# Biosynthesis of Corydaline and of Ochotensimine[1]

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Radioactivity from [3-$^{14}$C]tyrosine and from [*methyl*-$^{14}$C]methionine is incorporated non-randomly into predicted positions of corydaline and ochotensimine in *Corydalis solida* and *C. ochotensis*, respectively. The methyl group of methionine supplies the *C*-methyl group of corydaline and the exocyclic methylene group of ochotensimine, as well as the "bridge" carbon atom and the exocyclic *O*- and *N*-attached one-carbon units of each alkaloid. Maintenance of the $^{3}H/^{14}C$ ratio of [*methyl*-$^{3}$H,$^{14}$C]methionine within these units of corydaline is consistent with incorporation of intact $CH_3$ groups.

Partial loss of $^{3}H$ relative to $^{14}C$ is observed in the course of a Schmidt reaction of [2-$^{3}$H,2-$^{14}$C] acetate.

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La radioactivité due à la [$^{14}$C-3] tyrosine et à la [$^{14}$C-méthyl]méthionine est respectivement introduite, d'une façon ordonnée, dans des positions prévues de la corydaline et d'ochotensimine dans la *Corydalis solida* et la *C. ochotensis*. Le groupe méthyle de la méthionine alimente le groupe *C*-méthyl de la corydaline, le méthylène exocyclique de l'ochotensimine ainsi que l'atome de carbone de pont et les groupements exocycliques à un atome de carbone relié à *O* ou *N* de chaque alcaloïde. La conservation du rapport $^{3}H/^{14}C$ de la [$^{3}$H-, $^{14}$C-méthyl] méthionine à l'intérieur de ces unités de corydaline est en accord avec l'hypothèse que les groupes $CH_3$ sont introduits d'une façon intacte. On observe une perte partielle de $^{3}H$ relative à $^{14}C$ durant la réaction de Schmidt effectuée sur de l'acétate [$^{3}$H-2, $^{14}$C-2].

[Traduit par le journal]

## Introduction

Chemical models which have been proposed (1) for the biogenesis of ochotensine (**1**) and ochotensimine (**2**), spirobenzylisoquinoline alkaloids which contain an exocyclic methylene group (2,3), envisage a skeletal rearrangement of a suitably functionalized protoberberine precursor in which a preformed *C*-methyl group is already present at the corresponding position, C-13, and is converted into the exocyclic methylene group in the course of the rearrangement (Scheme 1). Such a C-13 methylated protoberberine precursor would be closely related to the alkaloid corydaline (**5**). The simultaneous occurrence of corydaline and ochotensine in *Corydalis solida*[2] may then be more than coincidental.

The origin of the *C*-methyl group of corydaline has not been determined. The suggestion has been made (5) that this group arises from a one-carbon unit which is introduced into C-13 of a preformed protoberberine system (Scheme 2). An analogous chemical synthesis of the corydaline skeleton, by reaction of formaldehyde (6) or of methyl iodide (7) with a dihydroprotoberberine, has been accomplished.

An alternative proposal (8) for the biogenesis of corydaline (Scheme 3) does not invoke an intermediate containing a preformed protoberberine system, but envisages that the corydaline skeleton is generated by *de novo* combination of amino acid fragments. In particular, the segment of corydaline containing the *C*-methyl group is thought to be derived from a branched chain $C_6$—$C_3$ unit (*e.g.*, **6**), analogous in structure to tropic acid (**13**).

It was the aim of the present study to establish the origin of the *C*-methyl group of corydaline and the exocyclic methylene group of ochotensimine. The evidence to be presented demon-

[1]This paper is dedicated to Professor Otto Hoffmann-Ostenhof, Professor of Biochemistry, Institute of General Biochemistry, University of Vienna, Austria, on the occasion of his 60th birthday, October 18, 1974.

[2]C. K. Yu, H. L. Holland, D. B. MacLean, R. H. F. Manske, and R. Rodrigo. Unpublished observation (1972).

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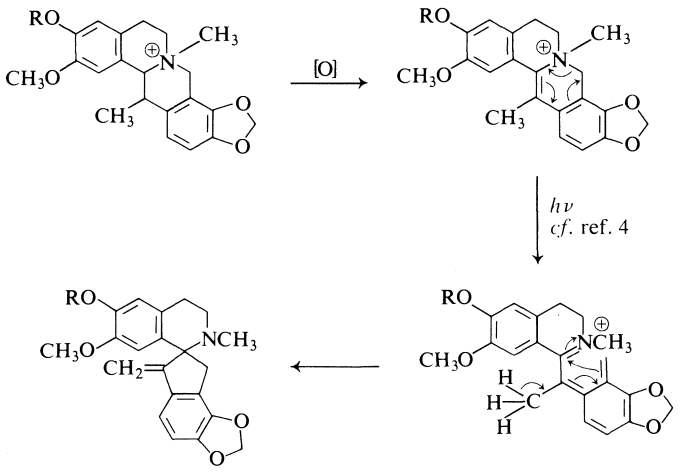


**1** R = H (Ochotensine)
**2** R = $CH_3$ (Ochotensimine)

SCHEME 1 Biogenesis of ochotensimine. One of several possible routes (*cf.*, refs. 1, 4) for the transformation of the protoberberine into the spirobenzylisoquinoline skeleton

strates unequivocally that in each case a one-carbon unit serves as the precursor. The findings lend support to the view that the spirobenzylisoquinoline and the 13-methylprotoberberine systems are further structural variants among the alkaloids related to norlaudanosoline (**3**), as had been widely assumed.

## Methods and Results

In six tracer experiments, specifically labelled samples of methionine, tyrosine, and phenylalanine were administered by the wick method to intact plants of *Corydalis solida* (L.) Swartz (Expts. *S*1, *S*2, *S*3) and of *Corydalis ochotensis* Turcz. (Expts. *O*1, *O*2, *O*3). Samples of corydaline and of ochotensine and ochotensimine, respectively, were isolated from these plants. Corydaline was purified to constant radioactivity by repeated crystallization and sublimation at reduced pressure. Ochotensine was purified by crystallization. Ochotensimine was converted into the methiodide which was crystallized to constant radioactivity. The details of these experiments are recorded in Tables 1 and 2.

The alkaloids from the feeding experiments with methionine and tyrosine were highly radioactive (Expts. *S*1, *S*2, *S*3, *O*1, *O*3). Label from phenylalanine was not incorporated to a significant extent into the alkaloids of *C. ochotensis* (Expt. *O*2).

The labelled samples of corydaline and of ochotensimine, obtained from individual feeding experiments, were diluted with inactive carrier and degraded by reactions, described in the Experimental section, into the degradation products shown in Schemes 4 and 5. The specific activities of the degradation products are listed in Tables 3 and 4 (indicated limits are standard deviation of the mean).

The samples of corydaline and of ochotensimine, derived from the experiments with [3-$^{14}$C]tyrosine (Expts. *S*3 and *O*3) yielded acetic acid containing one-half of the activity of the intact alkaloid (Tables 3 and 4). Since the methylamine obtained by further degradation of the acetic acid was devoid of activity in each case, it was the carboxyl group of the acetate, and hence the ring carbon of corydaline and of ochotensimine to which the *C*-methyl and the *C*-methylene group, respectively, is attached, which contained one-half of the activity of each alkaloid molecule.

The sample of ochotensimine, derived from the experiment with [*methyl*-$^{14}$C]methionine

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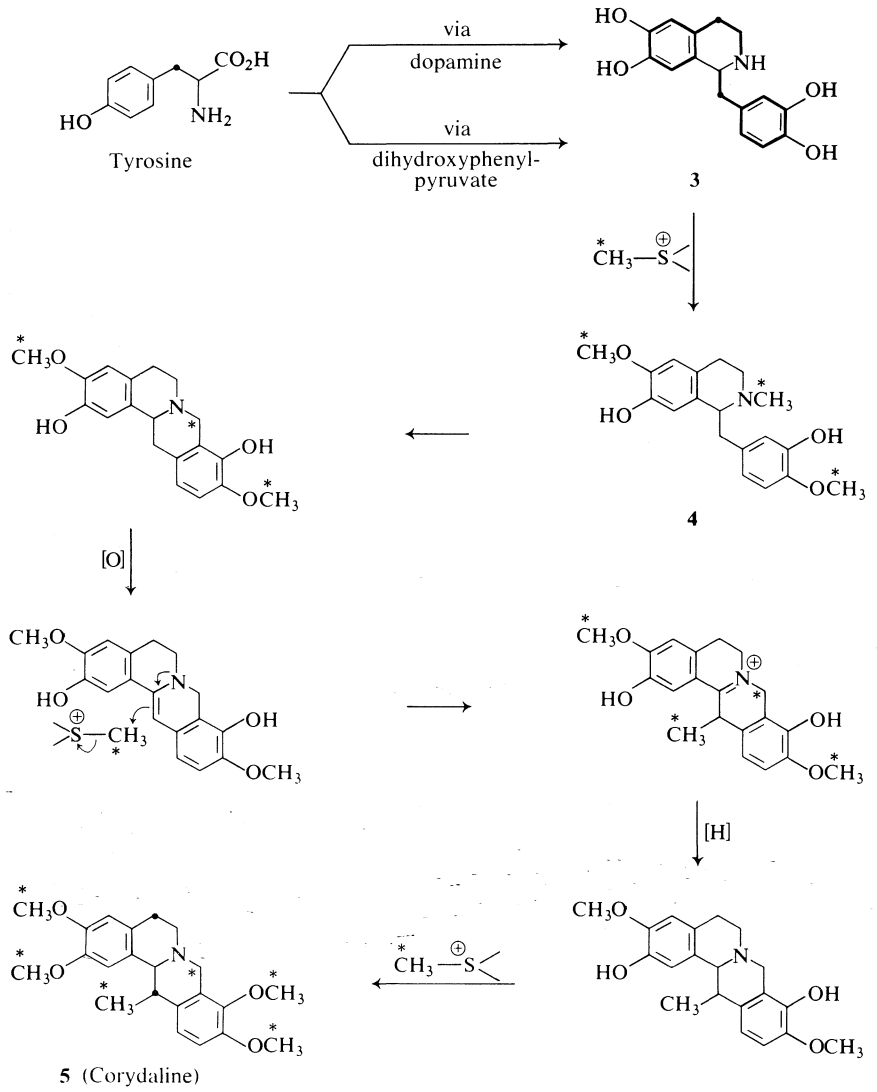


SCHEME 2. Protoberberine route to corydaline

(Expt. *O*1) contained approximately one-sixth of its activity within the exocyclic methylene group (Schmidt methylamine), and one-sixth within the methylenedioxy group (formaldehyde). The phthalic acid derivative (**11**) obtained from rings C and D contained one third of the total activity. Half of this activity, *i.e.* one-sixth of the total, is accounted for by the methylenedioxy group (Table 4).

The corydaline derived from [*methyl*-$^{14}$C]methionine (Expt. *S*1 and Expt. *S*2, $^{14}$C activity) contained one fifth of its $^{14}$C-activity at the *C*-methyl group (Schmidt methylamine) and one fifth at the berberine bridge (C-8) (benzoic acid). Des-*O*-methylcorydaline (**7**) accounts for two fifths of the $^{14}$C-activity of corydaline. The relative specific activity of tetramethylammonium chloride, representing the average $^{14}$C activity of four *O*-methyl groups, was one sixth that of the intact alkaloid. The four *O*-methyl groups thus

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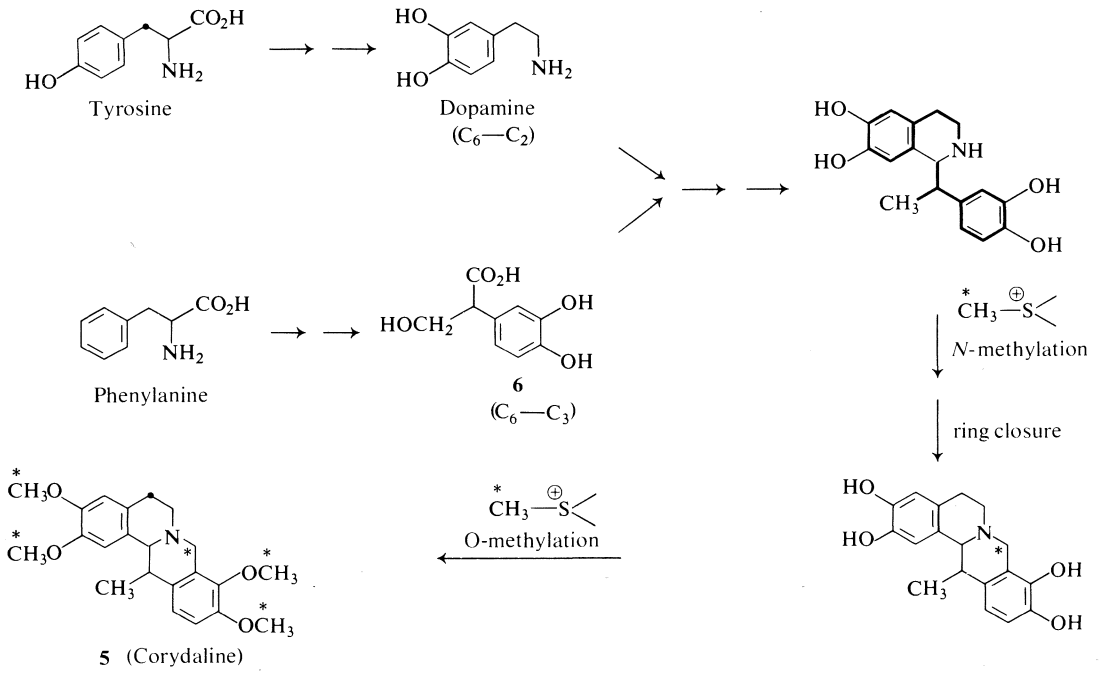


SCHEME 3. Biogenesis of corydaline. Route from a branched $C_6$—$C_3$ precursor (now disproved)

account for two thirds of the $^{14}C$ activity of the alkaloid (Table 3).

The $^{3}H/^{14}C$ ratio found within the corydaline derived from [*methyl*-$^{3}H$,$^{14}C$]methionine (Expt. *S*2) is compared with the $^{3}H/^{14}C$ ratio of the precursor and with that observed within the degradation products of corydaline (Table 5). Some loss of $^{3}H$, relative to $^{14}C$ (10–20%) in corydaline and in all but one of its degradation products is observed, compared to the $^{3}H/^{14}C$ ratio of the doubly labelled methionine.

Schmidt degradation of [2-$^{3}H$,2-$^{14}C$]acetate under three different experimental conditions (sulfuric acid (98 and 85% w/v) and phosphoric acid (85%)) leads to [$^{3}H$,$^{14}C$]-methylamine whose $^{3}H/^{14}C$ ratio is approximately 5% lower than that of the acetate from which it is derived (Table 6).

## Discussion

Several tests can be applied to invalidate one or the other of the two hypotheses which have been advanced to account for the origin of corydaline.

The simplest of these tests is a tracer experiment with a one-carbon donor such as [*methyl*-$^{14}C$]methionine. The protoberberine route (5) to corydaline demands that the *C*-methyl group is derived from such a precursor. The branched chain route (8) demands that it is not. In the case of the protoberberine route (9), and probably also in the other case, five other sites within the molecule, namely the "berberine bridge" (C-8) and four *O*-methyl functions, will incorporate the tracer if corydaline formation occurs at the time of the experiment, and this serves as internal evidence of active biosynthesis (Scheme 6).

Tyrosine would serve as a precursor of the two $C_6$—$C_2$ units which complete the skeleton of the molecule, if corydaline originated via the protoberberine route (10). In the case of the other postulated route to corydaline only one tyrosine derived $C_6$—$C_2$ unit is likely to be involved. The other fragment required to complete the corydaline skeleton by this route, a phenolic branched chain $C_6$—$C_3$ unit, might be derived from phenylalanine or tyrosine by rearrangement of the carbon chain. Such a rearrangement might be analogous to that which leads, by a carboxyl migration of unknown mechanism, from phenylalanine (**12**) to tropic acid (**13**) (11) or it might be similar to that which leads from phenylalanine, by phenyl migration, to the branched chain phenylpropanoid moiety of the isoflavones (*e.g.*,

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TABLE 1. Experiments with *Corydalis solida*

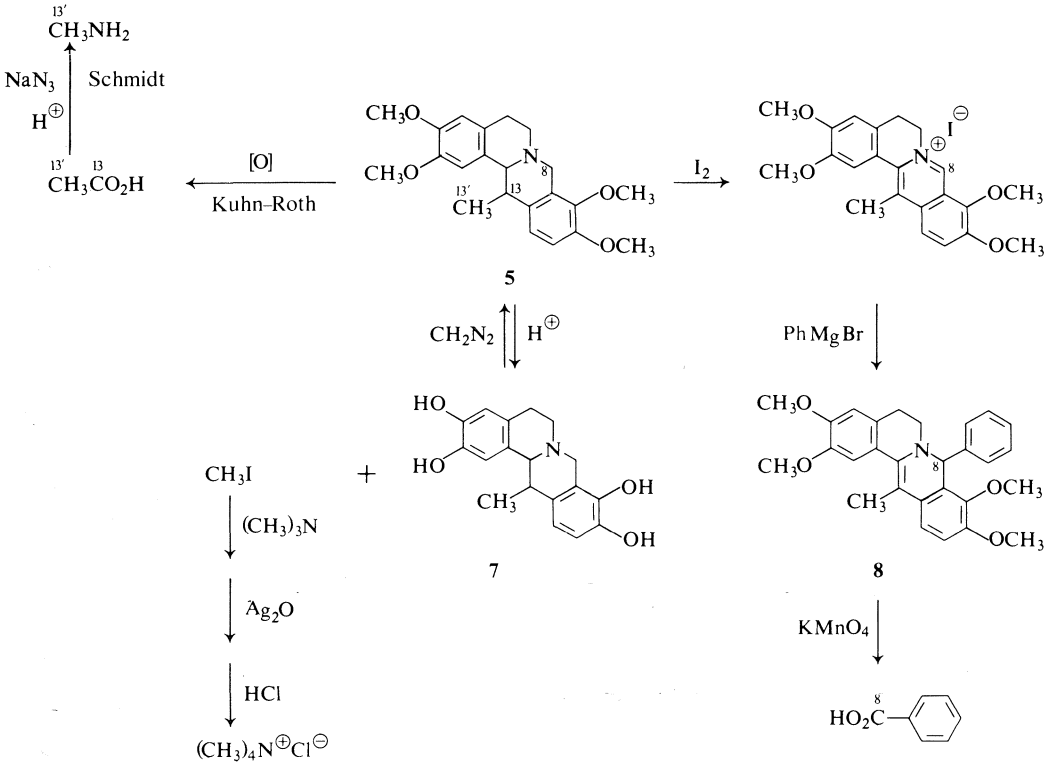
| Expt. No. | Substrate | Nominal activity: Specific (mCi/mmol) | Nominal activity: Total (mCi) | No. of plants | Date | Product, corydaline (**4**): (mg) | Product, corydaline (**4**): Specific activity ($^{14}$C) (d.p.m./mmol) |
|---|---|---|---|---|---|---|---|
| *S*1 | L-[*methyl*-$^{14}$C]Methionine[a] | 60 | 0.1 | 25 | April 1970 | 10 | $2.5 \times 10^7$ |
| *S*2 | L-[*methyl*-$^{14}$C]Methionine[a]<br>L-[*methyl*-$^{3}$H]Methionine[a] | 56<br>5000 | 0.1<br>1.0 | 16 | May 1972 | 9 | $5.5 \times 10^7$ |
| *S*3 | DL-[3-$^{14}$C]Tyrosine[b] | 11 | 0.1 | 16 | May 1972 | 12.5 | $4.1 \times 10^7$ |

[a]Amersham/Searle.
[b]New England Nuclear.

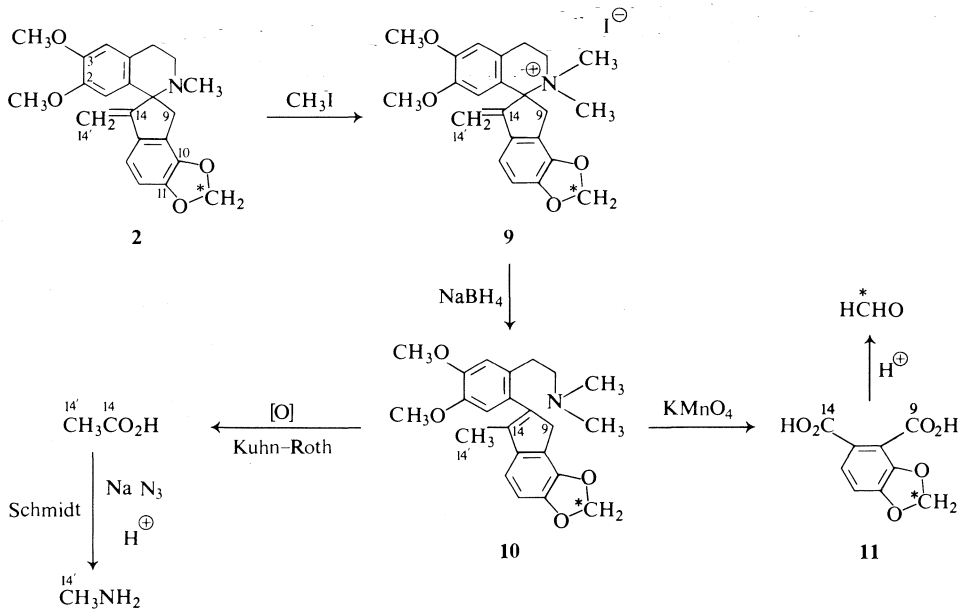
TABLE 2. Experiments with *Corydalis ochotensis*

| Expt. No. | Substrate | Nominal activity: Specific (mCi/mmol) | Nominal activity: Total (mCi) | No. of plants | Date | Products: Ochotensimine (**6**): Yield (mg) | Products: Ochotensimine (**6**): SA (d.p.m./mmol) | Products: Ochotensine (**5**): Yield (mg) | Products: Ochotensine (**5**): SA (d.p.m./mmol) |
|---|---|---|---|---|---|---|---|---|---|
| *O*1 | L-[*methyl*-$^{14}$C]Methionine[a] | 56 | 0.1 | 4 | July 1972 | 95 | $7.6 \times 10^6$ | 16 | $5.8 \times 10^4$ |
| *O*2 | DL-[3-$^{14}$C]Phenylalanine[b] | 50 | 0.1 | 5 | July 1971 | 62 | $5.8 \times 10^3$ | 20 | $2.5 \times 10^3$ |
| *O*3 | DL-[3-$^{14}$C]Tyrosine[b] | 50 | 0.1 | 8 | August 1972 | 272 | $2.3 \times 10^5$ | 24 | $1.1 \times 10^6$ |

[a]Amersham/Searle.
[b]Commissariat à l'Énergie Atomique, France.




SCHEME 4. Degradation of corydaline




SCHEME 5. Degradation of ochotensimine

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TABLE 3. Distribution of label within corydaline

| Products | Carbon atoms of corydaline | Substrates: L-[*methyl*-$^{14}$C]Methionine Expt. *S*1 SA[a] | RSA[b] | L-[*methyl*-$^{14}$C]Methionine Expt. *S*2 SA[ac] | RSA[b] | DL-[3-$^{14}$C]Tyrosine Expt. *S*3 SA[a] | RSA[b] |
|---|---|---|---|---|---|---|---|
| Corydaline (diluted) (**4**) | All | 10.11 ± 0.22[d] | 100 ± 2 | 90.43 ± 0.87[d] | 100 ± 1 | 15.64 ± 0.19[d] | 100 ± 1 |
| Kuhn–Roth acetate (as α-naphthylamide) | C-13,13′ | 1.91 ± 0.05 | 19 ± 1 | 18.61 ± 0.25 | 21 ± 1 | 7.84 ± 0.16 | 50 ± 1 |
| Schmidt methylamine (as β-naphthoyl derivative) | C-13′ | | | 17.30 ± 0.16 | 19 ± 1 | 0.01 ± 0.02 | 0 |
| Corydaline (diluted) (**4**) | All | 10.11 ± 0.22[d] | 100 ± 2 | 76.20 ± 0.53[d] | 100 ± 1 | | |
| Methyl iodide (as tetramethylammonium chloride) | Average of four $OCH_3$ groups | 1.67 ± 0.04 | 17 ± 1 | 11.96 ± 0.52 | 16 ± 1 | | |
| Corydaline (diluted) (**4**) | All | | | 98.01 ± 0.94[d] | 100 ± 1 | | |
| Benzoic acid | C-8 | | | 17.37 ± 0.48 | 18 ± 1 | | |
| Corydaline (diluted) (**4**) | All | | | 72.85 ± 0.30[d] | 100 ± 1 | | |
| Des-*O*-methylcorydaline(**7**) | All except four $OCH_3$ groups | | | 28.10 ± 0.81 | 39 ± 1 | | |

[a]SA = specific activity (d.p.m. per mmol) × $10^{-4}$.
[b]RSA = relative specific activity (%) (intact corydaline = 100).
[c]Specific activity due to $^{14}$C only.
[d]Obtained from the original labelled corydaline (Table 1) by dilution with inactive corydaline.

TABLE 4. Distribution of label within ochotensimine

| Product | Carbon atoms of ochotensimine | Substrates: Expt. *O*1 L-[*methyl*-$^{14}$C]Methionine SA[a] | Expt. *O*1 RSA[b] | Expt. *O*3 DL-[3-$^{14}$C]Tyrosine SA[a] | Expt. *O*3 RSA[b] |
|---|---|---|---|---|---|
| Reduction product (**10**) of ochotensimine methiodide[c] | All | 64.01±0.57 | 100±1 | 9.24±0.11 | 100±1 |
| Kuhn–Roth acetate (as α-naphthylamide) | C-14,14′ | 10.30±0.19 | 16±1 | 4.53±0.08 | 49±1 |
| Schmidt methylamine (as β-naphthoyl derivative) | C-14′ | 10.42±0.16 | 16±1 | 0.05±0.03 | 1±1 |
| 3,4-Methylenedioxyphthalic acid (**11**) (as *N*-ethylimide) | C-9 to 14, —$OCH_2O$— | 21.36±0.26 | 33±1 | | |
| Formaldehyde (as dimedone derivative) | —$OCH_2O$— | 10.47±0.11 | 16±1 | | |

[a]SA specific activity (d.p.m. per mmol) × $10^{-4}$.
[b]RSA = relative specific activity (%) (reduction product of ochotensimine methiodide = 100).
[c]Obtained from the original labelled ochotensimine (Table 2) by dilution with inactive ochotensimine, followed by methylation and borohydride reduction.

TABLE 5. The $^3H/^{14}C$ ratio at the one-carbon sites of corydaline derived from L-[*methyl*-$^3$H,$^{14}$C]methionine (Expt. *S*2)

| | | $^3H/^{14}C$ ratio | % retention of $^3H$, relative to $^{14}C$: Observed | Calculated |
|---|---|---|---|---|
| Precursor L-[*methyl*-$^3$H,$^{14}$C]methionine | | 10.4±0.1 | 100±1 | |
| Products | One-carbon units | | | |
| Corydaline (**4**) | All six | 9.6±0.1 | 92±1 | 17/18 = 94.4 |
| Des-*O*-methylcorydaline (**7**) | C-8 plus C-13′ | 8.6±0.1 | 82±1 | 5/6 = 83.3 |
| Methyl iodide (as tetramethylammonium chloride) | Average of four $OCH_3$ | 10.7±0.1 | 103±1 | 3/3 = 100 |
| Kuhn–Roth acetate (as α-naphthylamide) | C-13′ | 9.8±0.1 | 93±1 | 3/3 = 100 |
| Corrected[a] | | 10.5±0.2 | 101±2 | |
| Schmidt methylamine (as β-naphthoyl derivative) | C-13′ | 9.1±0.1 | 87±1 | 3/3 = 100 |

[a]See text.

TABLE 6. Schmidt reaction of [2-$^3$H,2-$^{14}$C]acetate

| Solvent | $^3H/^{14}C$ ratio of acetate (as α-naphthylamide) | $^3H/^{14}C$ ratio of methylamine (as β-naphthoyl derivative) | Tritium loss (%) |
|---|---|---|---|
| Sulfuric acid (98% w/v) | 21.0±0.1 | 20.1±0.1 | 4.3 |
| Sulfuric acid (85% w/v) | 21.0±0.1 | 19.5±0.1 | 7.2 |
| Phosphoric acid (85%) | 21.0±0.1 | 20.3±0.1 | 3.4 |

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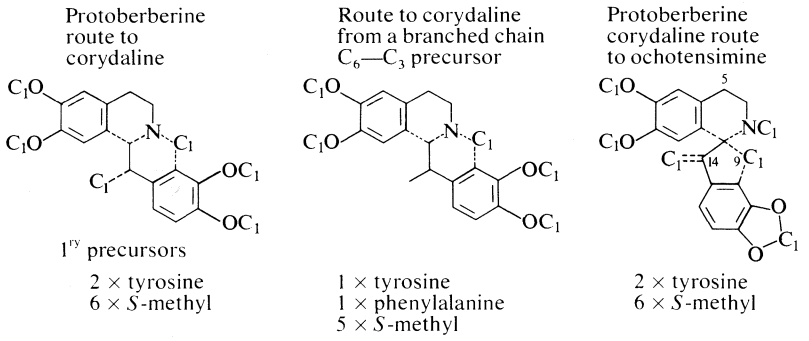
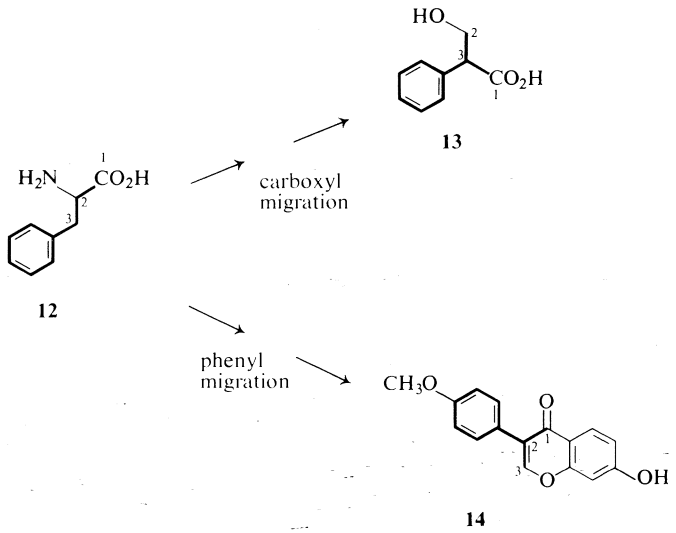



SCHEME 6. Construction of the skeletons of corydaline and ochotensimine from precursor fragments




SCHEME 7. Rearrangement of phenylalanine to tropic acid and to the branched phenylpropanoid unit of the isoflavones (numbers refer to C-atoms of phenylalanine)

formononetin (**14**)) (12) and rotenoids (13) (Scheme 7).

Since phenolic $C_6$—$C_3$ units which participate in alkaloid biosynthesis appear to be derived from phenylalanine (*e.g.*, colchicine (14), decodine, and decinine (15)), whereas tyrosine supplies phenolic $C_6$—$C_2$ units, phenylalanine would be a more likely precursor than tyrosine. In most higher plants phenylalanine is not convertible into tyrosine (16).

In the event, the *C*-methyl group (C-13′) of corydaline was found to originate from the *S*-methyl group of methionine (Table 3, Expts. *S*1 and *S*2). The Kuhn–Roth acetate (C-13′, -13) obtained from [*methyl*-$^{14}$C]methionine-derived corydaline contained 20% of the total activity of the molecule, all of which, within experimental error, was shown to reside in the *C*-methyl group (C-13′) recovered as methylamine in a Schmidt reaction. The berberine bridge (C-8), recovered as benzoic acid, contained 18% of the total label. That no carbon atom within the carbon skeleton of corydaline, other than C-8 and C-13′, contained radioactivity is shown by the observation that des-*O*-methylcorydaline (**7**) contained 39 ± 1% of the total activity of the alkaloid. The label within des-*O*-methylcorydaline (**7**) is fully accounted for, within experimental error, by C-8 plus C-13′ ((19 ± 1) + (18 ± 1) = 37 ± 1%). The four *O*-methyl groups contain the rest of the activity (4 × (16 ± 1) = 64 ± 4%).

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It is noteworthy that the six one-carbon units do not each contain exactly one sixth of the activity of the alkaloid. This is to be expected, since, even though the six units arise from the same precursor, they are introduced into the product at different stages of biosynthesis. However, even though it might be tempting to do so, it is not possible on the basis of the observed data to draw conclusions regarding the sequence in which the units enter the product.

The distribution of activity within corydaline, derived from [3-$^{14}$C]tyrosine, is in accord with prediction and consistent with the protoberberine route of biosynthesis. Half of the activity within the molecule resides at C-13, the benzylic carbon of one of the $C_6$—$C_2$ units of the protoberberine system (Kuhn–Roth acetate (C-13′, -13) minus Schmidt methylamine (C-13′)) (Expt. *S*3, Table 3). The other half of the activity was not extruded, but can be predicted to be localized at C-5, the benzylic carbon of the other $C_6$—$C_2$ unit (*cf.* ref. 10).

The distribution of activity within corydaline derived from [*methyl*-$^{14}$C]methionine and [3-$^{14}$C]tyrosine is clearly consistent with the protoberberine route (Scheme 2) and inconsistent with the route from a branched chain $C_6$—$C_3$ precursor (Scheme 3).

A similar conclusion was arrived at on the basis of the incorporation into corydaline of label from [*N-methyl*-$^{14}$C]reticuline (**4**) (17). Since the site of activity (presumably C-8) within the corydaline was not determined the experiment was indicative rather than conclusive.

If the spirobenzylisoquinoline system of ochotensimine were indeed derived by rearrangement of a corydaline-type skeleton (Scheme 1), whose *C*-methyl group yields the exocyclic methylene group of the product, label from [*methyl*-$^{14}$C] methionine and [3-$^{14}$C]tyrosine must enter sites within the ochotensimine skeleton which correspond to those now established for corydaline. The results of the tracer experiments with ochotensimine, summarized in Table 4, agree with this prediction.

One half of the activity of ochotensimine derived from [3-$^{14}$C]tyrosine resides at C-14 (Kuhn–Roth acetate (49 $\pm$ 1) minus Schmidt methylamine (1 $\pm$ 1) = 48 $\pm$ 1) (Expt. *O*3). The other half of the label, which was not isolated, is presumably located at C-5.

Ochotensimine, derived from [*methyl*-$^{14}$C] methionine, contains one-sixth of its activity at the exocyclic methylene (Schmidt methylamine) and one-sixth at the methylenedioxy group (formaldehyde). C-14, the C-atom to which the exocyclic methylene group is attached, is free of activity (Kuhn–Roth acetate (16 $\pm$ 1) minus Schmidt methylamine (16 $\pm$ 1) = 0 $\pm$ 1%). Since the 3,4-methylenedioxyphthalic acid derivative (**11**) contained two-sixths of the total activity, half of which resides in the methylenedioxy group, and since one of its two carboxyl groups (that derived from C-14 of ochotensimine) is devoid of label, and since the nucleus is part of a tyrosine-derived $C_6$—$C_2$ unit, the remaining sixth of the total activity must be located at the other carboxyl group, derived from C-9, which thus corresponds to the "berberine bridge". Half of the total label of the alkaloid is thus accounted for in terms of three sites, namely the exocyclic methylene group, C-9, and the methylenedioxy group. The other half of the activity, presumably located at the *N*-methyl and the two *O*-methyl groups, was not separated. Label from [3-$^{14}$C] phenylalanine, a putative precursor of the branched chain $C_6$—$C_3$ unit (*vide supra*) was not incorporated significantly into ochotensine or ochotensimine (Expt. *O*2).

The tracer evidence is thus entirely consistent with the view that the skeleton of spirobenzylisoquinolines such as ochotensimine arises by rearrangement of a C-13 methylated protoberberine skeleton of the corydaline type, which, in turn, originates from a preformed protoberberine intermediate by introduction of a one-carbon unit.

That this one-carbon unit originates from a $CH_3$-group, presumably the *S*-methyl group of methionine, and that this group is incorporated intact, without C—H bond cleavage, is made likely by the results, presented in Table 5, of an experiment (Expt. *S*2) with L-[*methyl*-$^{3}$H,*methyl*-$^{14}$C]methionine.

Corydaline contains six one-carbon sites (Table 3). Five of these, the *C*-methyl group (C-13′) and the four *O*-methyl groups are each associated with three H atoms. The sixth, the berberine bridge (C-8), originates by oxidative cyclization of an *N*-methyl group (18), a reaction in the course of which one of the H atoms must be extruded.

Thus, if *S*-methyl groups supplied all six one-carbon sites as well as the H atoms which are

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attached to them, a maximum of 17 of the original 18 H atoms associated with the precursor methyl groups could be retained within corydaline. A maximum of 94.4% (17/18) of the tritium, relative to $^{14}C$, of the doubly [*methyl*-$^{3}H$,$^{14}C$]-labelled precursor would then be recovered within the alkaloid in the absence of an isotope effect. The observed value (92 ± 1%) (Table 5) agrees with this prediction. That the *O*-methyl groups represent intact precursor-methyls is shown by the quantitative retention of tritium, relative to $^{14}C$, within the tetramethylammonium chloride obtained as the degradation product representing these groups. The des-*O*-methylcorydaline (**7**), which retains the remaining two one-carbon sites, the *C*-methyl group (C-13′) (3 H) and the bridge (C-8) (2 H), retains 82% $^{3}H$, relative to $^{14}C$. The predicted value for retention of five of six H atoms is 83.3%.

The observed $^{3}H/^{14}C$ ratios are entirely consistent with and therefore support the view that the *O*-methyl and *C*-methyl groups of corydaline represent precursor $CH_3$-groups whose H atoms are entirely retained, and that in the formation of the berberine bridge two of the original three H atoms of the methyl precursor are preserved.

At first sight, the 7% loss, rather than quantitative retention of tritium, relative to $^{14}C$, within the Kuhn–Roth acetate, representing the one-carbon unit, C-13′, of corydaline together with the ring carbon to which it is attached (Table 5), appears to be inconsistent with the other results. However, not all the $^{14}C$ within this acetate resides within the methyl group. It is evident from Table 3 (Expt. *S*2) that of (18.61 ± 0.25) × $10^4$ d.p.m. per mmol (specific activity of Kuhn–Roth acetate) only (17.30 ± 0.16) × $10^4$ d.p.m. per mmol (specific activity of Schmidt methylamine), *i.e.* 93 ± 2% of the total activity of the acetate, resides within its methyl group. If the observed $^{3}H/^{14}C$ ratio of the Kuhn–Roth acetate (9.8 ± 0.1) is corrected to allow for this scatter of label

$$\left((9.8 \pm 0.1)\Big/\frac{(17.30 \pm 0.16) \times 10^4}{(18.61 \pm 0.25) \times 10^4} = 10.5 \pm 0.2\right)$$

retention of tritium, relative to $^{14}C$, within the methyl group of acetate, *i.e.*, within the *C*-methyl group of corydaline, is indicated, in conformity with the other results.[3]

Schmidt degradation of the Kuhn–Roth acetate ($^{3}H/^{14}C$ ratio observed 9.8 ± 0.1, corrected 10.5 ± 0.2) yielded methylamine whose $^{3}H/^{14}C$ ratio (9.1 ± 0.1) was significantly lower than that of the acetate. This apparent tritium loss under the conditions used in the Schmidt reaction (concentrated $H_2SO_4$) is presumably due to exchange. That the loss is indeed inherent in the Schmidt reaction of the doubly labelled acetate is indicated by the results of a series of experiments in which [2-$^{3}H$,2-$^{14}C$]acetate of known $^{3}H/^{14}C$ ratio was subjected to this reaction under different experimental conditions (Table 6). In each case a small but significant loss of tritium, relative to $^{14}C$, was observed. It may well be that, by a more extensive search, conditions for quantitative tritium retention in the course of this reaction can be found. Until such a search is successful, however, the present results serve to indicate that caution is advisable in the interpretation of $^{3}H/^{14}C$ ratios obtained in the products of a Schmidt reaction.

## Experimental

*Administration of Labelled Compounds to Corydalis solida (L.) Swartz and to Corydalis ochotensis Turcz*

*Plants*

*Corydalis solida*, a perennial, and *Corydalis ochotensis*, a biennial, were propagated out of doors. *Corydalis solida* was potted and transferred to the greenhouse in April, and kept at a temperature not exceeding 18 °C. The experiments were carried out in May. *Corydalis ochotensis* was potted and transferred to the greenhouse in May. The feeding experiments were carried out 2 months later. Voucher specimens of both species are deposited in the herbarium of the Royal Botanical Gardens, Hamilton.

*Administration of Labelled Compounds*

The labelled compounds were administered to the plants by infusion into the stem, using the cotton wick technique described elsewhere (10). The plants were kept in contact with the radioactive tracers for 3–4 days. The details of the individual feeding experiments are summarized in Tables 1 and 2.

*Extraction of the Alkaloids*

*Corydaline*

After exposure to the radioactive amino acid for 4

[3]Correction for scatter of label of the $^{3}H/^{14}C$ ratios of corydaline and des-*O*-methylcorydaline does not affect the ratios significantly, since the amount of $^{14}C$, at centers other than those derived from one-carbon units, represents a much smaller fraction of the total $^{14}C$ than in the case of the Kuhn–Roth acetate. Such correction would increase the ratios and bring them closer to the calculated values.

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days, the plant material from 16 plants (aerial parts and corms, fresh weight, typically 80 and 40 g, respectively), of *Corydalis solida* (L.) Swartz was dried at 40° for 72 h. In a typical feeding, the yield of dry plant material was 20 g. The corydaline was extracted by a method similar to that used by Manske (19); the finely ground plant material was extracted with methanol in a Soxhlet apparatus for 48 h. After this time, the solution was evaporated and the residue dissolved in hydrochloric acid (5%, 50 ml), the solution filtered, and the filtrate extracted with chloroform. The chloroform extract was dried over $Na_2SO_4$, evaporated, and the residue redissolved in 5% hydrochloric acid (30 ml). The acidic solution was then washed with ether, basified with 10% KOH and then thoroughly re-extracted with ether. The ethereal extract was dried and evaporated to give a residue (Manske fraction BC, typically 200 mg) which was redissolved in the minimum quantity (*ca.* 5 ml) of methanol.

A sample of the methanolic solution was examined by t.l.c. (Merck silica gel F-254; 2% methanol in chloroform). Five components, at $R_f$ 0.1, 0.2, 0.3, 0.5, and 0.65, were detected under u.v. light. Most of the radioactivity of the fraction was shown by radioscanning (Radiochromatogram Scanner, Model 7201 Packard Instrument Company) to be associated with the major component, corydaline, at $R_f$ 0.5. Inactive corydaline (typically 200–400 mg) was added to the methanol solution and the total corydaline allowed to crystallize from this solution. Recrystallization from methanol gave corydaline (typically 200–400 mg) melting at 135–136° (lit. (19) m.p. 135°), which was sublimed at 100–110° and $5 \times 10^{-3}$ mm to give the sample used in the degradation experiments.

*Ochotensine and Ochotensimine*

The plant material (aerial parts and roots, fresh weight, typically 160 and 30 g, respectively) from five plants of *Corydalis ochotensis* Turcz used in each radioactive feeding was dried and ground to a fine powder (typically 20 g) which was extracted as outlined above to give fraction BC of the Manske extraction procedure (20). Radioscanning of a thin-layer chromatogram (Merck silica gel F-254; 2% methanol in chloroform) of the material isolated from the methionine and tyrosine feedings showed most of the activity to be associated with ochotensimine at $R_f$ 0.4. A similar scan of the crude product obtained from the phenylalanine feeding experiment failed to detect significant activity on the thin-layer plate. The crude fraction BC was dissolved in a small quantity of benzene and applied to a silica gel column (Woelm activity 1, 100 g). A 5% stepwise elution from benzene to chloroform afforded ochotensimine as a pale yellow oil. Further elution of the column with 5% methanol in chloroform gave ochotensine, which on crystallization from chloroform melted at 247–250° (lit. (20) m.p. 252°). Radioactive ochotensimine was diluted with inactive material before degradation.

*Degradation of Corydaline (5) (Scheme 4)*

*Carbon-13 and the C-Methyl Group as Acetic Acid*

Chromium trioxide (4 g) was added to a solution of corydaline (200 mg) in sulfuric acid (4 *M*, 20 ml) and the mixture subjected to the Kuhn–Roth oxidation procedure (21). The acetic acid formed was converted to sodium acetate by titration with 0.1 *M* sodium hydroxide and the solution divided into two equal portions. One portion was evaporated to give sodium acetate (8 mg) which was redissolved in water (1 ml) and reacted with α-naphthylamine hydrochloride (15 mg) as described (22) to give *N*-acetyl-α-naphthylamine (3 mg), m.p. 158–160° after sublimation at 80–90° and $1 \times 10^{-3}$ mm (lit. (22) m.p. 159–160°).

*The C-Methyl Group as Methylamine*

The second portion of the sodium acetate solution from the Kuhn–Roth oxidation (see above) was evaporated to dryness to give sodium acetate (8 mg), which was reacted with sodium azide (50 mg) and concentrated sulfuric acid (1 ml) as described (21). The methylamine evolved upon basification of the reaction mixture was trapped by passage through three traps each containing 1 ml of 0.1 *M* HCl and the resulting solution evaporated at 90° to give methylamine hydrochloride (4 mg). This was dissolved in dry dimethylsulfoxide (0.5 ml) and added to a solution of β-naphthoyl chloride (40 mg) in pyridine (1 ml). The solution was kept at room temperature for 12 h and was then poured onto water (4 ml) and extracted with ether ($3 \times 5$ ml). The ethereal extract was washed with aqueous sodium bicarbonate (2 *M*, $3 \times 5$ ml) and with water ($3 \times 5$ ml), was dried over sodium sulfate, and evaporated to give a residue which was crystallized from benzene and sublimed at 75–80° and $5 \times 10^{-3}$mm. The resulting *N*-methyl-β-naphthoamide (3 mg) melted at 107–109° (sealed capillary) (lit. (23) m.p. 108–109.5°).

*Carbon-8 as Benzoic Acid*

The procedure used followed the published method (9) for the isolation of C-8 of berberine as benzoic acid. Iodine (380 mg) was added to a solution of corydaline (280 mg) in anhydrous ethanol (50 ml) and the solution was stirred at room temperature for 12 h. The solvent was then removed and the resulting dehydrocorydaline iodide dried *in vacuo* for 12 h at room temperature and $5 \times 10^{-3}$ mm. The salt was then suspended in dry ether (50 ml) and the stirred suspension treated with a solution of phenylmagnesium bromide (0.05 mol) in ether (50 ml). After 2 h at reflux temperature, the solution was cooled and excess phenylmagnesium bromide decomposed by the careful addition of ice (100 g). The resulting solution was extracted with ether, and the ethereal extract re-extracted with 5% hydrochloric acid. The acid extract was then basified with 10% aqueous ammonia and extracted with ether. The ether extract was washed with water, dried ($Na_2SO_4$), and evaporated to give crude 8-phenyl-13,14-didehydrocorydaline (**8**) (200 mg) which was used in the next stage without further purification.

Potassium permanganate (1.5 g) was added at room temperature over a period of 72 h to a stirred suspension of 8-phenyl-13,14-didehydrocorydaline (**8**) (200 mg) in water containing potassium carbonate (1.5 g). Work-up of the reaction by the procedure outlined (9) gave benzoic acid (6 mg, 10.5%), which after sublimation at 80–90° and $1 \times 10^{-2}$mm, and recrystallization from water, melted at 119–120°.

*The O-methyl Groups as Tetramethylammonium Chloride*

Corydaline (200 mg) was reacted with freshly distilled hydriodic acid (*d* 1.7, 10 ml) and the evolved methyl iodide converted to tetramethylammonium iodide (0.13 g) by the described procedure (9). The product was recrystallized from methanol and converted into tetramethylammonium chloride as follows. Freshly precipita-

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ted silver oxide (250 mg) was added to a solution of the iodide (100 mg) in water (10 ml), and the resulting suspension was stirred at room temperature for 20 h. The suspension was filtered and the filtrate neutralized with 0.1 *M* HCl. The neutral solution was evaporated to dryness at 80° and the residue was crystallized from methanol yielding tetramethylammonium chloride (44 mg).

*Des-O-methylcorydaline (7) and its Treatment with Diazomethane*

A solution of corydaline (50 mg) in freshly distilled hydrobromic acid (5 ml, 48%) was heated under reflux for 7 h, the methyl bromide produced being swept away in a slow stream of nitrogen. After this period, the solution was cooled, basified with ammonia, and extracted with ether. The ethereal extract was washed with sodium metabisulfite solution (5% w/v, 10 ml) and with water, dried ($Na_2SO_4$) and evaporated. The residue (37 mg) was dissolved in methanol (5 ml), and the solution mixed with a large excess of diazomethane dissolved in ether (100 ml) and the mixture allowed to stand at room temperature for 48 h.

Evaporation of the ether gave a solid residue which, on purification by preparative layer chromatography (Merck silica gel F-254, 2% methanol in chloroform), afforded corydaline which was sublimed at 100–110° and $5 \times 10^{-3}$ mm (1.2 mg), m.p. 135–136° (lit. (19) m.p. 135°).

*Degradation of Ochotensimine (2) (Scheme 5)*

*Ochotensimine Methiodide (9)*

A solution of iodomethane (0.3 ml) in ether (3 ml) was added to ochotensimine (365 mg) dissolved in methanol (3 ml). After 48 h at room temperature the deposited crystals were filtered off and dried *in vacuo* to give a pure sample of methiodide (470 mg, 84%), melting with decomposition at 225–228° (lit. (20) m.p. 225°).

*1-Methyl-2[2′-(2′′-dimethylaminoethyl)-4′,5′-dimethoxyphenyl]-4,5-methylenedioxy-1-indene (10)*

Sodium borohydride (370 mg) was added to a stirred suspension of ochotensimine methiodide (510 mg) in 2-propanol (30 ml) and the resulting mixture stirred at room temperature for 24 h. Water (200 ml) was then added and the solution extracted with chloroform. The extract was dried and evaporated to give a residue which, on recrystallization from hexane, afforded the title compound (**10**) (250 mg, 66%), melting at 87–89° (lit. (3) m.p. 91–92°). For degradation purposes, the compound was sublimed at 70–75° and $5 \times 10^{-3}$mm to give a sample melting at 87–89°.

*The C-Methyl Group and Carbon-1 of (10) as Acetic Acid*

1-Methyl-2[2′-(2′′-dimethylaminoethyl(-4′,5′-dimethoxyphenyl]-4,5-methylenedioxy-1-indene (**10**) (200 mg) was subjected to Kuhn–Roth oxidation as outlined above for corydaline. A portion of the sodium acetate (7 mg) from this reaction was converted to *N*-acetyl-α-naphthylamine (3.8 mg, m.p. 158–160°) as described above.

*The C-Methyl Group of (10) as Methylamine*

Another portion (7 mg) of the sodium acetate obtained from the Kuhn–Roth oxidation described above was converted to methylamine by the Schmidt degradation procedure and the methylamine was characterized as *N*-methyl-β-naphthoamide (2 mg) by the procedure described above.

*Rings C and D of Ochotensimine as the N-Ethylimide of 3,4-Methylenedioxyphthalic Acid (11)*

Potassium permanganate solution (50 ml, 2% w/v) was added over a period of 1 h to a suspension of 1-methyl-2[2′-(2′′-dimethylaminoethyl)-4′,5′-dimethoxyphenyl]-4,5-methylenedioxy-1-indene (**10**) (120 mg) in water (5 ml) containing potassium carbonate (1.0 g) at 60°. When addition was complete, the solution was acidified (5% HCl), decolorized with sodium bisulfite and extracted with ether. The ethereal extract was re-extracted with 2 *M* sodium bicarbonate solution and the basic extract acidified (concentrated HCl) and extracted with ether. This extract was dried and evaporated to give a residue (220 mg) to which an excess of a solution of ethylamine in ethanol (5 ml, *ca.* 30% w/v) was added. The resulting solution was evaporated, and the residue reacted twice more with ethanolic ethylamine. The final residue (310 mg) was sublimed at 90° and 0.4 mm to give *N*-ethyl-3,4-methylenedioxyphthalimide (15 mg) melting at 126–128° after recrystallization from ether (lit. (24) m.p. 127–130°).

*The Methylenedioxy Group of Ochotensimine as Formaldehyde (Dimedone Derivative)*

A suspension of *N*-ethyl-3,4-methylenedioxyphthalimide (10 mg) and dimedone (5,5-dimethylcyclohexan-1,3-dione) (30 mg) in dilute sulfuric acid (3 ml, 33% v/v) was refluxed for 24 h. The mixture was cooled, water (5 ml) added, and the solution extracted with ether. The extract was washed with water, dried, and evaporated to give a residue which on crystallization from aqueous methanol and sublimation at 75–80° and $3 \times 10^{-1}$mm afforded the dimedone derivative of formaldehyde (3 mg, 20%) melting at 188–190°.

*Conversion of [2-$^3$H,2-$^{14}$C]Acetic Acid into [$^3$H,$^{14}$C]Methylamine by the Schmidt Reaction*

A solution of sodium [2-$^3$H,2-$^{14}$C]acetate was prepared by mixing samples of sodium [2-$^{14}$C]acetate (nominal total activity 5 μCi, nominal specific activity 2 mCi per mmol, New England Nuclear), sodium [2-$^3$H]acetate (nominal total activity see footnote 4, nominal specific activity, 1.3 Ci per mmol, New England Nuclear), and inactive sodium acetate (100 mg). The mixture was dissolved and the volume of the solution adjusted to 10 ml.[4]

[4]In a preliminary experiment solutions containing sodium [2-$^3$H]acetate and [2-$^{14}$C]acetate were mixed in proportions calculated to yield a $^3$H/$^{14}$C ratio of 12.0. A sample of the solution of the resulting sodium[$^3$H,$^{14}$C] acetate did indeed show a $^3$H/$^{14}$C ratio close to this predicted value. However, when the α-naphthylamide derivative of this [$^3$H,$^{14}$C]acetate was prepared, the $^3$H/$^{14}$C ratio of the derivative was found to be much lower (~4) than that given by the solution of the sodium acetate from which it had been obtained. That this discrepancy was due to the presence of exchangeable tritium in the sample of sodium [$^3$H]-acetate (presumably as tritiated water of crystallization) was confirmed as follows.

A sample of known total activity of the original solution of sodium [2-$^3$H]acetate, to which a weighed amount of carrier sodium acetate had been added, was repeatedly evaporated and redissolved in distilled water. Sodium [$^3$H]acetate was ultimately obtained whose specific activity was less than 30% of that calculated for the original sample.

For each of the following experiments a 2-ml portion of the solution, containing approximately 20 mg sodium acetate, was used.

*Determination of the $^3H/^{14}C$ Ratio of the Doubly Labelled Acetate*

A portion of the sodium[2-$^3$H,2-$^{14}$C]acetate (approximately 20 mg) was converted into *N*-acetyl-α-naphthylamine (22), which was purified by sublimation at $5 \times 10^{-3}$mm.

This purified derivative was used for the determination of the $^3H/^{14}C$ ratio[4] of the sodium acetate which was to be converted to methylamine by the Schmidt reaction.

*Methylamine by the Schmidt Reaction*

In each of three separate experiments a sample (20 mg) of sodium [2-$^3$H,2-$^{14}$C]acetate, obtained by evaporation of 2 ml of the stock solution at 80°, was dissolved in the appropriate solvent (2 ml) (Table 6). Sodium azide (100 mg) was added and the Schmidt reaction performed in the usual manner (21). The methylamine formed in the reaction was converted into *N*-methyl-β-naphthoamide (typically 10 mg) which was purified as described above (see section on the C-methyl group as methylamine).

*Determination of Radioactivity*

Radioactivity was assayed by liquid scintillation counting (Mark 1 liquid scintillation computer, Model 6860, Nuclear Chicago). Activity due to $^3$H and $^{14}$C was determined simultaneously, by external standardization counting with $^{133}$Ba. Samples were dissolved in methanol or water and the solution dispersed in a solution of Aquasol (New England Nuclear). Triplicate samples of each compound were counted under comparable conditions of quenching. For highly quenched samples the confidence limits of the quench correction curves were ±5%. Confidence limits shown in the tables are standard deviation of the mean.

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