Studies of Enzyme-mediated Reactions. Part 9.[1,2] Stereochemistry of Oxidative Ring Cleavage Adjacent to Nitrogen during the Biosynthesis of Chelidonine

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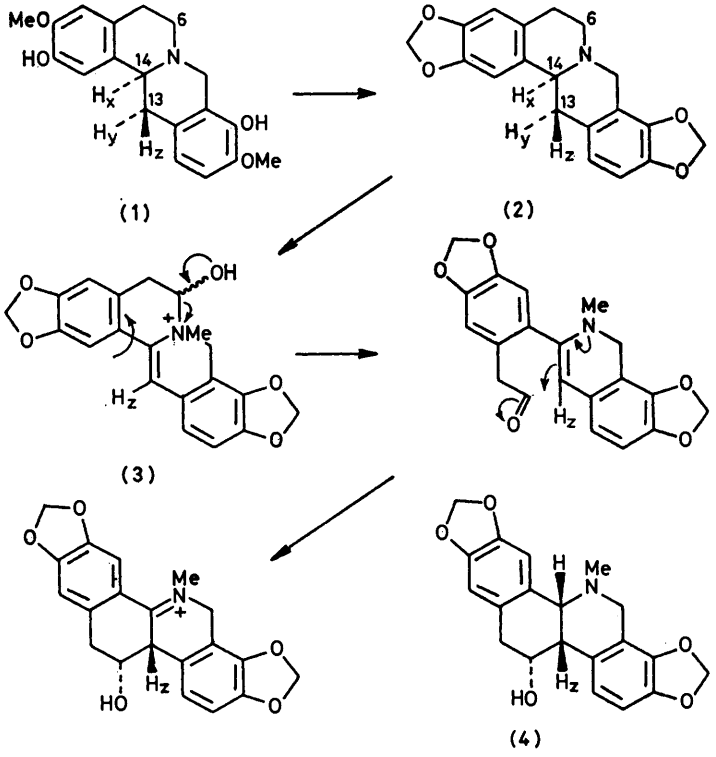
Synthetic routes are devised leading to phenethylamine derivatives which are chiral at C-1 due to ^3H-substitution. These (1*R*)- and (1*S*)-amines have high configurational purity (*ca.* 98% one enantiomer) and their absolute configurations are confirmed by studies with the amine oxidase from pea seedlings. (6*R*-$^3H_1$)-Scoulerine (37) and the (6*S*-$^3H_1$)-isomer (39) are synthesised from these phenethylamines and the products are used for incorporation experiments with *Chelidonium majus* plants. The results for tritium retention in the two series, and for the (6*RS*-$^3H_1$)-series, prove that cleavage of the bond between nitrogen and C-6 of stylopine (2) which finally leads to chelidonine (4), occurs with stereospecific loss of the hydrogen atom in *Si*-space.

THE biosynthesis of benzophenanthridines, exemplified by the plant alkaloid chelidonine (4), has been extensively studied [3] and the late stages of the pathway which emerged are shown in Scheme 1. The Scheme also illustrates the fate of hydrogen atoms at C-13 and C-14 of (—)-(14*S*)-scoulerine (1) as the protoberberine skeleton is converted by the living plants into the benzophenanthridine system.[4] In this connection, the events occurring at C-6 of scoulerine (1) are of special interest in that there is oxidation to the aldehyde level of a methylene group adjacent to nitrogen. Such oxidations (or dehydrogenations) occur widely in living systems and because of our broad interest in this area,[5] a study of the stereochemistry of the attack at C-6 was carried out for the conversion of scoulerine (1) into chelidonine (4).



SCHEME 1

RESULTS AND DISCUSSION

The exploratory experiments (Scheme 2) made use of (—)-(6*RS*,14*S*)-[6-^{14}C,6-$^3H_1$]scoulerine (5) ‡ and the corresponding (+)-(6*RS*,14*R*)-isomer (6); the synthesis of these labelled materials has already been described.[3] Incorporation experiments with (5) and (6) into chelidonine (8) were carried out using *Chelidonium majus* plants and we took the opportunity to investigate their incorporation into stylopine (7) and protopine (11) which are also present. The incorporation results and reten-

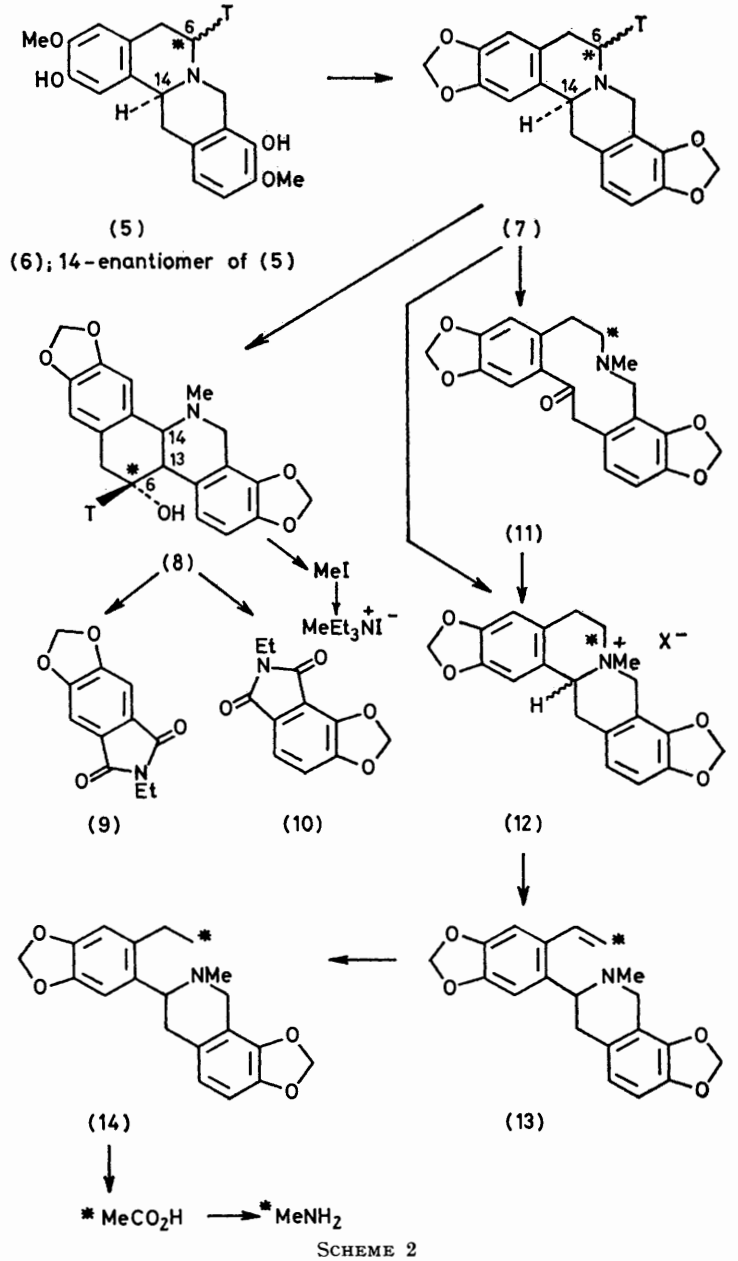
† Dedicated to our colleague Professor E. Lederer, Gif-sur-Yvette, France, on the occasion of his seventieth birthday.

‡ Note that (1) represents the unlabelled form and (5) a labelled form of scoulerine; the same holds for the pairs (2) and (7), and (4) and (8).

tions of tritium are collected in Table 1, experiments 1 and 2. Clearly, the (−)-(14*S*)-isomer (5) is converted far more efficiently by the plants into the three alkaloids than is the (+)-(14*R*)-enantiomer (6), in agreement with previous findings.[3]

and since they account for every carbon atom of chelidonine (8) apart from C-6, the ^{14}C-label is shown by difference to be located entirely at C-6.

Hydride reduction of protopine (11) followed by treatment of the product with phosphorus oxychloride



SCHEME 2

The next step was to establish that the incorporations of (5) into chelidonine (8), stylopine (7), and protopine (11) had occurred without randomisation of the label, and Scheme 2 sets out the reaction sequences used. Radio-labelled chelidonine (8) was (*a*) oxidised as earlier [3] to yield two fragments isolated as the imides (9) and (10), and (*b*) *N*-demethylated, the generated methyl iodide being trapped as methyltriethylammonium iodide. These three products were all radio-inactive yielded the salt (12) which was also obtained by quaterisation of (14*RS*)-stylopine (7).* The two samples of (12) were degraded separately by Hofmann's method to give the methine (13); this was hydrogenated and the product (14) was oxidised (Kuhn–Roth) to yield acetic acid. Schmidt degradation then gave methyl-

* The labelled stylopine from the plants, which contains [6] an excess of the (14*S*)-isomer (7), was isolated using (*RS*)-material as added carrier.

amine which, as for acetic acid, was purified as a crystalline derivative (see Table 2). The results reported above for chelidonine (8) and those collected in Table 2 for stylopine (7) and protopine (11) demonstrate specific incorporation of (−)-(14*S*)-scoulerine (5) into all three alkaloidal types. Confirmation is therefore given to earlier findings [3] for chelidonine.

TABLE 1

Tracer experiments on *Chelidonium majus* plants

Experiment no.	Precursor	Incorporation (^{3}H-retention %) Chelidonine (8)	(*RS*)-Stylopine (as 7)	Protopine (1)
1	(−)-(6*RS*,14S)-[6-^{14}C,6-$^3H_1$]Scoulerine (5)	0.81 (63)	0.18 (109)	0.72 (109)
2	(+)-(6*RS*,14*R*)-[6-^{14}C,6-$^3H_1$]Scoulerine (6)	<0.01	<0.01	<0.01
3	(6*R*,14*RS*)-[6-^{14}C,6-$^3H_1$]Scoulerine (37)	0.22 (100)	0.14 (104)	*
4	(6*S*,14*RS*)-[6-^{14}C,6-$^3H_1$]Scoulerine (39)	0.21 (5)	0.10 (97)	*
5	(6*RS*,14*RS*)-[6-^{14}C,6-$^3H_1$]Scoulerine [(37) + (39)]	0.29 (64)	0.12 (98)	*

* Not examined.

TABLE 2

Degradation of labelled stylopine and protopine from experiment 1, Table 1

	Relative molar activities (^{14}C) Stylopine (7)	Protopine (11)
Starting alkaloid	1.00	1.00
Vinyl compound (13)		0.98
Ethyl compound (14)	0.98	0.98
Acetic acid (as *p*-bromophenacyl ester)	0.97	0.95
Methylamine (as *N*-methylphthalimide)	0.94	0.93

With these aspects established, we can consider the results for the tritium labelling. If oxidative attack at C-6 of stylopine (7) is stereospecific, eventually to produce (3), or a close relative, then retention of 50% of the tritium would be expected.[7,8] In fact, an apparent retention of 63% of the tritium was observed and though the accuracy of these early double-labelling experiments cannot be claimed to be better than *ca.* ±5%, it is evident that there has been a small increase in the ^{3}H : ^{14}C ratio over the expected one. Such small increases in ^{3}H : ^{14}C ratio have been observed many times and the probable cause has been discussed previously.[3] However, the important point is that these initial experiments indicated stereospecific attack at C-6 of the protoberberine skeleton (7).

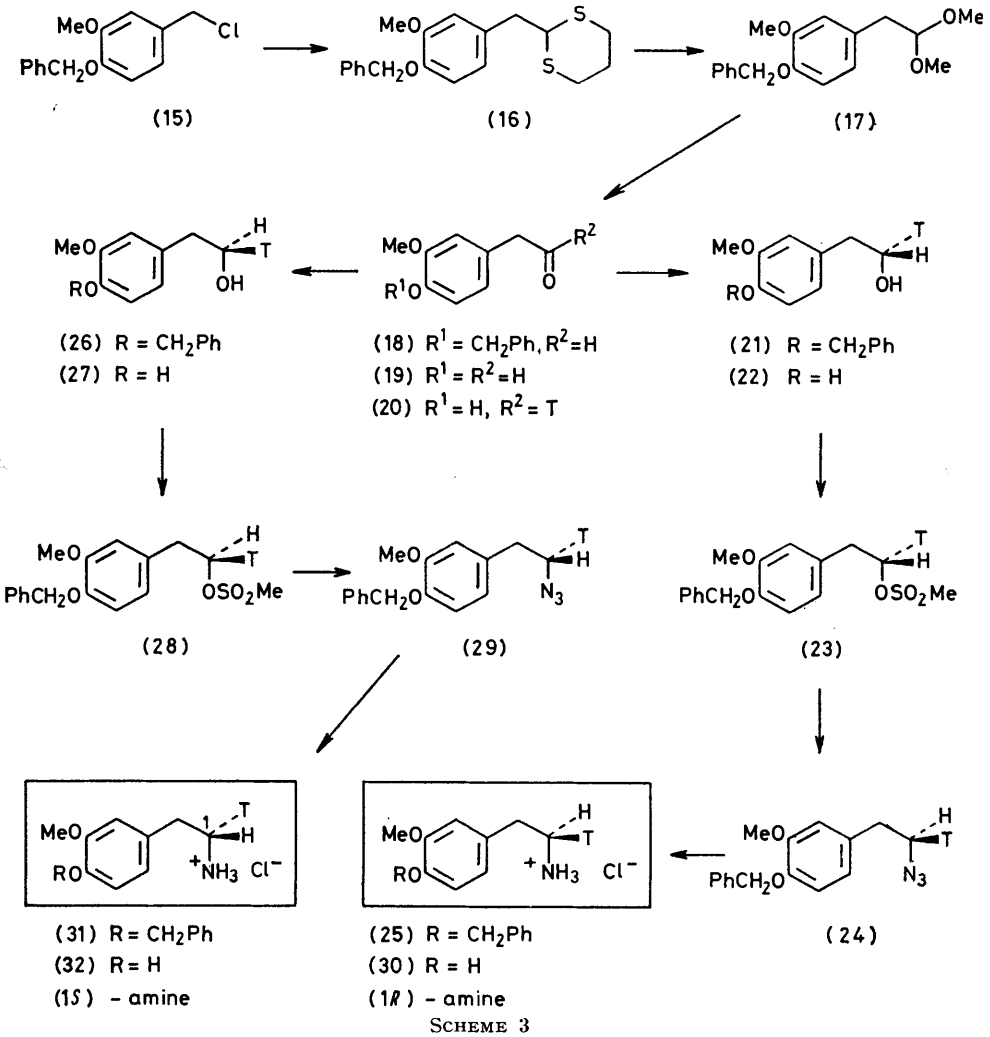
Further progress required the synthesis of two samples of doubly labelled scoulerine (5) in which C-6 had been made chiral by ^{3}H-substitution. The necessary labelled building blocks are the (1*R*)-[1-$^3H_1$]phenethylamine (25) and the corresponding (1*S*)-isomer (31) which can be converted into labelled scoulerine (5) by steps which do not affect the chiral centre. The plan was to prepare the chiral alcohols (21) and (26) and to convert them by strictly parallel series of reactions into the chiral amines (25) and (31). This approach [9] ensures that the final results from the *R*- and *S*-series will be *complementary*, even if some loss of configurational purity does occur during the synthetic operations.

One of the required alcohols (22) should be available by enzymic reduction of the [*formyl*-^{3}H]phenylacetaldehyde (20) by liver alcohol dehydrogenase and NADH generated *in situ*. Conversely, formation of the other alcohol (27) involves transfer of tritium from NAD^{3}H to unlabelled aldehyde (19). However, the instability of the phenylacetaldehydes necessitated synthesis of a protected form of the aldehyde (17) and the route used runs from the chloride (15) to the dithian (16) which was converted into the acetal (17) by mercuric oxide and mercuric chloride in methanol as in Scheme 3. Mild acid hydrolysis of (17) followed by reduction with borotritiide gave the (1*RS*)-[1-$^3H_1$]-phenethyl alcohol [(21) + (26)], and the corresponding *O*-methanesulphonate [(23) + (28)] was converted under S_N2 conditions (*e.g.* ref. 4) into the azide [(24) + (29)].

The possibility of aryl participation in the azide displacement step was eliminated as described later. Therefore we could with confidence reduce the azide [(24) + (29)] to give the (1*RS*)-[1-$^3H_1$]phenethylamine hydrochloride [(25) + (31)]. Some was reserved for conversion into labelled scoulerine and the remainder was catalytically debenzylated to yield the phenolic water-soluble amine ready for treatment with the amine oxidase from pea seedlings.[5] The generated [*formyl*-^{3}H]phenylacetaldehyde (20) was reduced *in situ* by liver alcohol dehydrogenase and NADH in catalytic quantity, the latter being continuously regenerated from NAD^+ and cyclopentanol. This use of cyclopentanol as the source of ' hydride ' is a valuable extension of the earlier method using cyclohexanol [9] in that it allows readier separation of the reducing alcohol from the product in this, and no doubt, other reductions. After *O*-benzylation of the alcohol (22), the product (21) was converted as above *via* (23) and (24) into the (1*R*)-[1-$^3H_1$]phenethylamine hydrochloride (25).

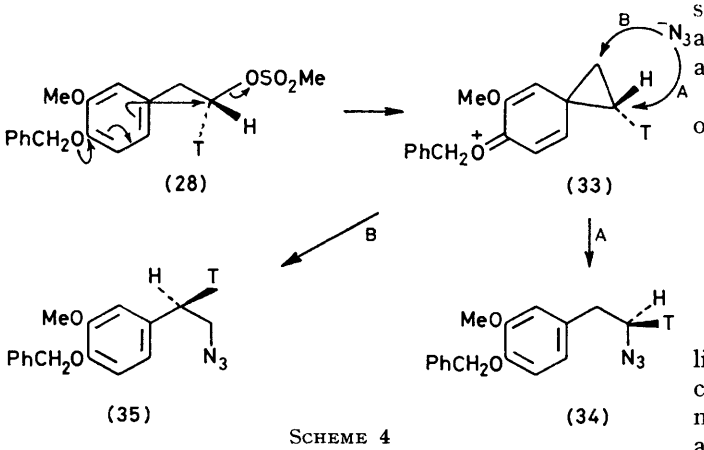
For preparation of the (1*S*)-enantiomer (31), the phenolic aldehyde (19) arising from catalytic *O*-debenzylation and hydrolysis of the acetal (17) was reduced with liver alcohol dehydrogenase, NAD^+ and [1-^{3}H]cyclopentanol. The product (27) was re-benzylated and the ether (26) was then converted as before, (28)→(29) and finally into the (1*S*)-[1-$^3H_1$]amine hydrochloride (31).

It is now important to consider the possibility of aryl participation in the above azide displacements. Par-



SCHEME 3

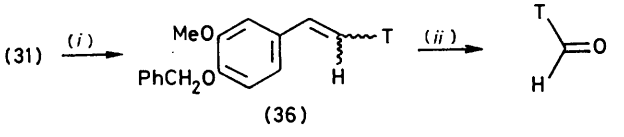
ticipation might occur either as the total reaction path or in competition with straightforward displacement.



SCHEME 4

Either event would cause serious problems as illustrated in Scheme 4. Any spiro-intermediate (33) can be opened by attack as A or as B; the former produces azide (34) having the same chirality as the starting alcohol whereas B involves rearrangement to produce (35). Accordingly, the amine (31) was degraded *via* the styrene (36) by the sequence [3] in Scheme 5; the formaldehyde carried all the ^{3}H-activity. Rearrangement and thus aryl participation are thereby excluded.

The configurations illustrated in Scheme 3 are based on two assumptions: (*a*) that reduction of aldehydes by



SCHEME 5 (*i*) HCHO–HCO_2H, MeI, OH^- sequentially; (*ii*) O_3

liver alcohol dehydrogenase occurs on the *Re*-face of the carbonyl group [10] and (*b*) that displacement of the methanesulphonyl group by azide anion has occurred by an S_N2 process with inversion. Available experience strongly indicates that both assumptions are very safe ones. Additional confirmation was obtained, however,

by testing the expected configurations of the final amines (25) and (31) using the amine oxidase from pea seedlings, which was shown [5] to be specific for the hydrogen atom of benzylamine which lies in *Si*-space.

For these experiments, a sample of [1-^{14}C]phenethylamine (25) was mixed with each of the amines (25) and (31) to act as an internal standard. The doubly-labelled phenolic amine (30) prepared by debenzylation of the (1*R*)-amine (25) should *retain* its tritium label when it is dehydrogenated by the pea enzyme whereas the ^{3}H-label should be *lost* from the phenolic amine (32) derived from the (1*S*)-isomer (31). The (1*RS*)-amine [(30) + (32)] was included in this same study.

The experimental approach was to trap the phenylacetaldehyde, produced by the amine oxidase, by reducing as it was formed; to this end, liver alcohol dehydrogenase, NAD^{+} and cyclopentanol were included in the reaction mixture. A stable phenolic alcohol was thus produced which was isolated and purified as its crystalline *O*-benzyl ether (21). The results in Table 3

TABLE 3

Dehydrogenation of phenethylamines by the amine oxidase from pea seedlings

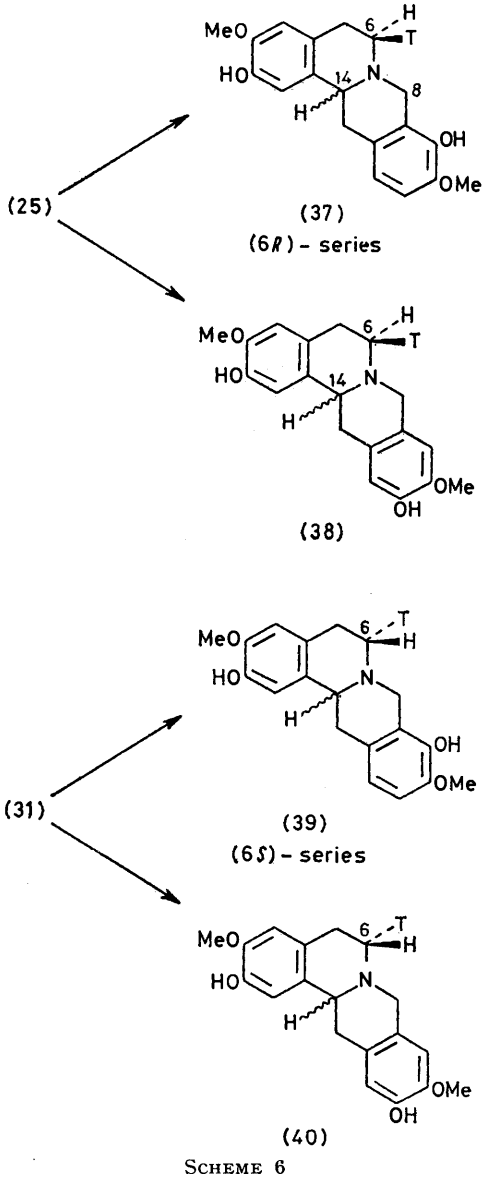
Phenethylamine *	^{3}H : ^{14}C ratio	Phenethyl alcohol * ^{3}H : ^{14}C ratio (% retention)
(1*R*)-[1-$^{3}H_1$,1-^{14}C]-2-(4-Hydroxy-3-methoxyphenyl)ethylamine (30)	7.8	Run 1 7.5 (96)
		Run 2 7.7 (99)
(1*S*)-[1-$^{3}H_1$,1-^{14}C]-2-(4-Hydroxy-3-methoxyphenyl)ethylamine (32)	10.2	Run 1 0.17 (1.7)
		Run 2 0.16 (1.6)
(1*RS*)-[1-$^{3}H_1$,1-^{14}C]-2-(4-Hydroxy-3-methoxyphenyl)ethylamine [(30) + (32)]	9.0	Run 1 4.6 (51)
		Run 2 4.8 (53)

* Isolated and assayed as the *O*-benzyl ether (*cf.* 21)

show (*a*) that the above expectations hold good and so the illustrated absolute configurations for the amines (25) and (31) are confirmed; (*b*) that these two amines have high configurational purities; and (*c*) that aryl participation is not significant in the azide displacement step of Scheme 3. Synthesis of the various labelled protoberberines could thus be undertaken with confidence.

The (1*R*)-amine (25) was converted by the route previously developed,[11] into a separable mixture of (6*R*,14*RS*)-[6-$^{3}H_1$]scoulerine (37) and (6*R*,14*RS*)-[6-$^{3}H_1$]-coreximine (38). Similarly the (1*S*)-amine (31) yielded the corresponding (6*S*,14*RS*)-isomers (39) and (40), and (6*RS*,14*RS*)-scoulerine [(37) + (39)] and (6*RS*,14*RS*)-coreximine [(38) + (40)] stemmed from the (1*RS*)-amine [(25) + (31)].

The foregoing samples of scoulerine (37), (39), and [(37) + (39)] were mixed in each case with an appropriate quantity of (14*RS*)-[8-^{14}C]scoulerine [3] to give a workable ^{3}H : ^{14}C ratio and a small portion of each was *O*-methylated with diazomethane.[4] This yielded tetrahydropalmatine which was diluted with unlabelled carrier and then was purified to constant ^{3}H : ^{14}C ratio. This established an accurate value for the original scoulerines.



SCHEME 6

It was not necessary for the incorporation experiments to resolve the labelled samples of (14*RS*)-scoulerine because it was shown here and earlier [3] that (14*R*)-scoulerine is essentially ineffective as a precursor of (14*S*)-stylopine (2) and chelidonine (4). Thus, (14*RS*)-scoulerine acts, for practical purposes, as the (14*S*)-isomer (1). Accordingly, the three doubly labelled scoulerine samples were administered separately to *Chelidonium majus* shoots and incorporation occurred into stylopine (2) and chelidonine (4).

The results in Table 1, experiments 3—5, establish

that (*a*) (14*S*)-stylopine (2) is biosynthesised from (14*S*)-scoulerine (1) as expected without C-6 being affected; stylopine thus acts as a valuable internal standard for the values found for chelidonine; and (*b*) more importantly, the biosynthesis of chelidonine (4) from scoulerine (1) involves oxidation of the methylene group at C-6 to the aldehyde level *with stereospecific removal of the hydrogen atom in* Si-*space*. It is interesting that the hydrogen atoms removed from C-13 and C-14 of stylopine during the biosynthesis of chelidonine [4] (see Scheme 1) also are lost from this same face of the molecule.

The foregoing precise results form a firm foundation on which future studies of the nitrogen–C-6 cleavage process can be built; any mechanistic proposals must accord with the present findings.

EXPERIMENTAL

General Directions.—Solutions in organic solvents were dried over anhydrous magnesium sulphate monohydrate unless otherwise stated. Anhydrous solvents were prepared as follows: ether, light petroleum or benzene distilled off P_4O_{10} and stored over sodium wire; tetrahydrofuran (THF) and dioxan stored over calcium hydride and distilled from $LiAlH_4$; dimethylformamide (DMF) distilled off calcium hydride and stored over a molecular sieve; pyridine distilled off calcium hydride; methylene chloride distilled and passed down basic alumina (Activity I).

The remaining general directions are given in ref. 12. For details of administration of precursors to *C. majus* plants and of extraction and degradation of relevant alkaloids, see ref. 6.

N-*Demethylation of Chelidonine* (8).—Chelidonine from experiment 1, Table 1 (50 mg, ^{14}C-activity 1.59×10^3 disintegrations s^{-1} $mmol^{-1}$), ammonium iodide (50 mg), phenol (0.5 g), tetrachloroauric acid (5 drops), and freshly distilled hydriodic acid (2 ml) were heated at 360 °C (oil bath) in a nitrogen stream in a Herzig–Meyer apparatus. The issuing gases were passed through a trap containing aqueous sodium thiosulphate (5% w/v) and cadmium sulphate (5% w/v) before entering a solution of triethylamine in ethanol (5% v/v, 5 ml). After the last solution had been kept at 20 °C for 16 h, it was evaporated and the residue was recrystallised from ethanol–ether to give methyltriethylammonium iodide (24 mg) which was radioinactive.

Oxidation of Chelidonine (8).—This was carried out exactly as earlier.[3] Chelidonine (250 mg, ^{14}C-activity 1.59×10^3 disintegrations s^{-1} $mmol^{-1}$) yielded the imide (9), m.p. 168—169 °C (12 mg) and the isomer (10), m.p. 123—125 °C (15 mg). Both were radioinactive.

Degradation of Stylopine (7).—A solution of stylopine from experiment 1, Table 1 (300 mg, ^{14}C-activity 0.99×10^3 disintegrations s^{-1} $mmol^{-1}$) in methanol (10 ml) and methyl iodide (25 ml) was heated under reflux for 2.5 h. The residue from evaporation was boiled with aqueous potassium hydroxide (20% w/v; 15 ml) for 3 h and, after addition of water, was extracted with ether. Crystallisation of the extracted material from ethanol gave the *vinyl compound* (13), m.p. 146—148 °C (96 mg) (Found: C, 71.2; H, 5.6. $C_{20}H_{19}NO_4$ requires C, 71.2; H, 5.7%). The vinyl compound (90 mg) was hydrogenated in ethanol (20 ml) over 5% palladium–carbon (20 mg) until uptake (1.0 mol) was complete. Removal of the catalyst and evaporation gave the *ethyl compound* (48 mg) (14), m.p. 138—139 °C after crystallisation from ethanol (Found: C, 70.9; H, 6.3. $C_{20}H_{21}NO_4$ requires C, 70.9; H, 6.2%). Carbon-14 activity 0.97×10^3 disintegrations s^{-1} $mmol^{-1}$. The ethyl compound (43 mg) was oxidised by the Kuhn–Roth method described earlier [13] to yield *p*-bromophenacyl acetate (16 mg), ^{14}C-activity 0.96×10^3 disintegrations s^{-1} $mmol^{-1}$. This ester was diluted with inactive material to 70 mg and was subjected to Schmidt degradation as described earlier [3] which yielded *N*-methylphthalimide (13 mg), m.p. 133—134 °C, ^{14}C-activity (corrected for dilution) 0.94×10^3 disintegrations s^{-1} $mmol^{-1}$.

Degradation of Protopine (11).—A stirred solution of protopine from experiment 1, Table 1 (370 mg, ^{14}C-activity 0.52×10^3 disintegrations s^{-1} $mmol^{-1}$) in anhydrous benzene (15 ml) was treated dropwise over 15 min with a solution of lithium aluminium hydride (70 mg) in dry ether (20 ml). The mixture was stirred at 20 °C for 18 h then acidified with 2M-hydrochloric acid (10 ml) and worked up by basification and extraction with chloroform. The extracted dihydroprotopine crystallised from chloroform–methanol (297 mg), m.p. 153—154 °C (lit.,[14] m.p. 152—153 °C).

All this product was heated under reflux for 10 min with phosphorus oxychloride (1.5 ml) and then evaporated. The residue was then boiled for 3 h with aqueous potassium hydroxide (20% w/v; 15 ml) to give material identical with the vinyl compound obtained above from stylopine (yield 98 mg), m.p. 146—148 °C; ^{14}C-activity 0.51×10^3 disintegrations s^{-1} $mmol^{-1}$.

The remaining steps of the degradation through to *N*-methylphthalimide followed exactly those described above for stylopine.

2-(4-*Benzyloxy*-3-*methoxybenzyl*)-1,3-*dithian* (16).—Freshly distilled thionyl chloride (6 ml) was added to a solution of 4-benzyloxy-3-methoxybenzyl alcohol (10 g) in anhydrous ether (50 ml) at 20 °C and the mixture was heated for 2 h under reflux. The residue from evaporation was crystallised from benzene–cyclohexane to give the chloride (15) (8.3 g) which was used directly for the next step; τ 2.6 (5 H, s, Ar-H), 3.1 (1 H, s, Ar-H), 3.15 (2 H, s, Ar-H), 4.88 (2 H, s, PhCH_2), 5.48 (2 H, s, ArCH_2), and 6.12 (3 H, s, OMe).

A solution of 1,3-dithian [15] (2.4 g) in anhydrous THF (75 ml) was treated at −78 °C during 20 min with a solution of n-butyl-lithium in hexane (1.84 mmol ml^{-1}, 11 ml). After the mixture had been kept at −30 °C for 6 h, it was treated with a solution of the above chloride (5 g) in THF. The mixture was warmed to 20 °C, set aside for 18 h, then poured into ice–water (80 g) and extracted thrice with chloroform. Chromatography of the extracted material on silica (250 g) in benzene (total 1 l) was followed by elution with ether. The ethereal fractions gave the required *dithian* (3.32 g), m.p. 105 °C from ethyl acetate (Found: C, 65.8; H, 6.3; S, 18.6. $C_{19}H_{22}O_2S_2$ requires C, 66.0; H, 6.4; S, 18.5%); τ 2.65 (5 H, s, Ar-H), 3.25 (3 H, s, Ar-H), 4.9 (2 H, s, PhCH_2), 5.85 (1 H, t, *J* 8 Hz, CH–S), 6.18 (3 H, s, OMe), 7.05 (2 H, d, *J* 8 Hz, ArCH_2), 7.2 (4 H, m, SCH_2), and 8.1 (2 H, m, CH_2); *m/e* 346 (M^+, 5%), 320 (80), 300 (15), 120 (100), and 91 (90).

1-(4-*Benzyloxy*-3-*methoxyphenyl*)-2,2-*dimethoxyethane* (17).—The foregoing dithian (1.4 g) was heated under reflux for 6 h with methanol (80 ml), mercuric oxide (1 g), and mercuric chloride (5.44 g). The methanol was then evaporated off and the residue was partitioned between water and chloro-

form. The organic layer from the filtered mixture was washed with water, dried, and evaporated. Chromatography [alumina (60 g), eluant chloroform] yielded the *dimethoxyethane* (17), m.p. 34—35 °C (Found: C, 71.4; H, 7.2. $C_{18}H_{22}O_4$ requires C, 71.5; H, 7.3%); τ 2.7 (5 H, s, Ar-H), 3.25 (3 H, s, Ar-H), 4.95 (2 H, s, PhCH_2), 5.52 [1 H, t, *J* 6 Hz, CH(OMe)$_2$], 6.2 (3 H, s, OMe), 6.7 (6 H, s, 2 OMe), and 7.2 (2 H, d, *J* 6 Hz, ArCH_2); *m/e* 302 (M^+, 5%), 271 (2), and 91 (100).

(1RS)-2-(4-*Benzyloxy*-3-*methoxyphenyl*)[1-$^3H_1$]*ethanol* [(21) + (26)].—The foregoing acetal (320 mg) was dissolved in THF (5 ml), treated with 2M-hydrochloric acid (5 ml) and set aside under nitrogen at 20 °C for 1 h. Evaporation of the solution at low temperature removed the THF and the crude aldehyde was extracted with chloroform (5 × 10 ml). The residue from the chloroform was treated in tetrahydrofuran–water (1 : 1, 8 ml) with sodium borohydride (6 mg) followed after 5 min by sodium borotritiide (100 mCi, 6.3 Ci mmol^{-1}). After the mixture had been kept at 20 °C for 16 h, an excess of borohydride was added and shortly thereafter, the THF was evaporated off. Chloroform extraction yielded the crude (1RS)-[1-$^3H_1$]*ethanol* which was purified by p.l.c. on silica with 5% methanol in ether and crystallisation from benzene–hexane (200 mg, total activity 50 mCi).

This sample was identical with an authentic one prepared by standard reduction of 4-benzyloxy-3-methoxyphenylacetic acid [16] with lithium aluminium hydride, m.p. 79 °C; τ 2.65 (5 H, s, Ar-H), 3.25 (3 H, s, Ar-H), 4.9 (2 H, s, PhCH_2), 6.1 (3 H, s, OMe), 6.2 (2 H, t, *J* 6 Hz, CH_2OH), and 7.2 (2 H, t, *J* 6 Hz, ArCH_2); *m/e* 258 (M^+, 100), 167 (50), 137 (40), and 91 (95).

(1RS)-2-(4-*Benzyloxy*-3-*methoxyphenyl*)[1-$^3H_1$]*ethylamine Hydrochloride* [(25) + (31)].—The procedure was developed with unlabelled material. 4-Benzyloxy-3-methoxyphenylethyl alcohol (200 mg) in methylene chloride (4 ml) containing triethylamine (120 mg) was treated during 10 min at —10 °C with methanesulphonyl chloride (100 mg) in methylene chloride [17] (2 ml). After a further 10 min at —10 °C the solution was washed with ice–water (2 × 10 ml), cold 3M-hydrochloric acid (2 × 5 ml), cold saturated sodium hydrogen carbonate (5 ml), then dried at 0 °C and evaporated to leave the *O*-methanesulphonate which was crystalline at 5 °C (265 mg); τ 2.65 (5 H, s, Ar-H), 3.25 (3 H, s, Ar-H), 4.95 (2 H, s, PhCH_2), 5.7 (2 H, t, *J* 7 Hz, CH_2O), 6.2 (3 H, s, OMe), 7.1 (2 H, t, *J* 7 Hz, ArCH_2), and 7.25 (3 H, s, SO_2Me); *m/e* 336 (M^+, 10%), 165 (20), 150 (30), 125 (40), and 91 (100).

This product (265 mg) was dissolved directly in acetone (2 ml) and treated with sodium azide (200 mg) in water (1 ml). After the mixture had been heated at 80 °C for 5 h, the acetone was evaporated off and the azide was extracted with methylene chloride from which it was recovered as a pale yellow oil (220 mg); τ 2.7 (5 H, s, Ar-H), 3.2 (3 H, s, Ar-H), 4.95 (2 H, s, PhCH_2), 6.2 (3 H, s, OMe), 6.6 (2 H, t, *J* 7 Hz, CH_2N_3), and 7.25 (2 H, t, *J* 7 Hz, ArCH_2); *m/e* 283 (M^+, 15%) and 91 (100).

The azide (220 mg) in anhydrous ether (30 ml) was then added to a stirred suspension of lithium aluminium hydride (150 mg) in ether (20 ml). The mixture was heated under reflux for 1 h, then worked up by the basic method [18] to give an ethereal solution of the amine. This was dried, and addition of ethereal hydrogen chloride precipitated the amine hydrochloride, which was recrystallised from methanol–ethyl acetate (104 mg), m.p. 174—175 °C, identical with an authentic sample; [19] τ 2.65 (5 H, s, Ar-H), 3.2 (3 H, s, Ar-H), 4.9 (2 H, s, PhCH_2), 6.2 (3 H, s, OMe), 7.16 (2 H, t, *J* 6 Hz, ArCH_2), and 7.4 (2 H, t, *J* 6 Hz, CH_2N); *m/e* 257 ([M — HCl]$^+$, 11%), 228 (18), 137 (61), and 91 (100).

The (1*RS*)-[1-$^3H_1$]-labelled sample was prepared exactly as above from the whole sample of [1-$^3H_1$]alcohol [(21) + (26)]; total activity obtained 30 mCi.

(1R)-2-(4-*Benzyloxy*-3-*methoxyphenyl*)[1-$^3H_1$]*ethylamine Hydrochloride* (25).—Part of the foregoing (1*RS*)-[1-$^3H_1$]-amine hydrochloride (80 mg, 20 mCi) was shaken in ethanol (10 ml) with hydrogen and 10% palladium–charcoal (20 mg) until H_2 uptake ceased. The filtered solution was evaporated and a solution of the residue in 0.01M-phosphate buffer (pH 7.0, 75 ml) was incubated at 37 °C with liver alcohol dehydrogenase (5 mg), NAD^+ (20 mg), cyclopentanol (250 mg), and diamine oxidase from pea seedlings [5] (5 ml of solution). The oxidase solution had specific activity at 25 °C of 0.026 × 10^{-6} mol benzylamine oxidised min^{-1} mg^{-1}. The total incubation lasted 24 h with further additions of NAD^+ (10 mg) and liver alcohol dehydrogenase (5 mg) after 10 h and 20 h. Radio-inactive alcohol [unlabelled (22)] was then added as carrier and the mixture, after saturation with sodium chloride, was extracted with chloroform (4 × 20 ml). A solution of the extracted material in methanol (20 ml) containing anhydrous potassium carbonate (250 mg) and benzyl chloride (300 mg) was heated under reflux for 16 h then poured onto ice–water (100 g) and extracted with chloroform (3 × 50 ml). The resultant (1*S*)-[1-$^3H_1$]alcohol (21) was purified as above by p.l.c. on silica as for the (1*RS*)-alcohol; total activity 2 mCi. This was converted into the (1R)-[1-$^3H_1$]*amine hydrochloride* (25) exactly as earlier; total activity 0.8 mCi.

(1*S*)-2-(4-*Benzyloxy*-3-*methoxyphenyl*)[1-$^3H_1$]*ethylamine Hydrochloride* (31).—The aldehyde (19) was prepared from the acetal (17) (340 mg) by hydrogenation in methanol (15 ml) over 10% palladium–charcoal (50 mg) followed by acidic hydrolysis of the product as described above for a related case.

A solution of all the phenolic aldehyde so obtained, in 0.01M-sodium phosphate buffer (45 ml, pH 7.0) was incubated with liver alcohol dehydrogenase (30 mg), NAD^+ (30 mg), and [1-^{3}H]cyclopentanol (*ca.* 70 mCi total, see below). After 6 h at 37 °C, the product was worked up and *O*-benzylated as in the previous experiment. Addition of carrier material [unlabelled (26)] (200 mg) and purification by p.l.c., with recrystallisation to constant activity, gave the (1*R*)-[1-$^3H_1$]alcohol (26), m.p. 81 °C (222 mg, total activity 15 mCi) (lit.,[20] m.p. 80—81 °C). This product was converted into the (1S)-[1-$^3H_1$]*amine hydrochloride* (31) by the sequence already described; total activity obtained 5 mCi.

[1-^{3}H]*Cyclopentanol*.—Sodium borohydride (22 mg) was added to a solution of cyclopentanone (800 mg) in aqueous 0.01M sodium hydroxide (200 ml) and set aside for 1 h. An aliquot (2 ml) was then transferred directly onto sodium borotritiide (100 mCi; 6.3 Ci mmol^{-1}) and after 16 h, an excess of borohydride was added. The mixture was left to stand for 1 h and the aqueous solution of [1-$^3H_1$]cyclopentanol was removed by vacuum transfer and used directly.

Configurational Assay on [1-$^3H_1$,1-^{14}C]*Phenethylamines* (25) *and* (31).—The procedure involving *O*-debenzylation, treatment with liver alcohol dehydrogenase and diamine oxidase followed by re-benzylation, followed exactly that

used for preparation of the (1*S*)-[1-$^3H_1$]-alcohol (21). The quantities used in a typical run were 4-hydroxy-3-methoxyphenethylamine hydrochloride (7 mg), 0.01M sodium phosphate buffer (15 ml), liver alcohol dehydrogenase (2 mg), NAD^+ (5 mg), cyclopentanol (45 mg), and standard diamine oxidase preparation (1 ml). The incubation time was 24 h at 37 °C and sufficient radio-inactive (4-hydroxy-3-methoxyphenyl)ethanol was added as carrier to allow ready handling and purification to constant ^3H : ^{14}C ratio. The necessary [1-^{14}C]phenethylamine hydrochloride required for admixture with the ^{3}H-labelled material was prepared as described below.

2-(4-*Benzyloxy*-3-*methoxyphenyl*)[1-^{14}C]*ethylamine Hydrochloride*.—To a stirred solution of the chloride (15) (140 mg) in dry DMF (10 ml) was added potassium cyanide (20 mg). After 1 h, potassium [^{14}C]cyanide (2 mCi, 57 mCi mmol^{-1}) was added and after the mixture had been stirred for 2 d, unlabelled potassium cyanide (50 mg) was added, and stirring was continued for 1 day. The mixture was poured onto ice–water (50 g) and the ^{14}C-nitrile was extracted into chloroform, the extracts were washed with water and dried.

The nitrile from the chloroform in anhydrous THF (20 ml) was treated with sodium borohydride (400 mg) and boron trifluoride–diethyl ether (2.5 ml). After the mixture had been heated under reflux for 5 h, it was acidified with 2M-hydrochloric acid, diluted with water and extracted with chloroform (2 × 20 ml). The aqueous phase was adjusted to pH 9 with sodium hydroxide and the amine was extracted with methylene chloride. The recovered crude amine in ether was treated with ethereal hydrogen chloride and the precipitated [1-^{14}C]phenethylamine hydrochloride was recrystallised from methanol–ether (98 mg, 1.3 mCi).

Synthesis of (6R,14RS)-[6-$^3H_1$]*Scoulerine* (37), (6S,14RS)-[6-$^3H_1$]*Scoulerine* (39), *and the Corresponding* (6RS)-*Base* [(37) + (39)].—These materials were synthesised from the amine salts (25), (31), and [(25) + (31)] by the route developed earlier (ref. 11, see also refs. 3 and 6). The only significant change was replacement of ether by benzene for the preparation of the necessary substituted phenylacetyl chloride using oxalyl chloride. The final products were identified by comparison with authentic samples from the previous syntheses.

After each sample of [6-$^3H_1$]scoulerine had been mixed with (14*RS*)-[8-^{14}C]scoulerine, a small portion was diluted with unlabelled material and the product (250 mg) in methanol (10 ml) was treated at 5 °C with an excess of ethereal diazomethane. After 2 d, the solvents were evaporated off, the residue purified by p.l.c. on silica in ether and the (14*RS*)-tetrahydropalmatine (240 mg) was recrystallised to constant ^3H : ^{14}C ratio from aqueous methanol, m.p. 150—151 °C.

We thank Mr. J. K. Hulme (Ness Botanic Gardens, Wirrall) and Mr. J. Symonds (University Botanic Garden, Cambridge) for cultivation of the plants and the S.R.C. for a research studentship (M. C. S.), and the S.R.C. and the Nuffield Foundation for support.

[7/2032 *Received*, 18*th November*, 1977]

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