Lawrence Berkeley National Laboratory

Recent Work

Title

ROLE OF 1,2-DEHYDRORETICULINIUM ION IN THE BIOSYNTHETIC CONVERSION OF RETICULINE TO THEBAINE

Permalink

<https://escholarship.org/uc/item/4s34j5nc>

Author

Borkowski, P.R.

Publication Date

1977-08-01

Submitted to Journal of the American
Chemical Society

LBL-6745 C. /
Preprint

ROLE OF 1,2-DEHYDRORETICULINIUM ION IN THE
BIOSYNTHETIC CONVERSION OF RETICULINE TO THEBAINE.

Paul R. Borkowski, Jerold S. Horn, and
Henry Rapoport

August 1977

Prepared for the U. S. Energy Research and
Development Administration under Contract W-7405-ENG-48

For Reference

Not to be taken from this room

LBL-6745 C. /

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

1 Role of 1,2-Dehydroreticulium Ion in the Biosynthetic
2 Conversion of Reticuline to Thebaine.
3

4
5 Paul R. Borkowski, Jerold S. Horn, and Henry Rapoport*
6

7 Contribution from the Department of Chemistry and Lawrence Berkeley
8 Laboratory, University of California, Berkeley, California 94720.
9

10
11 Abstract:

12 The role previously assigned to 1,2-dehydroreticulium ion
13 as a precursor to the morphinan alkaloids in P. somniferum was
14 based on feeding experiments with a synthetic compound of uncertain
15 identity. We have now prepared authentic 1,2-dehydroreticulium
16 chloride and shown its efficient incorporation into the morphinan
17 alkaloids, supporting the previous hypothesis. Moreover, using a
18 double-label technique and steady-state $^{14}\text{CO}_2$ biosynthesis, we
19 have determined that 1,2-dehydroreticulium ion is a natural
20 product whose native pool size is about one-fifth that of reticuline.
21 These data clearly establish 1,2-dehydroreticulium ion as an
22 intermediate in morphinan alkaloid biosynthesis.
23

fn 1,2 1 Introduction. Reticuline (1) has been firmly established^{1,2}
2 as the biosynthetic precursor, via salutaridine (2) and salutari-
3 dinol (3), of thebaine (4) in Papaver somniferum. Although it
4 is the (-)-enantiomer of reticuline which corresponds in absolute
5 stereochemistry to the configuration found at that center in the
fn 3 6 morphinan alkaloids,³ both enantiomers, when fed separately, were
7 incorporated into thebaine essentially to the same extent.

8 Feedings with reticuline labeled with ³H at C-1 and ¹⁴C at other
9 positions showed that incorporation into thebaine was accompanied
10 by no loss of ¹⁴C but with considerable loss of ³H.^{1b,c}

11 These unexpected results were accommodated by proposing a
12 reversible oxidation-reduction side path to 1,2-dehydroreticulinium
13 ion (5), which would allow both for the loss of ³H and inversion of
14 configuration at C-1. Support for this proposal was found when
15 synthetic material characterized as 1,2-dehydroreticulinium
16 chloride was efficiently incorporated into morphine,^{1c} and dehydro-
17 reticulinium ion was assigned a role as a precursor of thebaine.

18 High incorporation is necessary and strong evidence for a
19 precursor-product relationship; however, by itself it is insuf-
20 ficient. An additional requirement is the natural occurrence of
21 the candidate precursor, a question which we set about to answer
22 for 1,2-dehydroreticulinium chloride (5). The only characterization
23 for 5 previously reported^{1c} was its mp (190-200°dec) and ultraviolet
24 absorption. Since the latter (λ_{max} 250, 323 nm) did not correspond
25 to that of similar 1-benzyl-3,4-dihydroisoquinolinium salts in our
n 4 26 experience nor to published spectra,⁴ it also was necessary to prepar
27 fully authenticated 1,2-dehydroreticulinium chloride and re-examine

1 its role as a precursor.

2 Discussion. Synthesis of 1,2-Dehydroreticulinium Chloride (5)

3 and its ^3H and ^{14}C Isotope-Isomers. 1,2-Dehydroreticulinium ion is

fn 5 4 a common intermediate in the many syntheses of reticuline⁵ and our
5 synthesis proceeded by standard methods from vanillin to 3-benzyloxy-
6 4-methoxyphenylacetic acid (6). For the other half of the
7 molecule, we chose 4-benzyloxy-3-methoxyphenylacetonitrile (7) as
8 the intermediate, anticipating the introduction via the cyano
9 group of ^{14}C and ^3H at C-3 of the final compound.

10 The conversion of nitrile 7 to amine 8 has been erratic in
11 the past using the more conventional (PtO_2/H_2 or LiAlH_4) methods.
12 We found that this reduction can be reliably performed and in good
fn 6 13 yield using sodium borohydride and cobalt chloride in methanol.⁶
14 The amide (9) then formed nearly quantitatively when acid (6) and
15 amine (8) were refluxed in xylene with removal of water. Cyclization
16 of the amide in refluxing toluene with POCl_3 gave the iminium
17 chloride 10 in 95% yield. When 10 as the free base was treated with
18 methyl iodide in methanol, a 92% yield of the methiodide 11 resulted.
19 To prepare the corresponding chloride, 11 was treated with excess
20 freshly prepared AgCl in aqueous methanol, giving quantitative
21 conversion to methochloride 12. Finally, debenzylation of 12
22 occurred quantitatively in refluxing ethanolic HCl to give pure
23 1,2-dehydroreticulinium chloride (5).

24 In our hands, 1,2-dehydroreticulinium chloride (5) is a stable
25 salt melting at 180-185° dec. The infrared spectrum shows clearly
26 a band at 1630 cm^{-1} characteristic of the conjugate iminium salt.
27 The ultraviolet spectrum (λ_{max} 370, 309, 250 nm) of 5 is in accord

1 with the earlier report on the ultraviolet spectra of dihydroiso-
2 quinolines of this type,⁴ but contrasts with the previous^{1c}
3 absorption (λ_{max} 323, 250 nm) assigned to structure 5. The NMR
4 spectrum accounts for all the hydrogens of 5 and confirms its
5 iminium salt character (as shown in the infrared) by the appearance
6 of the C-9 methylene hydrogens as a two-hydrogen singlet at δ 4.40.
7 The NMR spectrum can be regenerated sans the δ 4.40 signal if the
8 sample is treated with NaOD in D₂O to pH 13 and immediately quenched
9 by acidification to pH 2 with DCl in D₂O. Finally, 1,2-dehydro-
10 reticulium chloride (5) was reduced with sodium borohydride to
11 give an excellent yield of d,l-reticuline which was further charac-
12 terized by conversion to its perchlorate and picrate salts, in full
13 agreement with the reported data.⁵

14 To prepare [3-¹⁴C]-reticuline and [3-¹⁴C]-1,2-dehydroreticu-
15 linium chloride (5) Na¹⁴CN was used in the reaction with 4-benzyloxy-
16 3-methoxybenzyl bromide, and the resulting radioactive acetonitrile
17 7 was carried through the synthesis as above. To prepare the ³H
18 labeled phenylethylamine 8, we used the cobalt chloride-sodium boro-
19 hydride procedure, preforming and isolating the catalyst so formed.
20 However use of this isolated catalyst with tritium was troublesome
21 and led to significant dilution of the tritium with adsorbed hydrogen.
22 A more effective synthesis was found in the use of Ni₂B·2% CrB as
23 catalyst.^{6b} When applied with ³H₂ to acetonitrile 7, [α -³H₂]- β -(4-
24 benzyloxy-3-methoxyphenyl)ethylamine (8) was conveniently prepared.

25 Feeding Experiments. Of the various techniques used for
26 precursor feedings (smearing on the leaves, hydroponic feeding
27 through the roots, wick feeding through the stems or roots, direct

1

2, X, Y = O

3, X = H, Y = OH

4

5

10, X = Cl, R = H

11, X = I, R = CH₃

12, X = Cl, R = CH₃

9

7

8

6

1 injection) we have found that direct injection is the best method
2 for delivering a specific amount of precursor in a short period
3 with little wound or insult to the plant. The hypocotyl was chosen
4 as the site for injection because of the ease of entering the
5 mainstream of the plant through this fleshy region between root
6 and stem.

7 A solution of [3-¹⁴C]-1,2-dehydroreticulium chloride (5) was
8 fed to P. somniferum by direct injection over a one-hour period
9 using a motor driven syringe. As a control, parallel feedings were
10 carried out with [3-¹⁴C]-reticuline. Since the rates of incorpor-
11 ation were not known, the plants were then transferred to hydroponic
12 nutrient solutions and allowed to grow for periods varying from
13 20 hours to 14 days. The plants were then harvested, radio-
14 inactive carriers were added, and the alkaloids were obtained by
15 the usual isolation procedures.

fn 7 16 The results of the feeding experiments with P. somniferum⁷
17 are presented in Table I. Clearly, 1,2-dehydroreticulium ion
18 is incorporated into thebaine, codeine, and morphine with approxi-
19 mately the same high efficiency as is reticuline. These results
20 may be considered to confirm the previous^{1c} report of incorporation,
21 although the nature of the compound fed, and claimed to be 1,2-
22 dehydroreticulium ion, remains uncertain. It is of interest to
23 note that the relative incorporation as a function of time among
24 the various morphinan alkaloids roughly follows the previously
fn 8 25 established sequence:⁸ thebaine → codeine → morphine. Also,
26 decreased incorporation at long times, 14 days, is consistent
27 with the metabolism of morphine to normorphine and the conversion

fn 9 1 of the latter to unknown metabolites.⁹

2 Natural Occurrence of 1,2-Dehydroreticulinium Ion. The
3 demonstration that authentic dehydroreticulinium chloride acts as
4 an effective precursor of the morphinan alkaloids in P. somniferum
5 is clearly of significance; however, it is conceivable that in the
6 feeding experiments dehydroreticulinium chloride entered the
7 natural biosynthetic pathway (i.e. was converted to reticuline) via
8 some nonspecific reductase rather than by actually occupying a
9 legitimate position in the scheme. Thus the question of whether
10 1,2-dehydroreticulinium ion exists as a natural product becomes
11 important. If the dehydroreticulinium species is naturally occurring
12 and if its role is to provide a means of interconverting the two
13 enantiomeric forms of reticuline, then its pool size might be so
14 small as to escape detection. Additionally, the polar, ionic nature
15 of dehydroreticulinium chloride precludes the use of gas chroma-
16 tography and the common thin layer techniques for its separation
17 and purification. The problem is further compounded by the insta-
18 bility of this iminium salt under even mildly alkaline conditions.

19 One possible approach which seemed attractive would involve the
20 conversion of 1,2-dehydroreticulinium chloride to reticuline, an
21 easily isolable derivative. Further, the use of plants grown in a
22 ¹⁴CO₂ atmosphere would lead to labeled material and even very small
23 quantities would be readily detectable. However, endogenous
24 reticuline would be indistinguishable from reticuline derived by
25 reduction of 1,2-dehydroreticulinium chloride. This problem
26 could be overcome if a method were available for separating a
27 mixture of dehydroreticulinium chloride and reticuline.

1 Such a method was developed based on the following principles:
2 a) powdered plant material was extracted with 1N HCl to remove both
3 reticuline and dehydroreticulium ion; b) separation from most
4 of the other 1N HCl soluble plant substituents was effected by ion
5 exchange, leaving reticuline and dehydroreticulium ion finally
6 in solution at pH 7; c) this pH 7 solution was extracted thoroughly
7 with chloroform and chloroform/isopropanol to yield the organic
8 extract fraction, further described below; d) ion exchange of the
9 pH 7 aqueous phase and elution with 12N HCl gave dehydroreticulium
10 chloride which was reduced to reticuline with sodium borohydride.

11 The organic extracts, step c, contained reticuline appreciably
12 contaminated with dehydroreticulium chloride. These were
13 separated by extraction into HCl and then removal of the reticuline
14 at pH 8.5 into chloroform. This step c gives the endogenous
15 reticuline and step d gives dehydroreticulium ion, isolated as
16 reticuline. In both cases, the reticuline was thoroughly purified
17 by TLC and GC.

18 To rigorously evaluate this method, three experiments were
19 performed, each with P. somniferum plant material to which different
20 labeled carriers were added. In experiment 1, [3-³H]-reticuline was
21 added; in experiment 2, [3-³H]-1,2-dehydroreticulium chloride was
22 added; and in experiment 3, both [3-¹⁴C]-reticuline and [3-³H]-
23 dehydroreticulium chloride were added. Each mixture was then
24 subjected to the separation method. Both the distribution and
25 recovery of reticuline and dehydroreticulium chloride were
26 determined and are summarized in Table II.

27 The relatively low overall recovery of activity in experiments

1 1 (83%) and 2 (75%) was due primarily to loss of significant
2 amounts of reticuline and dehydroreticulium to the resin beads
3 in each of the two ion exchange processes. Subsequently we found
4 that this material could be recovered after the initial elution
5 with 12N HCl by allowing the resin to stand in contact with 12N HCl
6 for 16 hours followed by further elution with fresh 12N HCl. This
7 technique was used in experiment 3 and accounts for the excellent
8 activity recovery (98, 97%) found there.

9 The second question which had to be answered about the separatio
10 method was its ability to avoid contamination of one component by
11 the other. The results of this evaluation are found in experiment 3,
12 Table II. Although the organic extract (step c) contains [^{14}C]-
13 reticuline accompanied by considerable [^3H]-dehydroreticulium
14 chloride, this contaminant can be removed easily and totally by sub-
15 jecting the organic extracts to another distribution at pH 8.5 as
16 described above. The result was pure [^{14}C]-reticuline containing no
17 ^3H activity above background. However, some reticuline did remain in
18 the aqueous phase at pH 7 and it accompanied the dehydroreticulium
19 chloride throughout to the end (step d). This was shown by 4% of
20 the added ^{14}C activity appearing in the final [^3H]-reticuline
21 resulting from the borohydride reduction.

22 To account for any endogenous reticuline which would be
23 carried along and might confuse the dehydroreticulium assay, a
24 method was devised involving use of a double label. P. somniferum
25 plants exposed to $^{14}\text{CO}_2$ under short term steady state conditions¹⁰
26 will carry ^{14}C labels in all carbon-containing compounds. If
27 [$3\text{-}^3\text{H}$]-reticuline and inactive 1,2-dehydroreticulium chloride

are added as carriers then the endogenous reticuline will carry a double label whereas an endogenous dehydroreticulinium (if any is present) should carry only the ^{14}C label. Moreover, the $^3\text{H}/^{14}\text{C}$ ratio in the endogenous reticuline is fixed at the moment labeled carrier is added. In the separation method, the endogenous doubly-labeled reticuline can be obtained pure from step c and its $^3\text{H}/^{14}\text{C}$ ratio can be determined. After reduction of the dehydroreticulinium ion (step d) the resulting reticuline will also carry a double label by virtue of the small amount of endogenous reticuline left in the pH 7 aqueous phase after chloroform-isopropanol extraction. However, if dehydroreticulinium ion is a natural product, the $^3\text{H}/^{14}\text{C}$ ratio for this reticuline will be less than the $^3\text{H}/^{14}\text{C}$ ratio found for the purely endogenous reticuline.

Eighteen P. somniferum plants (105 days old; 1.5 kg, wet weight) were exposed for 4 hours to $^{14}\text{CO}_2$ under steady state conditions. The plants were ground to a fine powder in liquid nitrogen and inactive 1,2-dehydroreticulinium chloride (0.36 mmol) as well as [$3\text{-}^3\text{H}$]-reticuline (0.31 mmol, 2.45×10^8 dpm) were added. The entire mix was extracted with 1N HCl to provide the extract that was submitted to the separation procedure described above. The results are summarized in Table III and clearly demonstrate the presence of 1,2-dehydroreticulinium ion as a natural product in P. somniferum by virtue of the difference in $^3\text{H}/^{14}\text{C}$ ratios. Thus 1,2-dehydroreticulinium ion can be placed in the biosynthetic pathway leading to morphine with assurance.

Consideration of the ^{14}C specific activities shown in Table III also allows a further conclusion to be reached regarding pool sizes.

The reticuline derived from dehydroreticulinium chloride had a ^{14}C specific activity of 1362 dpm/mg. Of this activity, 90.7 dpm/mg (i.e., $\frac{2.06 \times 10^4}{227} = 90.7$) are from endogenous reticuline not removed in the separation. Thus 1272 dpm/mg are derived from endogenous dehydroreticulinium chloride. Using these values and the data in Table III, the following relationships may be drawn:

$$7145 \text{ dpm/mg} = \frac{^{14}\text{C activity from endogenous reticuline}}{\text{mg of endogenous + carrier reticuline}} \quad (1)$$

$$1272 \text{ dpm/mg} = \frac{^{14}\text{C activity from endogenous dehydroreticulinium ion}}{\text{mg of endogenous + carrier dehydroreticulinium ion}} \quad (2)$$

which on a millimolar basis become:

$$2.35 \times 10^6 \text{ dpm/mmol} = \frac{^{14}\text{C activity of endogenous reticuline}}{\text{mmol endogenous + carrier reticuline}} \quad (3)$$

$$0.418 \times 10^6 \text{ dpm/mmol} = \frac{^{14}\text{C activity of endogenous dehydroreticulinium ion}}{\text{mmol endogenous + carrier dehydroreticulinium ion}} \quad (4)$$

In both cases the ^{14}C activity must come entirely from native material but it is reasonable to assume that the endogenous materials contribute negligibly (<5%) to the mass, relative to the amount of carrier added. With this simplifying assumption the equations become:

$$2.35 \times 10^6 \text{ dpm/mmol} = \frac{^{14}\text{C activity of endogenous reticuline}}{0.31 \text{ mmol carrier}} \quad (5)$$

$$0.418 \times 10^6 \text{ dpm/mmol} = \frac{^{14}\text{C activity of endogenous dehydroreticulinium ion}}{0.36 \text{ mmol carrier}} \quad (6)$$

1 From equations (5) and (6) we calculated that the ^{14}C activity found
2 in endogenous reticuline equals 7.28×10^5 dpm and that in endogenous
3 1,2-dehydroreticulinium ion is 1.50×10^5 dpm.

4 The proposed biosynthetic pathway presents a close relationship
5 between reticuline and dehydroreticulinium ion with no intervening
6 compounds. Under conditions of short term steady state $^{14}\text{CO}_2$ bio-
7 synthesis it is reasonable to expect that the specific activities of
8 the two compounds should be equal. Thus the difference in the nor-
9 malized activities as calculated above can only arise from differences
10 in the native pool sizes of reticuline and dehydroreticulinium ion.
11 To a rough approximation our data suggest that the pool size of
12 reticuline is ~5 (i.e. $\frac{7.28 \times 10^5}{1.5 \times 10^5}$) times as large as the pool of
13 1,2-dehydroreticulinium ion in P. somniferum.

14

15

16

17

18

19

20

21

22

23

24

25

26

27

Table I. Incorporation of [3-¹⁴C]-Reticuline and [3-¹⁴C]-
1,2-Dehydroreticulium Chloride into Morphinan
Alkaloids in P. somniferum.^a

<u>Precursor</u>	<u>Time</u> ^b	<u>Thebaine</u>	<u>Codeine</u>	<u>Morphine</u>
dl-[3- ¹⁴ C]-Reticuline	20h	3.8	3.6	1.4
	3d	3.3	4.9	5.2
	14d	0.7	0.4	0.8
[3- ¹⁴ C]-1,2-Dehydro- reticulium chloride	20h	2.7	4.9	1.6
	3d	0.8	2.8	6.2
	14d	0.5	1.3	1.8

^aPlants were 83-92 days old and had just started elongating.

^bAmount of time plants were allowed to grow, including the
initial hour of injection, before harvesting.

Table II. Recovery and Distribution of Activity^a from Separation Scheme Applied to P. somniferum^b to which Reticuline and Dehydroreticulium Chloride were added.

	<u>Experiment 1</u>	<u>Experiment 2</u>	<u>Experiment 3</u>
Reticuline Added	1.43×10^8 dpm [^3H] (100%)	none	5.15×10^4 dpm [^{14}C] (100%)
Dehydroreticulium chloride added	none	5.07×10^8 dpm [^3H] (100%)	4.23×10^8 dpm [^3H] (100%)
Recovery from Total Organic Extracts (Step c)	1.19×10^8 dpm (83%)	1.18×10^8 dpm (22%)	4.81×10^4 dpm [^{14}C] (94%) 1.03×10^8 dpm [^3H] (24%)
Recovery from Aqueous after NaBH_4 Reduction (Step d)	7.15×10^4 dpm (0.05%)	2.69×10^8 dpm (53%)	0.20×10^4 dpm [^{14}C] (4%) 3.08×10^8 dpm [^3H] (73%)

^aAll radioactivity is at C-3 in all compounds. ^bFour plants, each 150 days old, were used for each experiment.

Table III. Activity Relationships of Reticuline Isolated from $^{14}\text{CO}_2$ Exposure.

<u>Compound</u>	^3H Spec. Act. <u>(dpm/mg)</u>	^{14}C Spec. Act. <u>(dpm/mg)</u>	$^3\text{H}/^{14}\text{C}$ <u>ratio</u>
Endogenous Reticuline ^a	1.62×10^6	7145	227
Reticuline ^a from reduction of 1,2-dehydroreticulium ion	2.06×10^4	1362	15

^aPurified by TLC and GC to constant specific activity and homogeneity.

1 Experimental Section

2 General. All melting points were determined on a Büchi
3 melting point apparatus and are uncorrected. Infrared spectra were
4 determined on a Perkin-Elmer Model 337 Spectrometer in KBr pellets.
5 Ultraviolet spectra were determined in 95% ethanol (unless otherwise
6 noted) on a Cary Model 14 Spectrometer. Nuclear magnetic resonance
7 spectra were determined on a Varian T-60 instrument in CDCl_3
8 (unless otherwise noted) with absorptions recorded in parts per
9 million downfield from internal TMS. Mass spectra were determined
10 on CEC-103 and 110B spectrometers. All organic extracts were
11 dried over MgSO_4 and evaporated from a Berkeley Rotary Evaporator.
12 Elemental analyses were provided by the Analytical Laboratory,
13 Department of Chemistry, University of California, Berkeley, and
14 agree with calculated values within $\pm 0.4\%$. For determination of
15 ^3H and ^{14}C in the same sample, a Packard Sample Oxidizer was used;
16 radioactivity determinations were performed on a Packard Tri-Carb
17 Liquid Scintillation Counter.

18 Thin layer chromatography, both analytical and preparative,
19 was carried out on Camag silica gel using solvent systems a,
20 $\text{C}_6\text{H}_6/\text{CH}_3\text{OH}$ (4/1); b, $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{NH}_3\text{OH}$ (75/25/1); and c, $\text{CHCl}_3/$
21 CH_3OH (5/1). R_f values are: a, thebaine 0.65, codeine 0.40;
22 b, codeine 0.60, reticulene 0.45, morphine 0.30; c, reticulene 0.30.

23 Gas chromatography was performed on a Hewlett-Packard Model
24 402B Gas Chromatograph with a hydrogen flame detector and using
25 glass columns, 6 ft x 6 mm od, on 3% OV-17 on Varaport 30 (100-120
26 mesh). Preparative GC was carried out at 260° using He at
27 60 mL/min as carrier gas; analytical GC was performed at 230° using

1 He at 40 mL/min. The analytical retention times are: thebaine,
2 14 min; codeine, 8 min; morphine, 12 min; reticuline, 23 min.

3 Synthetic Experiments. 4-Benzyloxy-3-methoxyphenylacetonitrile

4 (7). To a stirred solution of 4-benzyloxy-3-methoxybenzyl bromide
5 (22.0 g, 72 mmol) in 320 ml dimethylformamide was added powdered
6 sodium cyanide (15.6 g, 320 mmol) and the suspension was stirred
7 under nitrogen for 24 hr. Fresh sodium cyanide (1.00 g) was
8 added every hour for 5 h and the reaction mixture was then poured
9 into 600 ml of water and the product separated as a yellowish
10 precipitate which was extracted with one 200 ml portion and three
11 100 ml portions of benzene. The combined extracts were washed with
12 400 ml of water, dried, and evaporated to leave a residue which
13 was crystallized from chloroform/carbon tetrachloride (1:9);
14 yield of 7, 15.6 g (86%), mp 70-71° (lit.¹¹ mp 67°).

15 β-(4-Benzyloxy-3-methoxyphenyl)ethylamine (8). To a stirred
16 solution of nitrile 7 (6.0 g, 24 mmol) and cobalt(II) chloride
17 hexahydrate (10.0 g, 41 mmol) in methanol (900 ml) was added
18 portionwise sodium borohydride (9.0 g, 237 mmol). A black pre-
19 cipitate formed and hydrogen was evolved. The resulting suspension
20 was stirred for 2 hr at room temperature under nitrogen, then 3N
21 HCl (200 ml) was poured into the reaction mixture and stirring
22 continued until the precipitate dissolved. The methanol was
23 evaporated and the resulting aqueous solution was extracted with
24 two 100 ml portions of ether. The aqueous phase was basified with
25 conc. aq. NH₃ and extracted with four 100 ml portions of ether and
26 the combined ether extracts were washed with an equal volume of
27 satd sodium chloride solution and dried. Evaporation of the ether

1 gave the primary amine as an oil in 4.86 g, 81% yield: NMR δ 1.25
 2 (s, 2H, $-\text{NH}_2$), 2.85 (c, 4H, $-\text{CH}_2\text{CH}_2-$), 3.83 (s, 3H, $-\text{OCH}_3$), 5.12 (s,
 3 2H, $\text{C}_6\text{H}_5\text{CH}_2$), 6.80 (c, 3H, OArH), 7.35 (c, 5H, C_6H_5); MS m/e 257
 4 (M^+), 91 (C_7H_7^+ , 100). Anal. ($\text{C}_{15}\text{H}_{19}\text{NO}_2$): C, H, N.

5 3-Benzyloxy-N- $[\beta$ -(4-benzyloxy-3-methoxyphenyl)ethyl]-4-
 6 methoxyphenylacetamide (9). The phenylacetic acid 6¹¹ (11.9 g,
 7 43 mmol), the amine 8 (11.1 g, 43 mmol) and xylene (175 ml) were
 8 refluxed together under nitrogen for 18 hr with azeotropic removal
 9 of water. The solution was cooled to room temperature, hexane
 10 (100 ml) was added, and the precipitated product was recrystallized
 11 from methanol to yield 20.2 g of pure amide, mp 143-144° (lit.¹¹
 12 mp 140°).

13 7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-3,4-
 14 dihydroisoquinoline Hydrochloride (10). A solution of the amide 9
 15 (10.0 g, 18.9 mmol) in toluene (700 ml) was refluxed with phosphorus
 16 oxychloride (10 ml) for 12 minutes and then quickly cooled to
 17 room temperature. The toluene and excess POCl_3 were evaporated,
 18 and the residue was washed with petroleum ether and then dissolved
 19 in 200 ml of ethanol. Upon cooling to 5°C, 10 ml of conc. HCl was
 20 added followed by 300 ml of ether whereupon the product crystal-
 21 lized. Recrystallization from ethanol/ether provided analytically
 22 pure 10 (9.8 g, 95% yield): mp 211-214°C (dec) (lit.¹¹ mp 203-205°);
 23 NMR δ 2.75 (t, 2H, C-4 CH_2), 3.75 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3);
 24 3.82 (t, 2H, C-3 CH_2), 4.40 (s, 2H, C-9 CH_2), 5.10 (s, 4H, $\text{C}_6\text{H}_5\text{CH}_2-$),
 25 6.55, 6.73, 7.25 (15H, ArH); MS m/e 493 M^+-HCl , 91 (C_7H_7^+); IR
 26 1630 cm^{-1} ($^+\text{>C=N<}^+$). Anal. ($\text{C}_{32}\text{H}_{32}\text{NO}_4\text{Cl}$): C, H, N.

1 7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-2-
 2 methyl-3,4-dihydroisoquinolinium Iodide (11). To a stirred solution
 3 of the imine hydrochloride 10 (25.0 g, 47 mmol) in 800 ml methanol
 4 was added 48 ml of 0.97 M methanolic potassium hydroxide solution
 5 and iodomethane (71.0 g, 500 mmol) in that order. The solution
 6 was refluxed under nitrogen for 3 h, cooled, then poured into
 7 1200 ml of ether where the product crystallized overnight at 0°C.
 8 Recrystallization from methanol/ether gave pure methiodide 11
 9 (27.4 g, 92%): mp 203-204°C (lit.¹² mp 198-201°); IR 1630 cm⁻¹
 10 ($\text{>C=N}^+\text{<}$); NMR δ 3.27 (t, 2H, C-4 CH₂), 3.65 (s, 3H, NCH₃), 3.85 (s,
 11 3H, OCH₃), 3.94 (t, 2H, C-3 CH₂), 4.02 (s, 3H, OCH₃), 4.42 (s, 2H,
 12 C-9 CH₂), 5.05 (s, 2H, C₆H₅CH₂-), 5.10 (s, 2H, C₆H₅CH₂-), 6.52,
 13 6.70, 6.92, 7.20 (15H, ArH); UV λ_{max} 362 nm ($\epsilon=7,300$), 309(6,700),
 14 247(13,000); MS m/e 507 (M⁺-HI), 492 (M⁺-HI-CH₃), 91 (C₇H₇⁺, 100).
 15 Anal. (C₃₃H₃₄NO₄I): C, H, N.

16 7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-2-
 17 methyl-3,4-dihydroisoquinolinium Chloride (12). To a stirred
 18 solution of the methiodide 11 (7.60 g, 12 mmol) in 800 ml methanol
 19 and 500 ml water was added freshly prepared silver chloride (35.8 g,
 20 250 mmol) and the resulting suspension was vigorously stirred
 21 under nitrogen for 2 hr at room temperature. The mixture was
 22 filtered and the clear yellow filtrate was concentrated to an oil
 23 which crystallized from acetone/ether, giving 6.21 g (95%) of the
 24 methochloride 12: mp 183-184°C (lit.^{1c} mp 118-121°); IR 1630 cm⁻¹
 25 ($\text{>C=N}^+\text{<}$); UV λ_{max} 362 nm ($\epsilon=7,300$), 309(6,700), 247(13,000) (lit.^{1c}
 26 λ_{max} 250, 317 in H₂O/C₂H₅OH, 1/1); MS m/e 507 (M⁺-HCl), 492 (M⁺-HCl-CH₃),
 27 91 (C₇H₇⁺, 100); NMR δ 3.27 (t, 2H, C-4 CH₂), 3.67 (s, 3H, NCH₃),

1 3.82 (s, 3H, OCH₃), 3.90 (t, 2H, C-3 CH₂), 4.00 (s, 3H, OCH₃), 4.40
 2 (s, 2H, C-9 CH₂), 5.05 (s, 2H, C₆H₅CH₂), 5.10 (s, 2H, C₆H₅CH₂-),
 3 6.49, 6.70, 6.90, 7.20 (15H, ArH). Anal. (C₃₃H₃₄O₄NCl): C, H, N.

4 1,2-Dehydroreticulium Chloride (5). The methochloride 12
 5 (6.00 g, 11 mmol) was dissolved in 600 ml ethanol which had
 6 previously been saturated at 0-5°C with HCl gas. The resulting
 7 solution was refluxed under nitrogen for 18 hr, and the volatile
 8 materials were evaporated, finally by heating at 78°C/.01 Torr
 9 for 12 h, to give 3.95 g (98%) of pure 1,2-dehydroreticulium
 10 chloride (5): mp 180-185°C (dec) (lit.^{1c} mp 190-200° dec);
 11 IR 1630 cm⁻¹ (⁺>C=N<); UV λ_{max} 370 nm (ε=7,000), 309(7,000), 250
 12 (14,000) (lit.^{1c} λ_{max} 250,323); NMR δ (CD₃OD) 3.13 (t, 2H, C-4 CH₂),
 13 3.70 (s, 3H, NCH₃), 3.79 (s, 3H, OCH₃), 3.96 (s, 3H, OCH₃), 4.05
 14 (t, 2H, C-3 CH₂), 4.40 (s, 2H, C-9 CH₂), 4.80 (br s, 2H, ArOH),
 15 6.63, 6.78, 7.02, 7.18, 7.37 (5H, ArH); MS m/e 327 (M⁺-HCl), 312
 16 (M⁺-HCl-CH₃, 100). Anal. (C₁₉H₂₂NO₄Cl): C, H, N.

17 d,l-Reticuline (1). To a stirred solution of 5 (1.02 g, 2.8
 18 mmol) in 250 ml methanol was added portionwise sodium borohydride
 19 (1.00 g, 25.5 mmol) and the resulting solution stirred at room
 20 temperature for 2 h. Solvent was evaporated and the residue was
 21 dissolved in 100 ml of 0.1N NaOH. This solution was quickly
 22 adjusted to pH 8.3 with 3N HCl and then extracted with one 200 ml
 23 and three 100 ml portions of chloroform. The combined chloroform
 24 extracts were washed with 500 ml of satd sodium bicarbonate and
 25 500 ml of satd sodium chloride, dried, and evaporated to leave an
 26 oil which crystallized from ether/hexane giving pure dl-reticuline
 27 (0.89 g, 97%), mp 144-145° (lit.¹¹ mp 144°). The picrate melted at

1 192-194°C (lit.¹¹ mp 191-192°) and the perchlorate melted at
2 143-145° (lit.¹³ mp 144°).

3 [α -³H₂]- β -(4-Benzoyloxy-3-methoxyphenyl)ethylamine (8).--

4 To 4-benzoyloxy-3-methoxyphenylacetonitrile (7, 253 mg, 1 mmol) and
5 3 ml of Ni₂B·2% CrB catalyst^{6b} in 30 ml of methanol was introduced
6 ³H₂ and the mixture was stirred at room temperature for 5 h.
7 Hydrogen was then introduced and stirring continued for 24 h after
8 which the solution was degassed by seven freeze-thaw cycles. The
9 catalyst was removed by filtration, the filtrate was diluted with
10 50 ml of 3N HCl and extracted with three 25 ml portions of xylene,
11 the aqueous phase was adjusted to pH 9 with conc. aq. NH₃, and
12 four 30 ml portions of xylene were used to remove the amine. This
13 distribution between acidic and alkaline aqueous phases and xylene
14 was repeated twice and the final xylene phase was washed with satd.
15 NaCl solution, leaving a solution of pure [³H]-amine 8 in xylene,
16 used directly as described below.

17 [3-³H₂]-1,2-Dehydroreticulium chloride (5) was prepared by
18 adding the phenylacetic acid 6 (272 mg, 1 mmol) directly to the
19 xylene solution of tritiated 8 prepared above and proceeding as
20 previously described to the preparation of 5 which contained 188 mCi
21 of tritium.

22 [3-¹⁴C]-1,2-Dehydroreticulium chloride was prepared as
23 described above, starting with 4-benzoyloxy-3-methoxybenzyl bromide
24 and Na¹⁴CN.

25 [3-³H₂]- and [3-¹⁴C]-Reticuline were prepared from the corres-
26 ponding 1,2-dehydroreticulium chloride by reduction with NaBH₄
27 as described above.

1 Feeding Experiments. Injection of Precursors.-- The [3-¹⁴C]-
2 reticuline solution was prepared by dissolving 25.7 mg in 0.5 ml of
3 1M H₃PO₄, then adjusting the pH to 6.4 with 8M KOH. Water was then
4 added to a final volume of 1.5 ml. The [3-¹⁴C]-1,2-dehydro-
5 reticulinium chloride solution was prepared by dissolving 39.3 mg
6 in 1.55 ml of deionized water, pH 4.0.

7 Aliquots were taken using a 250 µL gas-tight syringe and were
8 injected into the hypocotyl of P. somniferum via a Sage No. 341
9 motor-driven syringe at a rate of ~3 µL/min; injection time was
10 about one hour. Three plants, 83-92 days old, were used in each
11 experiment and were injected with 100-200 µL of precursor solution.
12 Plants were then allowed to grow for the time specified in nutrient
13 solution with aeration of the roots. In some cases, 2-10% of the
14 injected radioactivity was observed in the nutrient solution in
15 which the plants were growing. This activity was shown to be
16 leakage from the injection hole and not release from the roots. By
17 placing a band-aid over the injection hole, activity in the nutrient
18 solution decreased to <0.3% while the band-aid contained 4-8% of
19 the activity.

20 Isolation Procedures were carried out as previously described¹⁰
21 and the alkaloids were separated into a non-phenolic fraction con-
22 taining thebaine and codeine, and a phenolic fraction containing
23 reticuline and morphine. The thebaine and codeine were further
24 resolved on alumina (Woelm(III), basic) using successively C₆H₆,
25 C₆H₆/CHCl₃ (9/1), C₆H₆/CHCl₃/2-propanol (88.5/10/1.5), and C₆H₆/
26 CHCl₃/2-propanol/CH₃OH (87.5/10/1.5/1). Further purification was
27 effected by TLC using system a for thebaine system b for codeine,

1 and system b for reticuline and morphine. Each compound was
2 purified until it was >99% pure, then it was sublimed, washed from
3 the cold finger with CH_3OH and dried to constant weight and specific
4 activity at $60^\circ/0.01$ Torr.

5 Natural Occurrence Experiments.--- Four shredded P. somniferum
6 plants were frozen in liquid N_2 in a Waring blender and ground to
7 a fine powder by blending for 60 sec. The frozen, pulverized plant
8 material was then transferred to a 1L Erlenmeyer flask, carrier
9 alkaloids and 500 mL of 1N HCl were added, and the mixture was
10 shaken for 18 h. The solids were removed by centrifugation, washed
11 with five 100 mL portions of 1N HCl, and the combined acid solutions
12 were filtered and passed through an ion exchange column [Bio-Rad
13 AG50W-X4 (100-200 mesh), 18cm x 2.4 cm id] in the H^+ form. The
14 column was then successively eluted with 1N HCl (1L), 3N HCl (250 mL),
15 6N HCl (100 mL) and 12N HCl (500 mL) after which the resin was
16 allowed to stand in contact with 12N HCl for 16 h. and then further
17 eluted with 500 mL of 12N HCl. The 6N and 12N eluents were combined
18 and evaporated to 15 mL, 500 mL of satd. aq. NaCl was added, the pH
19 was adjusted to 7.0 with aq. NaOH, and the solution was extracted
20 with thirteen 100 mL portions of chloroform and two 100 mL portions
21 of chloroform/isopropanol (9/1), re-adjusting the pH to 7.0 after
22 each extraction as necessary. The combined organic extracts were
23 evaporated and the resulting residue set aside for subsequent
24 isolation and purification of the reticuline (see below).

25 The pH 7.0 aqueous solution was acidified to pH 1.0 with conc.
26 HCl and then passed through an ion exchange column [Bio-Rad AG 50W-X4
27 (100-200 mesh) 15 cm x 1.8 cm id] in the H^+ form. The column was
eluted with 1N HCl (1L), 3N HCl (250 mL), 6N HCl (100 mL) and 12N

1 HCl (500 mL), and the resin was allowed to stand in contact with 12N
2 HCl for 16 h and then was further eluted with 12N HCl (500 mL). The
3 6N and 12N eluents were combined and evaporated to dryness, and the
4 residue was dissolved in 250 mL methanol and treated with NaBH_4
5 (2.0 g, 0.05 mol) at room temperature for one h. The methanol was
6 then evaporated and the residue was dissolved in 100 mL 1N HCl.
7 This solution was adjusted to pH 8.5 with aqueous Na_2CO_3 and extracted
8 with five 100 mL portions of chloroform, the combined chloroform
9 extracts were dried and evaporated, and the residue was purified by
10 preparative TLC and finally by preparative GC to yield pure reticuline.
11 Further TLC and GC showed this reticuline to be of constant specific
12 activity.

13 The residue from the chloroform and chloroform/isopropanol
14 extracts was dissolved in 100 ml of 1N HCl, the solution was adjusted
15 to pH 8.5 with aq. Na_2CO_3 and extracted with five 100 ml portions of
16 chloroform, and the combined and dried chloroform extracts were
17 evaporated. From the residue was isolated pure reticuline of constant
18 specific activity by preparative TLC and GC.

19 20 Acknowledgements

21 We thank Dr. T. G. Waddell for help in the synthesis of ^{14}C
22 labeled compounds. This research was supported in part by the
23 National Institute on Drug Abuse and the Division of Biomedical and
24 Environmental Research of ERDA.

25

26

27

References and Notes

- (1) (a) A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin and H. Ramuz, J. Chem. Soc., 3600 (1964); (b) A. R. Battersby, T. A. Dobson, H. Ramuz, D.H.R. Barton, G. W. Kirby, W. Steglich and G. M. Thomas, ibid., 2423 (1965); (c) A. R. Battersby, D. M. Foulkes and R. Binks, ibid., 3323 (1965); (d) A. R. Battersby, D. M. Foulkes, M. Hirst, G. V. Parry and J. Staunton, J. Chem. Soc. (C), 210 (1968); (e) D.H.R. Barton, Proc. Chem. Soc., 203 (1963); (f) A. R. Battersby, R. Binks, D. M. Foulkes, R. J. Francis, D. J. McCaldin and H. Ramuz, ibid., 203 (1960).
- (2) R. O. Martin, M. E. Warren and H. Rapoport, Biochemistry, 6, 2355 (1967).
- (3) J. Kalvoda, P. Buchschacher, and O. Jeger, Helv. Chim. Acta, 38, 1847 (1955); G. Kartha, F. R. Ahmed, and W. H. Barnes, Acta. Cryst., 15, 326 (1962).
- (4) J. L. Bills and C. R. Noller, J. Am. Chem. Soc., 70, 957 (1948).
- (5) Summarized and referenced in T. Kametani, "Chemistry of the Isoquinoline Alkaloids", Elsevier Publishing Co., New York, 1969.
- (6) (a) T. Satoh and S. Suzuki, Tetrahedron Lett., 4555 (1969); (b) R. Paul, P. Buisson, N. Joseph, Ind. & Eng. Chem., 44, 1006 (1952); (c) C. A. Brown and H. C. Brown, J. Am. Chem. Soc., 85, 1003 (1963).
- (7) Similar feeding experiments have been conducted with [3-¹⁴C]-reticuline and [3-¹⁴C]-1,2-dehydroreticulium chloride in Papaver bracteatum. Both compounds were incorporated into thebaine, demonstrating a common biosynthetic path with P. somniferum to this stage. Details of these experiments will be reported in the future.

- 1 (8) H. Rapoport, F. R. Stermitz and D. R. Baker, J. Am. Chem. Soc.,
2 82, 2765 (1960); F. R. Stermitz and H. Rapoport, ibid., 83,
3 4045 (1961).
- 4 (9) R. J. Miller, C. Jolles and H. Rapoport, Phytochemistry, 12,
5 597 (1973).
- 6 (10) H. I. Parker, G. Blaschke, and H. Rapoport, J. Am. Chem. Soc.,
7 94, 1276 (1972).
- 8 (11) I. Baxter, L. T. Allan, and G. A. Swan, J. Chem. Soc., 3645
9 (1965).
- 10 (12) A. H. Jackson and J. A. Martin, ibid., 2061 (1966).
- 11 (13) K. W. Gopinath, T. R. Govindachari and N. Viswanathan,
12 Chem. Ber., 92, 776, 1657 (1959).
- 13
14
15
16
17
18
19
20
21
22
23
24
25
26
27

This report was done with support from the United States Energy Research and Development Administration. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the United States Energy Research and Development Administration.

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720