-$^2H_1$]benzyl chloride as for the dideuterio-analogue above. This was then converted by the hydride method (a) above into (2*R*)-1,2-bis-(3-benzyloxy-4-methoxyphenyl)-[2-$^2H_1$]ethylamine (50). Mass spectrometry and n.m.r. confirmed the preservation of the $^2H_1$ label throughout.

This product (1·32 g), sodium carbonate (1·26 g), and methyl iodide (6 ml) in 1 : 1 water–dioxan (90 ml) were heated under reflux for 4 h in the dark. The solution was concentrated and extracted with chloroform to give a gum (2 g) which was percolated in 1 : 1 water–methanol through Amberlite IRA-400 (Cl^- form), and the percolate was evaporated to give the methochloride (1·42 g).

A solution of this salt in aqueous methanol (25 ml) was treated with sodium amalgam (1·73 g Na; 8·6 g Hg) in portions until reaction was complete (t.l.c.). The suspension, decanted from mercury, was diluted with water (50 ml) and extracted with chloroform to give a residue (729 mg) which in 1 : 2 benzene–methanol (150 ml) was hydrogenated over palladised carbon at 760 mmHg and 20 °C until uptake ceased (2 mol. equiv.). The filtered solution was evaporated and the *ethane* (from methanol) had m.p. 179° (225 mg, 31%) (Found for undeuteriated compound: C, 69·9; H, 6·8; $C_{18}H_{18}O_4$ requires C, 70·05; H, 6·6%); $\nu_{max.}$ 3520 cm^{-1}; $\lambda_{max.}$ 278 nm, shifting to 293 nm with base.

An authentic unlabelled sample of this product was prepared as follows. 1,2-Bis-(3-benzyloxy-4-methoxyphenyl)-acetaldehyde was prepared from 3-benzyloxy-4-methoxybenzaldehyde by the standard method used for deoxybenzoin. This product was reduced with borohydride in

[14] E. J. Corey and B. W. Erickson, *J. Org. Chem.*, 1971, **36**, 3553.

methanol to yield 1,2-*bis*-(3-*benzyloxy*-4-*methoxyphenyl*)-*ethanol*, m.p. 124° (from methanol) (Found: C, 76·4; H, 6·7. $C_{30}H_{30}O_5$ requires C, 76·6; H, 6·4%).

This alcohol was heated under reflux for 8 h with 2N-sulphuric acid; extraction with chloroform gave 3,3′-*dibenzyloxy*-4,4′-*dimethoxystilbene*, m.p. 187—189° (from ethanol) (Found: C, 79·6; H, 6·4. $C_{30}H_{28}O_4$ requires C, 79·6; H, 6·2%).

A solution of the stilbene (0·53 g) in 1 : 1 methanol–chloroform containing acetic acid (5 ml) was shaken with 10% palladised carbon and hydrogen at 760 mmHg and 20 °C. The filtered solution on evaporation gave the required ethane, m.p. 179°, identical with the above degradation product.

Degradation of the Ethane (52) *to Succinic Acid.*—Ozonised oxygen was passed for 10 h at 18—20 °C through a solution of the foregoing [$^2H_1$]ethane (160 mg) in chloroform (10 ml) and methanol (20 ml) and the solvents were evaporated off. The residue in water (1 ml) was treated with formic acid (1 ml) and hydrogen peroxide (1 ml; 30 vol) at 100 °C for 10 h; another portion of the peroxide was added after 2 h. The solution was warmed with palladised carbon (20 mg) until effervesence ceased, then filtered, and evaporated. The residue insoluble in chloroform was sublimed (120°; 0·05 mmHg) and recrystallised from ethyl acetate (27 mg, 39%); m.p. 184—185°. It was recrystallised twice more (charcoal) for o.r.d.[15] and mass spectrometry.[16] O.r.d. measurements

TABLE 4

O.r.d. measurements on the succinic acid

λ/nm	Standard [15] [α] (°)	Found [α] (°)	% Of standard
322	2·50	+0·71	29
312	3·13	+0·95	30
303	3·71	+1·1	30
294	4·22	+1·2	28
286	5·32	+1·6	30
278	6·38	+2·0	31
270	7·89	+2·5	32
263	10·3	+3·1	30
256	12·9	+4·1	32
250	17·4	+5·8	33
244	24·4	+8·3	34
238	34·7	+12·0	35

(Table 4) were performed on the acid (5·41 mg) in water (262 mg); the average over the range 312—244 nm was 31% of standard, corresponding (with allowance for 86% $^2H_1$ species) to 36% of standard, *i.e.* 68% of (+)-(2*S*)-acid and 32% of (−)-(2*R*)-acid. Mass spectrometry [16] was carried out on the dimethyl ester prepared by using diazomethane.

Degradation of the Amines (27) *and* (28) *to Aspartic Acid.*—The amine (or its oxalate) was acetylated by heating at 100° for 1 h with an excess of acetic anhydride and sodium acetate. The mixture was then diluted with water, basified with ammonia, and extracted with ethyl acetate to yield, from radioinactive material (1RS)-N-*acetyl*-1,2-*bis*-(3-*benzyloxy*-4-*methoxyphenyl*)*ethylamine* (95%), m.p. 168° (from methanol) (Found: C, 75·0; H, 6·4; N, 2·5. $C_{32}H_{23}NO_5$ requires C, 75·15; H, 6·5; N, 2·7%); $\nu_{max.}$ 3450 and 1675 cm^{-1}; $\lambda_{max.}$ 282 and 231 nm; *m/e* 511 (M^+, 2%), 451 (13), 284 (100), 242 (60), and 91 (85). In the ^3H series, the crude *N*-acetyl derivative was purified by p.l.c. on silica with 3% methanol in dichloromethane.

This amide (typically 50 mg) in methanol (50—100 ml) was treated with ozonised oxygen for 10 h at 20 °C before evaporation. Water (2 ml), formic acid (1 ml), and 30% hydrogen peroxide (1 ml) were added and the mixture was heated under reflux for 1 h. The excess of peroxy-acid was destroyed by warming with 10% palladised carbon. The solution was filtered and evaporated and the residue containing *N*-acetylaspartic acid was dissolved in water (1 ml); a drop of phenolphthalein was added followed by N-sodium hydroxide until the solution was pink. 0·067M-Phosphate buffer (pH 7) was then added until the solution was colourless and the solution kept for 16 h after addition of hog renal acylase (5 mg). The (2*S*)-aspartic acid formed was isolated as described previously; [9] ^{14}C-generally labelled (2*S*)-aspartic acid was added to give a suitable ^3H : ^{14}C ratio.

(2*S*)-N-(α-*Naphthylureido*)*aspartic Acid and its Dimethyl Ester.*—(2*S*)-Aspartic acid (29 mg) in water (1 ml) and N-sodium hydroxide (0·45 ml) was stirred for 2 h with α-naphthyl isocyanate (3 drops). Dinaphthylurea was removed and the filtrate acidified and extracted with ethyl acetate to give a foam on evaporation. This was crystallised from aqueous methanol to give the *urea* (35 mg, 50%), m.p. 155° (Found: C, 57·8; H, 5·3; N, 8·7%; equiv. wt., 165, 162. $C_{15}H_{14}NO_5$,0·75CH_3OH requires C, 58·0; H, 5·2; N, 8·6%; equiv. wt., 163); $\nu_{max.}$ (Nujol) 1725, 1705, 1670, 1640, and 1630 cm^{-1}; τ 1·12 (1H, s, aryl NH), 1·7—2·7 (7H, m, aryl H), 2·84 (1H, d, *J* 8·5 Hz, NH), 5·39 (1H, m, CH), 6·81 (*ca.* 2H, s, MeOH), and 7·19 (2H, m, CH_2); *m/e* (no M^+) 284, ($M^+ - H_2O$, 40%) 169 (18), and 143 (30).

The *dimethyl ester* was prepared as for the corresponding ethyl ester; [9] m.p. 158·5—159·5° (from benzene–hexane) (Found: C, 61·9; H, 5·3; N, 8·5. $C_{17}H_{18}N_2O_5$ requires C, 61·8; H, 5·45; N, 8·5%).

(2*S*,3*S*)-[*random*-^{14}C, 3-$^3H_1$]*Malic Acid.*—Nitrous fumes [from sodium nitrite (1 g) and 25% sulphuric acid (3 ml)] were carried with nitrogen into a suspension of the foregoing (2*S*,3*S*)-[*random*-^{14}C, 3-$^3H_1$]aspartic acid (58 mg) in water (5 ml). After 5 min the solution was degassed, 10% was removed for study of the malic acid therein, and the rest was treated with malate hydro-lyase (fumarase) as earlier.[9] The isolated fumaric acid (14 mg) as its sodium salt was heated with *p*-nitrobenzyl bromide (55 mg) in dry dimethylformamide (1 ml) for 6 h. The precipitate produced on addition of water was recrystallised to constant activity from chloroform–ether (charcoal) to give bis-*p*-nitrobenzyl fumarate, m.p. 153°.

N-(2,2-*Diethoxyethyl*)-1,2,3,4-*tetrahydro*-8-*hydroxy*-3-(3-*hydroxy*-4-*methoxyphenyl*)-7-*methoxyisoquinoline* (34), *its* 7-*Hydroxy*-8-*methoxy-isomer* (37) *and Labelled Forms* [(35), (36), (38), *and* (39)].—The *OO*-dibenzyl amino-acetal (33) (272 mg; 15 mCi) in ethanol (20 ml) and concentrated hydrochloric acid (4 drops) was shaken with 10% palladised carbon (75 mg) until uptake ceased. Basification with carbonate and chloroform extraction yielded the *phenolic base* (Found: M^+ 417·2136. $C_{21}H_{31}NO_6$ requires *M*, 417·2151). A radioscan of t.l.c. on silica showed the material to be virtually homogeneous. The whole in methanol (5 ml) was stirred under nitrogen with saturated aqueous sodium hydrogen carbonate (2 ml), aqueous 36% formaldehyde (2 ml), and

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[16] G. Popjak, D. S. Goodman, J. W. Cornforth, R. H. Cornforth, and R. Ryhage, *J. Biol. Chem.*, 1961, **236**, 1934.

water (2 ml). After 1·5 h, water (10 ml) was added and extraction with chloroform afforded the two isoquinolines (36) and (39); t.l.c. analysis showed *ca.* 40% of the mixture to be base (36), but p.l.c. did not clearly separate the two (combined radioactivity 11·8 mCi); *m/e* 417, 372, 326, 285, and 161.

The other labelled forms were prepared similarly.

(14RS,13S)-[13-$^3H_1$]*Scoulerine* (42), (14RS,13R)-[13-$^3H_1$]-*Scoulerine* (43), *and the Corresponding Coreximines* (46) *and* (47).—The foregoing mixture of isoquinolines (158 mg; 11·8 mCi; 0·16 mmol of 8-hydroxy-isomer) was dissolved in concentrated hydrochloric acid (6 ml) with warming and stirred in a hydrogenation flask overnight. Pallidised charcoal (100 mg; 10%) and 10% perchloric acid in acetic acid (15 ml) were added, and the mixture was hydrogenated at 760 mmHg and 20 °C for 24 h. The filtered solution and methanolic washings were concentrated to *ca.* 2 ml and an excess of saturated aqueous sodium hydrogen carbonate was added. Extraction with chloroform gave the two alkaloids, which were separated by p.l.c. on silica with ether; both were eluted with methanol (100 ml each). A sample of the solution of scoulerine was counted [0·83 mCi; 9·2 mg; 15% from (36)], acidified with methanolic hydrogen chloride, and evaporated to give the (14*RS*,13*R*)-[13-$^3H_1$]scoulerine hydrochloride. The labelled coreximine (47) salt was similarly isolated.

The same method was used for the synthesis of the (13*S*)-forms of [13-$^3H_1$]scoulerine (42) and [13-$^3H_1$]coreximine (46).

(14*RS*)-[8-^{14}C]Scoulerine hydrochloride[3] was added to each ^{3}H-labelled sample of scoulerine to give a convenient ^{3}H : ^{14}C ratio; the base was then recovered as above and was chromatographed (p.l.c.) on silica with ethyl acetate. The [^{3}H,^{14}C]scoulerines were recovered from the silica and converted into hydrochlorides as above.

Doubly Labelled [8-^{14}C,13-$^3H_1$]*Tetrahydropalmatines*.—A small sample of each of the foregoing labelled scoulerines was mixed with (1-*RS*)-nor-reticuline hydrochloride (*ca.* 100 mg) and treated with formaldehyde as for the preparation of scoulerine.[3] The scoulerine was isolated as above and was treated in methanol with an excess of ethereal diazomethane for 1 day. Purification of the labelled tetrahydropalmatine so obtained by p.l.c. on silica in ether was followed by crystallisation to constant ^{3}H : ^{14}C ratio and specific activity; m.p. 150—151° (lit.,[17] 151°).

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