1976

Biosynthesis of montanine and related compounds

Bjorn Olesen
Iowa State University

Biosynthesis of montanine and related compounds

by

Bjorn Olesen

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Chemistry
Major: Organic Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1976

TABLE OF CONTENTS

INTRODUCTION

During the last 15 years, much work has been done to determine the biosynthetic pathways of the Amaryllidaceae alkaloids. Early work centered on determining the origin of the carbon skeleton of the alkaloids, while the more recent work has focused on the late-stage modifications that take place after the basic carbon skeleton has been formed.

The stereochemistry of hydroxylation at C-11 of the crinine-ring system has been investigated with contradictory results. This thesis is concerned with the synthesis of O-methyl-(2R)-[2-^{3}H,1'-^{14}C]norbelladine in order to further study the stereochemistry of hydroxylation at C-11.

The biosynthesis of cherylline, a 4-phenyl-1,2,3,4-tetrahydroisoquinoline alkaloid, has been investigated. A second section of this thesis is concerned with the stereochemistry of hydroxylation at C-2 in the biological conversion of O-methylnorbelladine to cherylline.

HISTORICAL

Early Biosynthetic Investigations

Early biosynthetic work on the Amaryllidaceae alkaloids showed that the primary biosynthetic precursor, norbelladine (1), was derived from the amino acids phenylalanine (2) and tyrosine (3). Furthermore, it was found that phenylalanine (2) was incorporated only into the C_6 - C_1 moiety of norbelladine (1) *via* stepwise degradation to *trans*-cinnamic acid (4), *p*-coumaric acid (5), caffeic acid (6), and protocatechuic aldehyde (7) (1-8). Tyrosine (3) was found to be incorporated only into the C_6 - C_2 moiety of 1 by degradation to tyramine (8) (2,8,9-13) (Figure 1).

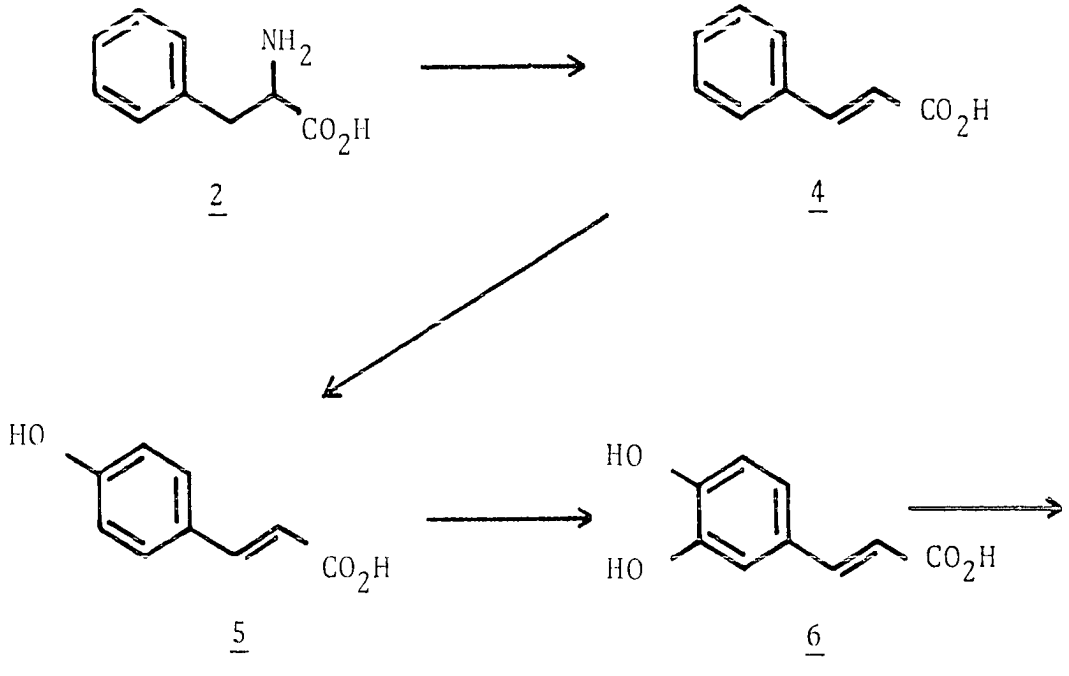


Figure 1. Biosynthesis of norbelladine (1).

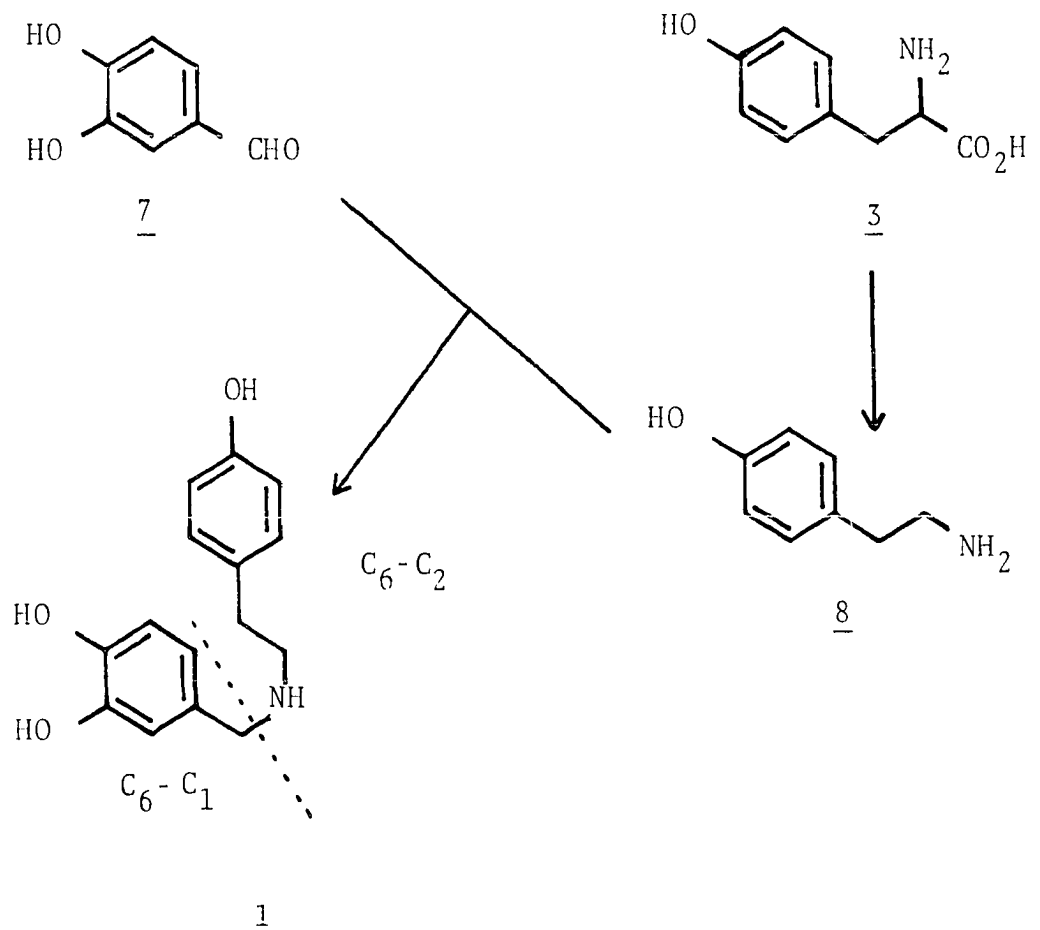


Figure 1. (Continued).

O-Methylnorbelladine (9) was proposed by Barton and Cohen (14) in 1957 to be converted into the major skeletal types of the Amaryllidaceae alkaloids *via* oxidative phenyl-phenyl coupling. Subsequent work by Barton and co-workers (15-18) provided experimental evidence for the theory. Thus *p*-*o*' coupling of 9 yields the galanthamine-type alkaloids, *p*-*p*' coupling yields the crinine-type alkaloids and *o*-*p*' coupling yields the lycorine-type alkaloids (Figure 2).

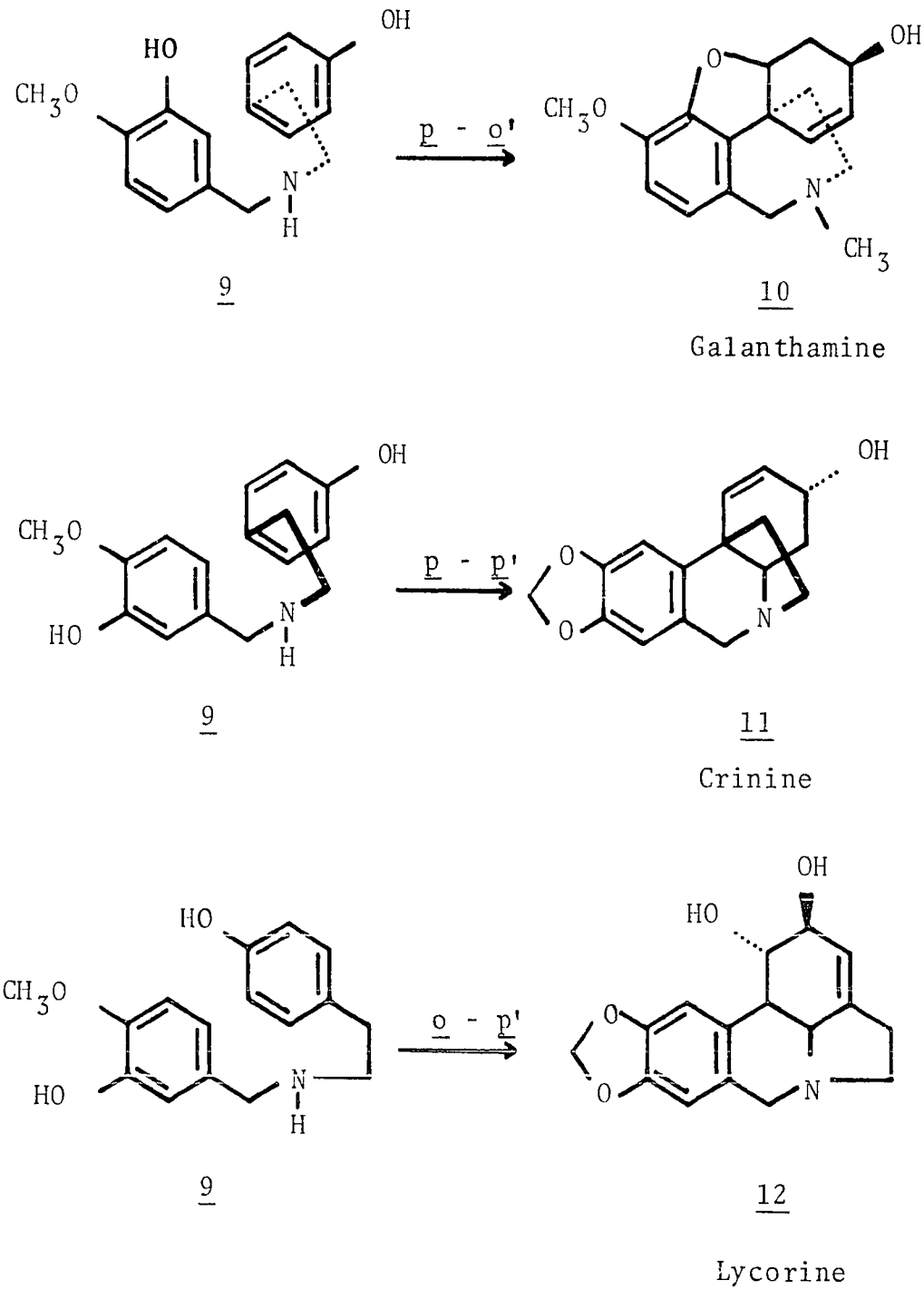


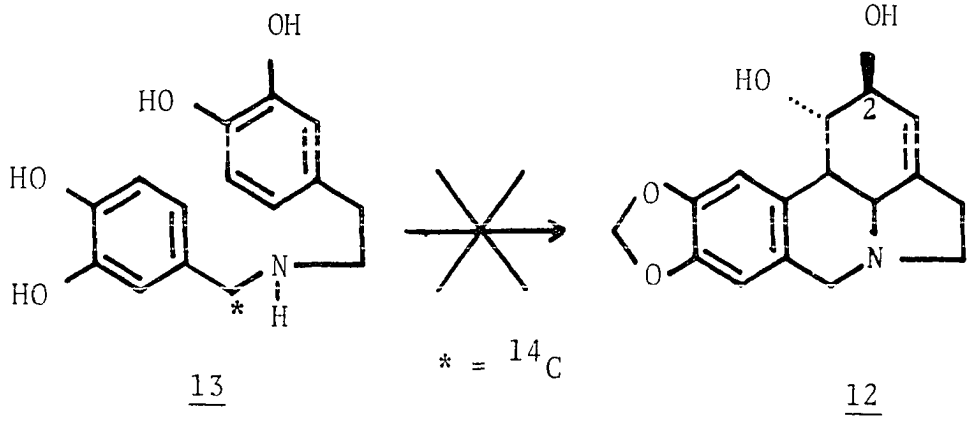
Figure 2. Biosynthetic pathways in the Amaryllidaceae alkaloids.

Late-stage Modifications

More recently biosynthetic work has been directed toward elucidating the steps involved in the late-stage modifications of the basic carbon skeletons. This section will discuss late-stage hydroxylations.

Hydroxylation at C-2

The hydroxylation of lycorine (12) and related compounds at C-2 is a late-stage process. This was shown when hydroxy-[1'-^{14}C]norbelladine (13) was not a precursor for lycorine (12) when fed to "Twink" double Narcissus (4) (Figure 3).



13 12

Figure 3. Late-stage hydroxylation.

Norpluviine (14) tritiated in the aromatic ring by treatment with tritiated acetic acid and a palladium catalyst was fed to "Deanna Durbin" daffodils and was found

to be converted efficiently into lycorine (15) (8) (Figure 4). This indicated that hydroxylation at C-2 occurred after the lycorine-type skeleton had been formed.

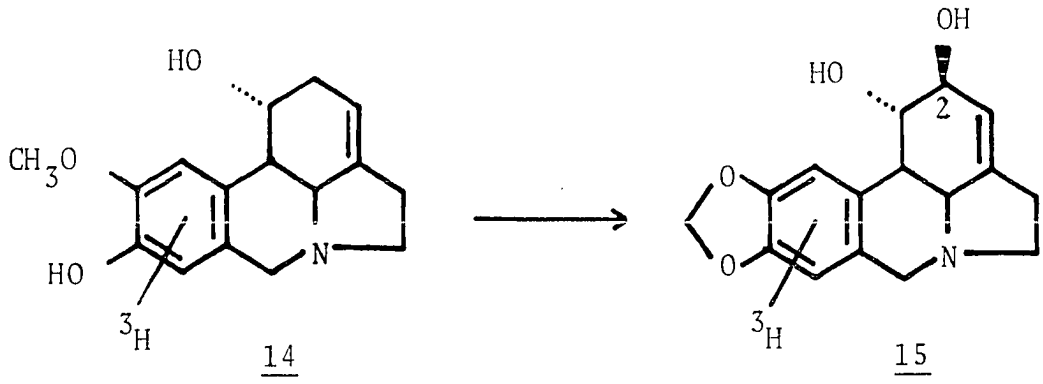
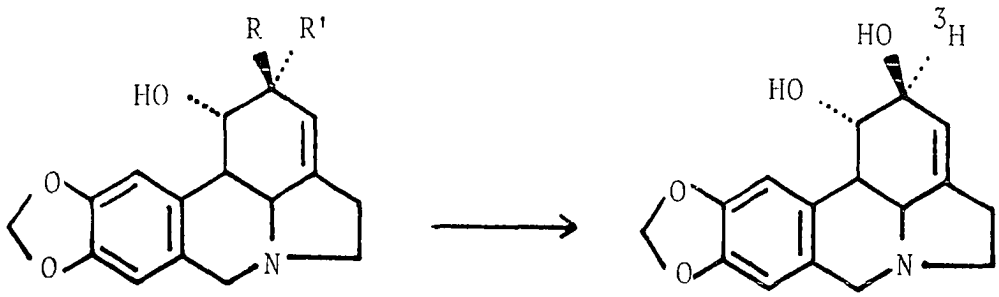


Figure 4. Conversion of norpluviine (14) into lycorine (15).

More recently the stereochemistry at C-2 in the conversion of caranine (16) into lycorine (12) had been studied with contradictory results.

Wildman and Heimer (19) synthesized [2β-^{3}H]caranine (17) and showed that it was converted by Zephyranthes candida into lycorine (18) with retention of the tritium at C-2 (Figure 5). From the data obtained it was concluded that the hydroxylation at C-2 occurred with at least partial inversion. The extent of the inversion could not be determined since the precursor (17) contained only the single label.



16 R = R' = H

17 R = ^{3}H, R' = H

18

Figure 5. Conversion of [2β-^{3}H]caranine (17) into lycorine (18).

Wildman and Heimer (19) synthesized [2β-^{3}H]caranine (17) as shown in Figure 6. Lycorine (12) was converted to chlorohydrin (19) with phosphorous oxychloride and sodium chloride. Chlorohydrin (19) formed epoxide (20) when chromatographed on Florisil. Reduction of 20 with [^{3}H]lithium aluminum hydride yielded 17 .

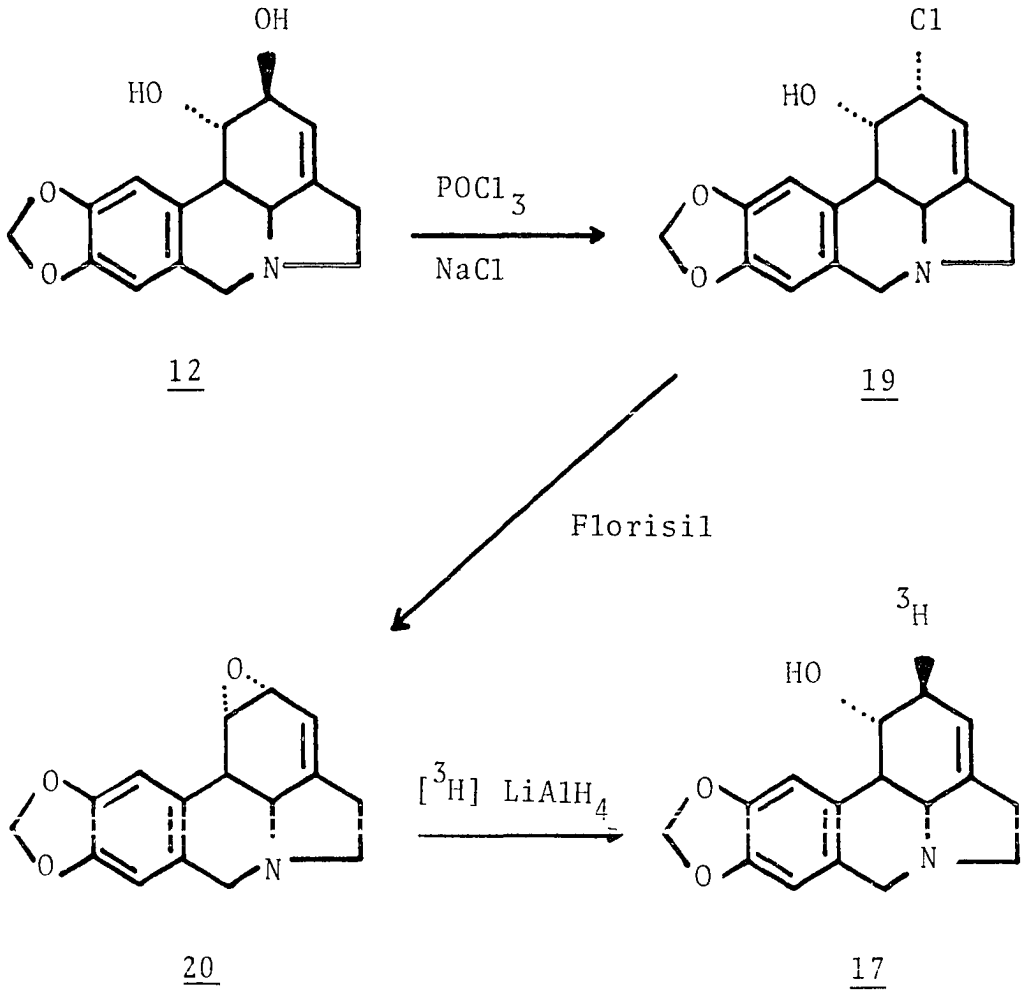


Figure 6. Wildman and Heimer's synthesis of [2β-^{3}H]caranine (17).

To further study the stereochemistry of hydroxylation at C-2, Bruce and Kirby (20,21) fed DL-[3,5-$^{3}H_2$;2'-^{14}C]tyrosine (21) to "Twink" daffodils. In agreement with earlier work (22) on the conversion of tritiated O-methylnorbelladine (22) into 23 (Figure 7), the conversion of 21 into

norpluviine (24) occurred with loss of half of the tritium present in the tyrosine precursor (21).

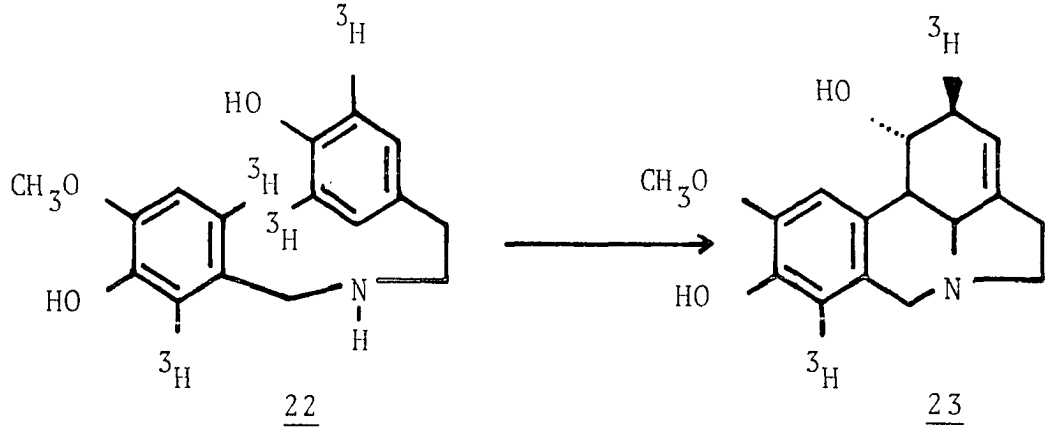


Figure 7. Conversion of O-methylnorbelladine (22) into norpluviine (23).

Of most importance, the lycorine (25) isolated in the tyrosine feeding had the same tritium content as norpluviine (24). Since the conversion of norpluviine (24) to lycorine (25) involves hydroxylation at a saturated carbon atom, this showed that the hydroxylation at C-2 occurred with inversion of configuration (Figure 8).

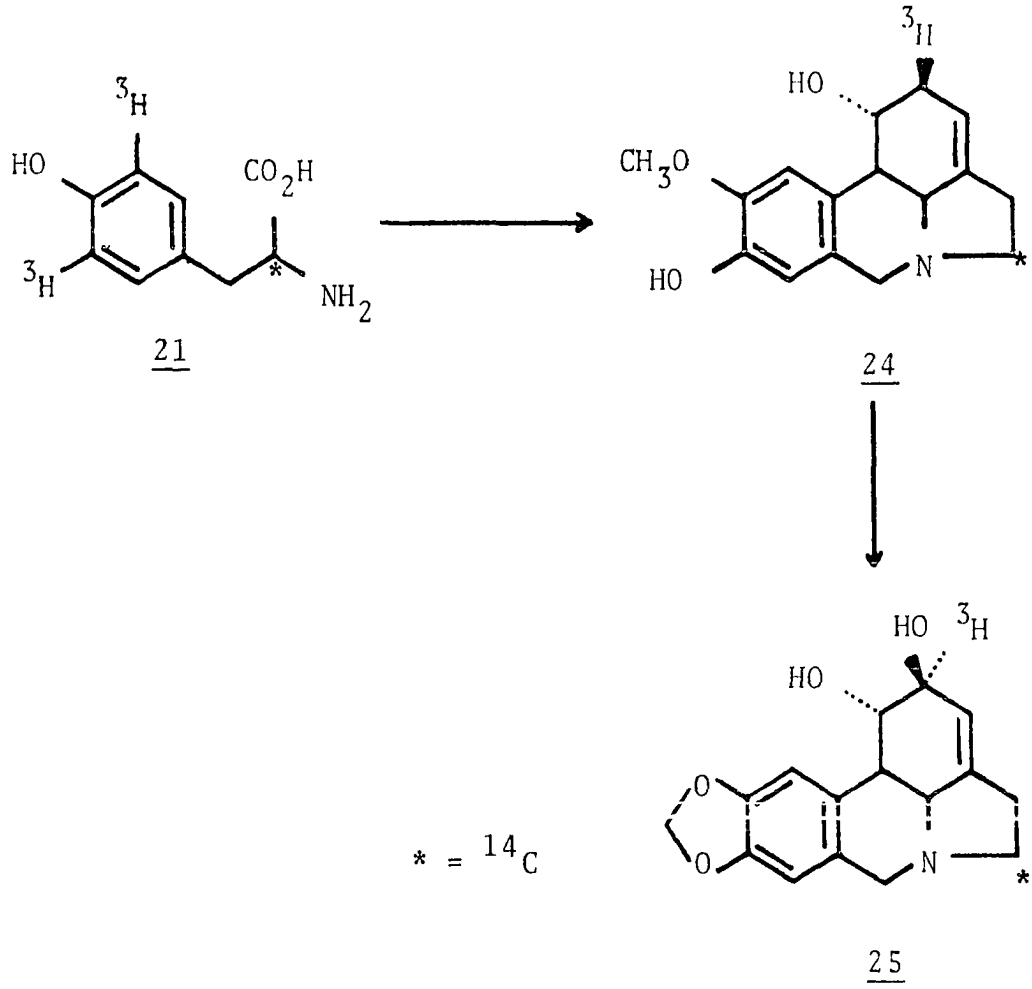
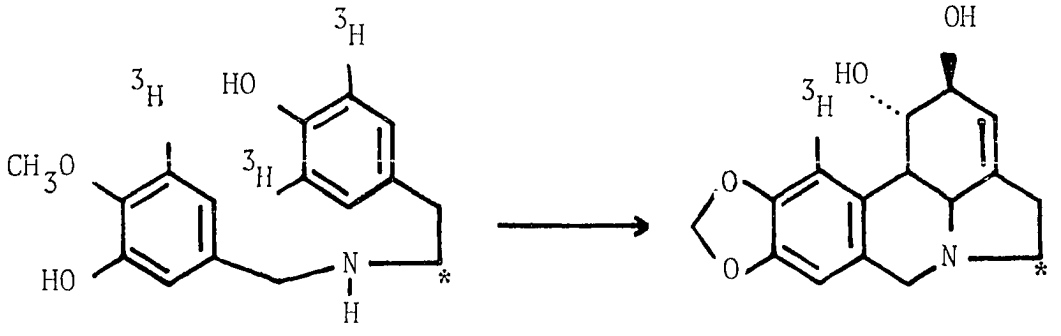


Figure 8. Conversion of tyrosine (21) into norpluviine (24) and lycorine (25).

Further studies on C-2 hydroxylation were performed by Fuganti and Mazza (23). O-Methyl-[3',5',5''-$^3H_3$;1-^{14}C]norbelladine (26) was fed to flowering Clivia miniata. The lycorine (27) isolated retained 37% of the tritium present in 26 (Figure 9). Degradation of the lycorine to

hydrastic acid showed that all the tritium present in lycorine was located on the aromatic ring. This data was consistent with hydroxylation at C-2 which took place with retention of configuration.



26 * = ^{14}C 27

Figure 9. Conversion of 0-methylnorbelladine (26) into lycorine (27).

Fuganti and Mazza (23) then synthesized [2α-^{3}H,5-^{14}C]-caranine (28) by the method of Wildman and Heimer (19). This was incorporated by C. miniata into lycorine (25) with 92% tritium retention. Finally [2β-^{3}H,5-^{14}C]norpluviine (24), isolated from "Twink" daffodils, was incorporated into lycorine (29) with 20% tritium retention. On the basis of these data, it was concluded that the conversion of 0-methylnorbelladine into lycorine through the intermediates norpluviine and caranine occurred without participation of

a 2-oxo derivative. Also hydroxylation at C-2 occurred with retention of configuration and protonation at C-2 of norpluviine occurred from the α-side of the molecule (Figure 10).

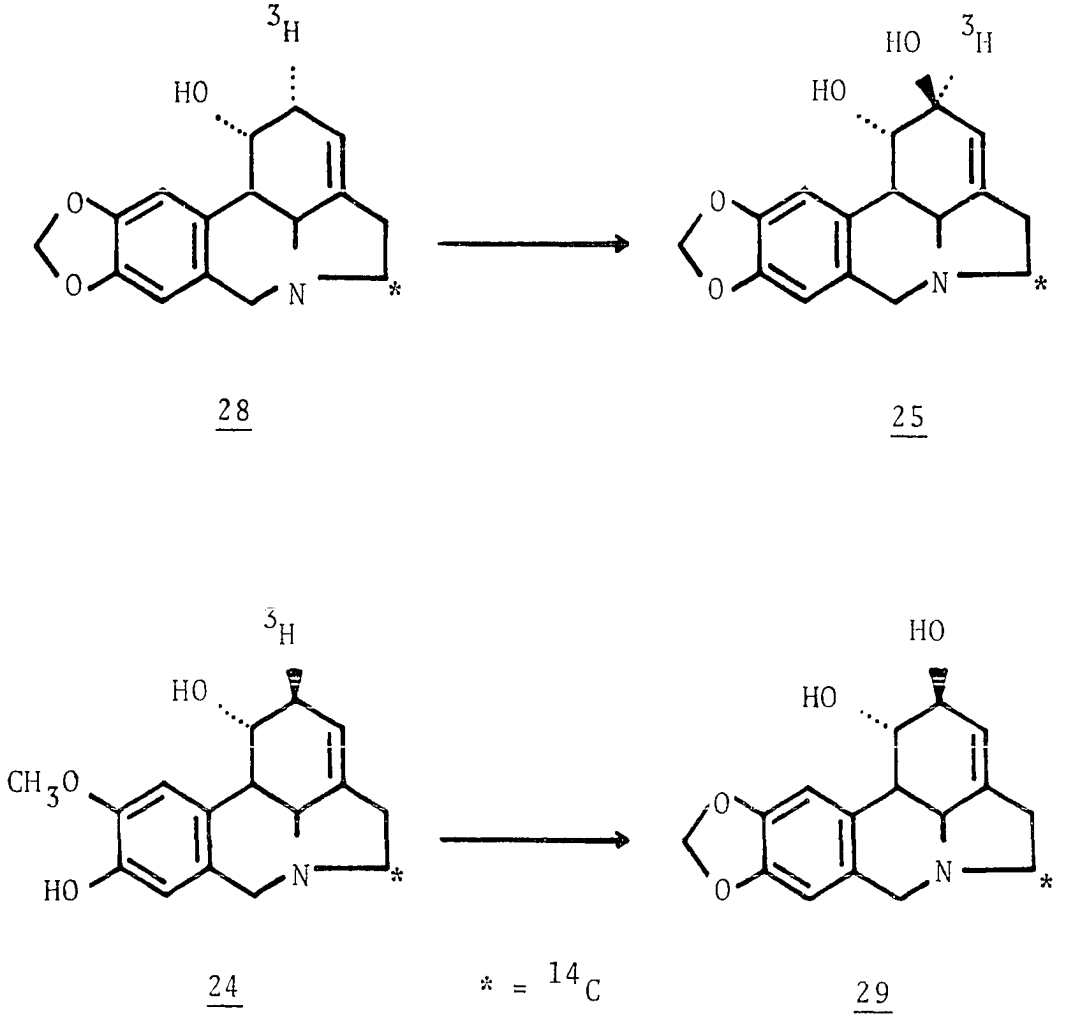


Figure 10. Biosynthesis of lycorine (25,29) in C. miniata.

In an effort to resolve these conflicting reports, Christenson (24) synthesized [9-O-methyl-^{14}C,2β-^{3}H]pluviine

(30) by the method of Wildman and Heimer (19). When 30 was fed to flowering "King Alfred" daffodils, it was converted into galanthine (31) with retention of the tritium present at C-2 in 30 . This required inversion of configuration at C-2 (Figure 11).

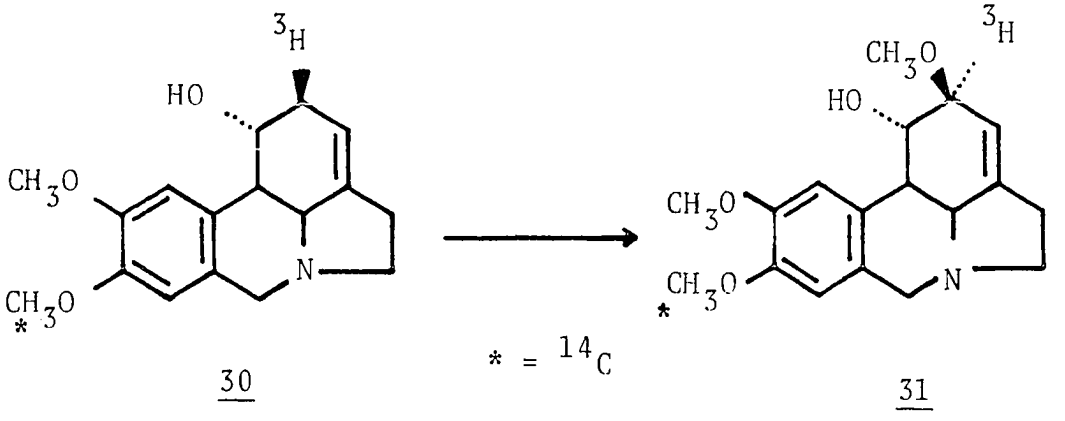


Figure 11. Conversion of pluviine (30) into galanthine (31) in daffodils.

The stereochemical course of hydroxylation at C-2 is still somewhat uncertain. A possible explanation for the different results would be that the stereochemical course varies with the different plants. However, Bruce and Kirby, Wildman and Heimer, and Wildman and Christenson each used different plants and all agree on hydroxylation with inversion of configuration. Fuganti and Mazza are the only investigators to claim hydroxylation with retention of configuration at C-2.

Hydroxylation at C-6 and C-7

[7-^{3}H]Norpluviine (32) and [6-^{3}H]haemanthamine (33) biosynthesized from [formyl-^{3}H]protocatechuic aldehyde (34) was converted into lycorenine (35) and haemanthidine (36) respectively in "Tresamble" and "Inglescombe" daffodils (25). Both 35 and 36 retained the tritium present in 34, and this indicated that the conversion of 34 into 35 and 36 are stereospecific processes and that the hydrogen introduced at C-1' in the biosynthesis of O-methylnorbelladine (37) was the hydrogen removed in the conversion into lycorenine (35) and haemanthidine (36) (Figure 12).

The stereochemistry of hydrogen removal at the benzylic position (C-7) was established by Fuganti and Mazza (26). O-Methyl-(1'R)-[1'-^{3}H,1-^{14}C]norbelladine (38) was converted by "Texas" daffodils into norpluviine (39) without loss of tritium. The norpluviine (39) was then fed to "Inglescombe" and "Tresamble" daffodils. The lycorenine (40) isolated had lost 82 $\pm$ 5% of the tritium present at C-7 in 39. Since 38 contained 75 $\pm$ 10% of the (1'R)-isomer this data indicated that the conversion of norpluviine into lycorenine takes place with the loss of the pro-R hydrogen from C-7 of 39 (Figure 13). This result combined with the earlier results established that the incorporation of protocatechuic aldehyde (7) into the

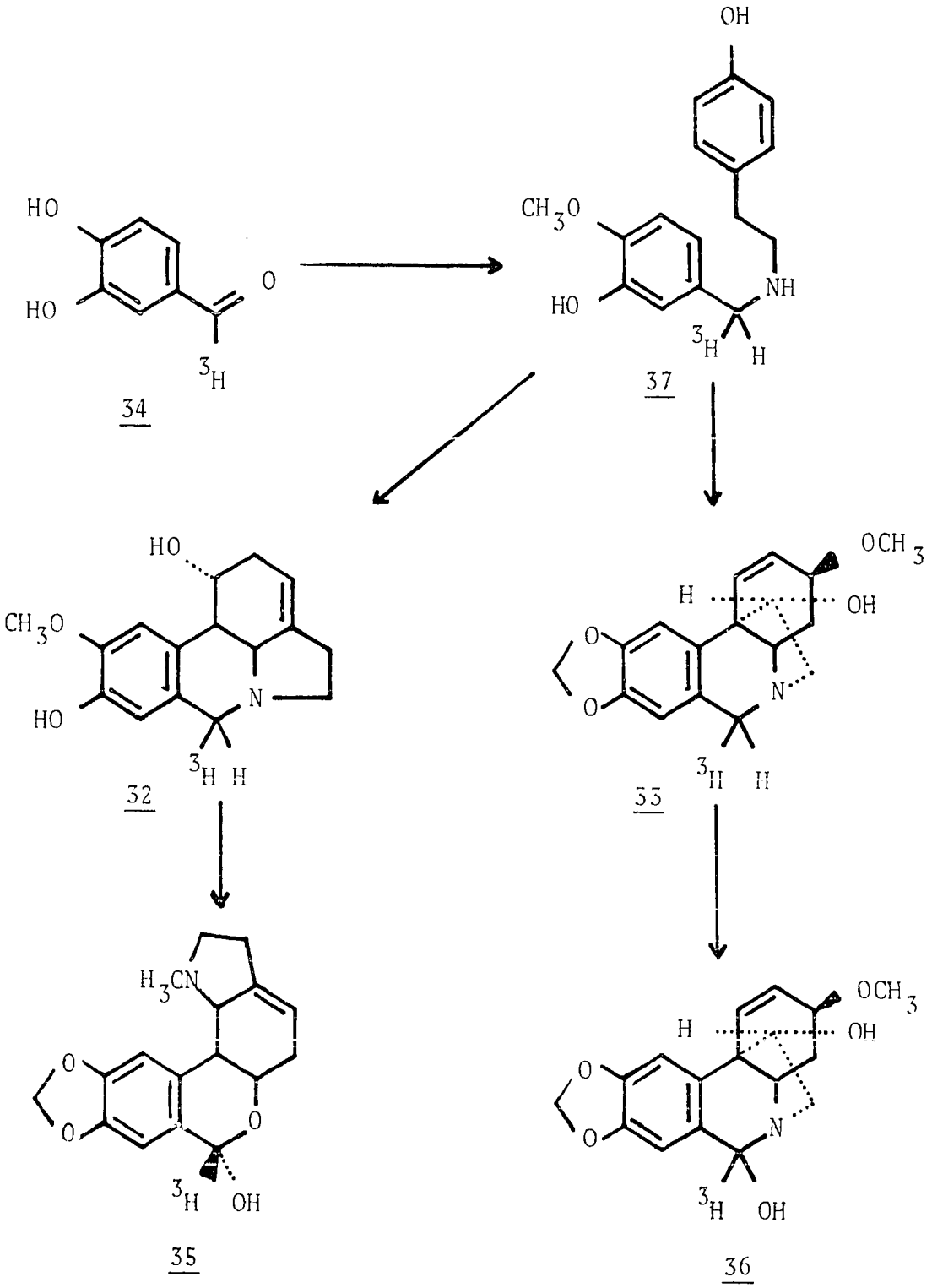


Figure 12. Biosynthesis of lycorenine (35) and haemanthidine (36).

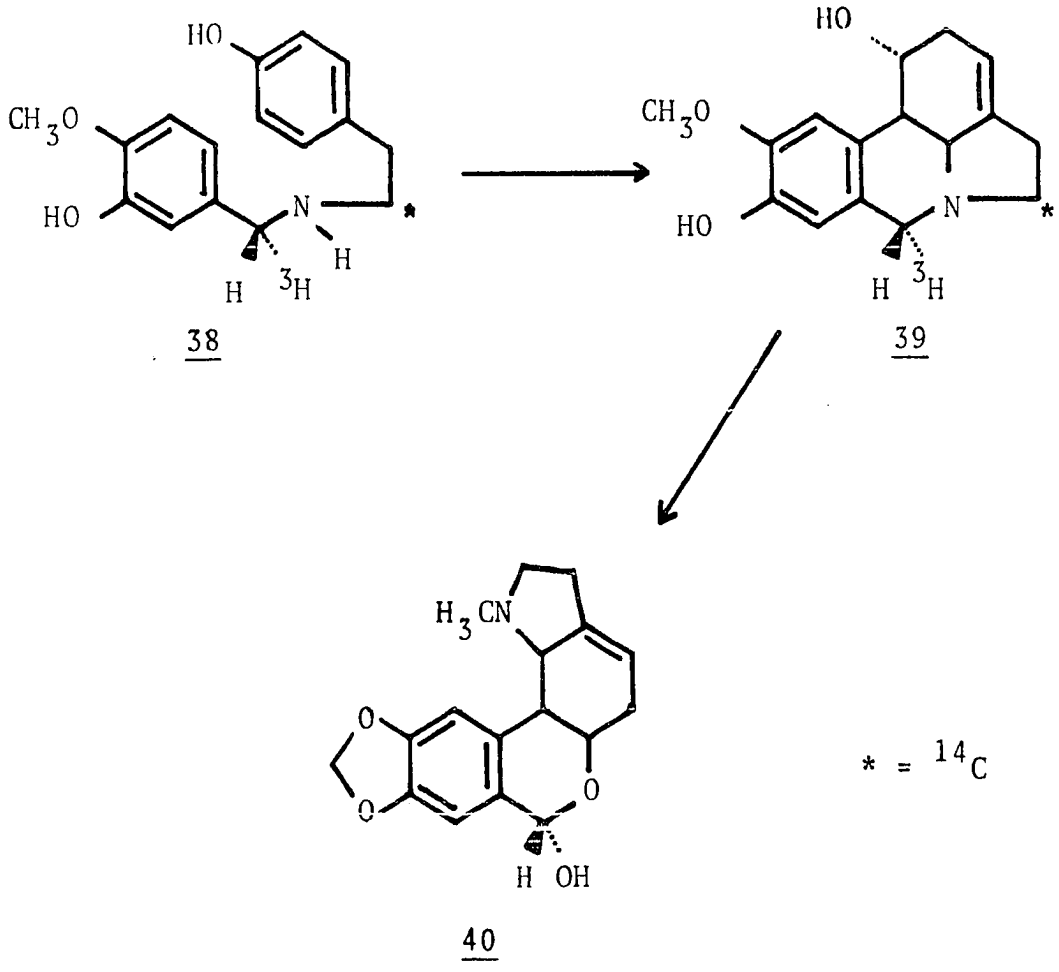


Figure 13. Biosynthesis of lycorenine (40).

C_6-C_1 unit of norbelladine (1) occurred with protonation from the re-face of the molecule.

Hydroxylation at C-6 was studied by Virnig (27) when O-methyl-(1'R)-[1'-^{3}H,1-^{14}C]norbelladine (38) was fed to Crinum erubescens. The isolated 6-hydroxycrinamine (41)

retained 64% of the tritium present at C-1' of 38. When 38 was fed to Nerine bowdenii, the isolated 6-hydroxybuphanidrine (42) also retained 64% of the tritium present at C-1' of 38 again indicating that the hydroxylation at C-6 occurred with the loss of the pro-S hydrogen from C-1' of 38 (Figure 14).

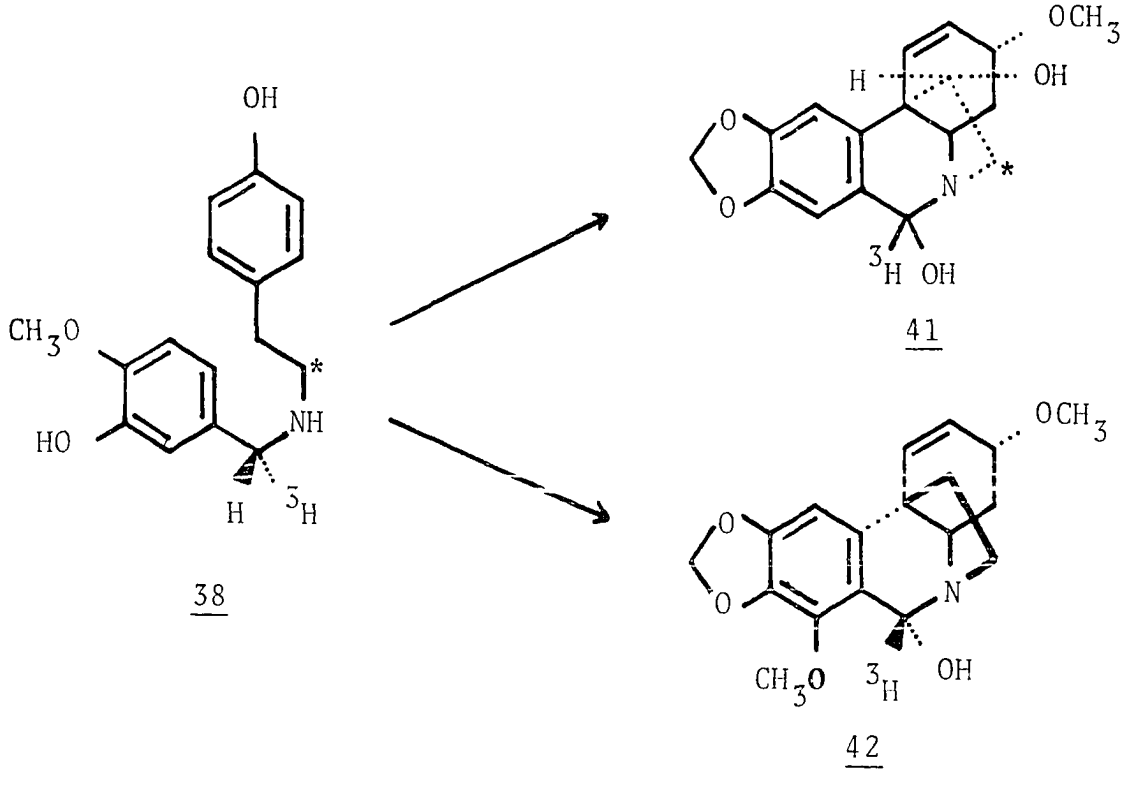
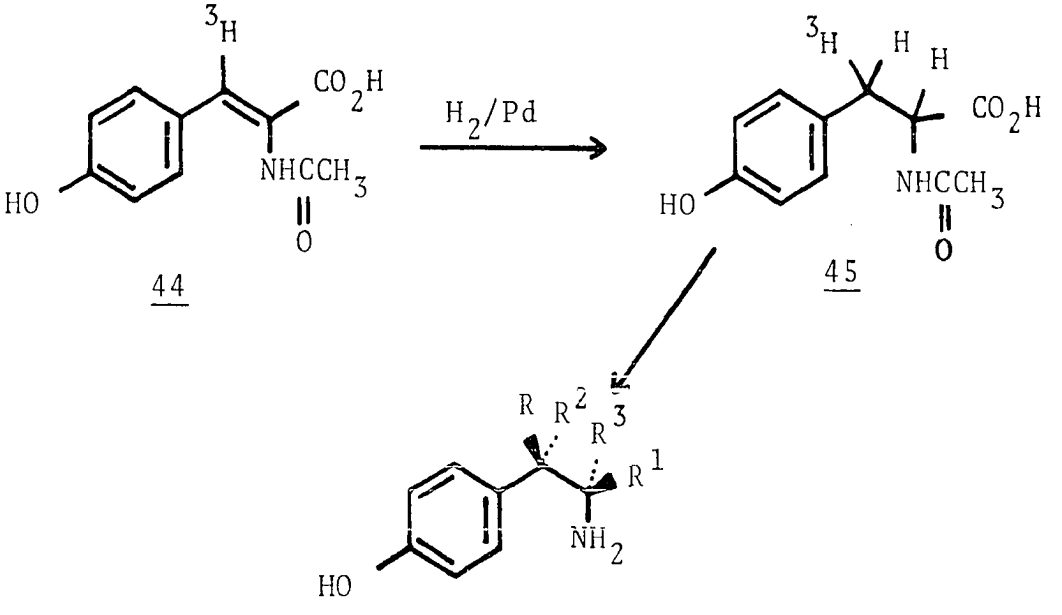


Figure 14. Biosynthesis of 6-hydroxycrinamine (41) and 6-hydroxybuphanidrine (42).

The data obtained by Virnig (27) are in direct contrast to those obtained by Fuganti and Mazza (25,26) and the conflict remains unsolved at the present time.

Hydroxylation at C-11

The stereochemistry of the hydroxylation at C-11 in the crinine-type alkaloids has also provided conflicting data. Kirby and Michael (28) synthesized L-[βR-^{3}H]tyrosine (43a) and D-[βS-^{3}H]tyrosine (43b) by resolving and deprotecting the racemic tyrosine derivative (45) obtained by hydrogenating 44 with a palladium catalyst (Figure 15).



44 45

43a R = ^{3}H, R^{1} = CO_2H, R^{2} = R^{3} = H

43b R = R^{1} = H, R^{2} = ^{3}H, R^{3} = CO_2H

Figure 15. Synthesis of tyrosine.

Biosynthetic investigations in growing "Texas" daffodils showed that L[βR-^{3}H]tyrosine (43a) was converted

into haemanthamine (46a) with the loss of 83% of the tritium present in 43a. D-[βS-^{3}H]tyrosine was converted into haemanthamine (46b) with retention of 87% of the tritium present in 43b. This showed that hydroxylation with predominant retention of configuration at C-11 had occurred (Figure 16).

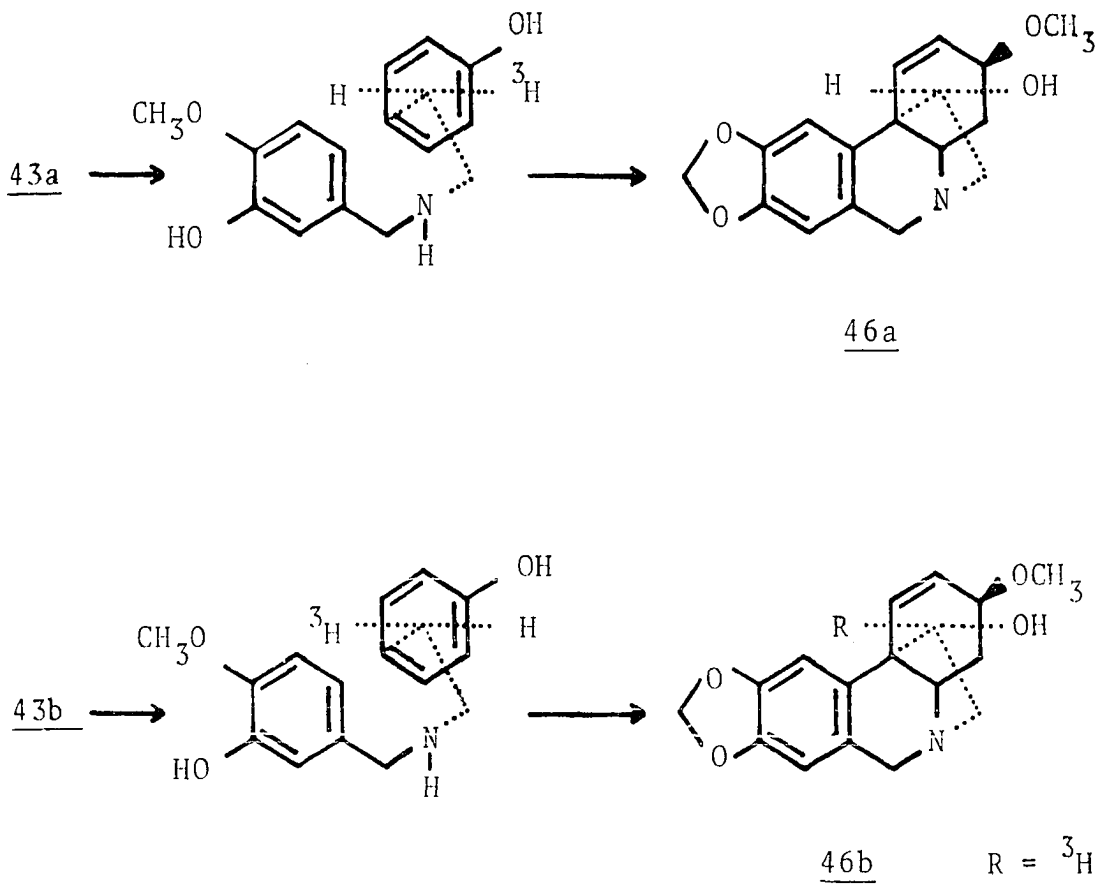


Figure 16. Biosynthesis of haemanthamine.

Hydroxylation at C-11 with retention of configuration was also observed by Battersby and co-workers (29,30). 0-Methyl-(2S)-[2-^{3}H]norbelladine hydrochloride (47a) was synthesized as shown in Figure 17. Aldehyde (48b) was reduced with equine liver alcohol dehydrogenase (LAD) and nicotinamideadenine dinucleotide (NADH) in an ethanol solution to yield alcohol (49) which was converted to 50 with triphenylphosphine/carbon tetrachloride. A malonate synthesis yielded 51 which was converted to amine (52) via an amide-hypochlorite rearrangement. Subsequent reductive amination with 53 followed by debenzylation afforded 0-methyl-(2S)-[2-^{3}H]norbelladine hydrochloride (47a). The (2R)-isomer was obtained by the same sequence by substituting thionyl chloride/dioxane for triphenylphosphine/carbon tetrachloride.

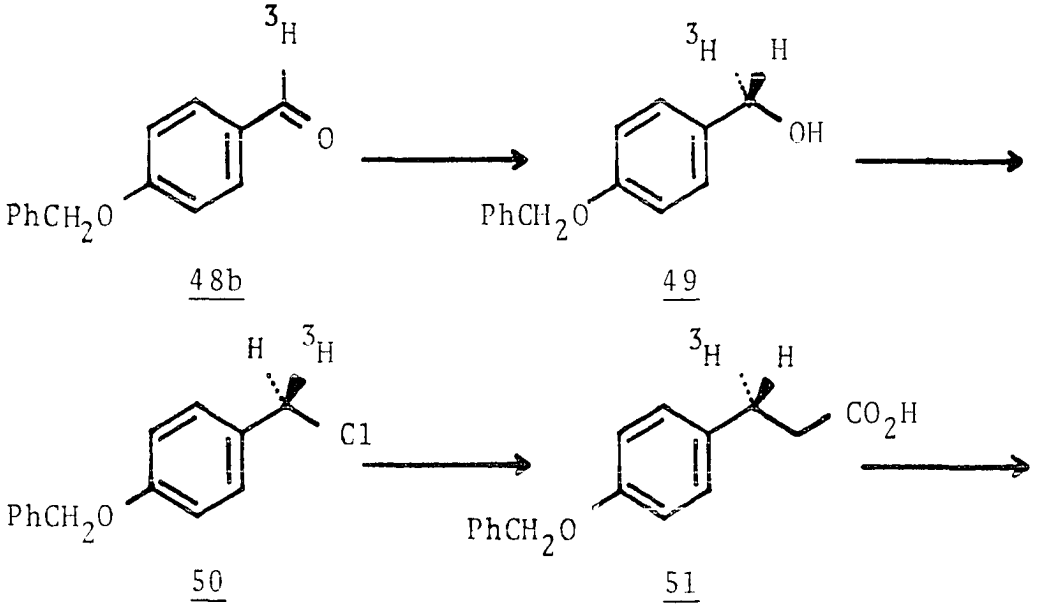
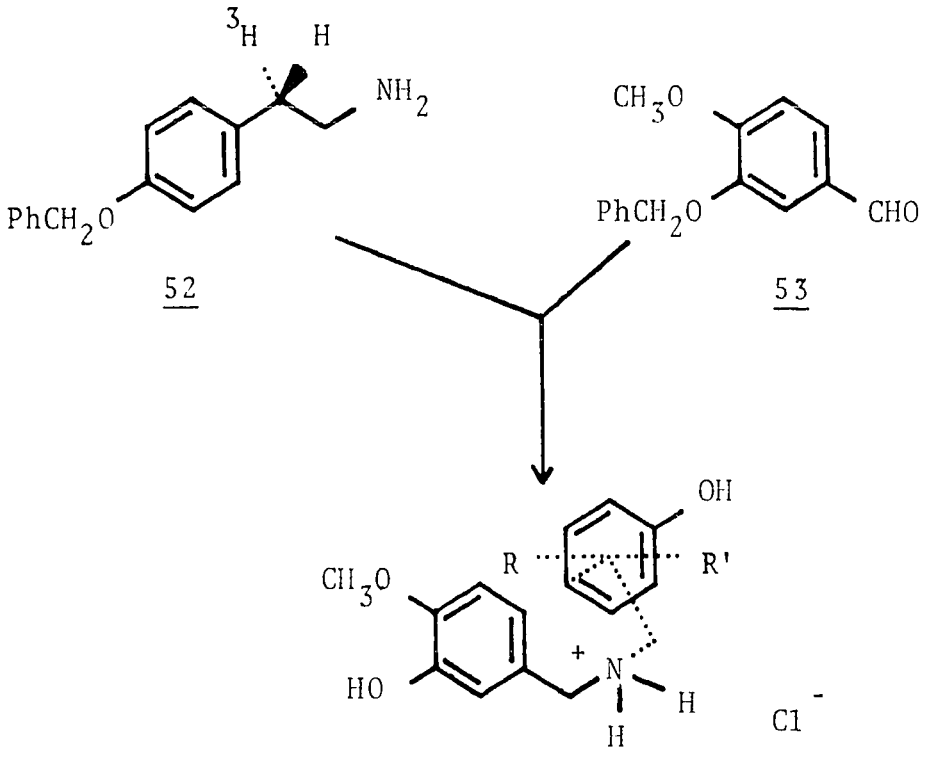


Figure 17. Synthesis of 0-methyl-(2S)-[2-^{3}H]norbelladine hydrochloride (47a).



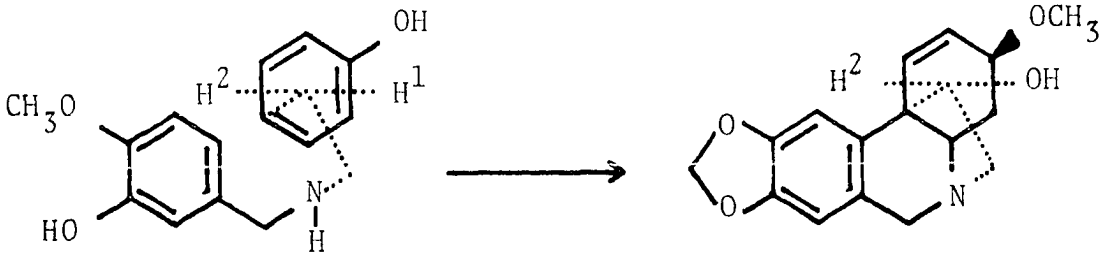
47a R = ^{3}H, R' = H

47b R = H, R' = ^{3}H

Figure 17. (Continued).

The (2R)- and (2S)-isomers were mixed with O-methyl-[1-^{14}C]norbelladine hydrochloride and were fed to Narcissus pseudonarcissus ("King Alfred" daffodils) and haemanthamine (46a) was isolated. The haemanthamine obtained from the (2S)-isomer retained 66 ± 4% of the tritium present at C-2 of 47a, whereas the haemanthamine obtained from the (2R)-isomer lost 69 ± 2% of the tritium present at C-2 of 47b. This showed that O-methylnorbelladine (9) was converted into

haemanthamine (46a) with the loss of the pro-R hydrogen from C-2 of 9. Also, the hydroxylation at C-11 occurred with retention of configuration (Figure 18).



9 46a

Figure 18. Biosynthesis of haemanthamine (46a).

Fuganti and co-workers (31) synthesized O-methyl-(2S)-[2-^{3}H]norbelladine hydrochloride (47a) and O-methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b) via the palladium-catalyzed ring opening of the corresponding optically active oxazines as shown in Figure 19 (shown only for (2R)-isomer).

Oxazine (54) was converted to 55 by refluxing in ethanol with 10% palladium/charcoal catalyst. Compound 55 was reduced with lithium aluminum hydride to yield 56 which underwent subsequent Hofmann elimination followed by

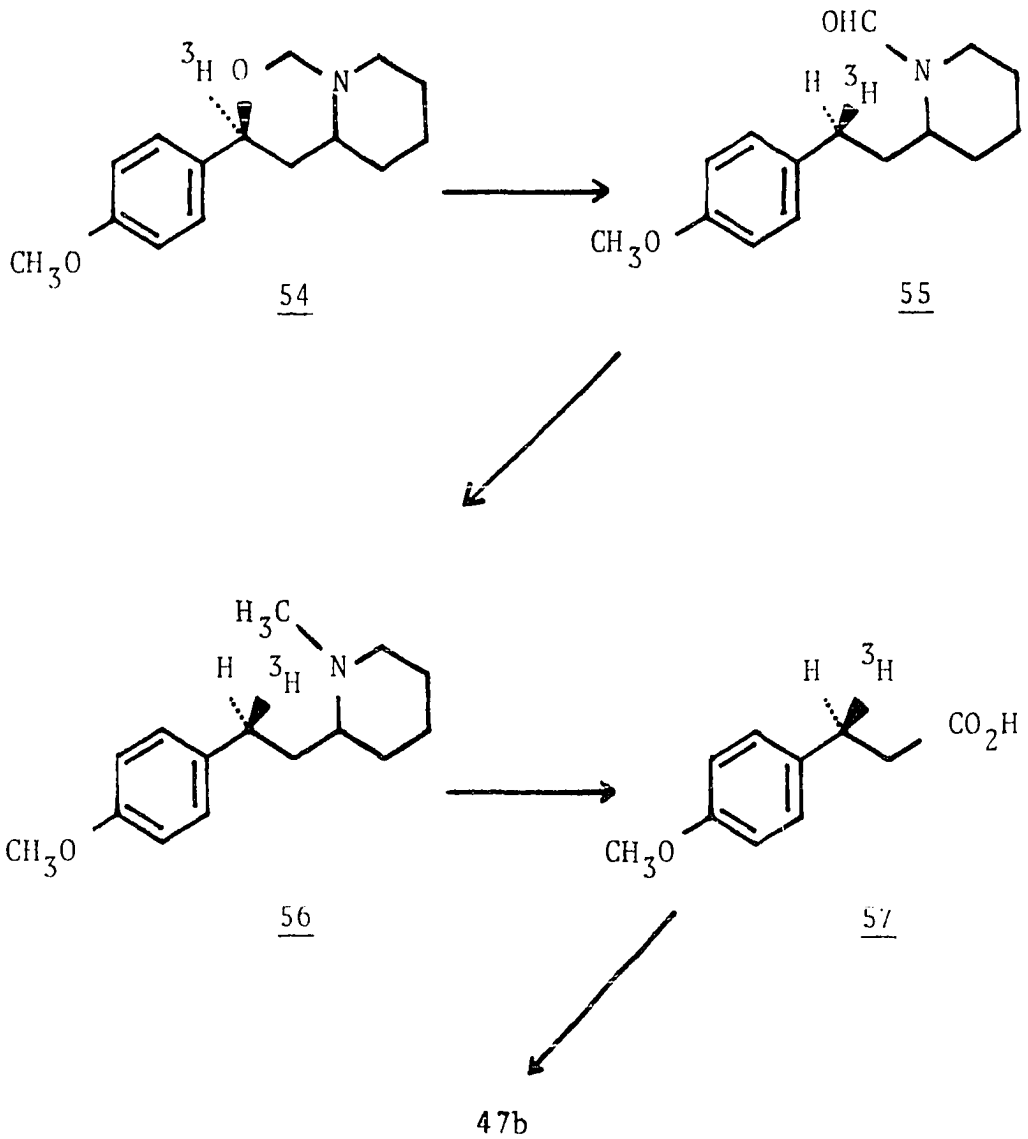
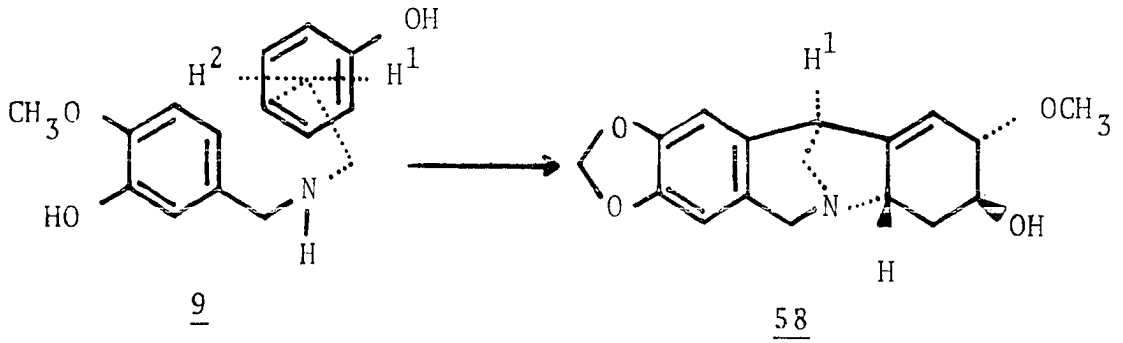


Figure 19. Synthesis of O-methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b).

ozonolysis utilizing an oxidative workup to yield the propionic acid derivative (57). Compound 57 was elaborated to 47b by well-established methods.

When the (2R)-isomer (47b) was mixed with 0-methyl-[1-^{14}C]norbelladine hydrochloride and fed to Sempre Avanti daffodils, the haemanthamine (46a) isolated retained only 5% of tritium present at C-2 of 47b. When the (2S)-isomer (47a) was mixed with 0-methyl-[1-^{14}C]norbelladine hydrochloride and fed to Ismene hybrid "Sulphur Queen", the haemanthamine isolated retained 94% of the tritium present at C-2 of (47a) (32). These data showed that 0-methyl-norbelladine (9) was converted into haemanthamine (46a) with loss of the pro-R hydrogen and that the hydroxylation at C-11 occurred with retention of configuration (Figure 18).

Fuganti and co-workers (33) also showed that Haemanthus coccineus converted 0-methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b) to montanine (58) with 104% tritium retention. The parallel feeding with the (2S)-isomer (47a) yielded montanine (58) with less than 5% tritium retention. From these data it was concluded that 0-methylnorbelladine (9) was converted to montanine (58) with the loss of the pro-S hydrogen from C-2 of 9 (Figure 20).



9 58

Figure 20. Biosynthesis of montanine (58) in H. coccineus.

Feinstein (34) showed that vittatine (59) tritiated in the aromatic ring was converted to both haemanthamine (61) and montanine (62) in Rhodophiala bifida with retention of tritium presumably via 11-hydroxyvittatine (60) (Figure 21). If such an intermediate is involved in the

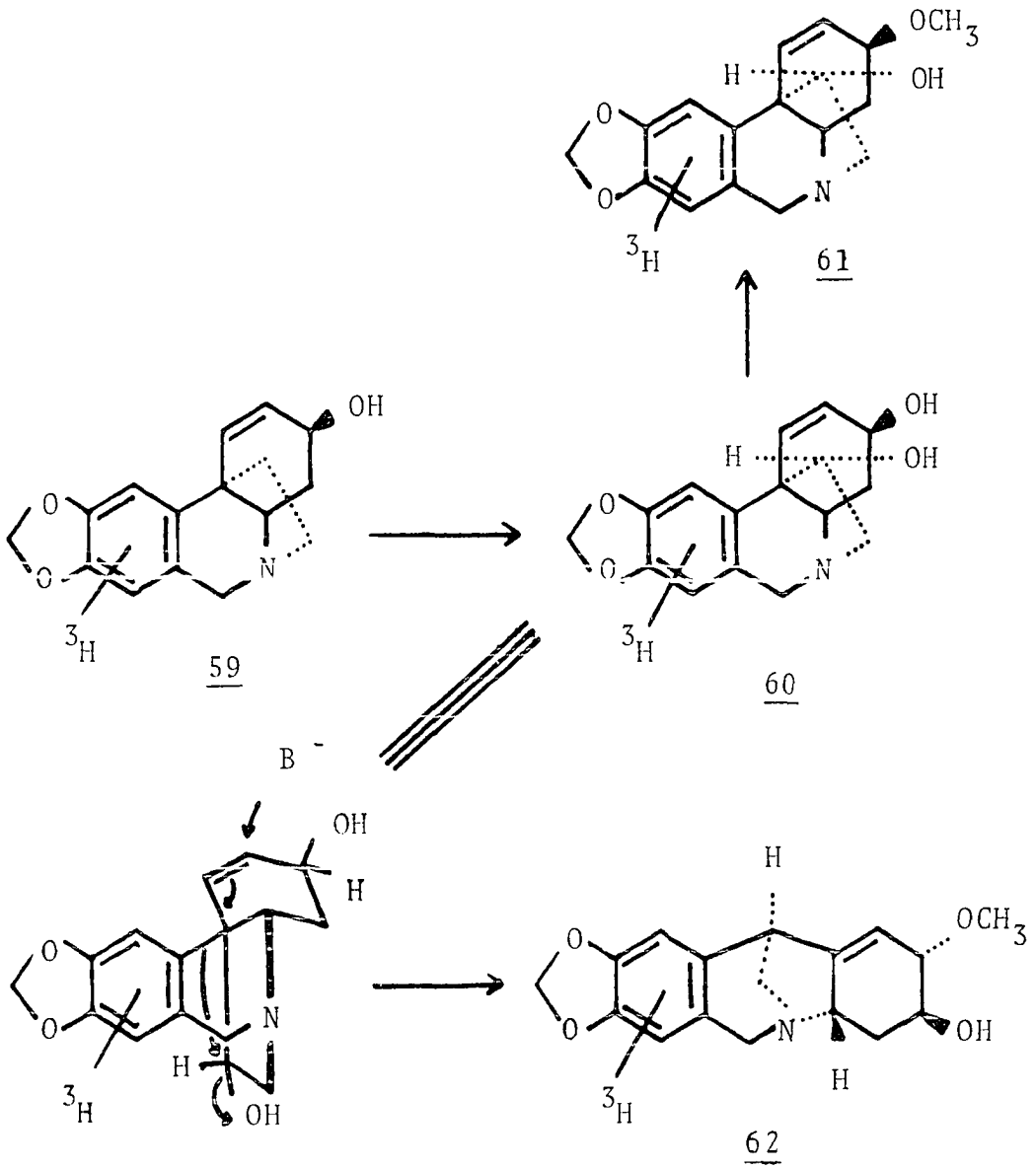


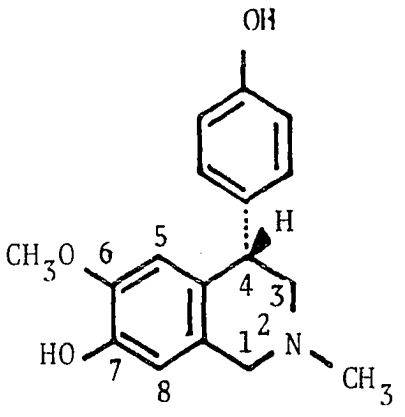
Figure 21. Proposed biosynthesis of haemanthamine (61) and montanine (62).

skeletal rearrangement, the stereochemistry of hydrogen removal at C-11 observed by Fuganti and co-workers (33) is opposite to that observed by Battersby and co-workers (29, 30).

The stereochemistry of hydroxylation at C-11 is still unclear, but could be clarified by feeding either (2R)- or (2S)-O-methyl-[2-^{3}H]norbelladine to R. bifida. If 60 is the common intermediate to 61 and 62 then one should observe incorporation of the label at C-2 of 9 into both 61 and 62 or one should observe the loss of the label at C-2 of 9 in both 61 and 62. If 61 is not the common intermediate, then one could observe incorporation of label into 61 and loss of label in 62 or vice versa.

Phenolic Alkaloids

To date, relatively few phenolic Amaryllidaceae alkaloids have been isolated. This section is concerned with one of the phenolic alkaloids, cherylline (63). Cherylline (63) was first isolated from Crinum moorei by Boit (35). The alkaloid was initially named crinin; however, to avoid confusion with the non-phenolic alkaloid crinine (11), the name was changed to cherylline.



63

Structure determination

The structure determination of cherylline (63) was based primarily on the nmr spectrum in DMSO-d_6. An AA'BB' pattern (δ6.91 and 6.64) characteristic of a 1,4-disubstituted aromatic ring, two one-proton singlets (δ6.49 and 6.23) indicative of two para-oriented protons on a second aromatic ring, and two three-proton singlets at δ3.51 (OCH_3) and δ2.24 (NCH_3) in addition to a few less well-defined signals were observed. The proton at δ6.23 was assigned to C-5 due to the shielding effect of the 4-phenyl group. The hydroxyl group was assigned to C-7 because when a drop of NaOD was added, the proton at δ6.49 shifted 26 Hz to δ6.06 whereas the proton at δ6.23 shifted only 10 Hz. The mass spectrum and elemental analysis indicated a molecular weight of 285 and an empirical formula of $C_{17}H_{19}NO_3$. These data are consistent with structure 63. Further proof that

cherylline contained the skeleton of 63 was obtained by methylating cherylline with diazomethane to yield 0,0-dimethylcherylline. The fully methylated derivative exhibited the same rf value on silica gel as synthetic 0,0-dimethyl-cherylline in several solvent systems, and the infrared spectra of the two were identical. The absolute configuration at C-4 was determined by comparison with the ORD and CD spectra of several 4-phenyl-1,2,3,4-tetrahydroisoquinolines and was assigned the S configuration. This was also confirmed by X-ray analysis (36,37).

Synthesis of cherylline

To date, four different synthetic routes to cherylline (63) have been reported. The first synthesis was performed by Brossi and Teitel (38). In their synthesis, phenethylamine (64) was resolved, and the (-)-isomer (65) was then transformed by N-formylation, Bischler-Napieralski cyclization, selective 0-demethylation, quarternization, and reduction to 63 (Figure 22).

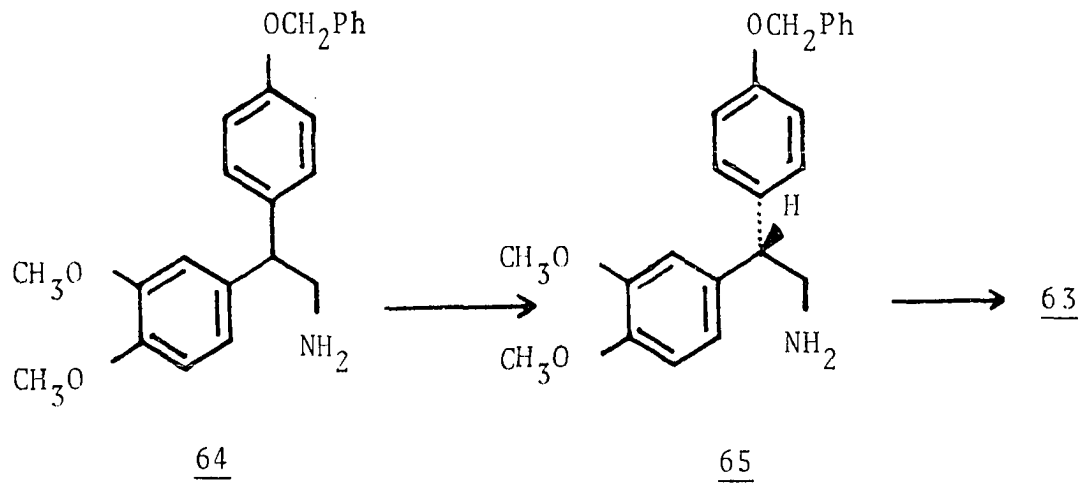


Figure 22. Brossi and Teitel's approach to cherylline (63).

Schwartz and Scott (39) synthesized cherylline (63) using a biogenetically patterned synthesis. Cherylline (63) was synthesized via base-catalyzed cyclization of 66, an intermediate proposed by Schwartz and Scott to have possible biogenetic significance (Figure 23).

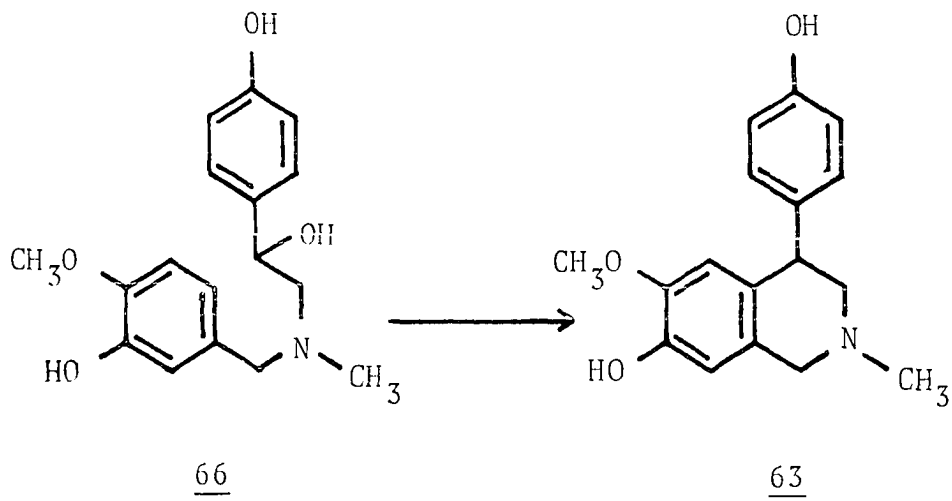


Figure 23. Schwartz and Scott's approach to cherylline (63).

Shaffer's (40) approach to cherylline was devised so that both C-1 and C-3 of cherylline (63) could be labelled. The key step in Shaffer's synthesis involved the reaction of cyanohydrin (67) with phenol and sulfuric acid to yield 68. Compound 68 was then reduced to the amine and elaborated to 63 by several standard steps (Figure 24).

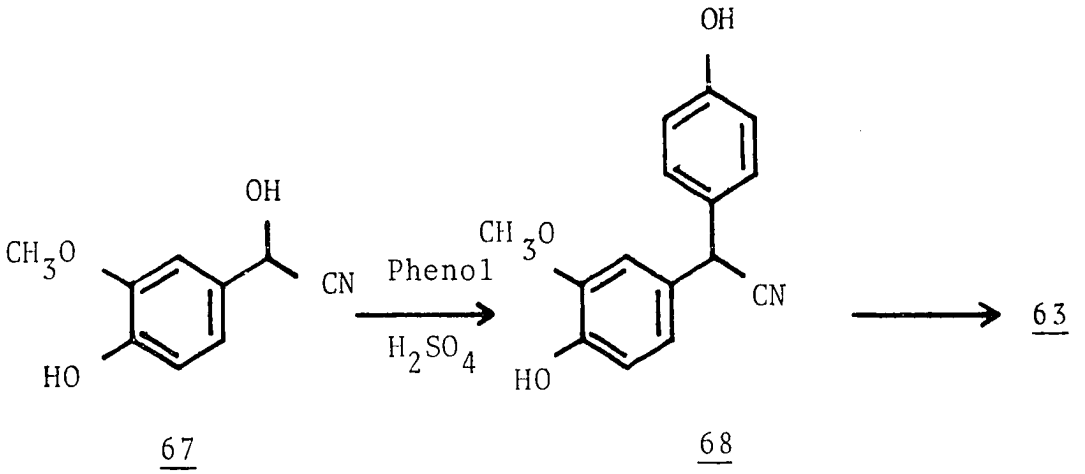
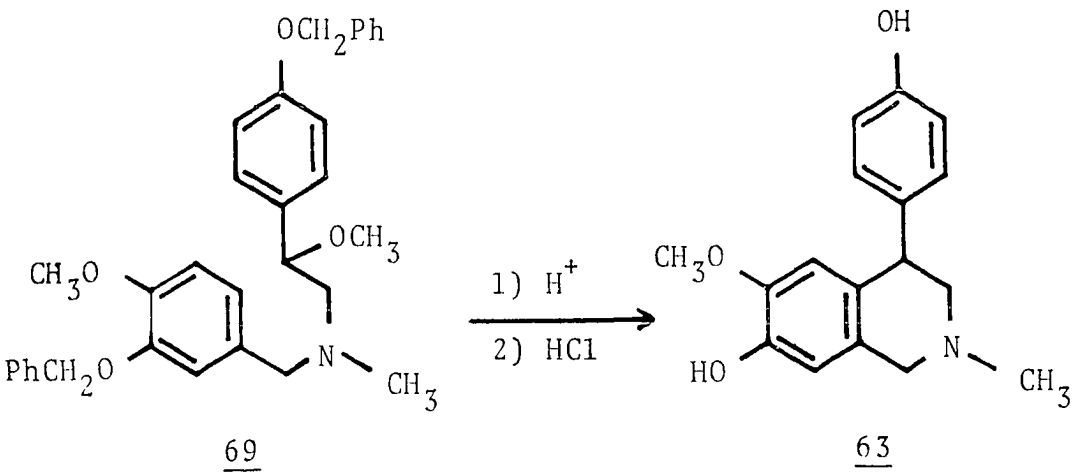


Figure 24. Shaffer's approach to cherylline (63).

The last synthesis to appear was by Kametani and coworkers (41). The key step in this synthesis of cherylline (63) was the acid-catalyzed cyclization of 69 to yield cherylline (63) (Figure 25).

Biosynthesis of cherylline

The biosynthesis of cherylline (63) cannot be rationalized in terms of the Barton and Cohen theory of phenyl-phenyl oxidative coupling since the phenyl rings are not



69 63

Figure 25. Kametani's approach to cherylline (63).

coupled. One possibility for the biogenesis of cherylline would be the oxidation of montanine-type alkaloids followed by elimination and N-methylation. However, this would lead to the wrong stereochemistry at C-4 (Figure 26).

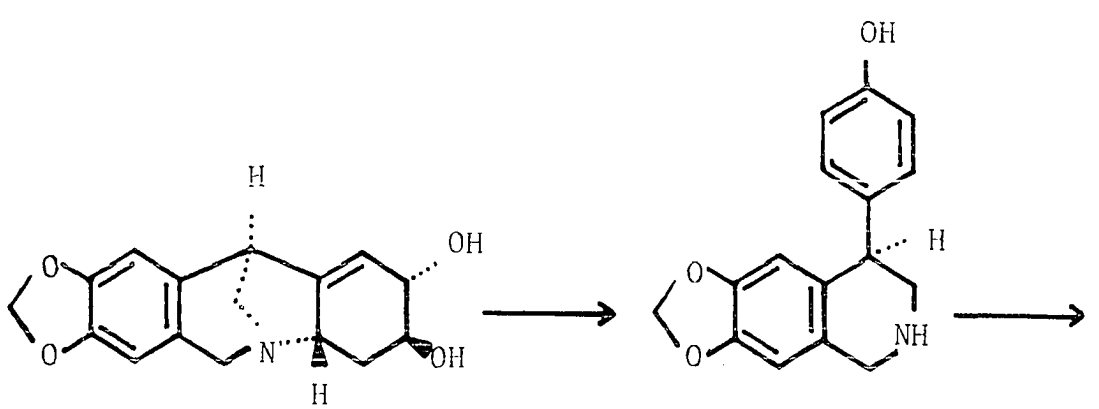


Figure 26. Possible route to cherylline skeleton.

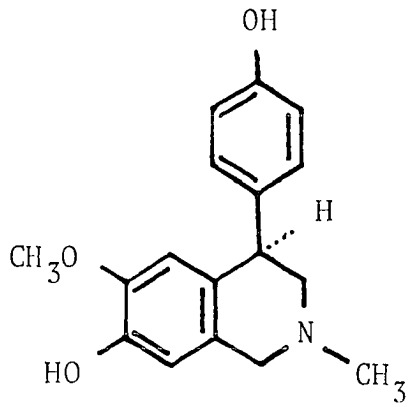
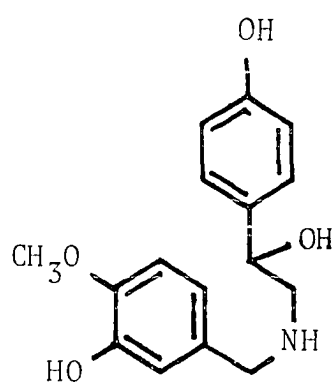


Figure 26. (Continued).

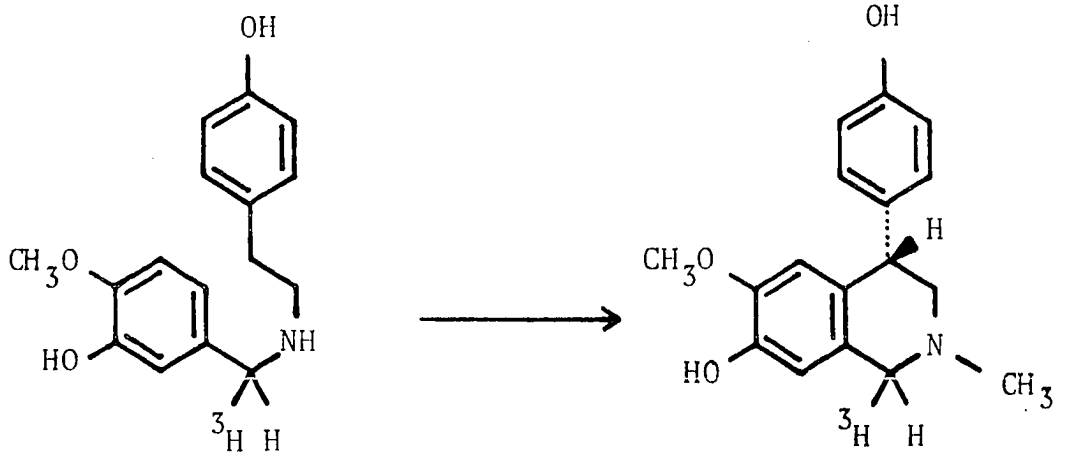
Since the structure of cherylline (63) is very similar to that of 0-methylnorbelladine (9), it was proposed by Schwartz and Scott (39) that 0-methylnorbelladine (9) or 2-hydroxy-0-methylnorbelladine (70) could be the key precursor to cherylline.



70

The biosynthesis of cherylline (63) was first investigated by Shaffer (40). Shaffer found efficient

incorporation of 0-methyl-[1'-^{3}H]norbelladine (37) into cherylline (71) in C. erubescens (Figure 27).



37 71

Figure 27. Biosynthesis of cherylline (71).

The biosynthetic mechanism for cherylline (63) was then elaborated further by Chan (42). The initial experiment by Chan involved feeding 0-methyl-[1'-^{3}H, 1-^{14}C]norbelladine (38) to C. powellii. In agreement with the earlier work, this precursor was found to be incorporated efficiently into cherylline (63). More importantly, Chan's work demonstrated the 0-methylnorbelladine (9) was incorporated intact into cherylline (63).

Chan also synthesized 0,N-dimethyl-[1'-^{3}H, 1-^{14}C]norbelladine (72), 2-hydroxy-0-methyl-[2-^{3}H, 1'-^{14}C]norbelladine (73), and N-demethyl-[1 -^{14}C, 4-^{3}H]cherylline (74) which were fed to C. powellii in separate experiments.

Since all of the above precursors were incorporated into cherylline (63), the following biosynthetic mechanism was postulated for cherylline (63) (Figure 28). In agreement with the theory of Schwartz and Scott, compound 67 was postulated as an intermediate even though it was not fed and shown to be incorporated.

The stereochemistry of the hydroxylation and subsequent ring closure was not studied, but this could be studied by feeding the appropriately labeled O-methylnorbelladine.

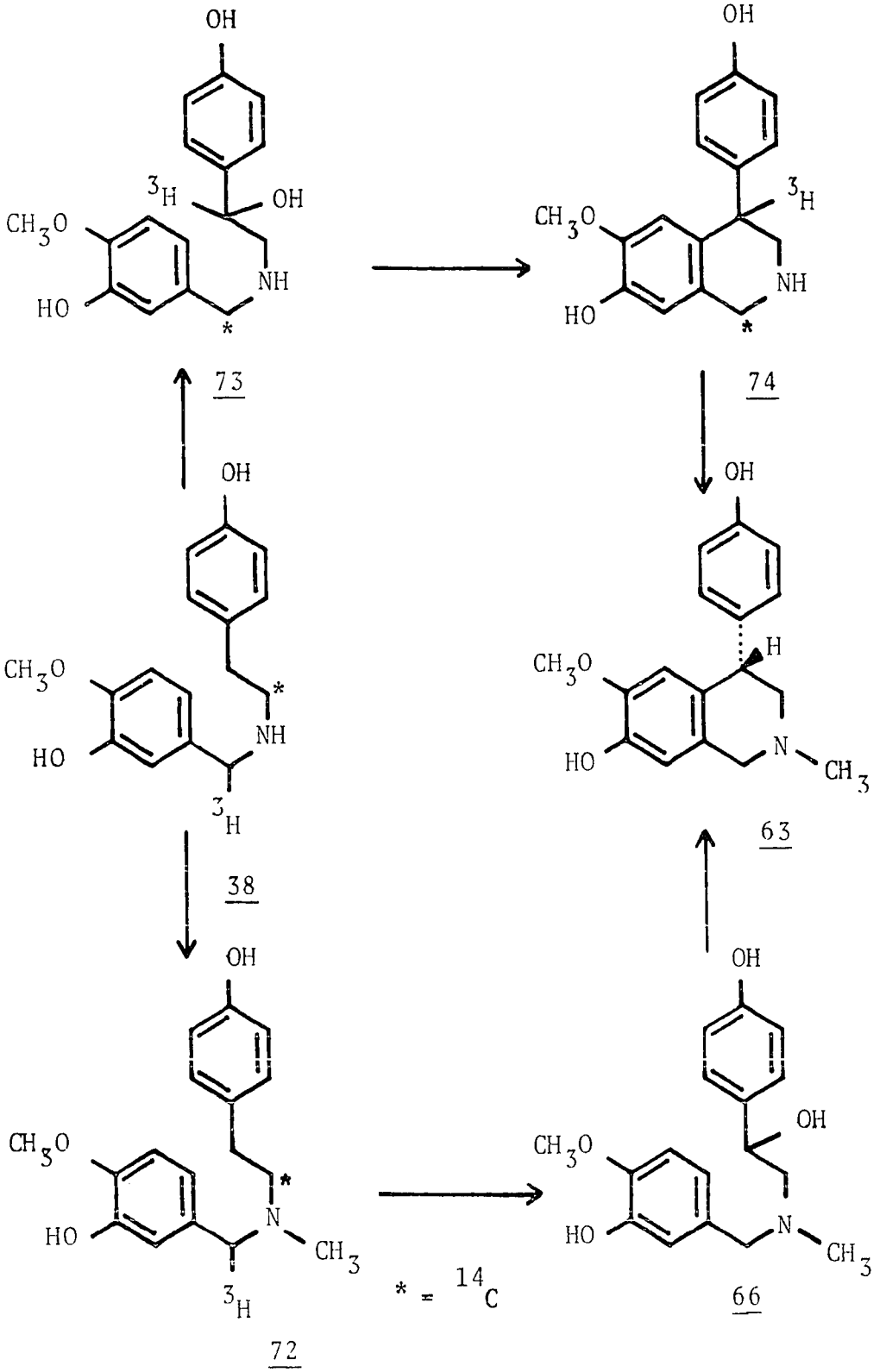


Figure 28. Biosynthesis of cherylline (63).

RESULTS AND DISCUSSION

Synthetic Investigations

This section is concerned with the synthesis of 0-methyl-(2R)-[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (85) and the subsequent biosynthetic investigations in R. bifida and C. moorii.

Synthesis of 0-methyl-[1'-^{14}C]norbelladine hydrochloride (75)

0-Methyl-[1'-^{14}C]norbelladine hydrochloride (75) was synthesized by the method of Chan (42) as shown in Figure 29.

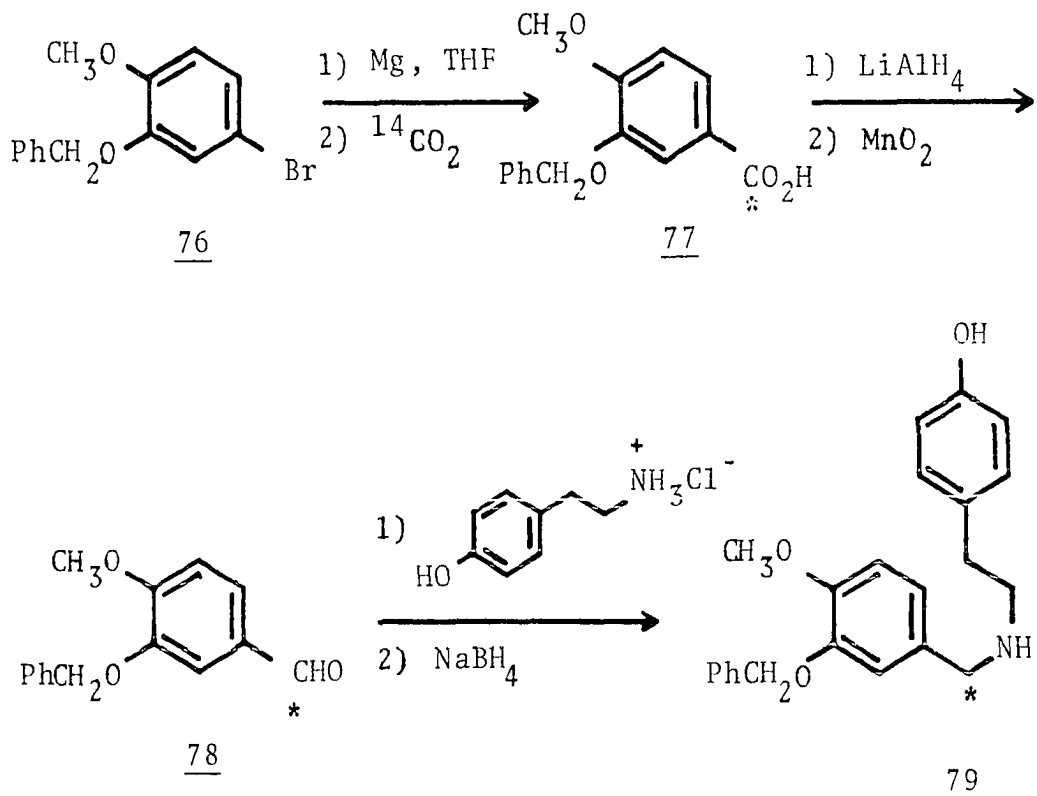
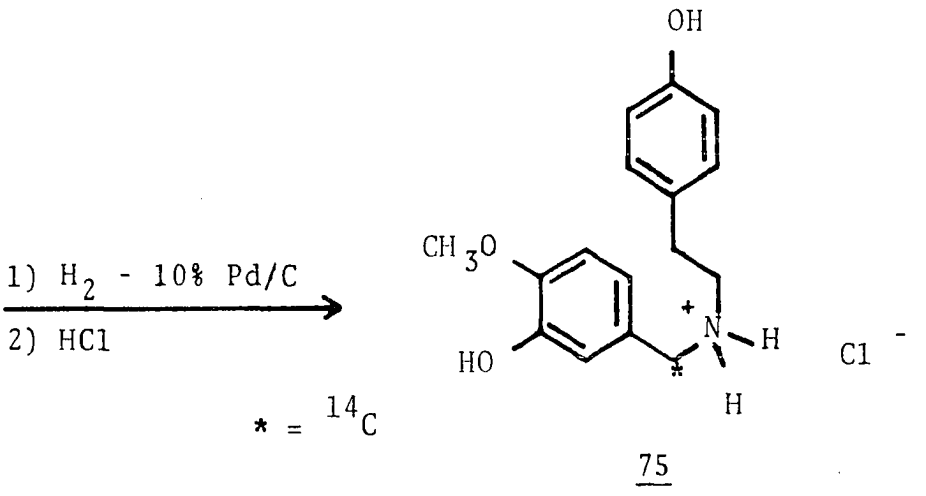


Figure 29. Synthesis of 0-methyl-[1'-^{14}C]-norbelladine hydrochloride (75).



75

Figure 29. (Continued).

Before the synthesis of the radioactive compounds were performed, each step was developed with non-radioactive compounds.

Synthesis of 0-methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b)

0-Methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b) was synthesized by a method that parallels that of Battersby and co-workers (29,30) as shown in Figure 30. Before synthesizing the radioactive compounds, all the steps in the synthetic scheme were developed with compounds containing deuterium.

The key step in this synthesis is the reduction of aldehyde (48b) to alcohol (49) using the enzyme equine liver alcohol dehydrogenase (LAD) and reduced nicotinamide-adenine dinucleotide (NADH). This afforded the (S)-alcohol (49) (29,30) which was elaborated further as shown.

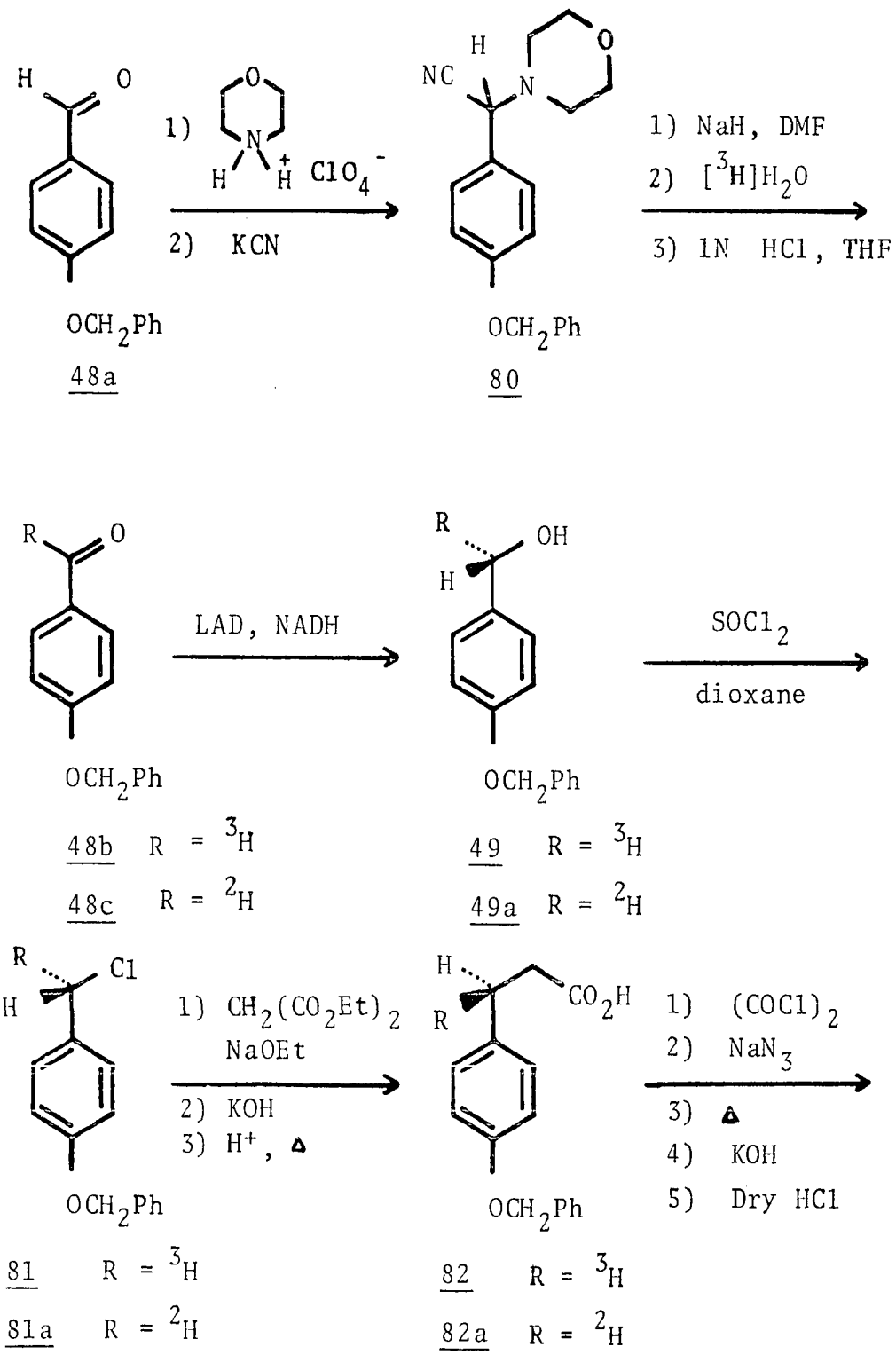
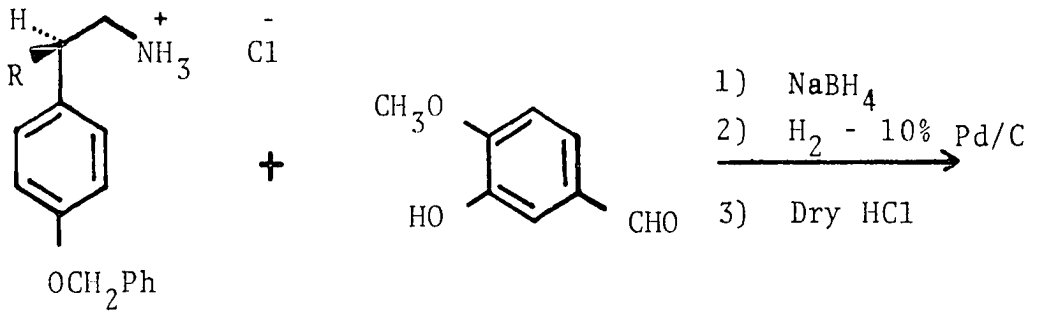
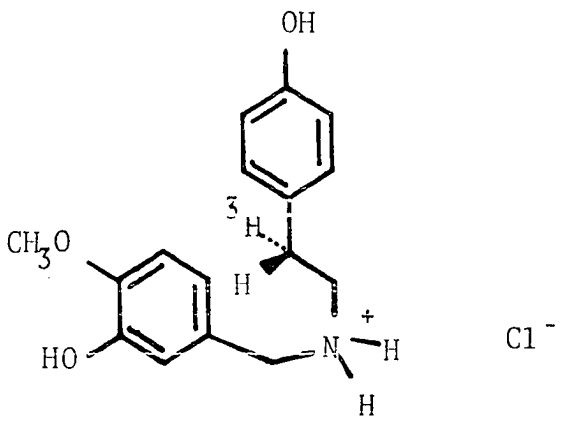


Figure 30. Synthesis of 0-methyl-(2R)-[2-^{3}H]-norbelladine hydrochloride (47b).



83 R = ^{3}H

83a R = ^{2}H



47b

Figure 30. (Continued).

Figure 31. NMR spectrum of 4-benzyloxybenzaldehyde (48a).

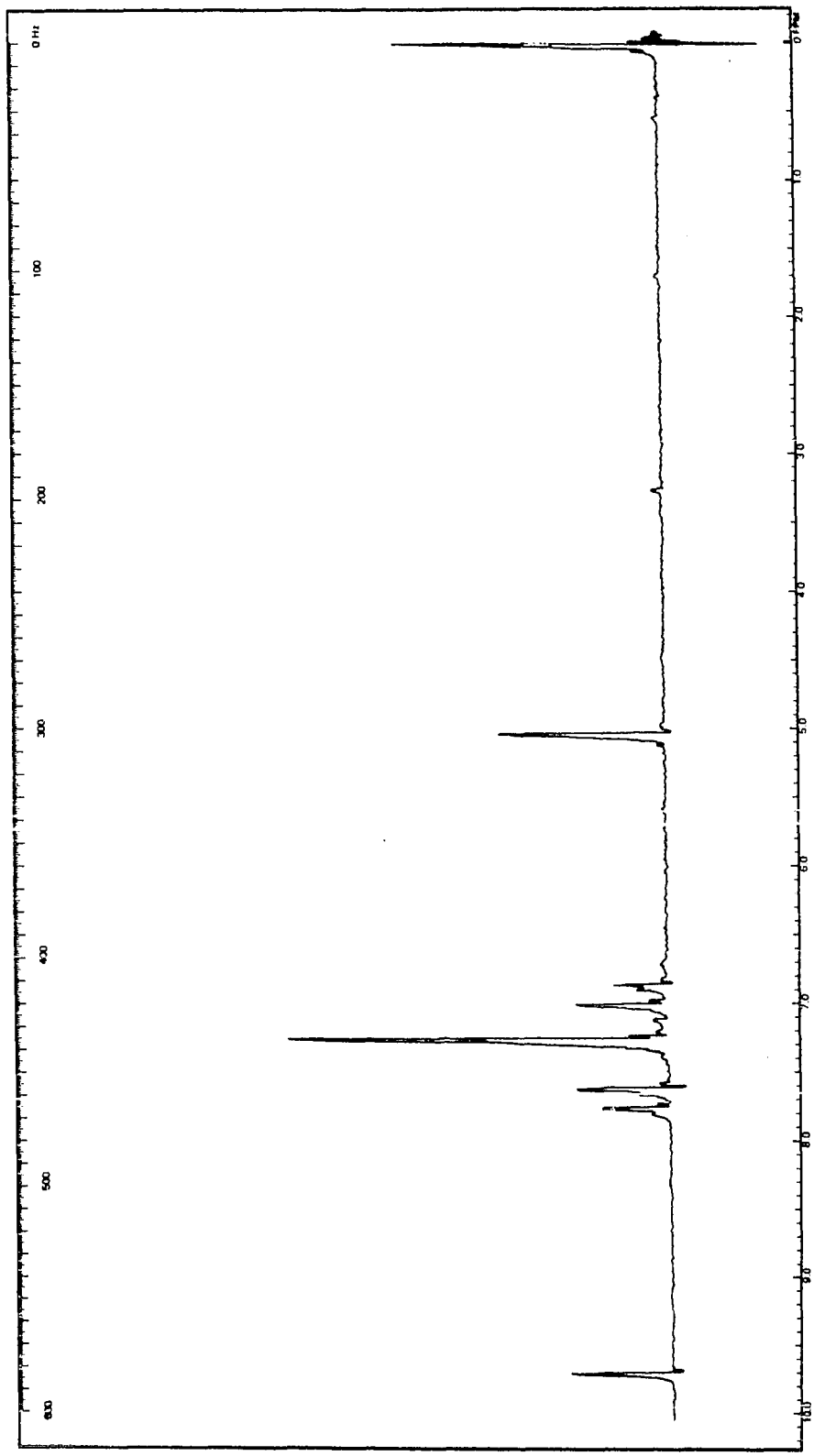

Hz
0
100
200
300
400
500
600
PPM
0.1
0
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1

Figure 32. NMR spectrum of 4-benzyloxyphenyl morpholine acetonitrile (80).

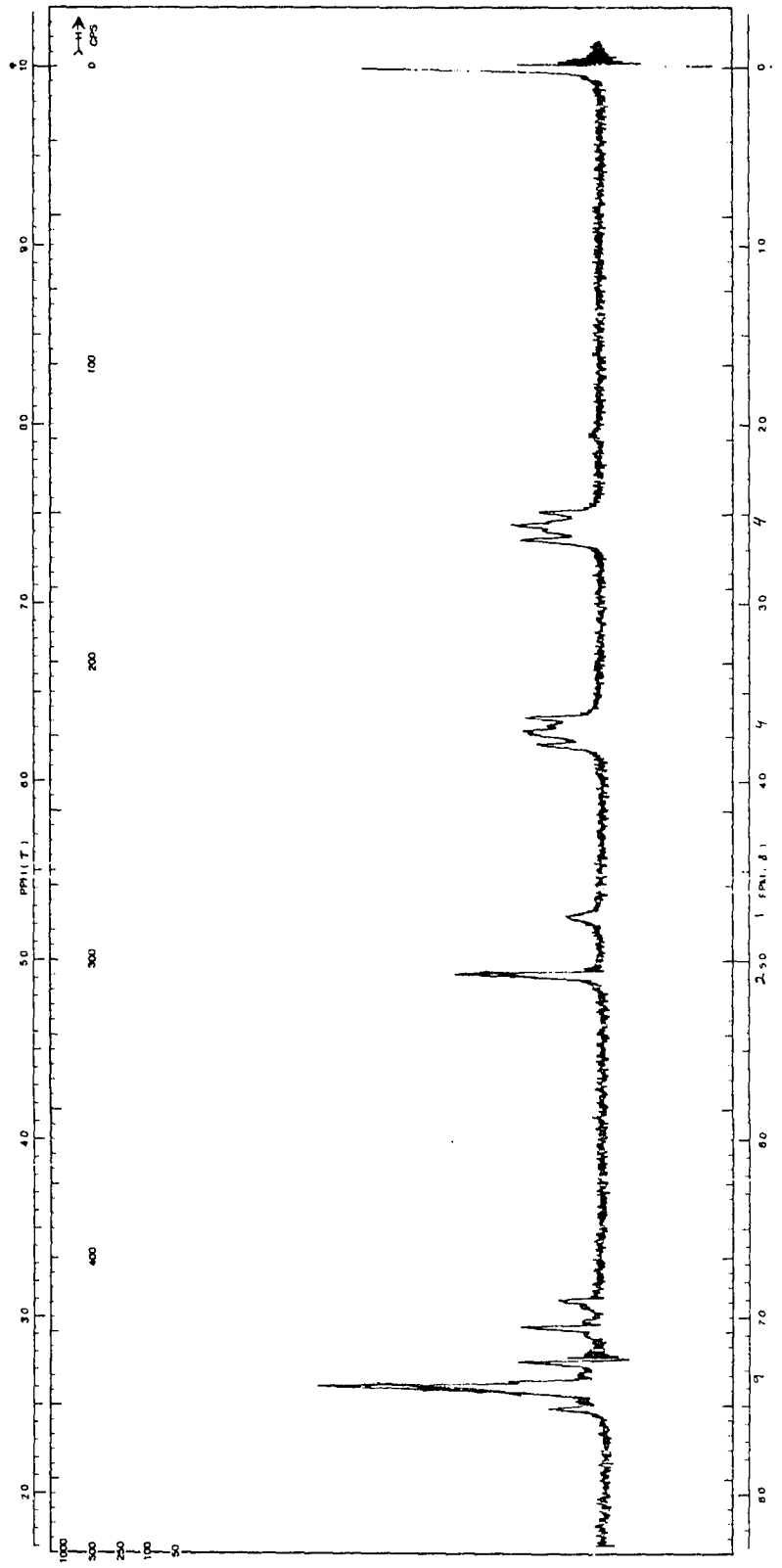


Figure 33. NMR spectrum of 4-benzyloxy-[7-^{2}H]benzaldehyde (48c).

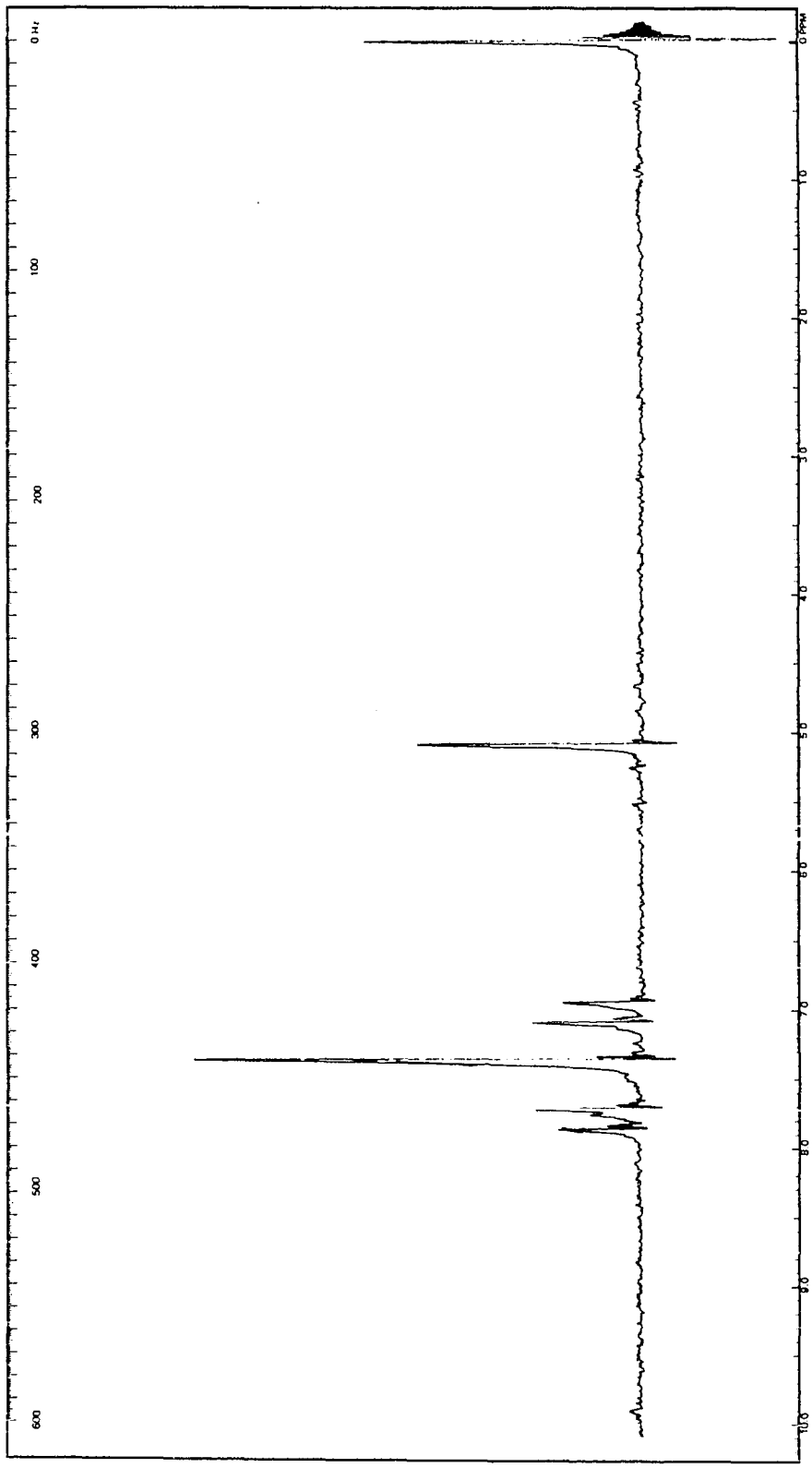

0 Hz
100
200
300
400
500
600
0 PPM
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0

Figure 34. NMR spectrum of 4-benzyloxy-(7S)-[7-^{2}H]benzyl alcohol (49a).

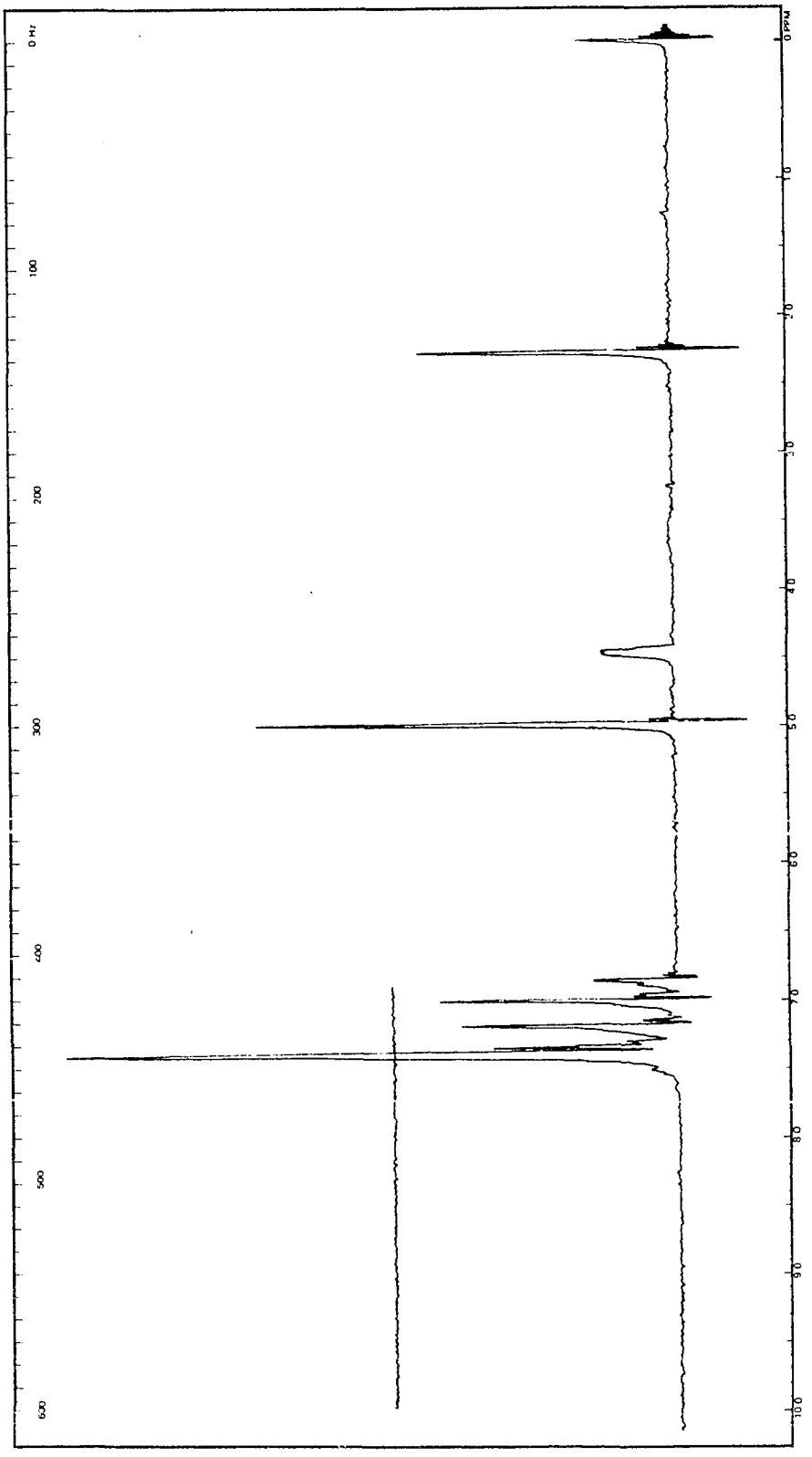

0 Hz
100
200
300
400
500
600
PPM
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0

Figure 35. NMR spectrum of 4-benzyloxy-(7S)-[7-^{2}H]benzyl chloride (81a).

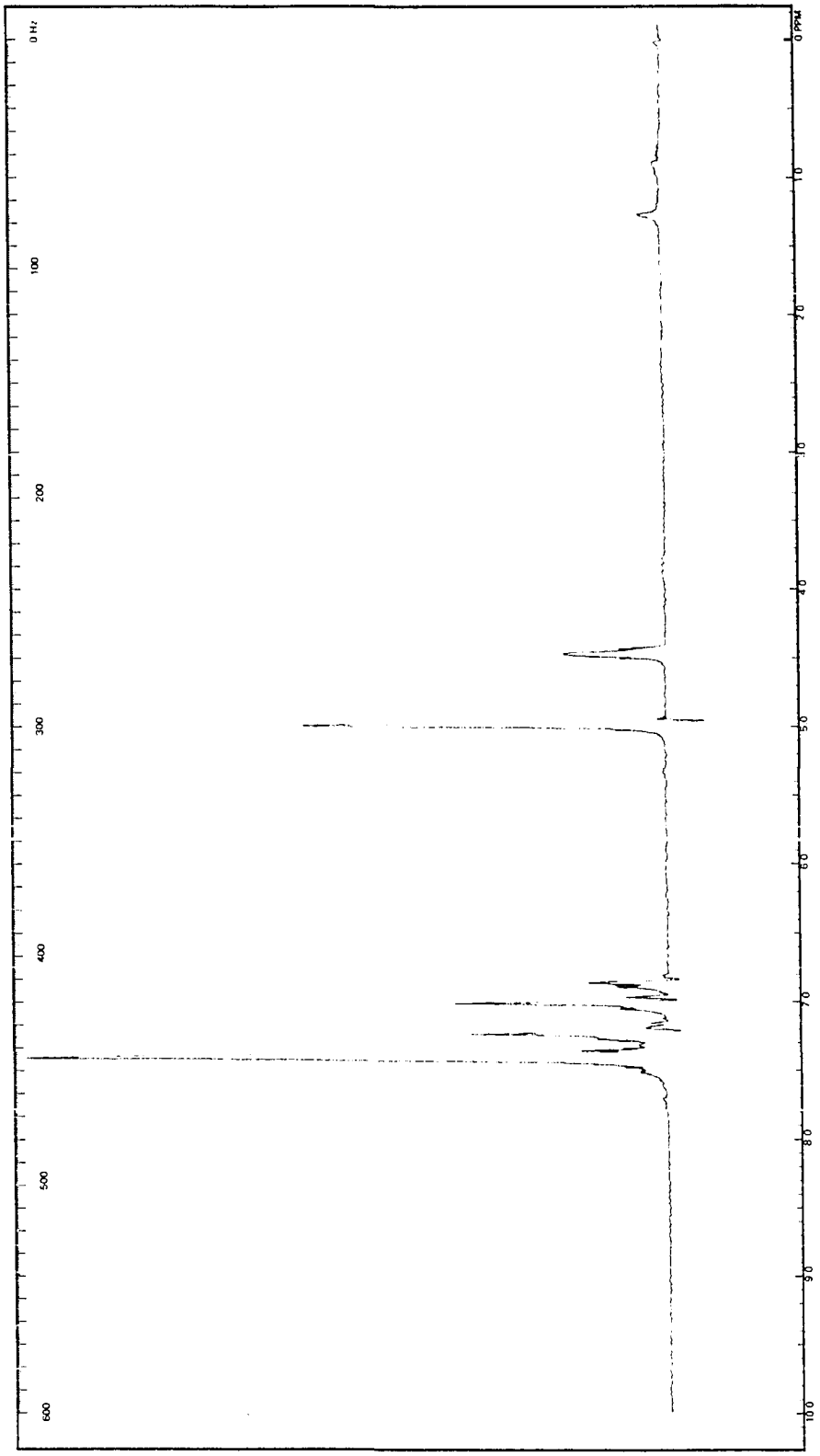

0 PPM
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0 HZ
100
200
300
400
500
600

Figure 36. NMR spectrum of 3-[4-(benzyloxy)phenyl]-[3-^{2}H]propionic acid (82a).

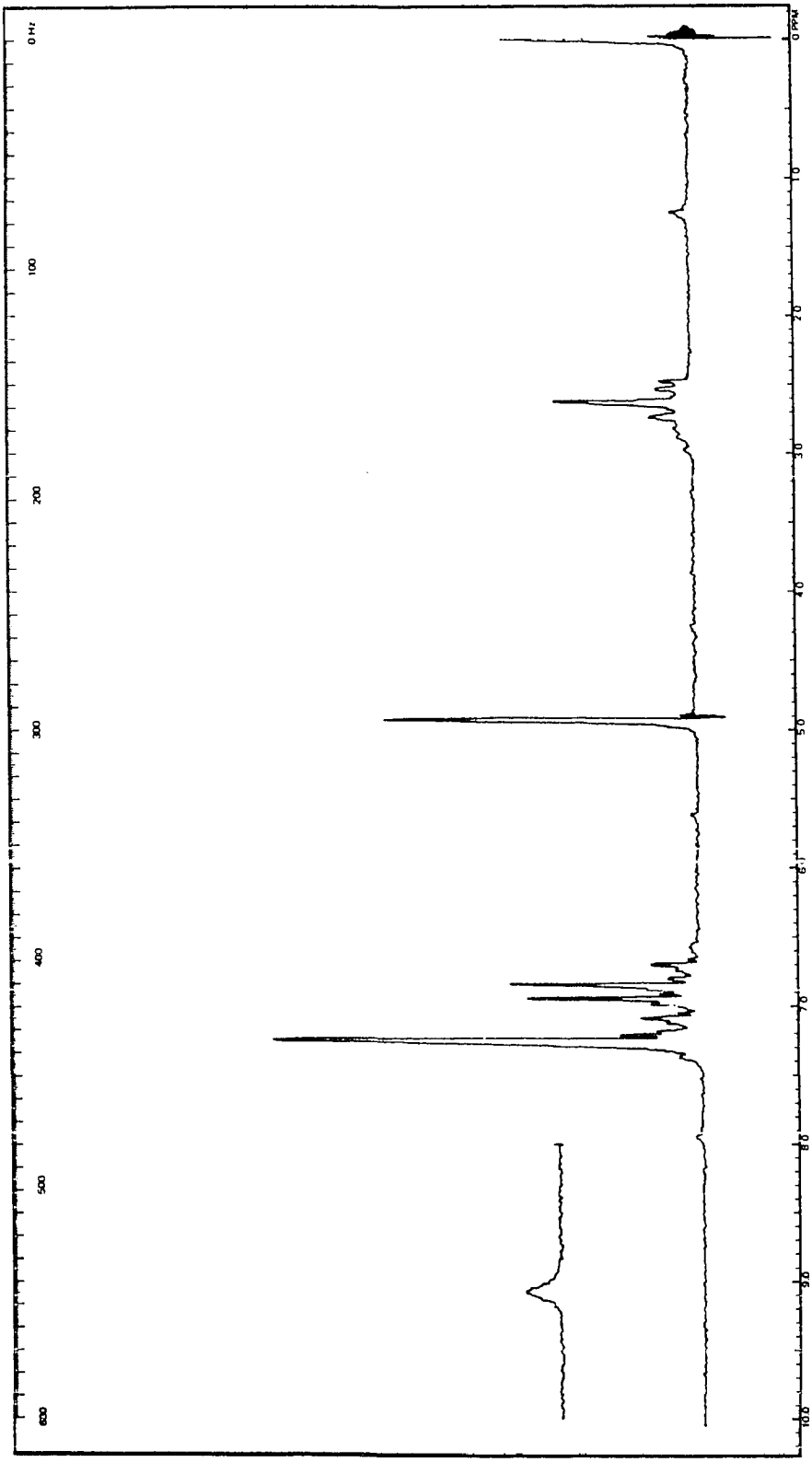
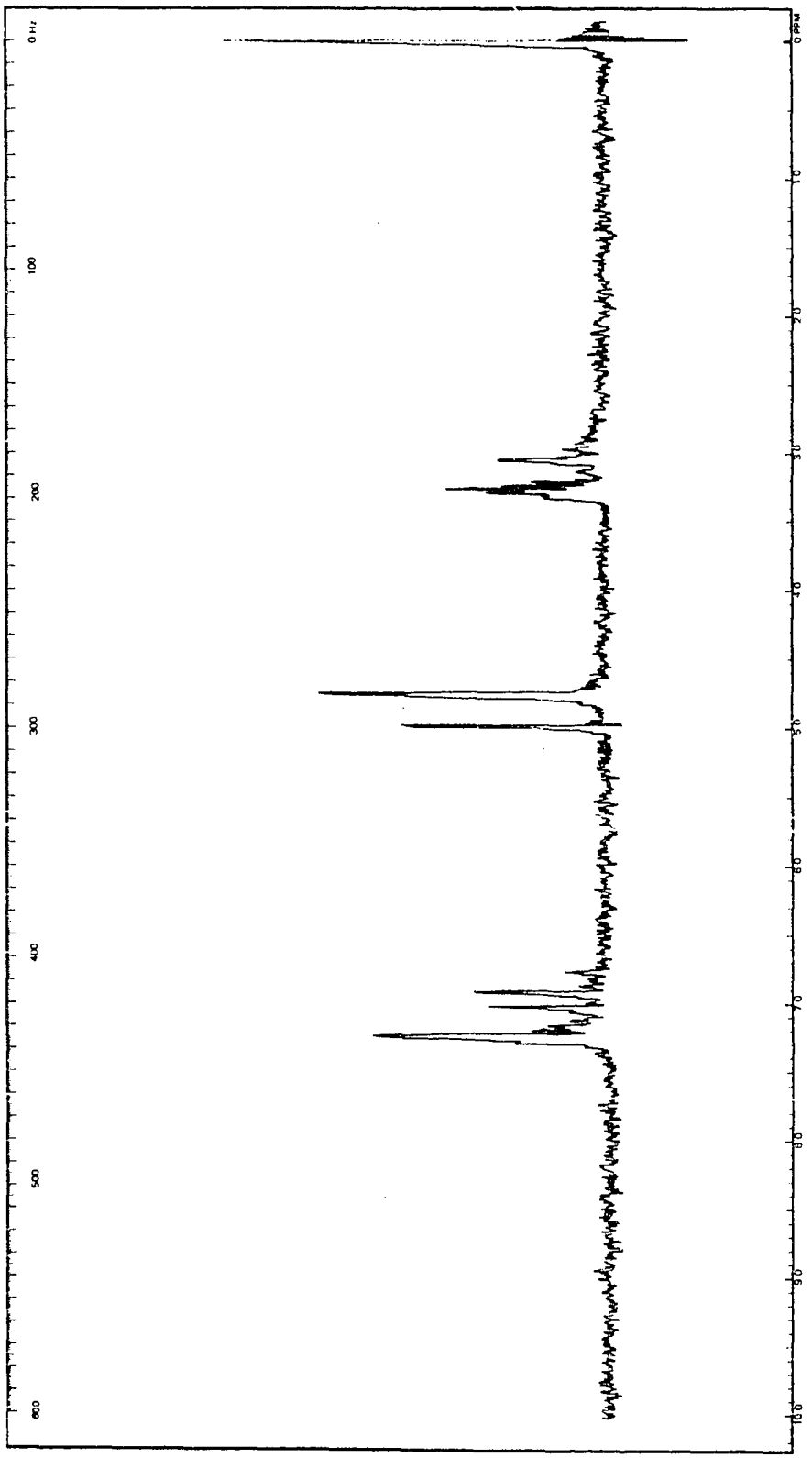
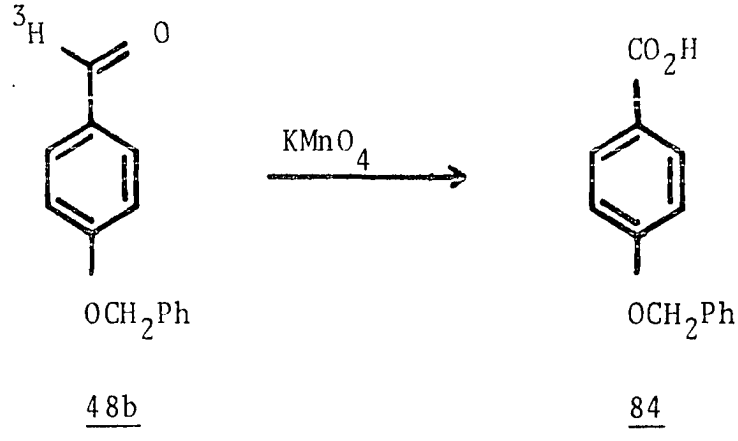

0 Hz
100
200
300
400
500
600
0 PPM
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0

Figure 37. NMR spectrum of 2-[4-(benzyloxy)phenyl]-[2-^{2}H]ethylamine (83a).


PPM
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
HZ
0
100
200
300
400
500
600

During the final trial run, the deuterated aldehyde (48c) was reduced with LAD/NADH and then elaborated to chloride (81a). The appropriate spectra were obtained on the optically active compounds. However, the conversion of chloride (81a) to carboxylic acid (82a) failed due to a drop in water pressure that shut off the water flowing through a condenser on what was to have been an overnight reflux. This caused the solvent to boil off leaving an intractable tar. Due to the expense and availability of the enzyme, the enzymatic reduction was not repeated on the deuterated compound and the data recorded for the deuterated acid (82a) and the deuterated amine (83a) are those obtained on the racemic compounds.

To insure that the tritium present in aldehyde (48b) was located only in the formyl group, aldehyde (48b) was oxidized with aqueous potassium permanganate to yield 4-benzyloxybenzoic acid (84) (Figure 38). The acid (84) lost



48b 84

Figure 38. Oxidation of 4-benzyloxy-[7-^{3}H]benzaldehyde.

greater than 99.6% of the tritium present in 48b thus establishing that the tritium was located in the formyl group.

The synthesis outlined in Figure 30 differs from that of Battersby and co-workers (29,30) in two of the transformations. Battersby and co-workers (29,30) introduced the tritium label *via* a dithiane derivative whereas the above synthesis introduced the tritium label *via* the morpholine acetonitrile derivative (80). These two methods were equally effective in introducing the tritium label, and both gave comparable yields. Battersby and co-workers (29,30) converted the propionic acid derivative (51) to amine (52) by the amide-hypochlorite method whereas the above synthesis converted acid (82) to amine (83) by a Curtius rearrangement. Both methods converted the acids to the corresponding amines in approximately 50% yields. Since the conversion of acid (82) to amine (83) did not involve any transformations at the chiral center, the optical purity obtained in this experiment should be comparable to that obtained by Battersby and co-workers (29,30). Therefore, the O-methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b) should contain approximately 70% of the (R)-isomer.

O-Methyl-[1'-^{14}C]norbelladine hydrochloride (79) and O-methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b) were

mixed and recrystallized to constant activity prior to performing the biosynthetic experiments (Table 1).

Biosynthetic Investigations

R. bifida feeding experiment

In order to study the biosynthesis of montanine (58) and to verify the biosynthetic mechanism proposed by Feinstein (34) for montanine (58) and haemanthamine (46a), O-methyl-(2R)-[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (85) was introduced by hypodermic syringe into the bulbs of R. bifida. R. bifida was chosen as the plant host since it contains both montanine (58) and haemanthamine (46a) in large enough quantities to isolate and count to constant activity. After a two-week period the bulbs were processed and montanine (58) and haemanthamine (46a) were isolated (Table 2).

The ^{3}H: ^{14}C ratio for the isolated montanine (58) and haemanthamine (46a) was 1.31 ± 0.06 and 1.36 ± 0.02, respectively. Since the precursor (85) had a ^{3}H: ^{14}C ratio of 5.61 ± 0.18, this represented a loss of 77% and 76% of the tritium present at C_2 of (85) when transformed into montanine (58) and haemanthamine (46a), respectively. From this it can be concluded that O-methyl-(2R)-[2-^{3}H, 1'-^{14}C]-norbelladine hydrochloride (85) is converted to haemanthamine (46a) and montanine (58) with the loss of the pro-R hydrogen of C_2 in (85).

Table 1. Summary of precursors used in the *R. bifida* and *C. Moorei* feedings.

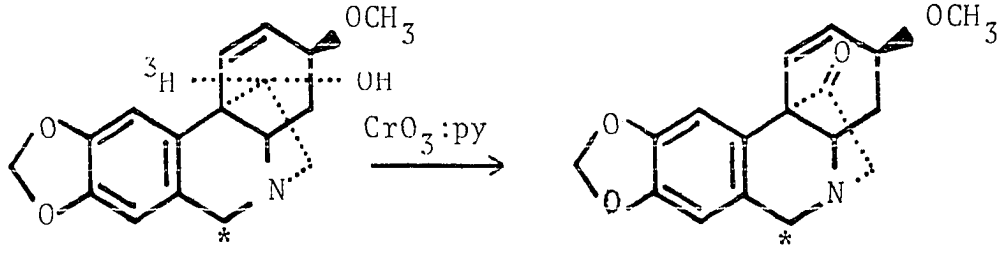
Precursor	Quantity (mg)	Specific Activity (mci/mmol)	Isotope	^{3}H:^{14}C Ratio
O-Methyl-(2R)-[2-^{3}H, 1'-^{14}C]-norbelladine hydrochloride (85) (*R. bifida* feeding)	7.48	14.9 2.66	^{3}H ^{14}C	5.61 ± 0.18
O-Methyl-(2R)-[2-^{3}H, 1'-^{14}C]-norbelladine hydrochloride (85) (*C. moorei* feeding)	8.75	14.3 3.44	^{3}H ^{14}C	4.15 ± 0.09

Table 2. Summary of incorporation of O-methyl-(2R)-[2-^{3}H, 1'-^{14}C]-norbelladine hydrochloride in a two-week feeding in *R. bifida*.

Alkaloid	Quantity (mg)	Specific Activity (mci/mmol)	Total Activity (mci)	Isotope	Percent Incorporation	^{3}H:^{14}C Ratio
Montanine Perchlorate (58)	210 mg	9.72×10^{-5}	5.09×10^{-5}	^{3}H	0.061[a]	1.31 ± 0.06
		7.48×10^{-5}	3.92×10^{-5}	^{14}C		
Haemanthamine (46a)	183 mg	3.28×10^{-4}	1.99×10^{-4}	^{3}H	0.23[a]	1.36 ± 0.02
		2.40×10^{-4}	1.46×10^{-4}	^{14}C		

[a]Based on Carbon-14.

The location of the residual tritium in haemanthamine (46b) was determined by oxidizing 46b with CrO_3:pyridine generated *in situ* (54) to yield oxohaemanthamine (86) (Figure 39). The oxohaemanthamine (86) retained only 1.7% of the tritium present in haemanthamine (46b), thus showing that the residual tritium in haemanthamine was located at C_{11}. Compared to the tritium present at C_2 in (85), oxohaemanthamine (86) retained only 0.4% of the tritium. This corresponds well with the amount of tritium retained when 4-benzyloxy-7-^{3}H benzaldehyde (48b) was oxidized to 4-benzyloxybenzoic acid (84).



46b * = ^{14}C 86

Figure 39. Oxidation of haemanthamine (46b).

The data obtained for montanine (58) and haemanthamine (46a) are consistent with the biosynthetic pathway proposed

by Feinstein (34). The proposed pathway can now be elaborated as shown in Figure 40.

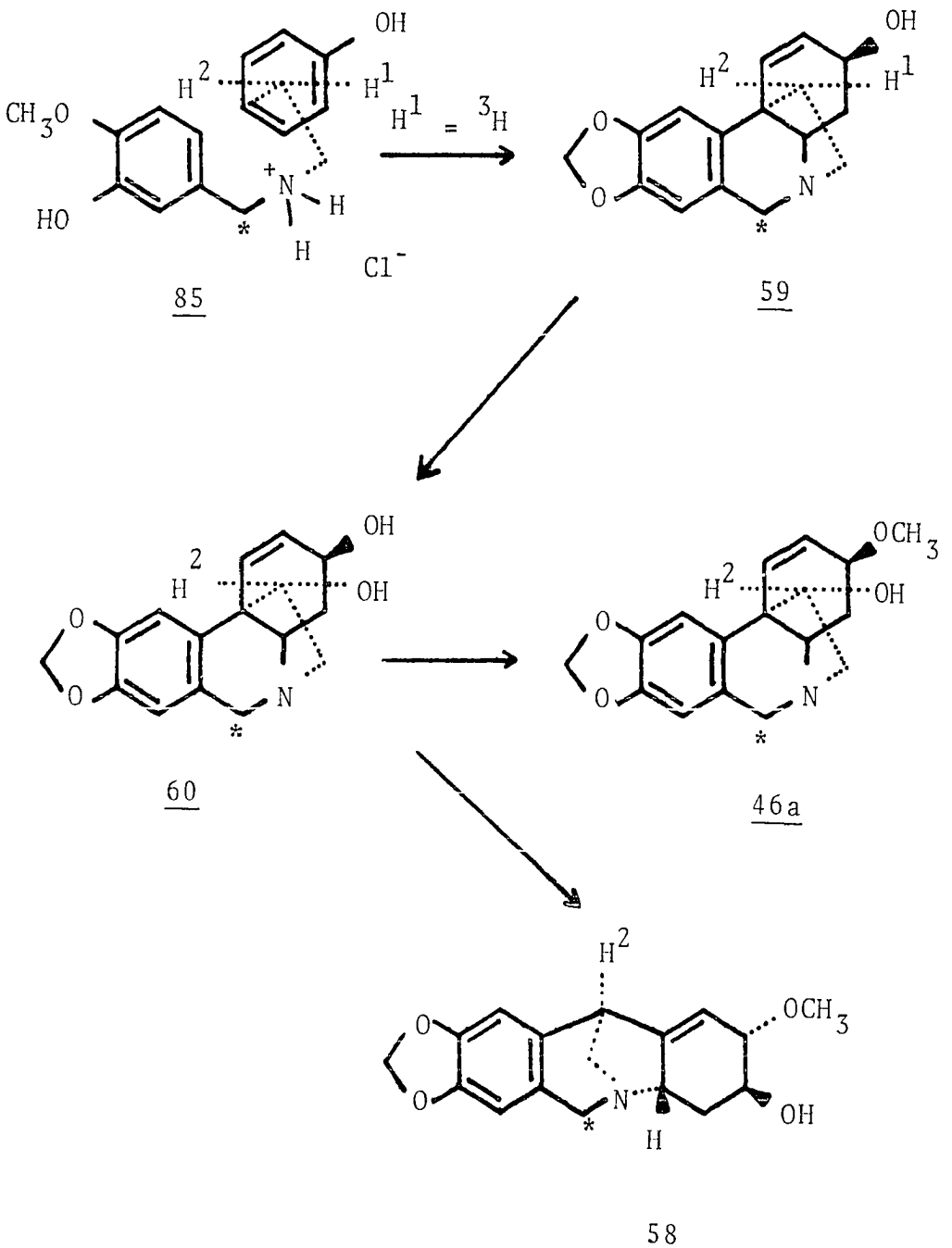


Figure 40. Proposed biosynthetic pathway for montanine (58) and haemanthamine (46a).

Although 11-hydroxyvittatine (60) is known to be present in R. bifida (43), it was not present in sufficient quantity to be isolated, purified, and counted to constant activity. However, the intermediacy of (60) is entirely consistent with the data obtained in this experiment. It is also consistent with the chemical evidence for the rearrangement of the crinine-ring system to the montanine ring system (44).

These data also agree with the reported biosynthesis of haemanthamine (46a) in "King Alfred" daffodils (29,30), in "Texas" daffodils (28), in Sempre Avanti daffodils (32), and in Ismene hybrid "Sulphur Queen" (32). In all cases the biological conversion of either tyrosine (3) or O-methylnorbelladine (9) into haemanthamine (46a) occurred with the loss of the pro-R hydrogen from C-2 of 9 .

The data obtained in this experiment contradict the reported biosynthesis of montanine (58) in Haemanthus coccineus (33). In this case, Fuganti and co-workers observed the biological conversion of (9) with the loss of the pro-S hydrogen from C-2 of (9).

C. moorei feeding experiment

The biosynthesis of cherylline (63) was first investigated in detail by Chan (42) (Figure 28). In order to probe the stereochemistry of the hydroxylations proposed in the conversion of O-methylnorbelladine (9) to

cherylline (63), O-methyl-(2R)-[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (85) was fed to C. moorei. C. moorei was chosen as the plant host for this experiment, since it was known to contain cherylline (63) and lycorine (12) in large enough quantities to isolate and count to constant activity. After a two-week period, the bulbs were processed and cherylline (63) and lycorine (12) were isolated and recrystallized to constant activity (Table 3).

Lycorine retained 95% of the tritium present at C-2 of (85). This showed that the precursor (85) was incorporated intact into lycorine (12). It also showed that the bulbs were active biosynthetically.

Cherylline (63) retained 70% of the tritium present at C-2 of (85). This indicated that the biological conversion of (85) into cherylline occurred with the loss of the pro-S hydrogen from C-2 of (85). These data are consistent with the mechanism shown in Figure 41.

The key step in the sequence of Figure 41 would be oxidation to yield (87) or (91) with loss of the pro-S hydrogen followed by displacement of the hydroxyl group in a S_N2 manner to yield cherylline (89).

Alternatively, carbon-carbon bond formation could occur in the manner proposed by Schwartz and Scott (39) as shown in Figure 42.

Table 3. Summary of incorporation of 0-methyl-(2R)-[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride in a two-week feeding in C. moorei.

Alkaloid	Quantity (mg)	Specific Activity (mci/mmol)	Total Activity (mci)	Isotope	Percent Incorporation	^{3}H:^{14}C Ratio
Cherylline (63)	355	3.67×10^{3}	4.57×10^{-3}	^{3}H	1.62[a]	2.92 ± 0.01
		1.26×10^{-3}	1.56×10^{-3}	^{14}C		
Lycorine (12)	1276	1.82×10^{-3}	8.09×10^{-3}	^{3}H	2.12[a]	3.93 ± 0.01
		4.64×10^{-4}	2.06×10^{-3}	^{14}C		

[a]Based on Carbon-14.

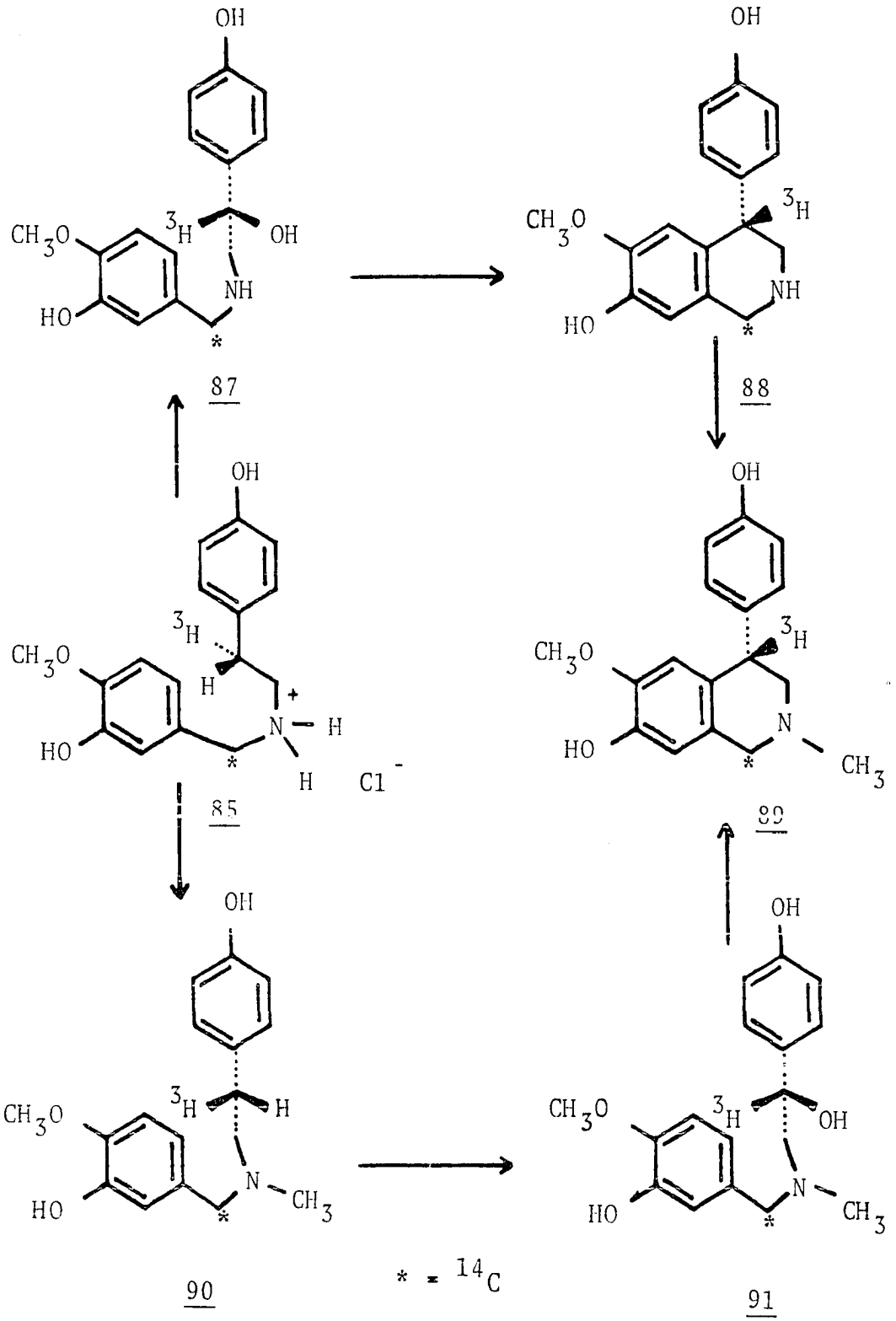


Figure 41. Proposed biosynthesis of cherylline (89).

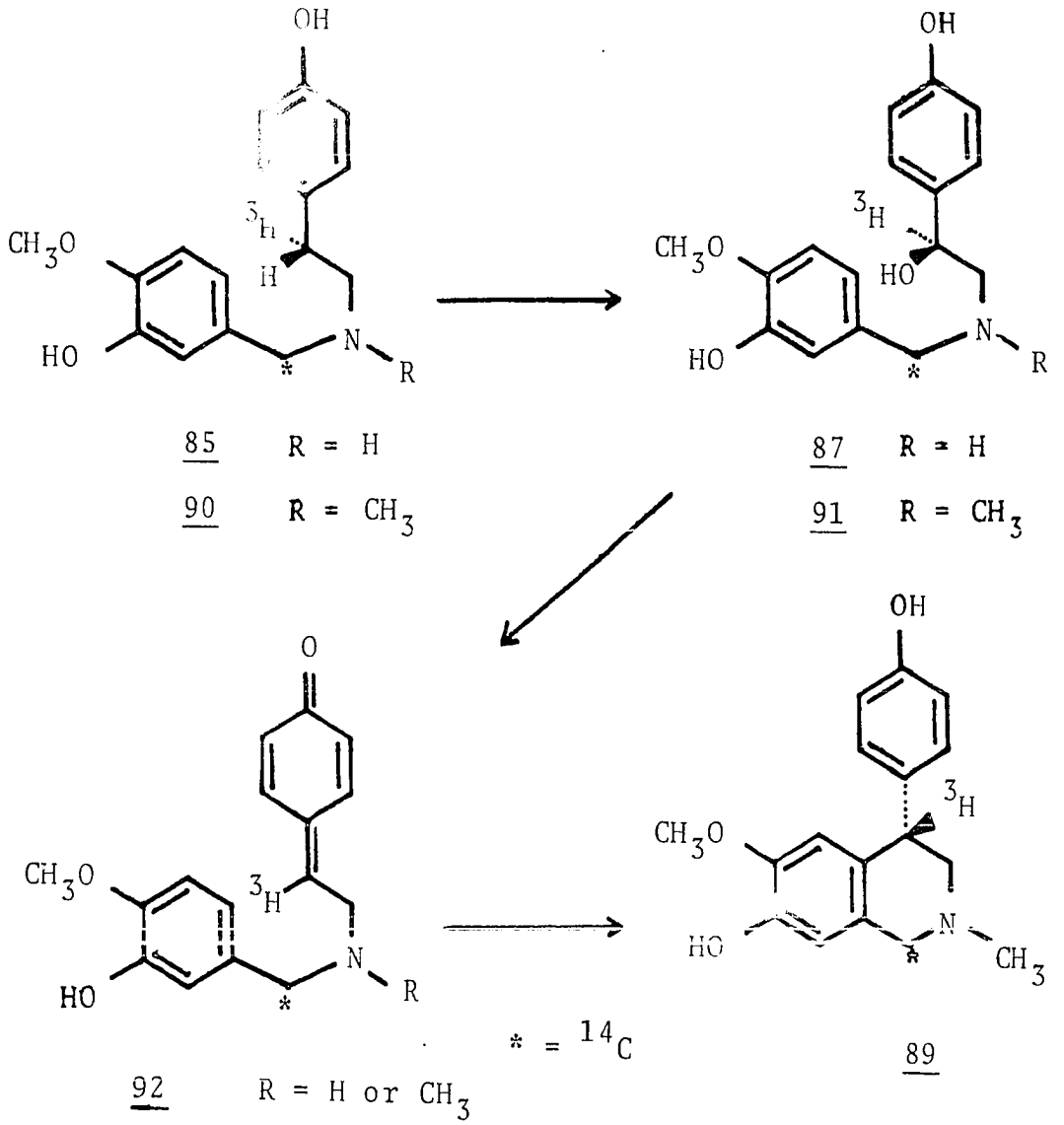


Figure 42. Alternate biosynthesis of cherylline (89).

The key step in the above scheme would be the dehydration of the alcohols (87) and (91) to yield 92 , which then cyclized to 89 . On the basis of the information obtained in this experiment, it would not be possible to distinguish between the mechanisms in Figures 41 and 42.

In order to determine the position of the remaining tritium, cherylline was degraded as shown in Figure 43. The resulting compound (93) was recrystallized to constant activity. The $^{3}H:^{14}C$ ratio indicated that 93 retained only 1.7% of the tritium present in cherylline (89) showing that the residual tritium in cherylline (89) was located at C-4 as expected.

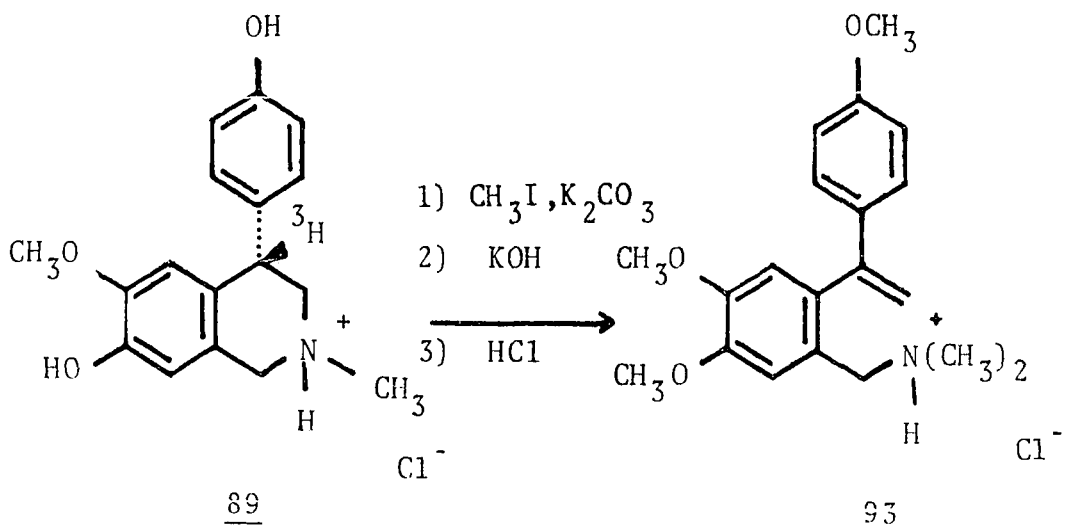


Figure 43. Degradation of cherylline (89).

The data obtained in the two feeding experiments are complementary. In the R. bifida feeding, O-methyl-(2R)-[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (85) lost 76% and 77% of the tritium present at C_2 of (85) in the biological conversion to haemanthamine (46a) and montanine (58), respectively. In the C. moorei feeding 85 retained 70% of the tritium present at C-2of 85 in the biological conversion

to cherylline (89). Since 85 was expected to contain approximately 70% of the (R)-isomer, this agrees well with the data obtained.

SUMMARY

O-Methyl-(2R)-[2-^{3}H,1'-^{14}C]norbelladine (85) was synthesized in order to study the stereochemistry of hydroxylation of the Amaryllidaceae alkaloids.

When O-methyl-(2R)-[2-^{3}H,1'-^{14}C]norbelladine (85) was converted by R. bifida into montanine (58) and haemanthamine (46a), the loss of the pro-R hydrogen from C-2 of O-methyl-norbelladine was observed. The relationship of these results with the earlier work was discussed, and a biosynthetic mechanism for the biological conversion was proposed.

When O-methyl-(2R)-[2-^{3}H,1'-^{14}C]norbelladine (85) was converted by C. moorei into cherylline (63), the loss of the pro-S hydrogen from C-2 of O-methylnorbelladine was observed. The relationship of this result with the earlier work was discussed, and two biosynthetic mechanisms were proposed.

EXPERIMENTAL

Instrumentation

The infrared spectra were taken in chloroform solution or as potassium bromide pellets on a Beckman Model IR-12, IR-18A, or IR 4250 spectrophotometer. The proton magnetic resonance spectra were run in the indicated solvent on a Hitachi Perkin-Elmer Model R-20B, a Varian Model A-60, or a Varian Model HA-100. The mass spectra were run on an A.E.I. MS 902 high-resolution mass spectrophotometer. Melting points were taken on a Köfler hot stage and are corrected. Radioactivity measurements were taken on a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3002).

Radioassay

All counting of radioactive samples was done in the same manner. Samples were weighed on a micro-analytical balance and were dissolved in enough methanol to make 5 ml of solution. Triplicate aliquots were counted using 15 ml toluene: POPOP, PPO scintillation cocktail (4.90 g 2,5-diphenyloxazole (PPO) (Packard Scintillation Grade) and 0.10 g 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) (Packard Scintillation Grade) dissolved in enough dry toluene (Fischer A.C.S. Reagent Grade) to make 1 l of solution). The tritium counting efficiency varied from 22% to 26%, and the carbon-14 counting efficiency varied from 61% to 70%.

Counting procedures and treatment of the data obtained were done by the method of Kobayashi and Maudsley (45).

The radioactive compounds were recrystallized and counted until constant activity was obtained. The percent incorporation was calculated as:

$$\% \text{ incorporation} = \frac{(\text{total activity of isolated alkaloid})}{(\text{total activity fed})} \times 100.$$

Preparative Thin Layer Chromatography

Preparative TLC was done on 20 cm x 20 cm glass plates coated with 0.6 mm silica gel (Merck, PF 254) which were dried in an oven for at least 24 hrs before use. The alkaloids were detected by U.V. light, and the bands were scraped off and soaked in methanol overnight. The methanol mixture was filtered and the solvent was evaporated *in vacuo*. The product was redissolved in chloroform, filtered, and the chloroform solution was evaporated to dryness. The alkaloids were further identified by melting point, comparative TLC, and comparison of infrared spectra with authentic samples.

Synthetic Procedures

3-Benzyloxy-4-methoxybenzoic acid (77)

3-Benzyloxy-4-methoxybenzoic acid (77) was synthesized by the method of Chan (42). Magnesium turnings (48 mg, 2.0 mmol) were added to a solution of 2-benzyloxy-4-bromoanisole (76) (586 mg, 2.0 mmol) and 10 ml tetrahydrofuran (freshly

distilled from lithium aluminum hydride). A small crystal of iodine was added to initiate the reaction. After refluxing for 1 hr , the solution was degassed. Carbon dioxide generated from barium carbonate (197 mg, 1.0 mmol) and 5 ml concentrated sulfuric acid was then trapped in the Grignard solution at -20°. After stirring for 20 min at -20°, the temperature was allowed to come to room temperature as stirring was continued for an additional 20 min. The reaction mixture was hydrolyzed with 6 N hydrochloric acid and then extracted with ether. The ether solution was then extracted with 10% sodium hydroxide, and the basic solution was neutralized with concentrated hydrochloric acid. The neutral solution was extracted with ether, and the ether solution was dried with anhydrous sodium sulfate, filtered, and evaporated *in vacuo*. The residue was crystallized from absolute ethanol to yield 3-benzyloxy-4-methoxybenzoic acid (*77*) (166 mg, 64% yield based on barium carbonate): mp 180-183° (lit. (46) 177-178°); IR ($CHCl_3$), 1695 (s) (C=O), 1275 (s); NMR ($CDCl_3$) δ3.92 (s,3,OCH_3), δ5.15 (s,2,OCH_2Ph), δ6.88-7.90 (m,8,aromatic).

In the ^{14}C-series, the Grignard reagent formed from 2-benzyloxy-4-bromoanisole (*76*) (396 mg) was carbonated with [^{14}C]carbon dioxide (0.45 mmol,50 mci/mmol, New England

Nuclear) and yielded 3-benzyloxy-4-methoxy-[7-^{14}C]benzoic acid (112 mg, which was used in the next step without further purification).

3-Benzyloxy-4-methoxybenzaldehyde (78)

To a solution of 3-benzyloxy-4-methoxybenzoic acid (63 mg,0.26 mmol) and 10 ml dry tetrahydrofuran (freshly distilled from lithium aluminum hydride) was added excess lithium aluminum hydride. After refluxing overnight under a nitrogen atmosphere, the excess lithium aluminum hydride was destroyed with saturated aqueous sodium potassium tartrate. The aqueous solution was extracted with ether, and the ether solution was dried with anhydrous sodium sulfate, filtered, and evaporated in vacuo. A chloroform solution of the residue was stirred overnight at ambient temperature with activated manganese dioxide (350 mg). The solution was filtered using a Celite pad and the filtrate was evaporated in vacuo. The residue was crystallized from methanol and yielded 3-benzyloxy-4-methoxybenzaldehyde (78) (49 mg,83% yield): mp 63.5-65.5° (lit. (47) 63°); IR ($CHCl_3$), 1690 (C=O), 1590, 1268, 1130, 1020, 910; NMR ($CDCl_3$), δ3.90 (s, 3, OCH_3), δ5.13 (s, 2, OCH_2Ph), δ6.83-7.47 (m,8,aromatic).

In the ^{14}C-series, 3-benzyloxy-4-methoxy-[7-^{14}C]benzoic acid (112 mg) yielded 3-benzyloxy-4-methoxy-[7-^{14}C]benzaldehyde (89 mg, 82% yield).

0-Methyl-[1'-^{14}C]norbelladine hydrochloride (75)

A mixture of 3-benzyloxy-4-methoxy-[7-^{14}C]benzaldehyde (81 mg, 0.33 mmol), tyramine hydrochloride (57.5 mg, 0.33 mmol), sodium bicarbonate (33 mg, 0.39 mmol), and 10 ml isopropyl alcohol was refluxed for 45 min. The water formed was removed as the benzene-water azeotrope. The solution was cooled and excess sodium borohydride was added. After stirring overnight at ambient temperature, the solution was evaporated in vacuo. The residue was dissolved in water, acidified with concentrated hydrochloric acid, and then neutralized with sodium carbonate. The neutral solution was extracted with ethyl acetate, and the ethyl acetate solution was evaporated in vacuo. Preparative TLC (ethyl acetate:methanol, 70:30, v/v) yielded 58 mg of product (rf 0.5) which was hydrogenolized in 20 ml ethanol with 50 mg 10% palladium on charcoal catalyst. After hydrogen uptake ceased, the solution was filtered and the filtrate was evaporated in vacuo. The residue was crystallized from ethanol and ethereal hydrogen chloride to give 0-methyl-[1'-^{14}C]norbelladine hydrochloride (25 mg, 53% yield based on reacted starting material): mp 203-205° (lit. (18) 205-207°). The sample was identical with authentic 0-methyl-norbelladine hydrochloride by TLC in chloroform: methanol: diethylamine (85:10:5, v/v) and ethyl acetate: methanol (70:30, v/v). Specific activity = 36.2 mci/mmol.

4-Benzyloxybenzaldehyde (48a)

A solution of p-hydroxybenzaldehyde (6.10 g, 0.05 mol), benzyl chloride (6.95 g, 0.05 mol), potassium carbonate (3.52 g, 0.025 mol), and 50 ml methanol was refluxed for 5 hours. The hot solution was filtered and upon cooling, needles formed. Recrystallization from absolute methanol yielded 6.65 g (63% yield) of colorless needles: mp 75-76° (lit. (48) 73.5-74°); IR ($CHCl_3$), 1700 (C=O), 1610, 1585, 1265, 1168 ; NMR ($CDCl_3$) Figure 31.

4-Benzyloxyphenyl morpholine acetonitrile (80)

The morpholino acetonitrile derivative (80) was synthesized by the method of Bennett, Kirby, and Moss (49). A solution of 4-benzyloxybenzaldehyde (48a) (4.85 g, 0.025 mol), morpholine perchlorate (4.26 g, 0.025 mol), and 25 ml morpholine was stirred at 60° for 1 hr. Potassium cyanide (1.49 g, 0.023 mol in 3 ml water) was added and the solution was stirred at 90° for 1 hr. The hot solution was then poured into 100 ml ice water and extracted with chloroform. The organic solution was dried with anhydrous sodium sulfate, filtered, and evaporated in vacuo leaving an oil. Crystallization of the oil from ethyl acetate-ether yielded 4.48 g of (80) (64% yield): mp 143-144°; IR ($CHCl_3$), 2240 (C≡N), 1512, 1118, 1250; NMR ($CDCl_3$) Figure 32; MS calculated for $C_{19}H_{20}N_2O_2$ 308.1525, found 308.1523.

4-Benzyloxy-[7-^{2}H]benzaldehyde (48c)

To a solution under a nitrogen atmosphere of 4-benzyloxyphenyl morpholino acetonitrile (80) (616 mg, 2 mmol) and 8 ml dry N,N-dimethylformamide was added sodium hydride (96 mg, 2 mmol, 50% oil dispersion). After stirring for 2 hrs at room temperature, 36 µl [$^{2}H_2$]H_2O (99.75% $^{2}H_2$) was added. After 15 min the solution was chilled in an ice bath, neutralized by adding one equivalent of thionyl chloride, diluted with 50 ml ice water, and extracted immediately with chloroform. The chloroform was dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The remaining N,N-dimethylformamide was removed by vacuum distillation. The residue was refluxed for 2.5 hrs with 16 ml tetrahydrofuran and 60 ml 1 N hydrochloric acid. The cooled solution was then extracted with chloroform. The chloroform solution was dried, filtered, and evaporated *in vacuo*. Preparative TLC (ether: hexane, 80:20, v/v) yielded 4-benzyloxy-[7-^{2}H]benzaldehyde (rf 0.8, 306 mg, 82% yield based on reacted starting material): mp 74.5-76°; IR (CCl_4), 2055, 2030, 1690 (C=O), 1600, 1260, 1240, 1230, 1165; NMR ($CDCl_3$), Figure 33; MS, calculated for $C_{14}H_{11}DO_2$ 213.0900, found 213.0900.

In the ^{3}H-series, similar steps converted 4-benzyloxyphenyl morpholino acetonitrile (80) (548 mg) to 4-benzyloxy-[7-^{3}H]benzaldehyde (48b) (358 mg, 95% yield).

4-Benzyloxy-(7S)-[7-^{2}H]benzyl alcohol (49a)

The alcohol (49a) was synthesized by the method of Virnig (27). To a solution of 350 ml deionized water, 50 ml pH 8.8 glycine buffer (50), nicotinamide-adenine dinucleotide (NADH) (220 mg, Calbiochem), 20 ml absolute ethanol, enough 3 N sodium hydroxide to adjust the pH to 8-9, and equine liver alcohol dehydrogenase (30 mg in 3 ml 0.02 M phosphate buffer, Calbiochem) at 37.5° was added 3.5 ml of a solution of 4-benzyloxy-[7-^{2}H]benzaldehyde (48c) (306 mg, 1.44 mmol in 15 ml of absolute ethanol). The remainder of the solution was added in equal aliquots at 30 min, 60 min, and 105 min. The reaction mixture was maintained at 37.5° overnight and was then extracted thoroughly with ethyl acetate. The organic solution was dried with anhydrous sodium sulfate, filtered, and evaporated *in vacuo*. Preparative TLC (ether:hexane, 80:20, v/v) yielded 4-benzyloxy-(7S)-[7-^{2}H]benzyl alcohol (49a) (rf 0.5, 234 mg, 85% yield based on reacted starting material) and 4-benzyloxy-[7-^{2}H]-benzaldehyde (48c) (rf 0.8, 32 mg). The alcohol (49a) was crystallized from hexane: mp 90.5-91° (lit. (30), 89-90°); IR ($CHCl_3$), 3610 (OH), 1515, 1244; NMR ($CDCl_3$), Figure 34; MS, calculated for $C_{14}H_{13}DO_2$ 215.1057, found 215.1055.

In the ^{3}H-series, 4-benzyloxy-[7-^{3}H]benzaldehyde (48b) (358 mg) was converted to 4-benzyloxy-(7S)-[7-^{3}H]benzyl alcohol (49) (308 mg, 85% yield).

4-Benzyloxy-(7S)-[7-^{2}H]benzyl chloride (81a)

A solution of 4-benzyloxy-(7S)-[7-^{2}H]benzyl alcohol (49a) (234 mg. 1.09 mmol), 15 ml purified dioxane (51), and colorless thionyl chloride (0.56 ml, 7.71 mmol) was stirred for 40 min at room temperature. The solvent was evaporated in vacuo and the residue was crystallized from hexane to yield 4-benzyloxy-(7S)-[7-^{2}H]benzyl chloride (200 mg, 79% yield): mp 80-81.5° (lit. (52) 79-80°); IR (KBr), 1520, 1258, 1014, 760, 745; NMR ($CDCl_3$), Figure 35; MS, calculated for $C_{14}H_{12}DClO$ 233.0718, found 233.0716.

In the ^{3}H-series, 4-benzyloxy-(7S)-[7-^{3}H]benzyl alcohol (49) (308 mg) was treated in a similar manner with thionyl chloride. However, after evaporating the solvent, the residue (81) was used directly in the next step without further purification.

3-[4-(benzyloxy)phenyl]-[3-^{2}H]propionic acid (82a)

4-Benzyloxy-[7-^{2}H]benzyl chloride (300 mg, 1.29 mmol) was dissolved in 4 ml tetrahydrofuran (freshly distilled from lithium aluminum hydride) and was added dropwise to a solution containing 14.4 ml of 0.0914 N sodium ethoxide (1.29 mmol) and ethyl malonate (0.30 ml, 1.96 mmol). After refluxing for 2.5 hrs under a nitrogen atmosphere, the solution was cooled, neutralized with 10% hydrochloric acid, and extracted with ether. The organic solution was concentrated in vacuo and the excess ethyl malonate was removed

by distillation at 50° and 1 torr. The residue was refluxed with 50 ml 50% aqueous potassium hydroxide for 2 hrs. After cooling, the basic solution was extracted once with ether and the ether solution was discarded. The solution was then acidified with concentrated hydrochloric acid and extracted thoroughly with ether. The organic solution was dried with anhydrous sodium sulfate, filtered, and evaporated *in vacuo*. The residue was dissolved in pyridine and the excess pyridine was removed as an azeotrope with toluene. The residue was then pyrolyzed under a nitrogen atmosphere for 2 hrs at 145°. Preparative TLC on silica gel (chloroform: methanol: diethylamine, 85:10:5, v/v) yielded 3-[4-(benzyloxy)phenyl]-[3-^{2}H]propionic acid (82a) (rf 0.5, 170 mg, 51% yield): mp 124-126.5° (lit. (30), 123-124°); IR ($CHCl_3$), 1718 (C=O), 1515, NMR ($CDCl_3$), Figure 36; MS, calculated for $C_{16}H_{15}DO_3$ 257.1162, found 257.1158.

In the ^{3}H-series, 4-benzyloxy-(7S)-[7-^{3}H]benzyl chloride (81) (334 mg) was converted to 3-[4-(benzyloxy)-phenyl]-(3S)-[3-^{3}H]-propionic acid (82) (225 mg, 61% yield).

2-[4-(Benzyloxy)phenyl]-[2-^{2}H]ethylamine (83a)

Oxalyl chloride (0.20 ml, 1.70 mmol) was added to a solution of 3-[4-(benzyloxy)phenyl]-[3-^{2}H]propionic acid (70 mg, 0.28 mmol) and 3 ml benzene (freshly distilled from sodium). After stirring at room temperature for 15 min, the

solution was refluxed for 1 hr and then evaporated *in vacuo*. The residue was dissolved in 5 ml dry acetone (dried with anhydrous sodium carbonate) and chilled in an ice bath. Sodium azide (100 mg, 1.54 mmol), dissolved in 8 drops water, was added. This was stirred for 15 min as the temperature was gradually allowed to rise to room temperature. Fifteen ml water was added and this solution was extracted with ether. The organic solution was dried with anhydrous sodium sulfate, filtered, and evaporated *in vacuo*. The residue was dissolved in 50 ml dry benzene and after refluxing for 1 hr the benzene was evaporated *in vacuo*. Ten ml 50% potassium hydroxide was added, and the mixture was warmed on a steam bath for approximately 10 min, then cooled and neutralized with concentrated hydrochloric acid. After extracting with chloroform, the organic solution was dried with anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. The residue was crystallized from absolute ethanol and ethereal hydrogen chloride to yield 2-[4-(benzyloxy)phenyl]-[2-^{2}H]-ethylamine (*83a*) (37 mg, 51% yield): mp 208-212° (lit. (8), 210-215°); IR (KBr), 3440 (broad), 1512, 1250; NMR (CD_3OD) Figure 37; MS, calculated for $C_{15}H_{16}DNO$ 228.1373, found 228.1369.

In the ^{3}H-series, 3-[4-(benzyloxy)phenyl]-(3S)-[3-^{3}H]-propionic acid (*82*) (130 mg, 0.507 mmol) was converted to

2-[4-(benzyloxy)phenyl]-(2R)-[2-^{3}H]ethylamine hydrochloride (83) (70 mg, 52% yield).

O-Methyl-(2R)-[2-^{3}H]norbelladine hydrochloride (47b)

A mixture of 2-[4-(benzyloxy)phenyl]-(2R)-[2-^{3}H]ethylamine hydrochloride (83) (70 mg, 0.276 mmol), isovanillin (41 mg, 0.31 mmol sodium bicarbonate (28 mg, 0.33 mmol), 4 Å molecular sieves (180 mg), and 6 ml dry isopropyl alcohol was refluxed for 1 hr. After cooling in an ice bath, sodium borohydride (21 mg, 0.55 mmol) was added and the mixture was allowed to stir for 1 hr at room temperature. An additional 31 mg sodium borohydride was added and stirring was continued for 2.5 hrs. The solution was filtered and evaporated in vacuo. The residue was dissolved in water, acidified with concentrated hydrochloric acid, neutralized with potassium bicarbonate, and extracted with chloroform. The organic solution was dried with magnesium sulfate, filtered, and evaporated in vacuo. The residue was dissolved in 6 ml absolute ethanol and hydrogenolized with 75 mg 10% palladium on charcoal catalyst. After hydrogen uptake ceased, the catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The residue was dissolved in 2 ml absolute ethanol, and ethereal hydrogen chloride was added until the solution became cloudy. The hydrochloride salt was isolated and recrystallized from ethanol-ether (44 mg, 52% yield): mp 204-206° (lit. (18)

205-207°); the sample was identical with authentic material by TLC in chloroform: methanol: diethylamine (85:10:5, v/v) and in ethyl acetate: methanol (70:30, v/v); specific activity = 37.8 mci/mmol.

Oxidation of 4-benzyloxy-[7-^{3}H]benzaldehyde (48b)

To a solution of 4-benzyloxy-[7-^{3}H]benzaldehyde (48b) (200 mg, 3.65 x 10^{5} dpm/mg) and 10 ml dioxane was added a solution of potassium permanganate (148 mg) and 10 ml water. After stirring for 1 hr, sulfur dioxide was bubbled through the solution to remove the manganese dioxide. The solution was then extracted with chloroform, and the chloroform was extracted with 10% sodium hydroxide. The basic solution was acidified and extracted with chloroform. Evaporation of the chloroform yielded 4-benzyloxybenzoic acid (84) which was crystallized from ethanol: mp 186-189° (lit. (53) 188°; 1.39 x 10^{3} dpm/mg.

Feeding Experiments

R. bifida feeding

O-Methyl-(2R)-[2-^{3}H, 1'-^{14}C]norbeliadine hydrochloride (85) (7.48 mg, ratio ^{3}H: ^{14}C = 5.61 ± 0.18) was dissolved in 0.75 ml deionized water and injected directly into the bulbs of growing R. bifida. After 2 weeks, the bulbs (787 g) were macerated in a Waring Blendor with 3.5 l 95% ethanol. After standing overnight, the mixture was filtered. The

solid material was extracted again with 1.5 l 95% ethanol, and the combined filtrates were evaporated *in vacuo* at 35°. The residue was treated with 400 ml 2 N hydrochloric acid. After standing for 2 hrs with occasional stirring, the solution was filtered using a Celite pad. The insoluble material was extracted twice with 100 ml portions of 2 N hydrochloric acid and, after filtering, the hydrochloric acid solutions were combined. The hydrochloric acid insoluble material was discarded. The acidic solution was extracted with benzene. The benzene solution did not give a precipitate with silicotungstic acid and was discarded. The acidic solution was basified to pH 10 with concentrated ammonium hydroxide and extracted with chloroform. The chloroform solution was dried with anhydrous sodium sulfate and evaporated *in vacuo*. 1.350 g of crude alkaloidal material was obtained.

Montanine (58) was isolated by dissolving the crude alkaloid mixture in a minimal amount of acetone. The acetone solution was then acidified with 85% perchloric acid. Ether was added until the solution became turbid. The crude montanine perchlorate (210 mg) was isolated by filtration and was crystallized to constant activity from acetone: mp 249-250° (lit. (54), 249-250°); ratio ^{3}H: ^{14}C = 1.31 ± 0.06.

The filtrate was basified with concentrated ammonium hydroxide and extracted with chloroform. After evaporation of the chloroform, the crude material (1.14 g) was chromatographed on 35 g neutral alumina (Woelm). Elution with benzene: chloroform (75:25, v/v) yielded haemanthamine (46a) (183 mg) which was crystallized to constant activity from acetone: mp 199-201° (lit. (54), 200-201°); ratio ^{3}H: ^{14}C = 1.36 ± 0.02.

Degradation of haemanthamine (46a)

To a solution of 25 ml methylene chloride and pyridine (158 mg, 2 mmol) was added chromium trioxide (110 mg, 1 mmol) (55). After stirring for 2 hrs, a solution of haemanthamine (48 mg, 0.16 mmol, ratio ^{3}H: ^{14}C = 1.36) and 3 ml methylene chloride was added and stirring was continued for 30 min. This solution was extracted with 0.5 N sodium hydroxide and washed with saturated aqueous sodium chloride. The organic solution was dried with anhydrous magnesium sulfate, filtered, and evaporated in vacuo. Preparative TLC (chloroform: methanol: diethylamine, 95:2.5:2.5, v/v) yielded oxohaemanthamine (86) (18 mg, 38% yield, ratio ^{3}H: ^{14}C = 0.023, rf 0.5) which was crystallized from ether: mp 164-166.5° (lit. (56), 164°); sample was identical with authentic material by TLC (chloroform: methanol: diethylamine, 90:5:5, v/v).

C. moorei feeding

O-Methyl-(2R)-[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (85) (8.75 mg, ratio ^{3}H: ^{14}C = 4.15 ± 0.09) was dissolved in 1.0 ml deionized water and was injected directly into the bulbs of growing C. moorei. After 2 weeks the bulbs (1300 g) were macerated in a Waring Blendor with 3.5 l 95% ethanol. After standing overnight, the mixture was filtered. The solid material was extracted again with 1 l 95% ethanol, and the filtrates were combined and evaporated in vacuo at 35°. The residue was treated with 1 l 5% hydrochloric acid and the acidic solution was filtered using a Celite pad. The insoluble material was discarded. The acidic solution was extracted with benzene. The benzene solution did not give a precipitate with silicotungstic acid and was discarded. The acidic solution was then basified to pH 9 with concentrated ammonium hydroxide and extracted with chloroform. Lycorine (12) (1276 mg) crystallized from the chloroform solution and was isolated by filtration. Lycorine was crystallized to constant activity as the hydrochloride salt: mp 211-214° (lit. (54), 212-214°); ratio ^{3}H: ^{14}C = 3.93 ± 0.01.

The filtrate was evaporated in vacuo, and the residue was dissolved in 150 ml chloroform. The chloroform solution was extracted with 0.5 N sodium hydroxide and the basic

solution was washed with chloroform. The chloroform solutions were combined and evaporated to yield 103 g of non-phenolic alkaloids.

The basic aqueous solution was neutralized with concentrated hydrochloric acid and extracted with chloroform. The organic solution was dried with anhydrous magnesium sulfate, filtered, and evaporated *in vacuo* to yield 504 mg of phenolic alkaloids.

Cherylline was isolated from the crude phenolic-alkaloid fraction by preparative TLC (ethyl acetate:methanol, 70:30, v/v, rf 0.6, 355 mg). Cherylline (63) was crystallized to constant activity as the hydrochloride salt: mp, first melted at 175°, solidified at 190°, remelted at 239-240°, (lit. (39), first melted at 175°, solidified at 190°, remelted at 240-243°). The sample was identical with authentic material by TLC in chloroform: methanol: diethylamine (85:10:5, v/v) and in ethyl acetate: methanol (70:30, v/v). Ratio ^{3}H: ^{14}C = 2.92 ± 0.01.

Degradation of cherylline (63)

A solution of cherylline hydrochloride (112 mg, 0.35 mmol, ratio ^{3}H : ^{14}C = 2.92), potassium carbonate (500 mg, 3.6 mmol), methyl iodide (5 ml, 0.08 mole), and 20 ml methanol was refluxed for 24 hrs. After evaporating to dryness, the residue was refluxed with 20 ml 20% potassium hydroxide for 18 hrs. The cooled solution was acidified

with benzene. The benzene solution was dried with anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. The product (*93*) (65 mg, 42% yield) was crystallized from absolute ethanol: mp 193-195° (188-189° for ^{1}H-compound); ratio ^{3}H: ^{14}C = 0.054.

LITERATURE CITED

1. R. J. Suhadolnik, A. G. Fischer, and J. Zulalian, J. Amer. Chem. Soc. 84, 4348 (1962).

2. W. C. Wildman, A. R. Battersby, and S. W. Breuer, J. Amer. Chem. Soc. 84, 4599 (1962).

3. R. J. Suhadolnik, A. G. Fischer, and J. Zulalian, Proc. Chem. Soc., 132 (1963).

4. D. A. Archer, S. W. Breuer, R. Binks, A. R. Battersby, and W. C. Wildman, Proc. Chem. Soc., 168 (1963).

5. R. J. Suhadolnik and J. Zulalian, Proc. Chem. Soc., 216 (1963).

6. R. J. Suhadolnik, A. G. Fischer, and J. Zulalian, Biochem. and Biophys. Research Commun. 11, 208 (1963).

7. J. Zulalian and R. J. Suhadolnik, Proc. Chem. Soc., 422 (1964).

8. A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, and W. C. Wildman, J. Chem. Soc., 1595 (1964).

9. D. H. R. Barton and G. W. Kirby, Proc. Chem. Soc., 392 (1960).

10. A. R. Battersby, R. Binks, and W. C. Wildman, Proc. Chem. Soc., 410 (1960).

11. A. R. Battersby, H. M. Fales, and W. C. Wildman, J. Amer. Chem. Soc. 83, 4098 (1961).

12. P. W. Jeffs, Proc. Chem. Soc., 80 (1962).

13. W. C. Wildman, H. M. Fales, and A. R. Battersby, J. Amer. Chem. Soc. 84, 681 (1962).

14. D. H. R. Barton and T. Cohen, "Some Aspects of Phenol Oxidation," in Festschrift: Arthur Stoll (Birkhauser, Basel, Switzerland, 1957), pp. 117-143.

15. D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, Proc. Chem. Soc., 254 (1961).

16. D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, Proc. Chem. Soc., 179 (1962).

17. D. H. R. Barton, G. W. Kirby, and J. B. Taylor, Proc. Chem. Soc., 340 (1962).

18. D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, J. Chem. Soc., 4545 (1963).

19. W. C. Wildman and N. E. Heimer, J. Amer. Chem. Soc. 89, 5265 (1967).

20. I. T. Bruce and G. W. Kirby, Chem. Commun., 207 (1968).

21. I. T. Bruce and G. W. Kirby, Chimia 22, 314 (1968).

22. G. W. Kirby and H. P. Tiwari, J. Chem. Soc. (C), 676 (1966).

23. C. Fuganti and M. Mazza, J. C. S. Chem. Commun., 936 (1972).

24. C. P. Christenson, Ph.D. thesis, Iowa State University of Science and Technology (1974) (unpublished).

25. C. Fuganti and M. Mazza, Chem. Commun., 1196 (1971).

26. C. Fuganti and M. Mazza, J. C. S. Perkin I, 954 (1973).

27. M. J. Virnig, Ph.D. thesis, Iowa State University of Science and Technology (1974) (unpublished).

28. G. W. Kirby and J. Michael, Chem. Commun., 415 (1971).

29. A. R. Battersby, J. E. Kelsey, and J. Staunton, Chem. Commun., 183 (1971).

30. A. R. Battersby, J. E. Kelsey, J. Staunton, and K. E. Suckling, J. C. S. Perkin I, 1609 (1973).

31. C. Fuganti, D. Ghiringhelli, and P. Graselli, J. C. S. Chem. Commun., 1152 (1972).

32. C. Fuganti, D. Ghiringhelli, and P. Graselli, J. C. S. Chem. Commun., 350 (1974).

33. C. Fuganti, D. Ghiringhelli, and P. Graselli, J. C. S. Chem. Commun., 430 (1973).

34. A. I. Feinstein, Ph.D. thesis, Iowa State University of Science and Technology (1967) (unpublished).

35. H. G. Boit, Chem. Ber. 87, 1704 (1954).

36. A. Brossi, G. Grethe, S. Teitel, W. C. Wildman, and D. T. Bailey, J. Org. Chem. 35, 1100 (1970).

37. V. Toome, J. F. Blount, G. Grethe, and M. Uskokovic', Tetrahedron Lett., 49 (1970).

38. A. Brossi and S. Teitel, Tetrahedron Lett., 417 (1970).

39. M. A. Schwartz and S. W. Scott, J. Org. Chem. 36, 1827 (1971).

40. R. D. Shaffer, Ph.D. thesis, Iowa State University of Science and Technology (1972) (unpublished).

41. T. Kametani, K. Takahashi, and C. Van Loc, Tetrahedron 31, 235 (1975).

42. J. A. Chan, Ph.D. thesis, Iowa State University of Science and Technology (1973) (unpublished).

43. W. C. Wildman, "Alkaloids of the Amaryllidaceae," in The Alkaloids, edited by R. H. F. Manske (Academic Press, New York, 1968), Vol. XI, pp. 307-405.

44. Y. Inubushi, F. M. Fales, E. W. Warnhoff, and W. C. Wildman, J. Org. Chem. 25, 2153 (1960).

45. Y. Kobayashi and D. V. Maudsley, Biological Applications of Liquid Scintillation Counting (Academic Press Inc., New York, 1974), pp. 196.

46. A. Lovecy, R. Robinson, and S. Sugasawa, J. Chem. Soc., 817 (1930).

47. R. Robinson and S. Sugasawa, J. Chem. Soc., 3161 (1931).

48. A. Arai and I. Ichikizaki, Bull. Chem. Soc. Japan 35, 504 (1962).

49. D. J. Bennett, G. W. Kirby, and V. A. Moss, Chem. Commun., 218 (1967).

50. G. Gomori, "Preparation of Buffers for Use in Enzyme Studies," in Methods in Enzymology, edited by S. P. Colowick and N. O. Kaplan (Academic Press Inc., New York, 1955), pp. 138-146.

51. L. F. Fieser and M. Fieser, Reagents for Organic Synthesis (John Wiley and Sons, Inc., New York, 1967), p. 333.

52. H. Burton and J. Praill, J. Chem. Soc., 522 (1951).

53. H. Pacheco and A. Grouiller, Bull. Soc. Chim. France, 779 (1965).

54. W. C. Wildman, "Alkaloids of the Amaryllidaceae," in The Alkaloids, edited by R. H. F. Manske (Academic Press, New York, 1960), Vol. VI, pp. 290-409.

55. R. Ratliffe and R. Rodehorst, J. Org. Chem. 35, 4000 (1970).

56. H. M. Fales and W. C. Wildman, J. Amer. Chem. Soc. 82, 197 (1960).

ACKNOWLEDGEMENTS

The author wishes to thank Dr. W. C. Wildman for suggesting the problem, and for the help and encouragement given during the course of this investigation.

The author also thanks the members of the research group for the many helpful discussions during the past four years.

A special thanks goes to my wife, Susan, for her patience and understanding, and for her help in the preparation of this manuscript.

The author wishes to thank the members of his family for the support and encouragement given through the many years of education.

Finally, the author wishes to thank Debbie Tully for an excellent job of typing this thesis.