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Biosynthesis of cherylline using doubly-labled norbelladine-type precursors

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by

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INTRODUCTION

The Amaryllidaceae alkaloids possess wide variations in structure and substituent functionality. Oxidation and cyclization of precursors of the norbelladine-type provide the unifying concept to biogenetically and biosynthetically interrelate such seemingly dissimilar ring systems.

Cherylline, a 4-phenyl-1,2,3,4-tetrahydroisoquinoline alkaloid has an entirely new ring structure and offers new problems in biogenetic theory and biosynthetic facts. Recently, a biogenetically possible synthesis of cherylline has been proposed.

To date, no published tracer studies have been reported to define the relationship of cherylline to the other Amaryllidaceae alkaloids. This thesis is concerned with the synthesis of four doubly-labeled precursors of the norbelladine-type and their incorporation into cherylline and other selected Amaryllidaceae alkaloids over various time periods.

Detection of previously unknown norbelladine-type alkaloids by isotopic dilution methods is also part of this thesis.

HISTORICAL

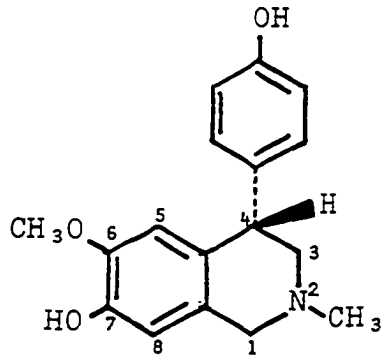
The Amaryllidaceae plant family has provided relatively few phenolic alkaloids, about fourteen have been isolated and characterized during the past twenty years (1). This section will focus on the chemistry of cherylline and the general biosynthesis of Amaryllidaceae alkaloids.

In 1954 Boit (2) isolated a phenolic alkaloid, crinin, from Crinum moorei by trituration of the phenolic fraction with acetone. The purified compound, mp 213-214°, had the molecular formula $C_{17}H_{19}NO_3$. Classical methods showed that it contained one N-methyl, one methoxyl, one or more phenolic groups but no methylenedioxy function. No final structure for this alkaloid was given. Brossi and coworkers (3) completed the structure elucidation of cherylline in 1970 and it was found to be identical to crinin. With the improvement in isolation techniques, especially preparative thin layer chromatography, cherylline can be isolated easily from many Crinum species.

Structure Elucidation of Cherylline

The structure determination of cherylline (1) was based primarily on the nmr spectrum in DMSO-d_6 (3), cherylline exhibits a three proton singlet at δ2.24 (NCH_3), another three proton singlet at δ3.51 (OCH_3), an AA'BB' pattern (δ6.91 and δ6.64) characteristic of a para

substituted aromatic ring, two one-proton singlets (δ6.49 and δ6.23) indicative of two <u>para</u> oriented protons on a second aromatic ring. The proton at δ6.23 was assigned to the proton at C-5 because of the shielding effect of the



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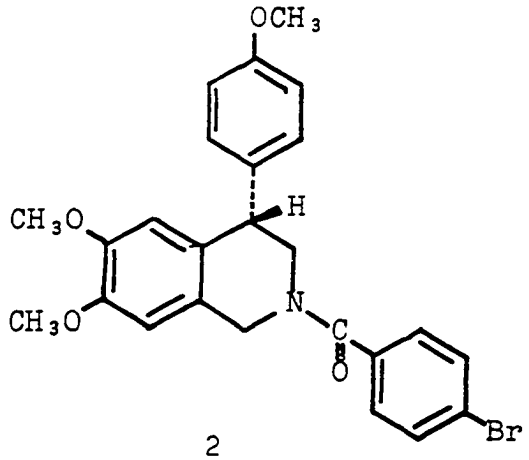
4-phenyl group, the hydroxyl group must be located at C-7, because upon addition of a drop of NaOD in D_2O, the one proton singlet at δ6.49 was shifted 26 Hz to δ6.06, while the proton singlet at δ6.23 was only shifted by 10 Hz. The rest of the protons were assigned as follows: multiplets at δ2.4-3.0 (methylene at C-3), singlet at δ3.42 (methylene at C-1), triplet at δ3.97 (methine at C-4).

O,O-Dimethylcherylline, which was obtained by treating cherylline with diazomethane, has the same TLC and spectral properties as the synthetic (±)-6,7-dimethoxy-4-(4'-methoxyphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline, thus verifying further the structure of cherylline.

The configuration at the chiral center C-4 was determined by comparing the CD and ORD curves of the resolved (±)-6,7-dimethoxy-4-(4'-methoxyphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline, it was found to have an S configuration at the chiral center C-4. The absolute configuration has been conclusively determined by the X-ray crystallographic study (4) of (±)-2-(4-bromobenzoyl)-6,7-dimethoxy-4(S)-(4-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (2).



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Total Syntheses of Cherylline

The first actual synthesis of racemic and optically active cherylline was accomplished by Brossi and Teitel (5, 6) by the route shown in Figure 1.

The (±) phenethylamine 3 could be resolved into its (+) and (-) isomers and the natural cherylline and its unnatural isomer were synthesized.

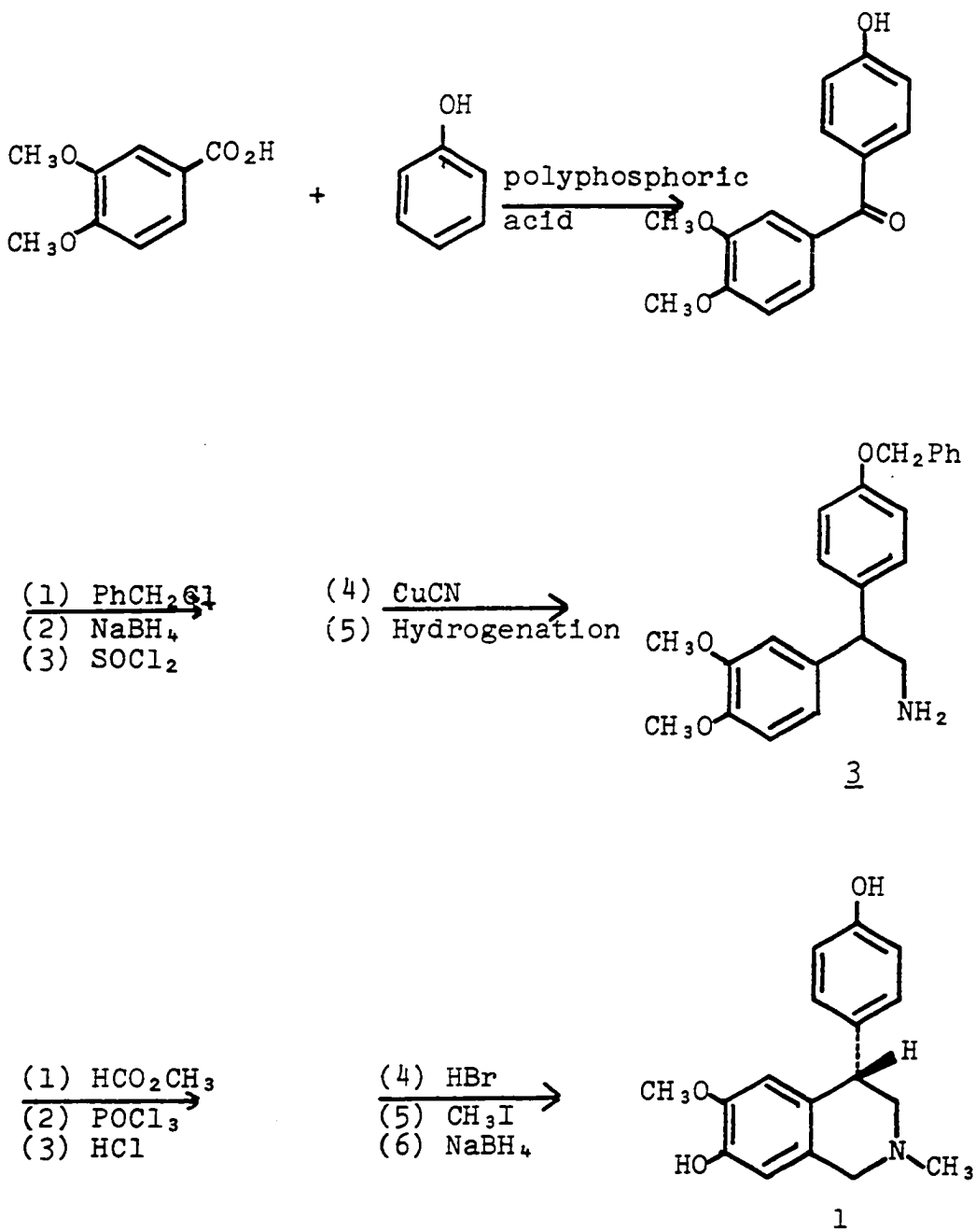


Figure 1. Brossi and Teitel's synthesis of cherylline

Schwartz and Scott (7) approached the total synthesis of racemic cherylline in a biogenetic-type oriented pathway with an overall yield of 33%. The route involved the condensation of (±) octopamine hydrochloride (4) with isovanillin (5) in absolute methanol in the presence of potassium carbonate, the resulting imine was then reduced by excess sodium borohydride without isolation and purification. The resulting reductive condensation product, 2-hydroxy-O-methylnorbelladine (6), was refluxed with ethyl formate in the presence of potassium carbonate to give an N-formylated compound, which was then directly reduced and cyclized to racemic cherylline by addition of excess lithium aluminum hydride using 1,2-dimethoxyethane as solvent, Figure 2.

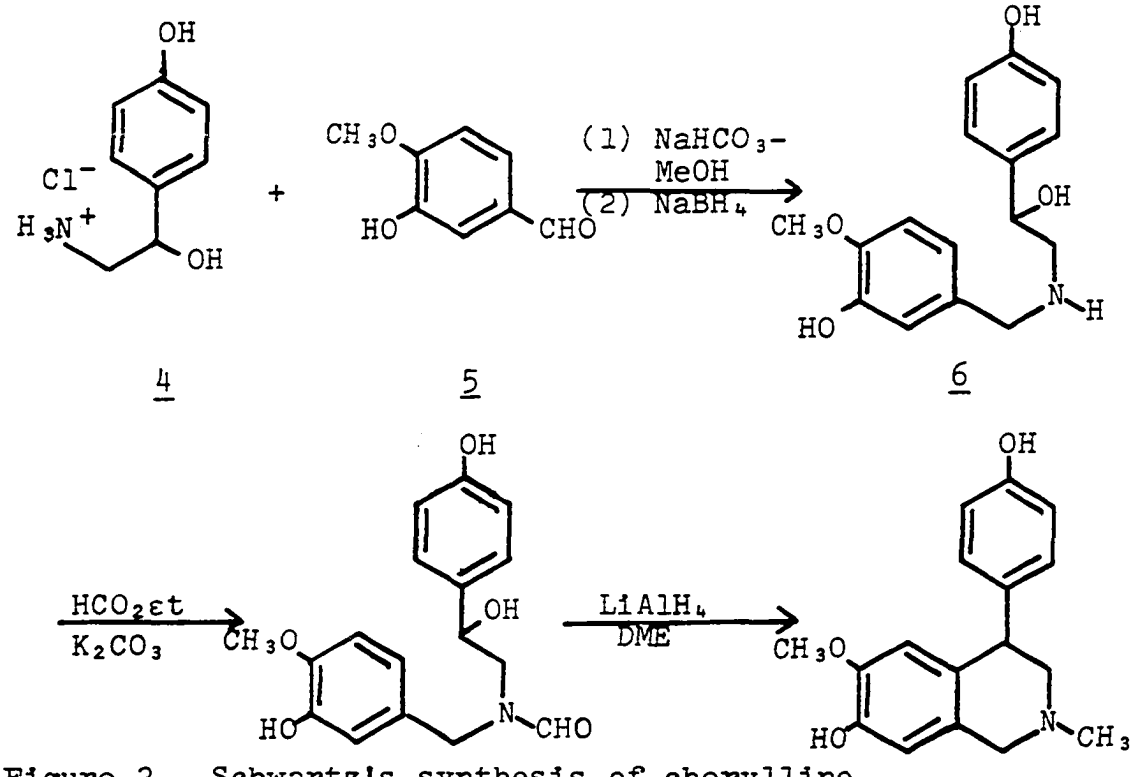


Figure 2. Schwartz's synthesis of cherylline

Shaffer's (1) approach to the total synthesis of cherylline was devised to provide a compound doubly-labeled at the C-1 and C-3 positions for biosynthetic studies. The route involved the preparation of phenethylamine 7 to form the ring system A and C, and then development of the hydroaromatic ring B. The synthetic sequence can be summarized as in Figure 3.

Biosynthesis of Amaryllidaceae Alkaloids

The first studies in the biosynthesis of Amaryllidaceae alkaloids (8) dealt with amino acid precursors like phenylalanine and tyrosine. These studies conclusively showed that phenylalanine is the precursor to the aromatic C_6-C_1 fragment, while tyrosine is the precursor to the hydroaromatic C_6-C_2 fragment of the alkaloids. The incorporations were highly specific in such a way that no phenylalanine has been incorporated into the C_6-C_2 fragment and no tyrosine has been incorporated into the C_6-C_1 fragment.

In 1957 Barton and Cohen (9) postulated that Amaryllidaceae alkaloids originated from the phenyl-phenyl oxidative coupling of the precursor norbelladine (8). *Para-ortho* coupling (Figure 4) providing lycorine (9); *ortho-para* coupling (Figure 5) providing galanthamine (10), *para-para* coupling (Figure 6) providing crinine (11). Subsequent isolation of belladine from *Amaryllis belladona* (10),

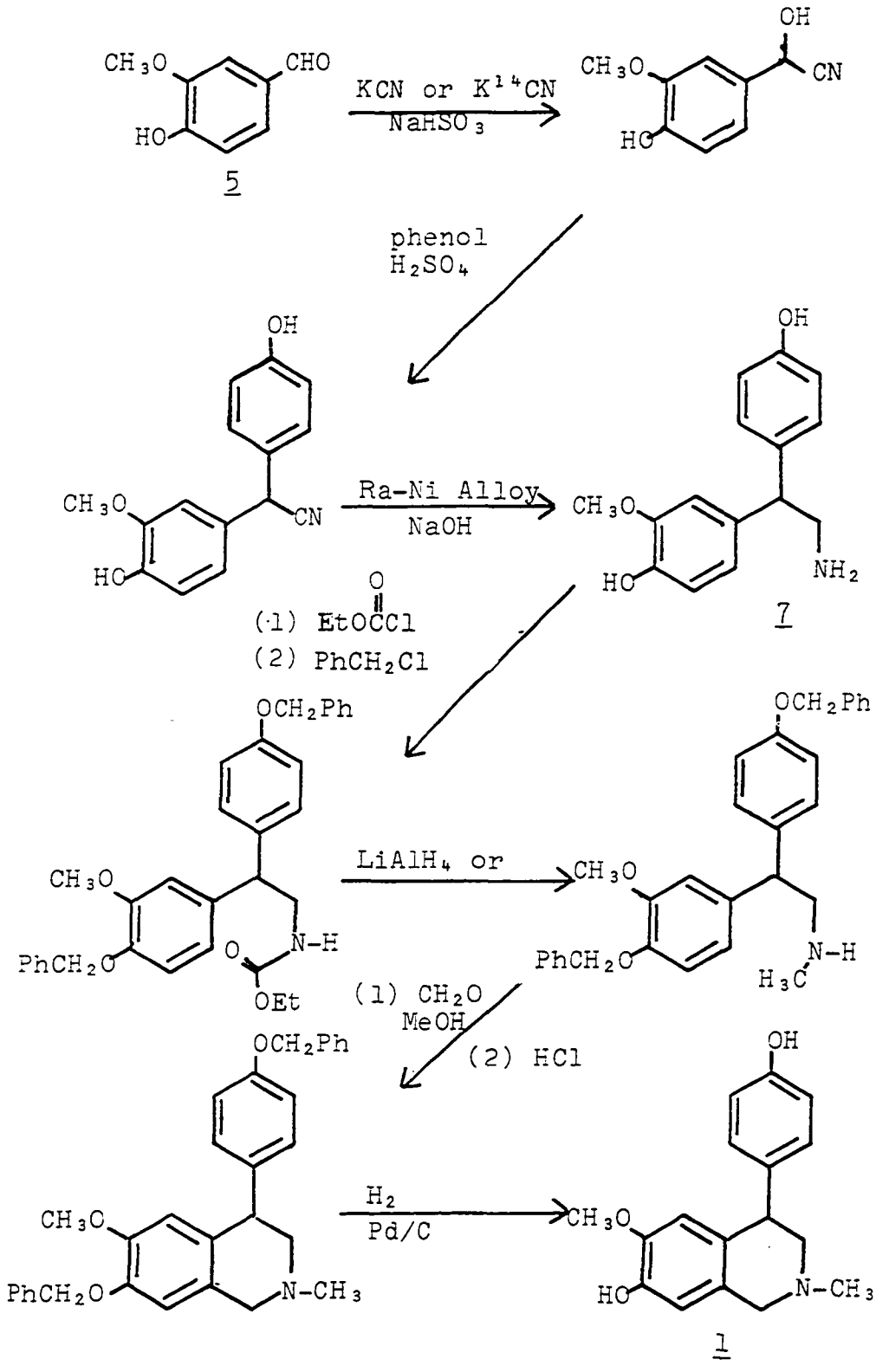


Figure 3. Shaffer's synthesis of cherylline

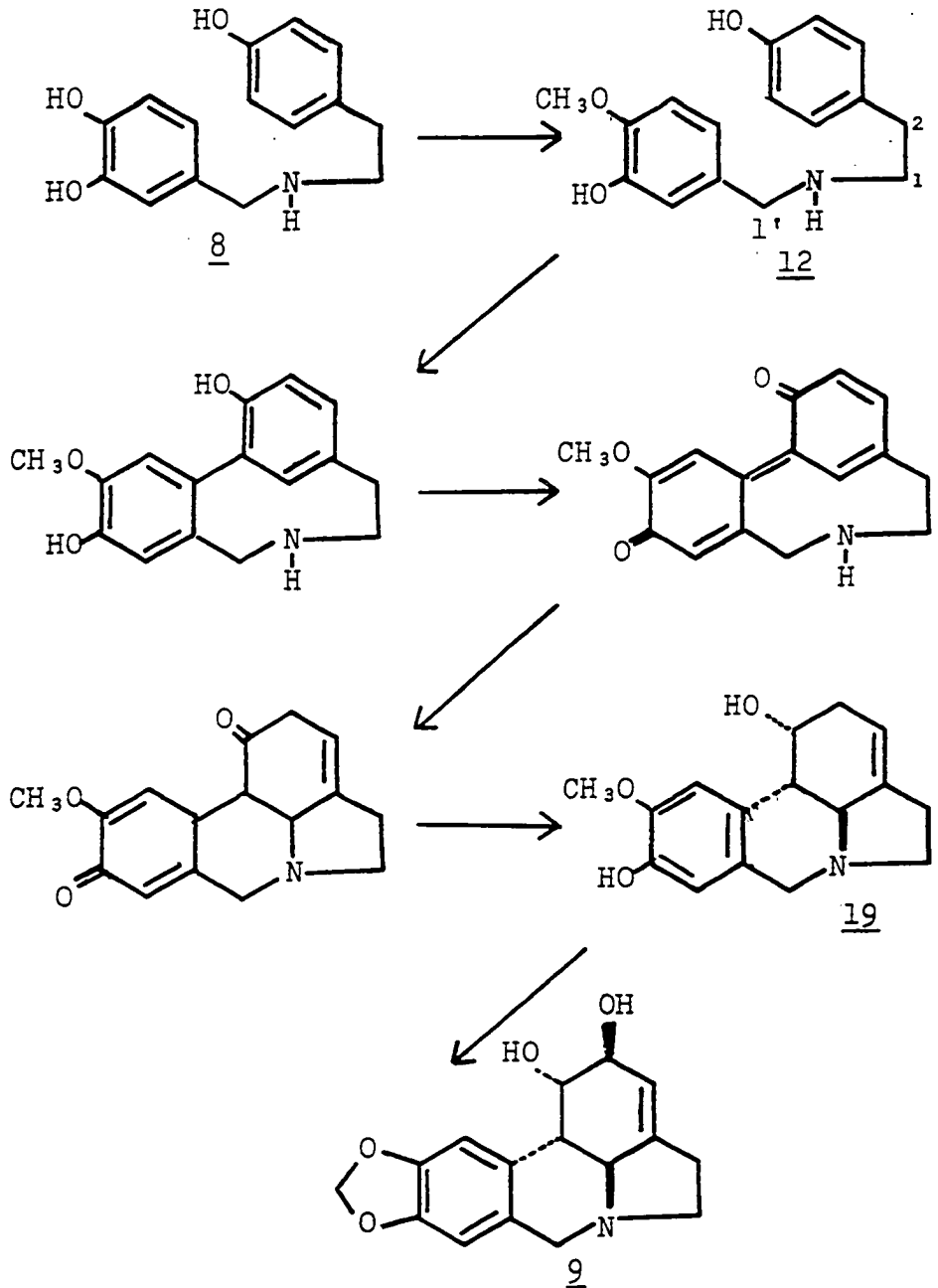


Figure 4. Biosynthesis of lycorine

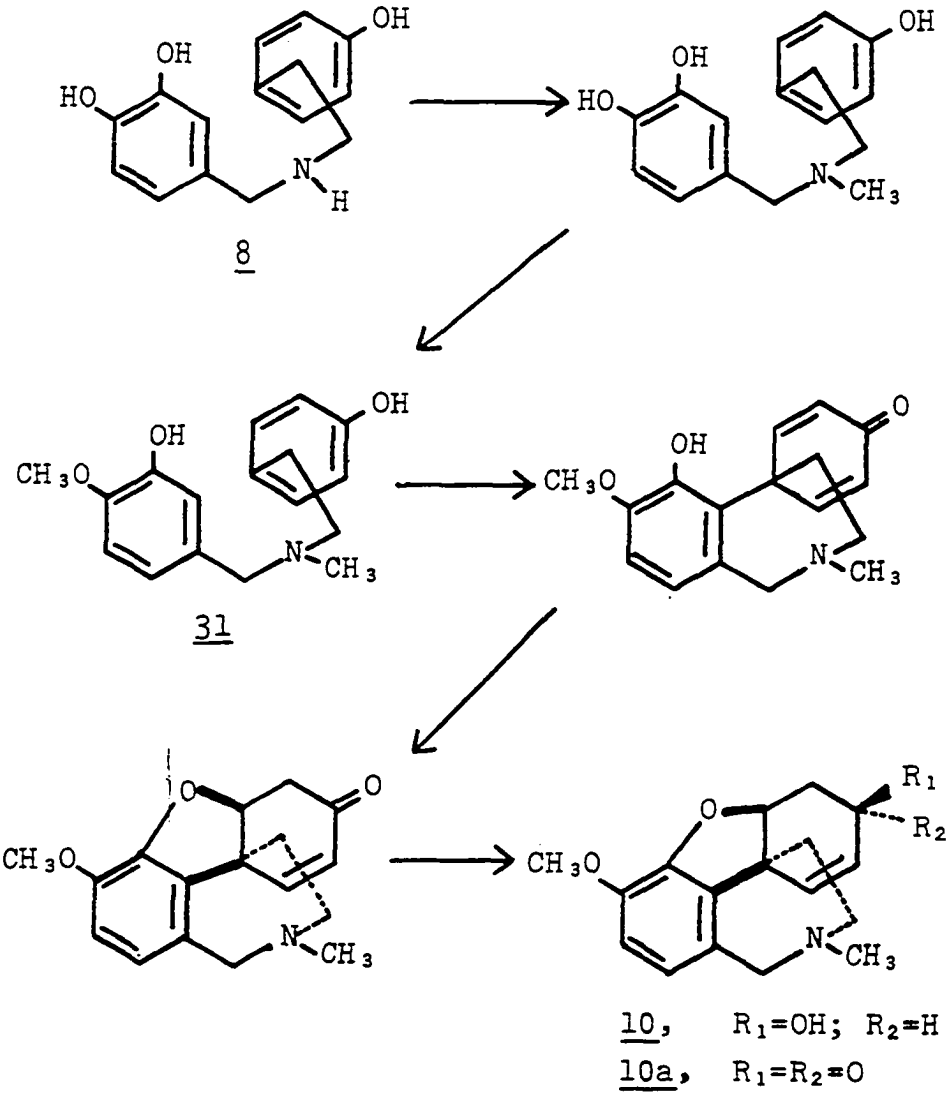


Figure 5. Biosynthesis of galanthamine

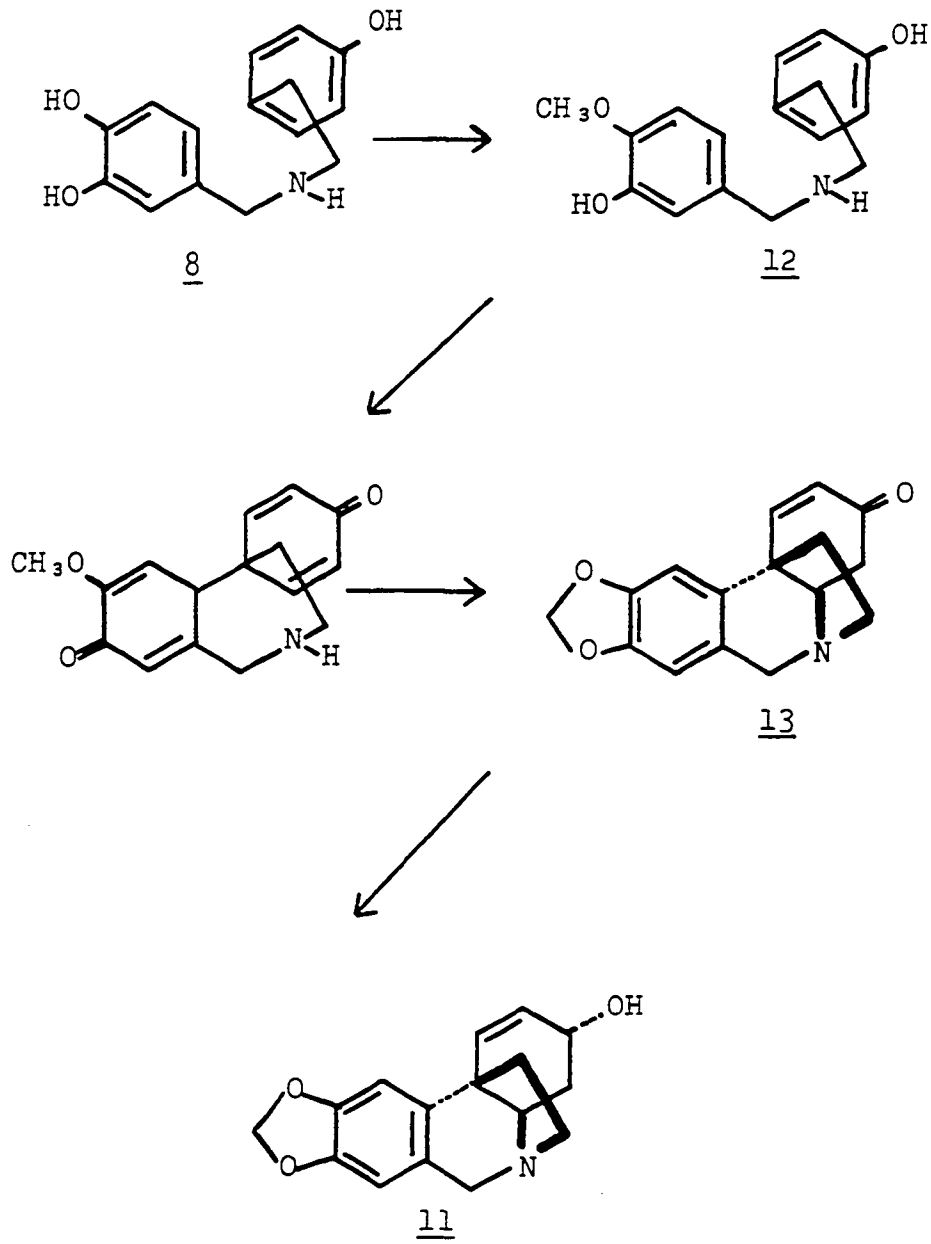
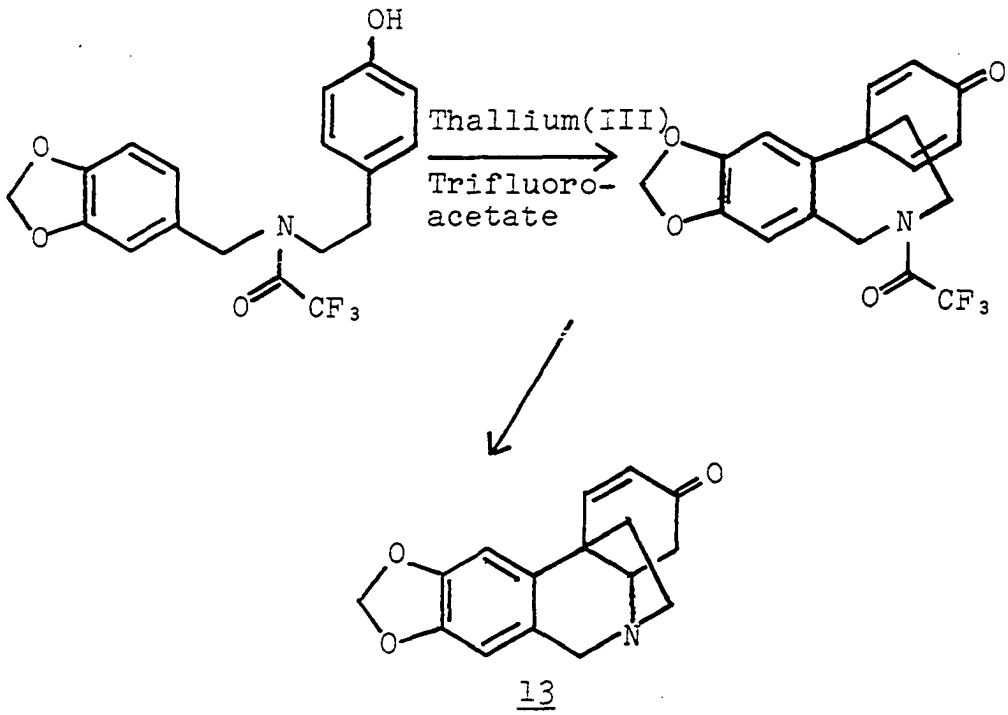


Figure 6. Biosynthesis of crinine

detection by isotopic dilution of O-methylnorbelladine (12) in Narcissus pseudonarcissus L. var. "Twink" (11) and isolation of O-methylnorbelladine in several Crinum species (1) further supported the Barton and Cohen oxidative coupling theory.

Several in vitro syntheses have been done to verify the theory (12). Recently, Schwartz and coworkers (13) were able to obtain (±)-oxocrinine (13) in an overall yield of 15% by using thallium (III) trifluoroacetate as two-electron oxidant.



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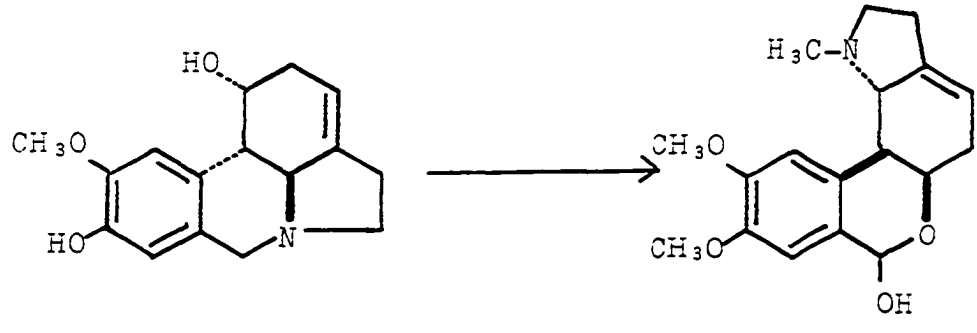
Tracer experiments showed that norbelladine (8) was synthesized in vivo in the plant by the condensation of protocatechuic aldehyde with tyramine. Protocatechuic

aldehyde is the degradation product of phenylalanine (14), which was shown to come from the following pathway: phenylalanine → trans-cinnamic acid → caffeic acid → protocatechuic aldehyde, while tyramine is the decarboxylated product of tyrosine (15). The biosynthesis of norbelladine is illustrated in Figure 7.

Doubly- and triply-labeled norbelladine-type derivatives were fed into the Amaryllidaceae and were found to incorporate intact into the alkaloids. Table 1 summarizes the result of the biosynthetic studies.

Late Stage Biosynthetic Modifications of the Amaryllidaceae Alkaloids

The lycorenine (16), pretazettine (17), and montanine (18) ring systems can not be accommodated within the framework of the phenol-phenol oxidative coupling theory.



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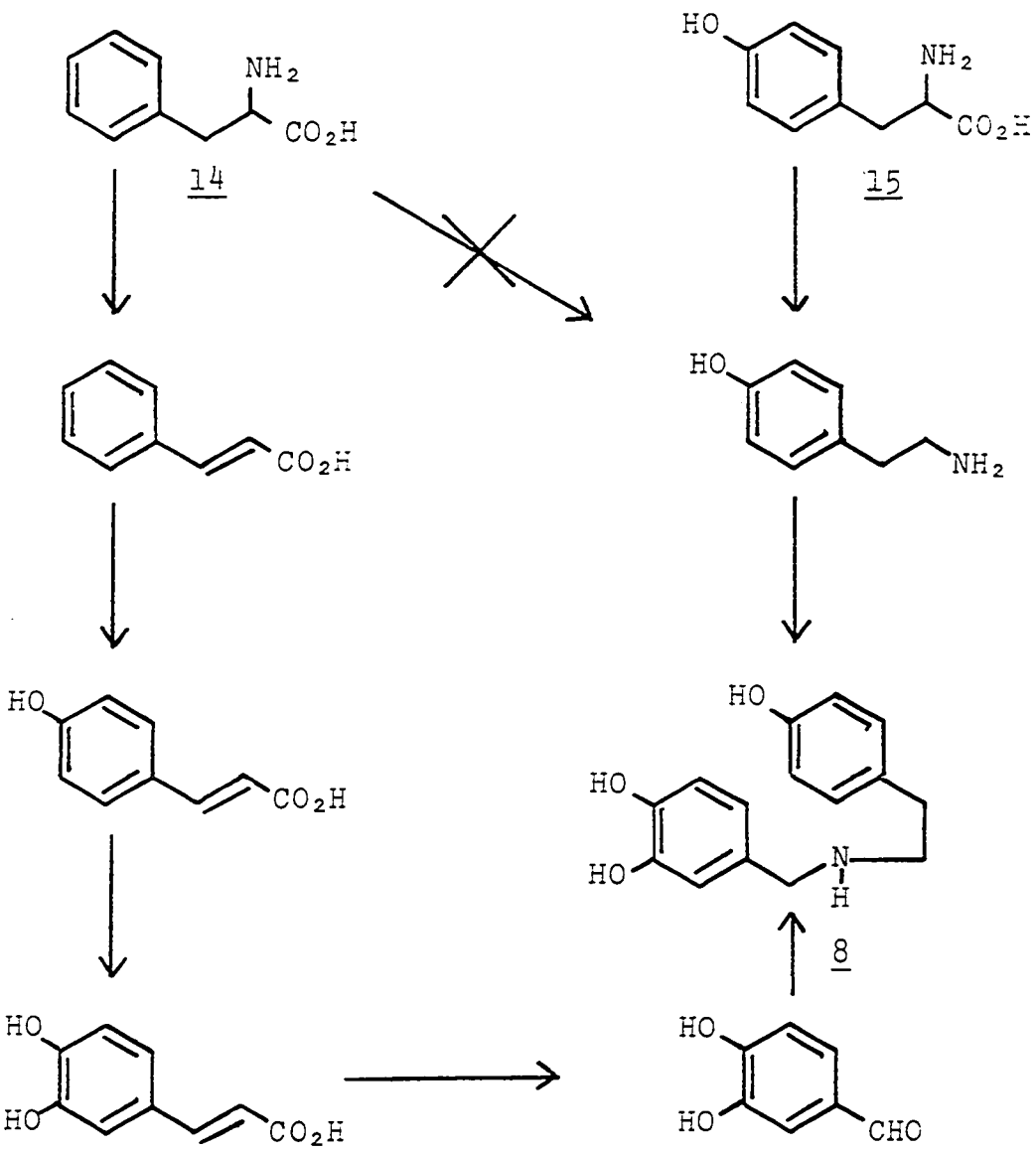


Figure 7. Biosynthesis of norbelladine

Table 1. Incorporation of norbelladine and derivatives into Amaryllidaceae alkaloids

Precursor	Plant	Alkaloid	% inc.	Ref.
[1-^{14}C]Norbelladine	Narcissus "Twink"	Lycorine	0.24	14
		Norpluvine	0.74	14
		Haemanthamine	0.15	15
	Narcissus "King Alfred"	Galanthamine	0.014	16
		Galanthine	0.003	16
		Haemanthamine	0.25	16
[1,1'-^{14}C]Norbelladine	Nerine bowdenii "King Alfred"	Belladine	2.64	17
		Crinamine	0.0009	17
		Lycorine	0.07	17
		Ambelline	0.17	18
[1'-^{14}C]Bisdeoxynorbelladine	Narcissus "Twink"	Tazettine	0.00	19
		Haemanthamine	0.00	19
[1'-^{14}C]Hydroxynorbelladine	Narcissus "Twink"	Lycorine	0.007	19
		Norpluvine	0.017	19
[N-^{14}C]Methylnorbelladine	Narcissus "King Alfred"	Galanthamine	0.18	16,20
		Galanthine	0.00	16,20
		Haemanthamine	0.00	16,20
O-Methyl[N-^{14}C]methylnorbelladine	Narcissus "King Alfred"	Galanthamine	0.14	16,20
		Galanthine	0.00	16,20
		Haemanthamine	0.00	16,20

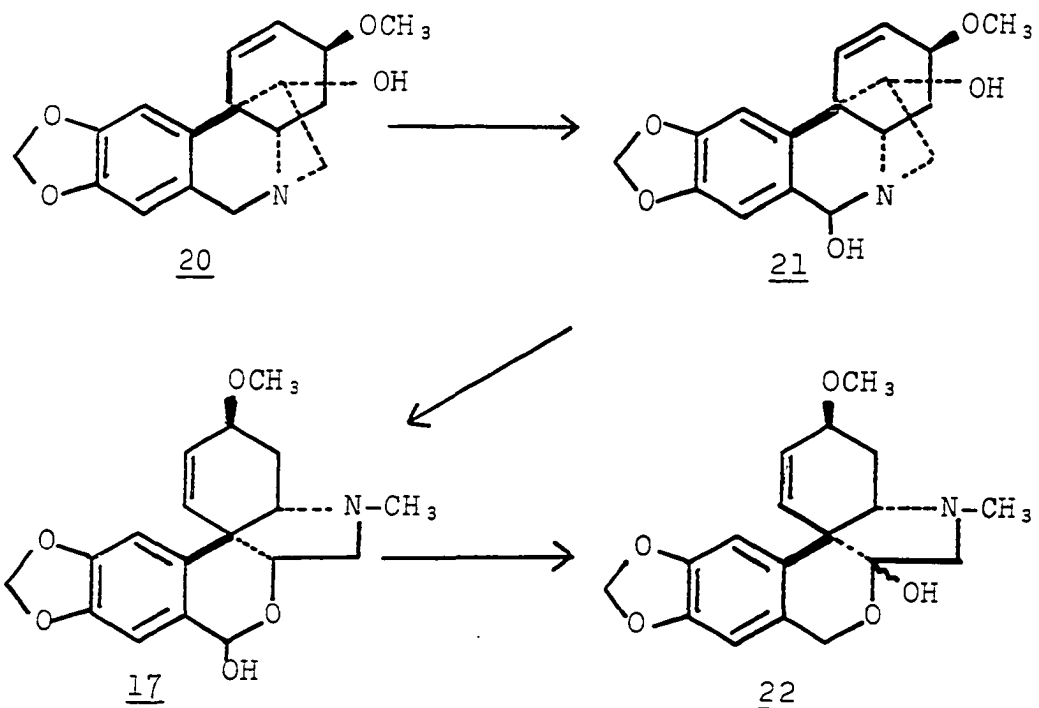
Table 1. (Continued)

Precursor	Plant	Alkaloid	% inc.	Ref.
O-[^{14}C]Methyl-[N-^{14}C]methyl-norbelladine	Narcissus "King Alfred"	Galanthamine	0.14	20,21
		Galanthine	0.00	20,21
		Haemanthamine	0.00	20,21
O-[^{14}C]Methyl-[N-^{14}C]-methyl-[1-^{14}C]norbelladine	Narcissus "King Alfred"	Galanthamine	0.018	21
		Galanthine	0.00	21
		Haemanthamine	0.00	21
O-[^{14}C]Methyl-norbelladine	Narcissus "King Alfred"	Galanthamine	0.00	20,22
		Galanthine	0.00	20,22
		Haemanthamine	0.036	20,22
O-[^{14}C]Methyl-[1-^{14}C]norbelladine	Narcissus "King Alfred"	Galanthamine	0.00	20,22
		Galanthine	0.00	20,22
		Haemanthamine	1.00	20,22
[1-^{14}C]-4-Hydroxy-N-(4-hydroxy-3-methoxybenzyl)-phenethylamine	Narcissus "King Alfred"	Galanthine	0.00	23
O-Methyl[$^{3}H_4$]norbelladine	Narcissus "Texas"	Norpluvine	1.40	23
	Narcissus "Deanna Durbin"	Lycorine	0.46	24
		Norpluvine	0.72	24
O-[^{14}C]Methyl[$^{3}H_2$]-norbelladine	Narcissus "Twink"	Lycorine	0.93	24
		Norpluvine	1.50	24

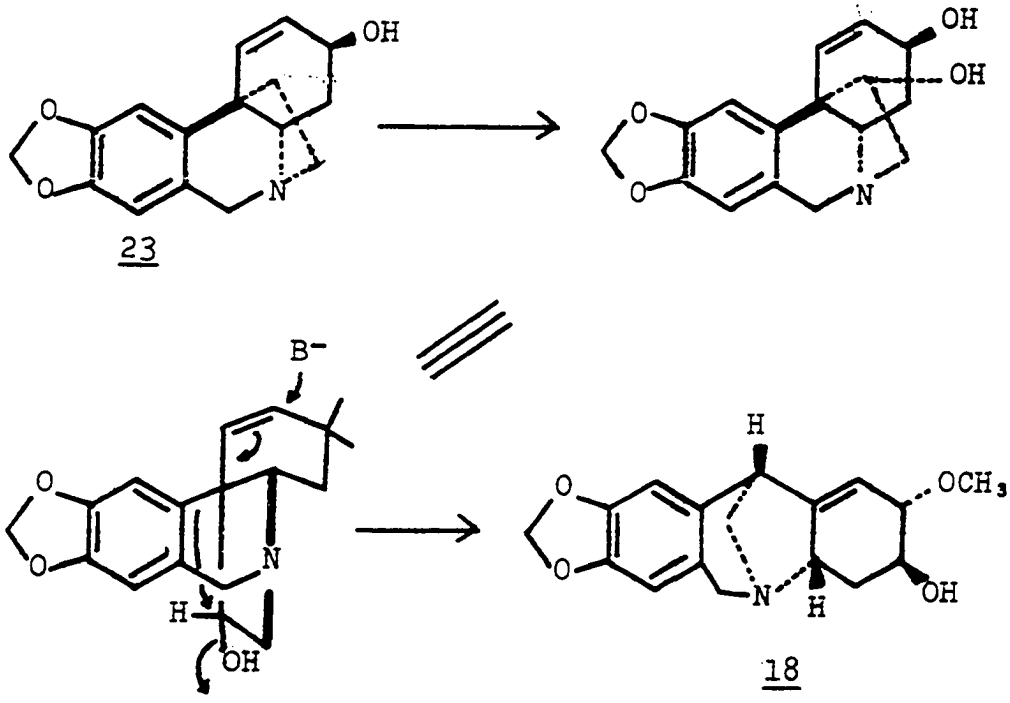
Table 1. (Continued)

Precursor	Plant	Alkaloid	% inc.	Ref.
O-Methyl[1'-^{3}H]-norbelladine	*Narcissus* "King Alfred"	Haemanthamine	4.1	25
		Oduline	0.04	25
		Masonine	0.00	25
		Lycoramine	0.02	25
	Crinum erubescens	Lycorine	4.47	1
		Cherylline	0.986	1
		Lyrechine	0.0165	1
		O-methylnor-belladine	0.126	1
O-[^{14}C]Methyl[$^{3}H_2$]-norbelladine	*Narcissus* "Twink"	Haemanthamine	0.11	26
		Norpluvine	0.73	26
		Lycorine	0.09	26
		Narciclasine	0.35	26

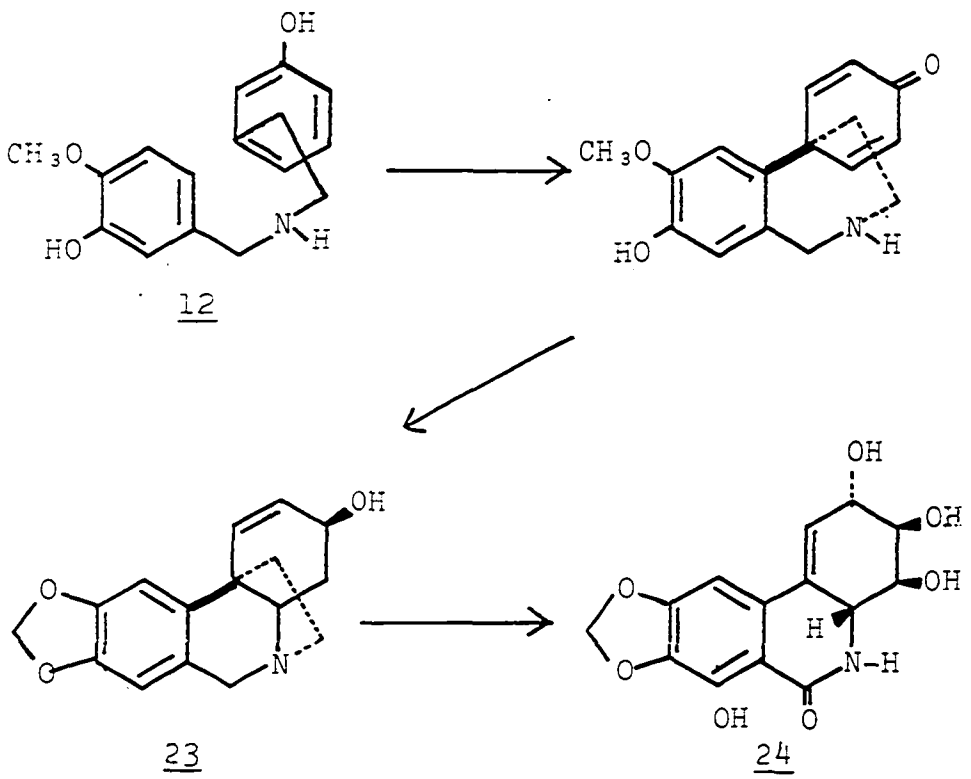
However, subsequent tracer feeding experiments (27) have shown that norpluvine (19) was converted *in vivo* into lycorenine by benzylic oxidation, N-methylation and skeletal rearrangement. Labeled haemanthamine (20) was incorporated into haemanthidine (21) and tazettine (22) in *Sprekelia formissima* (27), since it is known that tazettine is a rearrangement product of pretazettine (17) during isolation (28), the biosynthetic pathway of pretazettine can be rationalized as to involve the benzylic oxidation and subsequent skeletal rearrangement of haemanthamine (20). The montanine ring system has been



found by chemical methods to come from the rearrangement of the crinine nucleus (8). This was observed by the fact that aromatic tritiated vittatine (23) was incorporated intact into montanine (18) in Rhodophialia bifida (29). Thus, every ring system known thus far has been, in one way or another, related to some derivative of norbelladine. Even the unclassified lactam "alkaloid" narciclaisine (24), which contained two less carbon than the norbelladine ring system was found to come from the para-para coupling of



O-methylnorbelladine (12), through the intermediacy of the crinine ring system, followed by late elimination of two carbon atoms (30,31).



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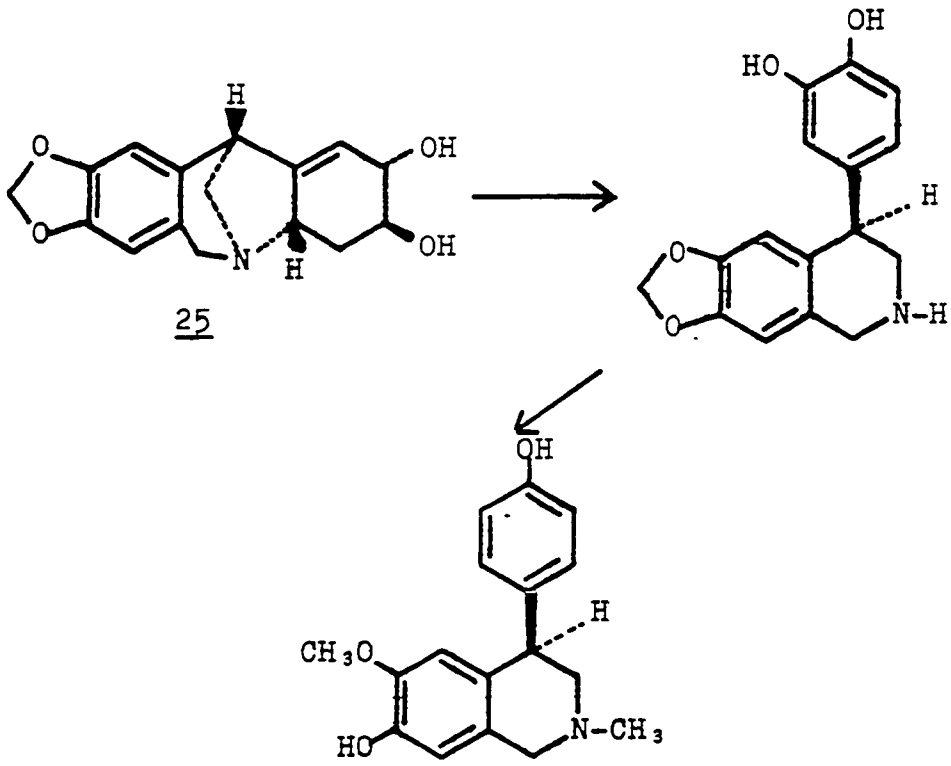
24

Biosynthetic Speculation of Cherylline

The biosynthesis of cherylline can not be rationalized in terms of the Barton and Cohen theory because the phenyl rings are not coupled. An obvious possibility for the biogenesis of cherylline would be the oxidation of

montainine-type alkaloids 25 followed by elimination and N-methylation. This would give the cherylline ring system, but opposite in configuration.



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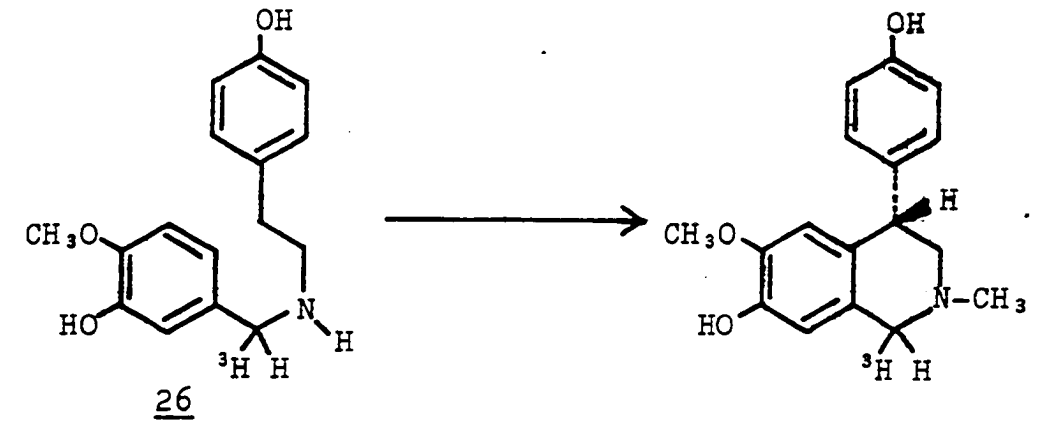
Since the structure of cherylline is very similar to that of the norbelladine ring structure, O-methylnorbelladine (12) can still be postulated to be the key intermediate. Thus, O-methyl[1'-^{3}H]norbelladine (26) was

found to be incorporated efficiently (0.99%) into cherylline. Surprisingly, N-demethyl[1-^{3}H, 3-^{14}C]cherylline (27) was not incorporated intact into cherylline and did not serve as precursor to the other types of ring systems.



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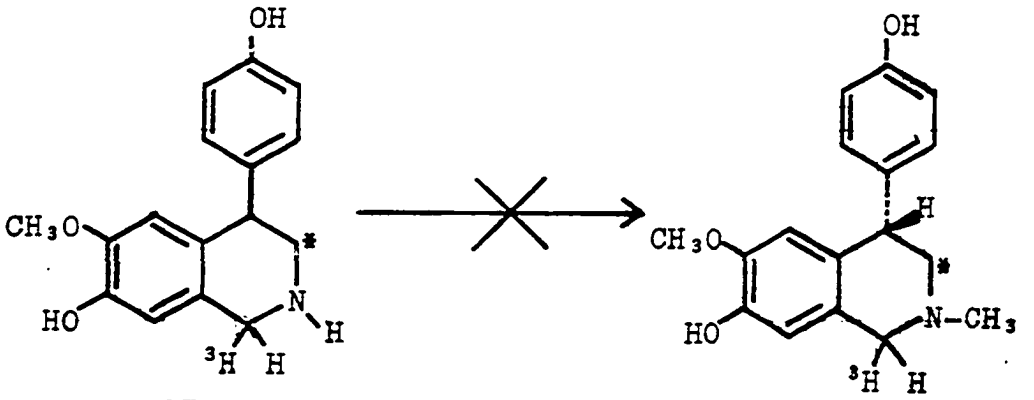

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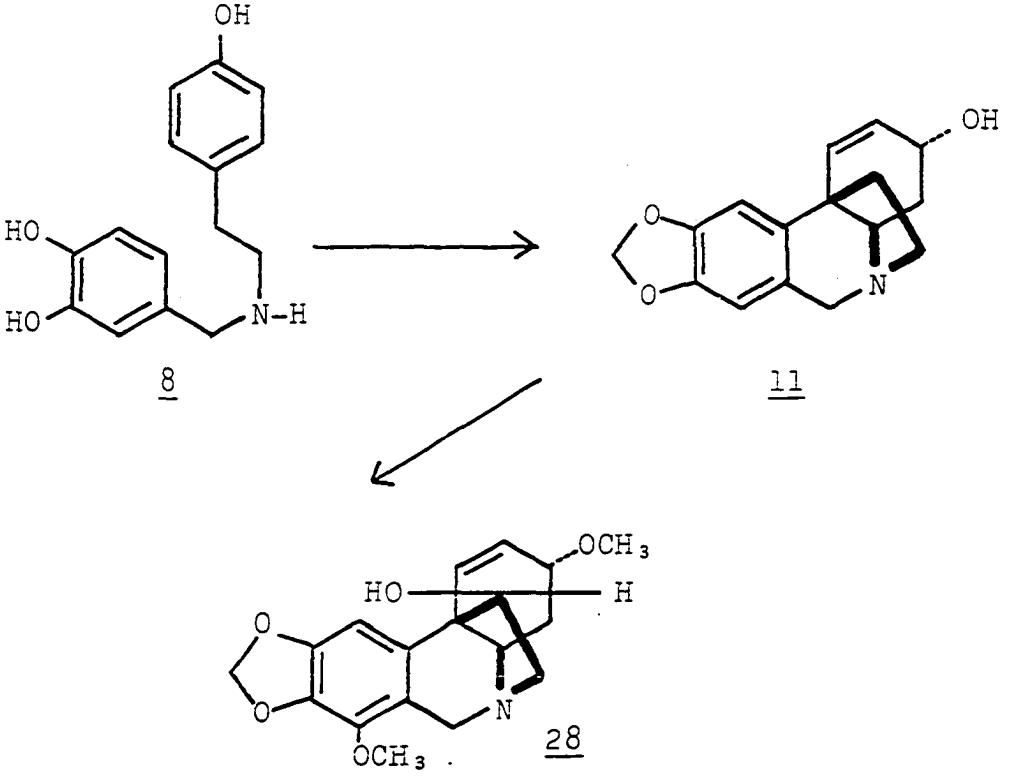
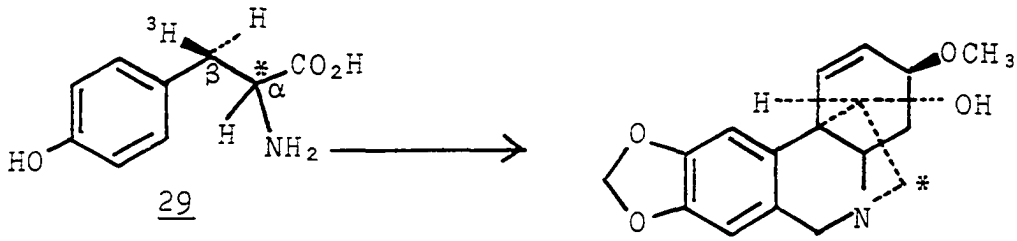
*Carbon-14.

C-11 Hydroxylation in Amaryllidaceae Alkaloids

Crinamine, 6-hydroxycrinamine, haemanthamine, haemanthidine, 11-hydroxyvittatine and ambelline (28) are crinine-type alkaloids which possess hydroxy group at the C-11 position. The conversion of [1,1'-^{14}C]norbelladine (18) and crinine (32) into ambelline in Nerine bowdenii proved that C-11 hydroxylation occurred at a late stage of the biosynthesis. This was substantiated by the fact that β-phenethanolamine derivatives such as octopamine and p-hydroxyphenylserine (29) were shown not to be incorporated into either the C_6-C_1 or C_6-C_2 units of 6-hydroxycrinamine or lycorine. They serve only as precursors to the methoxyl and methylenedioxy groups of lycorine and 6-hydroxy-crinamine.



When DL-[α-^{14}C, βR-^{3}H] tyrosine (29) and DL-[α-^{14}C, βS-^{3}H] tyrosine (30) were fed separately into "Texas" daffodils (33), radioactive haemanthamine was isolated in which the C-11 hydroxylation occurred with retention in configuration, the pro-R hydrogen being replaced by the hydroxy group. Narwedine (10a) was also isolated in which the radioactivity was retained in the C_6-C_2 fragment. This provided the first formal proof that tyrosine is not incorporated into the C_6-C_1 unit of the galanthamine (narwedine) group of ring systems.



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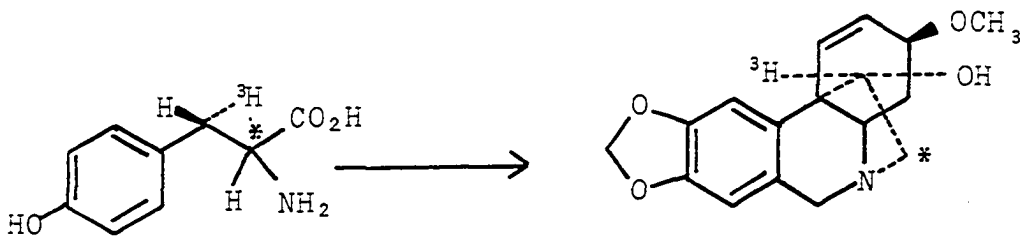

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Recently, Schwartz and Scott (7) postulated a biogenetic-type synthesis of cherylline which can be envisioned in the following manner, direct two electron oxidation of O,N-dimethylnorbelladine (31) into the quinone methide structure 32 followed by cyclization and tautomerization to cherylline or hydroxylation at the C-2 position to give 2-hydroxy-O,N-dimethylnorbelladine (33), which would give the same quinone methide upon dehydration. Figure 8.

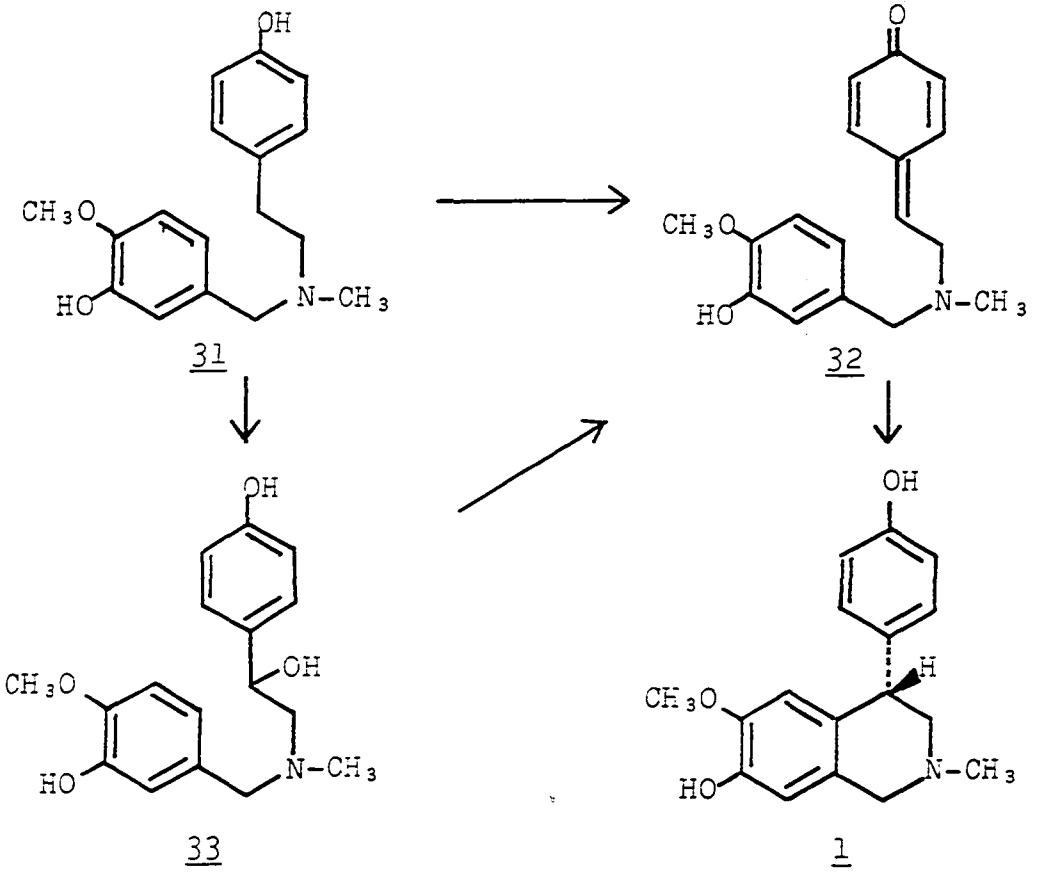


Figure 8. Proposed biosynthesis to cherylline (Schwartz)

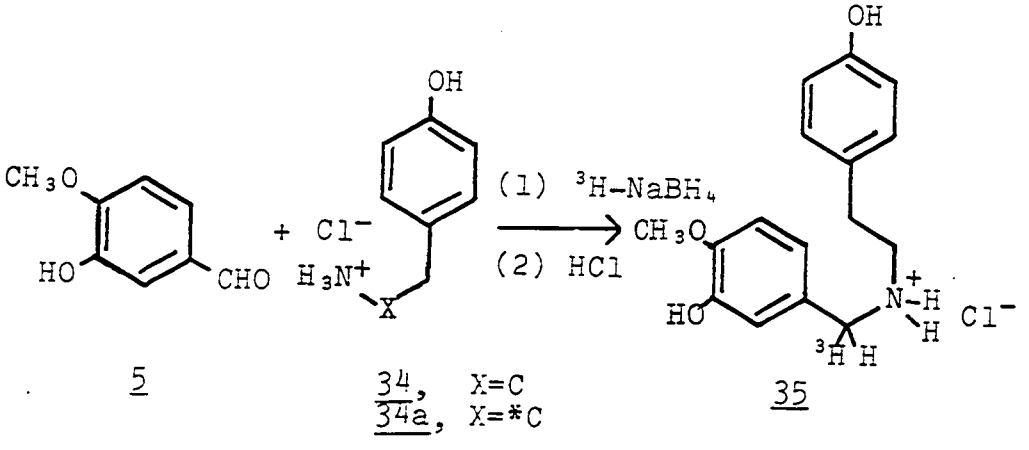
RESULTS AND DISCUSSION

This section will discuss the synthetic preparations of four doubly-labeled norbelladine-type alkaloids, their degradation and results of the biosynthetic feeding experiments.

Synthetic Investigations

Synthesis of O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride and O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride

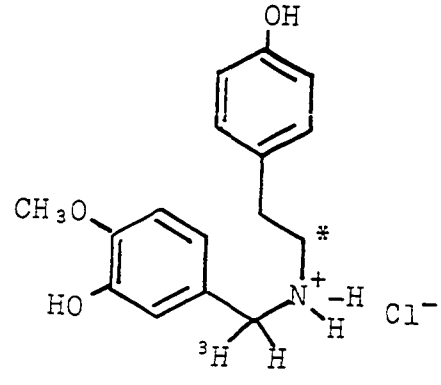
O-Methyl[1'-^{3}H]norbelladine hydrochloride (35) was prepared by the condensation of isovanillin (5) and tyramine hydrochloride (34) in the presence of sodium bicarbonate in isopropanol to form an imine (7). The imine was reduced *in situ* with ^{3}H-sodium borohydride to O-methyl[1'-^{3}H]norbelladine and converted into its hydrochloride salt 35.



5 34, X=C 34a, X=*C 35

O-Methyl[1-^{14}C]norbelladine hydrochloride (41) was synthesized as outlined in Figure 9. It was found that lithium aluminum hydride alone gave unsatisfactory yields due to the presence of active hydrogens. Thus, when a Lewis acid, aluminum chloride, was added to lithium aluminum hydride to give an acidic mixed hydride, the yield was increased appreciably.

Mixing of O-methyl[1'-^{3}H]norbelladine hydrochloride and O-methyl[1-^{14}C]norbelladine hydrochloride gave the doubly-labeled O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42).



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O,N-Dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43) was synthesized by reductive methylation of O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42)

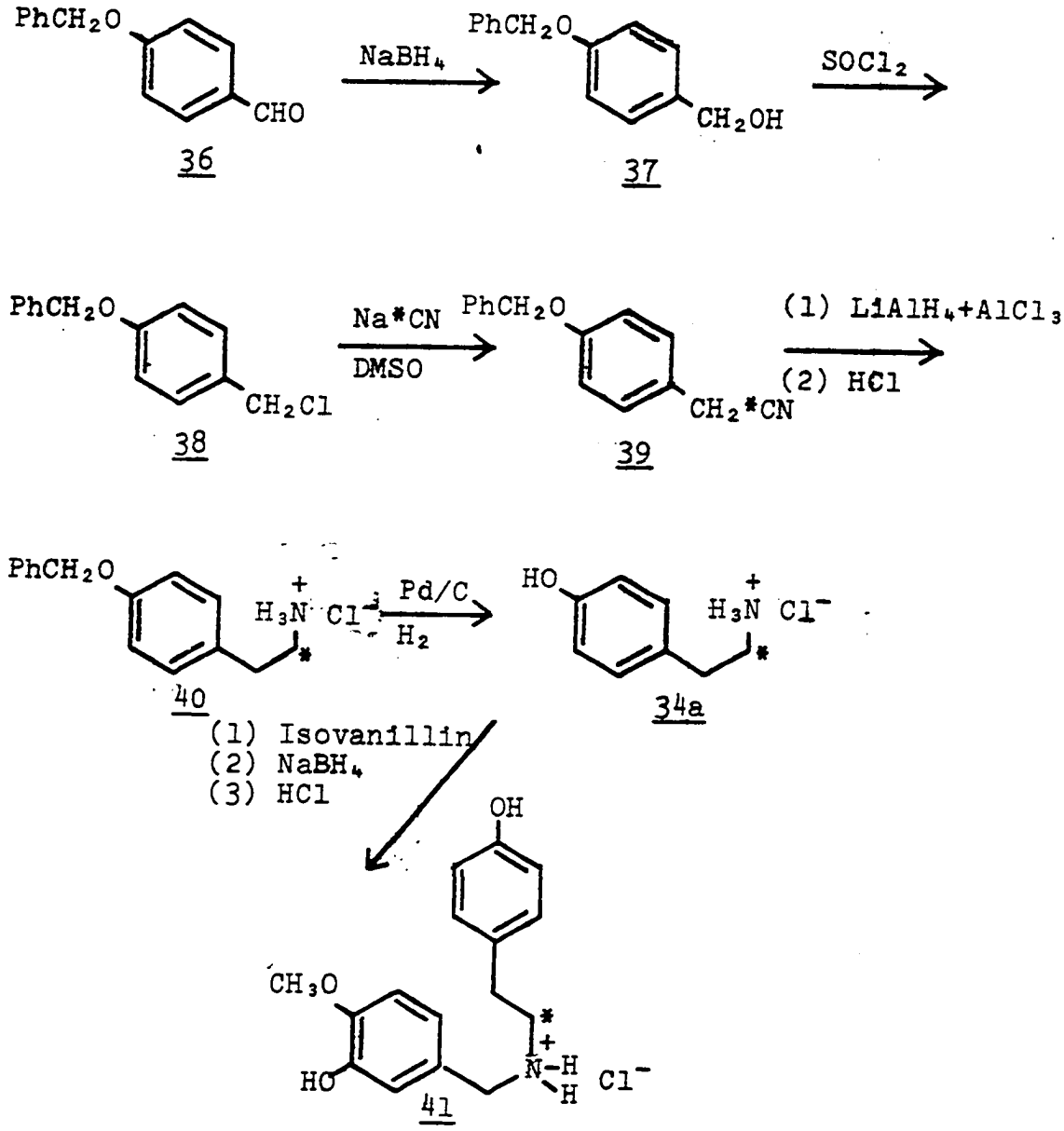
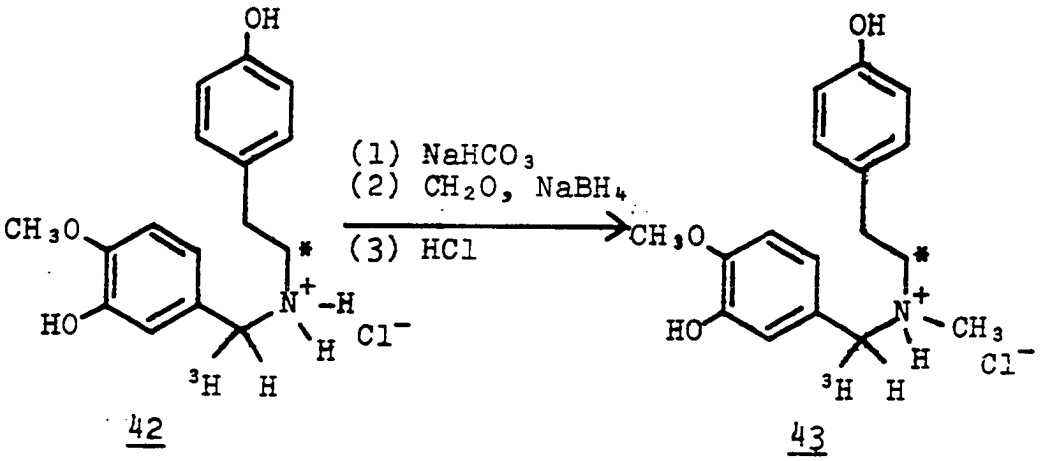


Figure 9. Synthesis of O-methyl[1-^{14}C]norbelladine hydrochloride



using excess formaldehyde and sodium borohydride, followed by conversion to its hydrochloride salt.

Degradation of O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride

In order to determine unambiguously the exact position of the ^{3}H and ^{14}C label, a chemical degradation was performed on O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43). Thus, O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride when treated with excess methyl iodide and potassium carbonate afforded [1'-^{3}H, 1-^{14}C]belladine methiodide (44) with no loss in activity. [1'-^{3}H, 1-^{14}C]-Belladine methiodide was cleaved by the Hofmann method using 20% KOH to give [1-^{3}H]N,N-dimethylveratrylamine (45)

and [1-^{14}C]p-methoxystyrene (46). [1-^{14}C]p-Methoxystyrene (46) was converted to [1-^{14}C]1,2-dibromo-2-(4-methoxyphenyl)-ethane (47) which retained 94% of the ^{14}C activity and was hydrolyzed to [1-^{14}C]1,2-dihydroxy-2-(4-methoxyphenyl)ethane (48) which retained 97% of the ^{14}C activity. [1-^{14}C]1,2-dihydroxy-2-(4-methoxyphenyl)ethane was cleaved by periodic acid into [^{14}C-formyl]formaldehyde (50) and p-anisaldehyde (49), and were counted as [^{14}C-methylene]2,2'-methylenebis-(5,5-dimethyl-1,3-cyclohexanedione) (53) and tetrahydro-9-(p-methoxyphenyl)-3,3,6,6-tetramethylxanthenedione (52) derivatives, respectively.

Compound 53 was found to retain the ^{14}C activity, while Compound 52 is non-radioactive. p-Anisaldehyde and formaldehyde were separated easily since 2,2'-(p-methoxy-benzylidene)bis[5,5-dimethyl-1,3-cyclohexanedione] (51) is not crystalline, while 2,2'-methylenebis(5,5-dimethyl-1,3-cyclohexanedione) (53) is crystalline. Also, 2,2'-(p-methoxybenzylidene)bis[5,5-dimethyl-1,3-cyclohexa-nedione) (51) is yellowish in color in silica gel column while 2,2'-methylenebis(5,5-dimethyl-1,3-cyclohexanedione) (53) is colorless. Therefore, it was easy to see where to cut fractions. [1-^{3}H]N,N-Dimethylveratrylamine hydro-chloride (54) was oxidized by potassium permanganate to veratric acid (55) and was found to be non-radioactive, thus confirming that all the ^{3}H label was retained in the

benzylic position. The degradation of O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43) is illustrated in Figure 10 and results of degradation are summarized in Table 2.

Synthesis of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride

Schwartz's synthesis (7) of 2-hydroxy-O-methyl-norbelladine (6) by the reductive condensation of (±)-octopamine hydrochloride (4) and isovanillin (5) is not useful for making doubly-labeled 2-hydroxy-O-methyl-norbelladine. Therefore, a suitable method has to be found to introduce a ^{14}C label in isovanillin and a ^{3}H label in octopamine in an efficient and convenient way. It has been well known in the literature (34) that carbonation of a Grignard reagent using $Ba^{14}CO_3$ as the source of carbon dioxide is one of the attractive ways of introducing the ^{14}C label. Therefore, experiments have been made in this direction to synthesize a suitable precursor for the Grignard reaction. Thus, treatment of guaiacol (56) with ethylchloroformate gave guaiacol carbonate (57), which upon bromination afforded 5-bromoguaiacol carbonate (58). Bromination occurs cleanly at the 5-position due to the para-directing effect of p-methoxy group, and is reinforced by the meta-directing effect of the carbonate functionality.

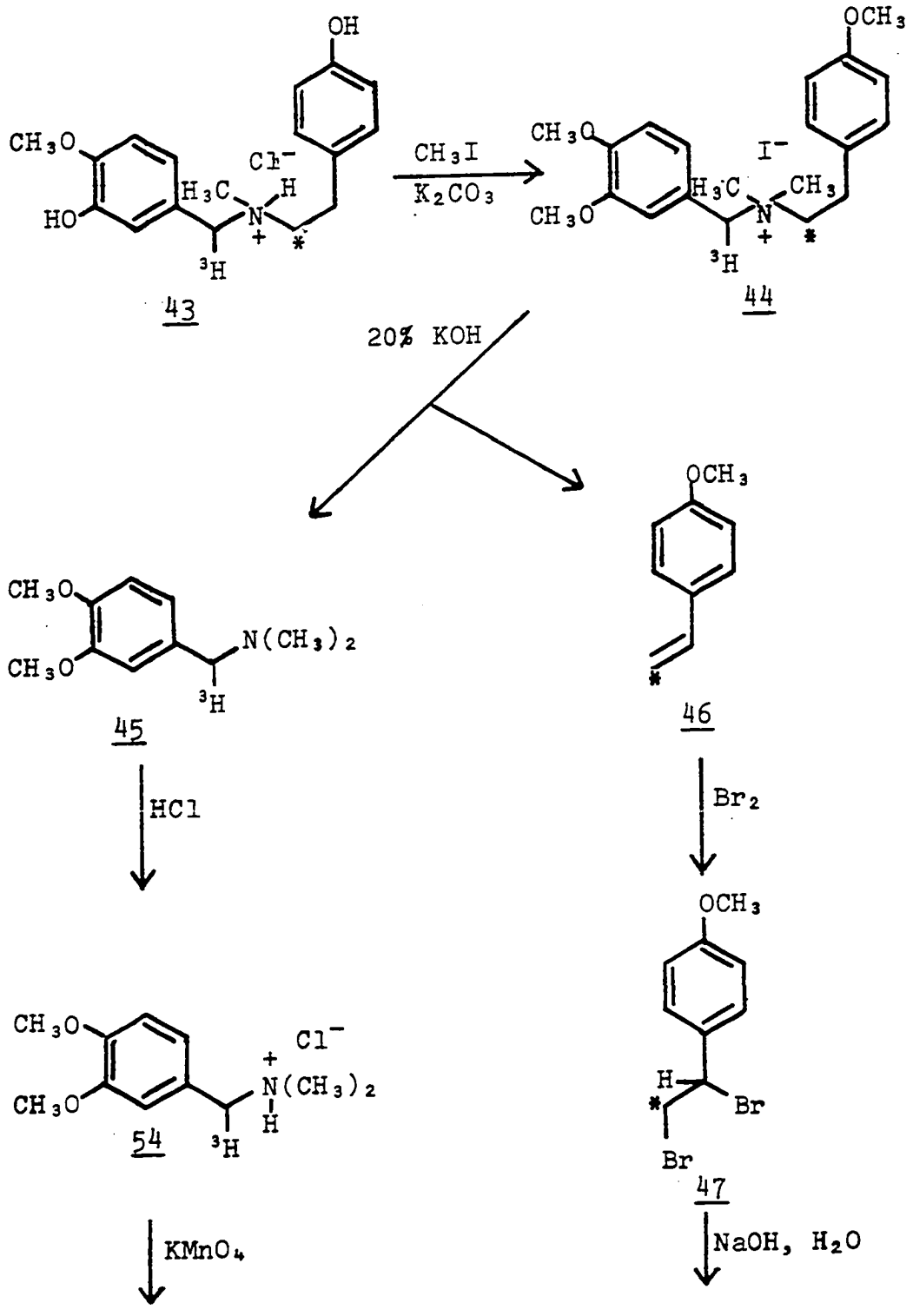


Figure 10. Degradation of O,N-dimethyl[1'-^{3}H, 1-^{14}C]-norbelladine hydrochloride

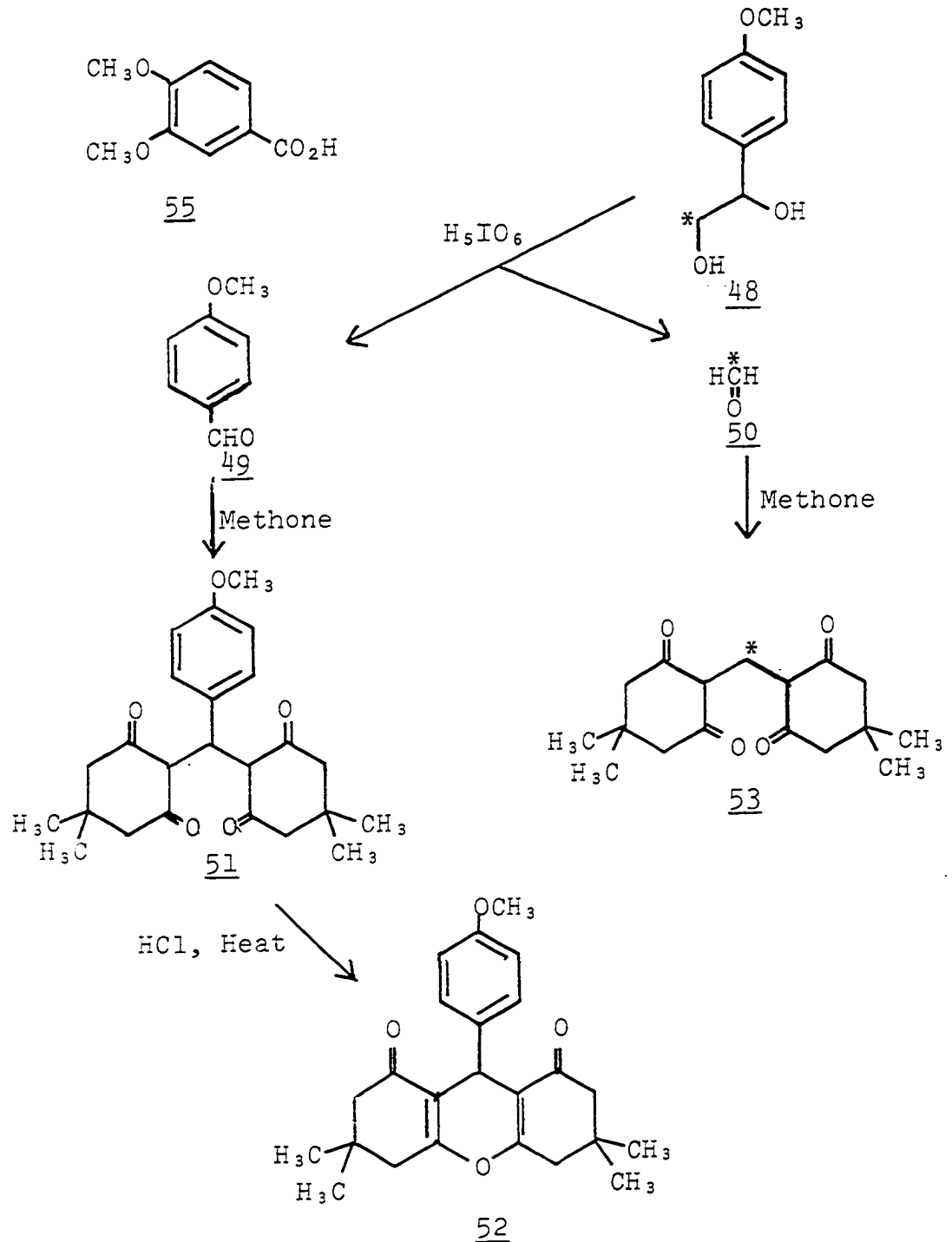


Figure 10. (Continued).

Table 2. Relative molar activities of O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43) and its degradation products

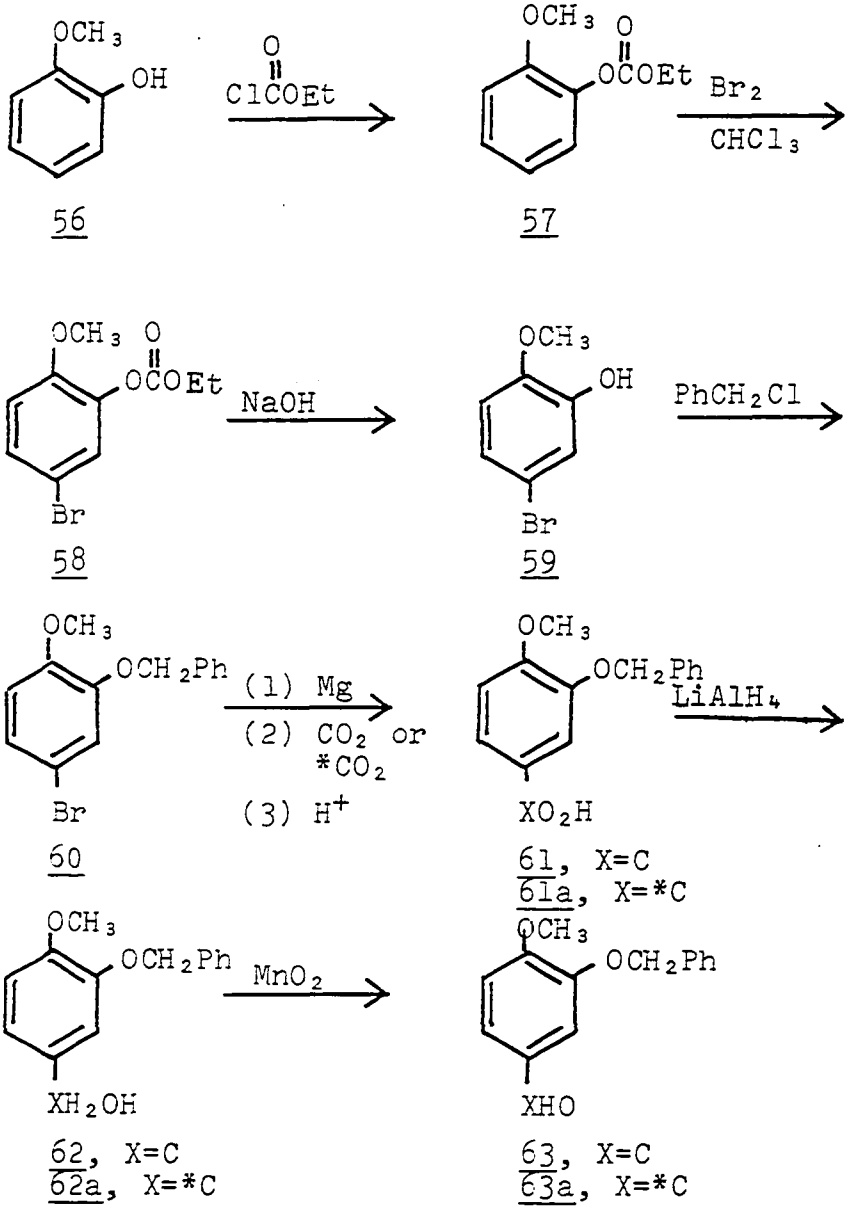
Product	Relative[a] molar activity		Specific Activity[b] µci/mmol	
	^{3}H	^{14}C	^{3}H	^{14}C
O,N-Dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride	1.00	1.00	11.70	3.56
[1'-^{3}H, 1-^{14}C]Belladine methiodide	0.98	0.96	11.50	3.40
[1-^{14}C]1,2-Dibromo-2-(4-methoxyphenyl)-ethane	----	0.94	-----	3.35
[1-^{14}C]1,2-Dihydroxy-2-(4-methoxyphenyl)-ethane	----	0.97	-----	3.44
[^{14}C-Methylene]2,2'-methylenebis(5,5-dimethyl-1,3-cyclohexanedione)	----	0.94	-----	3.35
Tetrahydro-9-(p-methoxyphenyl)-3,3,6,6-tetramethylxanthenedione	----	----	-----	0.00
[1-^{3}H]N,N-Dimethylveratrylamine hydrochloride	1.04	----	12.10	----
Veratric acid	----	----	0.00	----

[a]Relative molar activity = (specific activity of the product) divided by (specific activity of the precursor).

[b]Corrected for dilution factor.

Hydrolysis and decarboxylation of 5-bromoguaiacol carbonate using sodium hydroxide gave 5-bromoguaiacol (59). The next crucial step is to find a protecting group for the phenolic functionality. Benzyl group was found to be the protecting group of choice because the derivative formed is usually crystalline and can be easily removed by hydrogenolysis. Thus, treatment of 5-bromoguaiacol with benzyl chloride gave crystalline 2-benzyloxy-4-bromoanisole (60), which was then converted to the Grignard reagent. Carbonation, using $Ba^{14}CO_3$ as source of $^{14}CO_2$ and generated by a special apparatus designed originally by Dauben and coworkers (35), gave an intermediate which hydrolyzed in the presence of sulfuric acid to yield [^{14}C-carboxyl]3-benzyloxy-4-methoxybenzoic acid (61a). This was reduced to [^{14}C-benzyl alcohol]3-benzyloxy-4-methoxybenzyl alcohol (62a) using lithium aluminum hydride. Conversion of the acid 61 to an ester, followed by reduction, did not appreciably increase the yield. Compound 62a was oxidized to [^{14}C-carbonyl]p-benzyloxyisovanillin (63) in almost quantitative yield using manganese dioxide. The synthesis of [^{14}C-carbonyl]p-benzyloxyisovanillin is illustrated as in Figure 11.

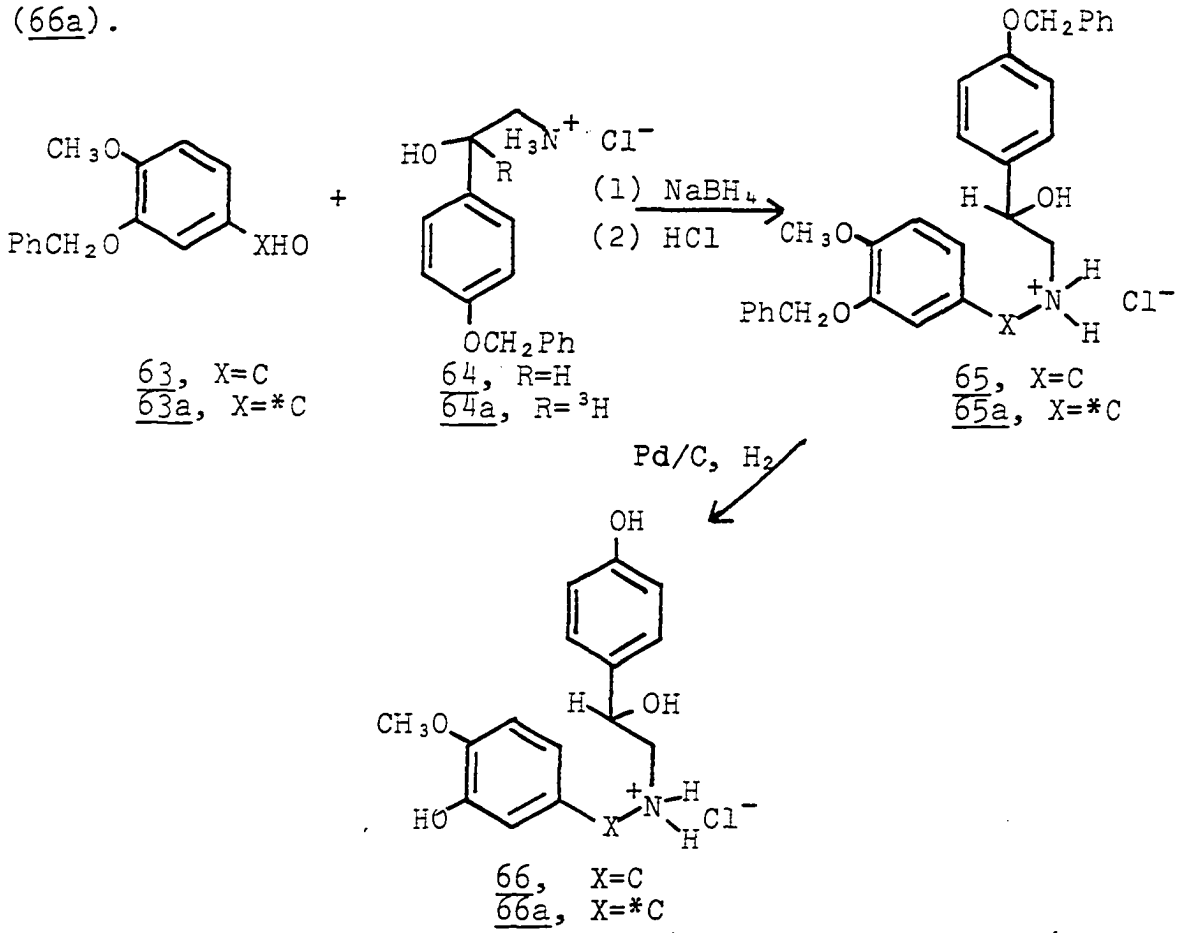
Attempt to make isovanillin by converting 3-benzyloxy-4-methoxybenzoic acid to acid chloride and Rosemund reduction was unsuccessful (36).



56 57 58 59 60 61, X=C 61a, X=*C 62, X=C 62a, X=*C 63, X=C 63a, X=*C

Figure 11. Synthesis of [^{14}C-carbonyl]p-benzyloxy-isovanillin

Reductive condensation of [^{14}C-carbonyl]p-benzyloxy-isovanillin with (±)-p-benzyloxyoctopamine hydrochloride gave the dibenzyloxy derivative 65a, which upon hydrogenolysis yielded 2-hydroxy-O-methyl[1'-^{14}C]norbelladine hydrochloride (66a).



63, X=C
63a, X=*C

64, R=H
64a, R=^{3}H

65, X=C
65a, X=*C

66, X=C
66a, X=*C

Although there are many ways of making octopamine (7, 37,38), not one of them is appropriate for introducing ^{3}H label at the benzylic position. Sodium borohydride has been well known to be a very useful reagent for the reduction of carbonyl compounds in protic solvents (39), and ^{3}H-sodium borohydride can be procured in very high specific activity. Thus, the goal for synthesizing [2-^{3}H]p-benzyloxyoctopamine

hydrochloride was to devise a way to introduce the ^3H label *via* reduction of a carbonyl functionality using ^3H-sodium borohydride. Bromination of p-benzyloxyacetophenone (67) afforded p-benzyloxy-ω-bromoacetophenone (68). Treatment of p-benzyloxy-ω-bromoacetophenone with hexamethylenetetramine gave the mixed salts of p-benzyloxy-ω-aminoacetophenone (69) which, upon reduction with ^3H-sodium borohydride, yielded [2-^3H]p-benzyloxyoctopamine hydrochloride (64a). The synthesis of [2-^3H](±)p-benzyloxyoctopamine hydrochloride is illustrated in Figure 12.

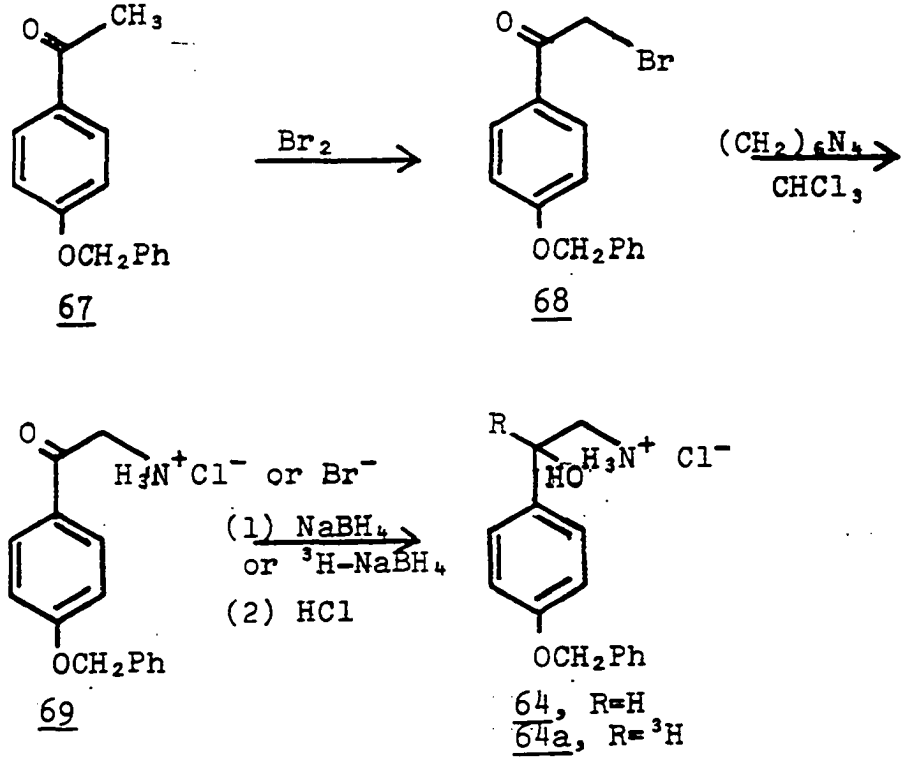
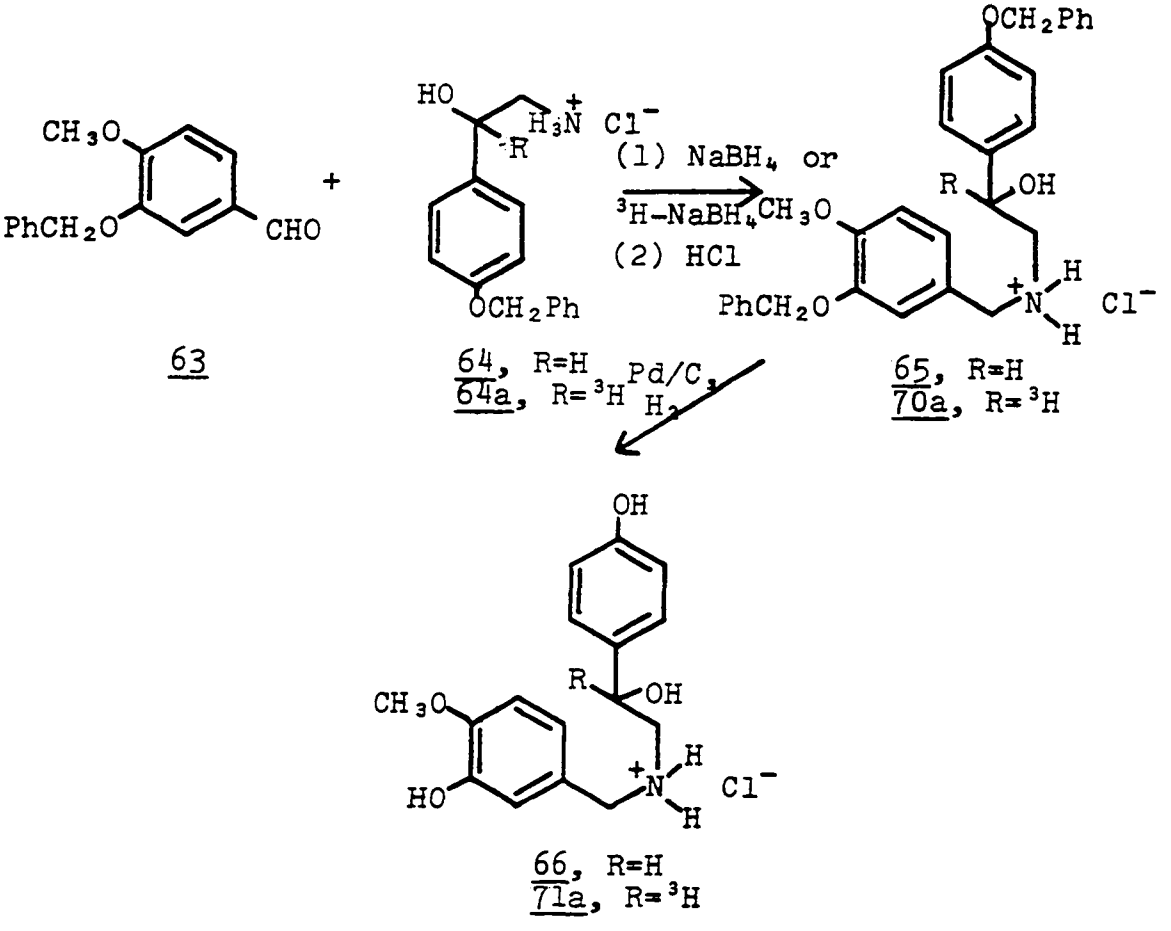
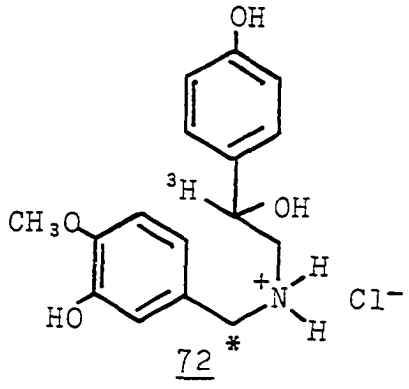


Figure 12. Synthesis of [2-^3H]p-benzyloxyoctopamine hydrochloride

Condensation of [2-^{3}H]p-benzyloxyoctopamine hydrochloride (64a) with p-benzyloxyisovanillin (63) gave a Schiff base which, upon reduction, provided the dibenzyloxy derivative 70a. Hydrogenolysis obtained 2-hydroxy-O-methyl[2-^{3}H]norbelladine hydrochloride (71a).



Mixing 2-hydroxy-O-methyl[1'-^{14}C]norbelladine hydrochloride (66a) and 2-hydroxy-O-methyl[2-^{3}H]norbelladine hydrochloride (71a) yielded 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]-norbelladine hydrochloride (72).



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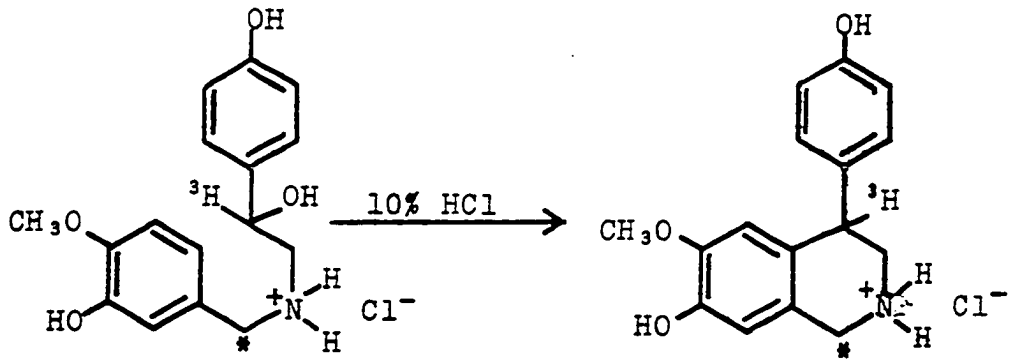
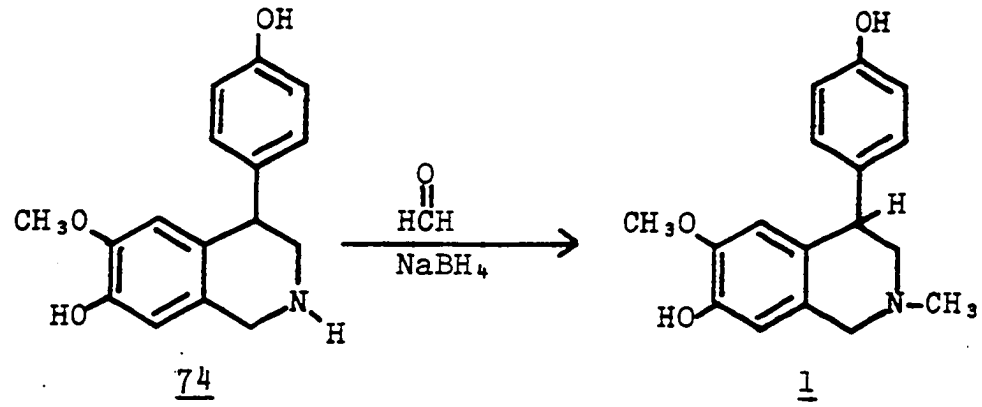
Synthesis of N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride

Shaffer's synthesis (1) of N-demethylcherylline involved the Pictet-Spengler cyclization. Since 2-hydroxy[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) was readily available, experiments were done to determine whether 2-hydroxy-O-methylnorbelladine hydrochloride (72) cyclized easily in the presence of base or acid. It was found that 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) indeed cyclized to N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride (73) in the presence of acid or base in 70% yield. The acid cyclization gave a cleaner product.

Although the hydrochloride 73 gave a different melting point than that reported by Shaffer (1), elemental and spectral



analysis gave satisfactory results. To further verify the structure, N-demethylcherylline (74) was converted to cherylline with excess formaldehyde and sodium borohydride. The cherylline obtained was identical in all respects (except optical activity) to natural cherylline. Further



evidence for the correctness of the structure was that the specific activity of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) was retained in N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride (73).

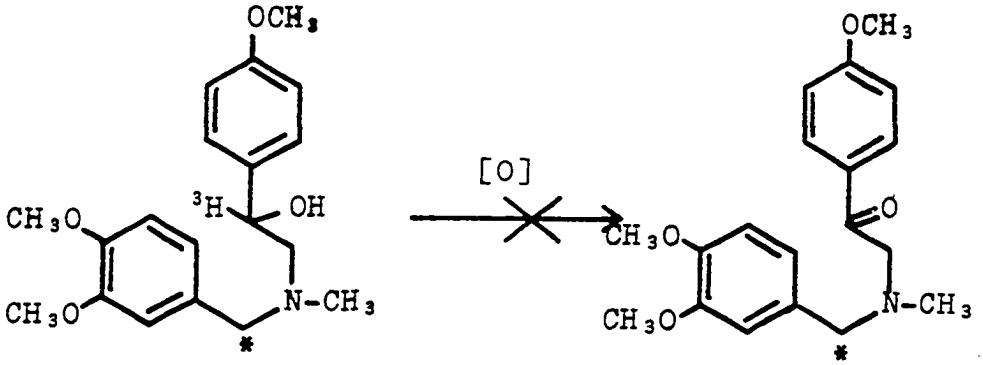
Up to now, Schwartz's synthesis (7) of cherylline is the synthesis of choice because it involved fewer steps and gave a high yield. This new route for the total synthesis of cherylline comparable in yield to Schwartz's synthesis has been accomplished. A 65% yield, starting from 2-hydroxy-O-methylnorbelladine hydrochloride, was realized. The advantage of this new synthetic approach, especially for a large scale reaction, is the absence of the time consuming operation of N-formylation (8 hours) and reduction with excess lithium aluminum hydride in dry 1,2-dimethoxyethane (50 hours) under dry atmospheric conditions. The synthesis can be accomplished in 10 hours with no special care in reaction conditions.

Degradation of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride

In order to identify the exact position of the ^{3}H and ^{14}C labels in 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72), a chemical degradation had to be devised.

One obvious possibility for determining the position of the ^{3}H label would be the oxidation of 2-hydroxy-[2-^{3}H,

1'-^{14}C]belladine (75) to a compound 76 devoid of ^{3}H activity. Attempts to oxidize this benzylic alcohol functionality using several oxidizing agents only resulted in unidentifiable degradation products.



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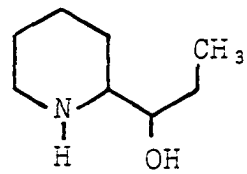
A chemical degradation procedure similar to that of O,N-dimethylnorbelladine hydrochloride was accomplished. Treatment of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) with excess diazomethane followed by reductive methylation and addition of an excess methyl iodide afforded 2-hydroxy [2-^{3}H, 1'-^{14}C]belladine methiodide (71) with no loss in specific activity. Hofmann degradation of 2-hydroxy [2-^{3}H, 1'-^{14}C]belladine methiodide using freshly prepared silver oxide gave [1-^{14}C]N,N-dimethylveratrylamine (79) and [2-^{3}H]1,2-dihydroxy-2-(4-methoxyphenyl)ethane (81). Hofman degradation utilizing 20% KOH similar to that for belladine methiodide (44) was found to be unsatisfactory,

because the intermediate p-methoxystyrene epoxide (80) polymerized in the presence of excess strong base. The mechanism for the Hofmann degradation step was precedented by the case of dl-conhydrine (86) (40). The degradation



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of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) is illustrated in Figure 13 and results summarized in Table 3.

To further confirm the position of the ^{3}H label and to show that no ^{3}H was exchanged during the hydrogenolysis step, a mass spectral study of 2-hydroxy-O-methylnorbelladine (6) and 2-hydroxy-O-methyl[2-^{2}H]norbelladine (89) was undertaken. 2-Hydroxy-O-methyl[2-^{2}H]norbelladine was synthesized under conditions similar to those used in the preparation of 2-hydroxy-O-methyl[2-^{3}H]norbelladine (71a). The mass spectrum of 2-hydroxy-O-methylnorbelladine (6) gave a peak at m/e 271 ($M^{+}-H_2O$) and 2-hydroxy-O-methyl[2-^{2}H]norbelladine (89) gave a peak at m/e 272 ($M^{+}-H_2O$). The intensity ratio

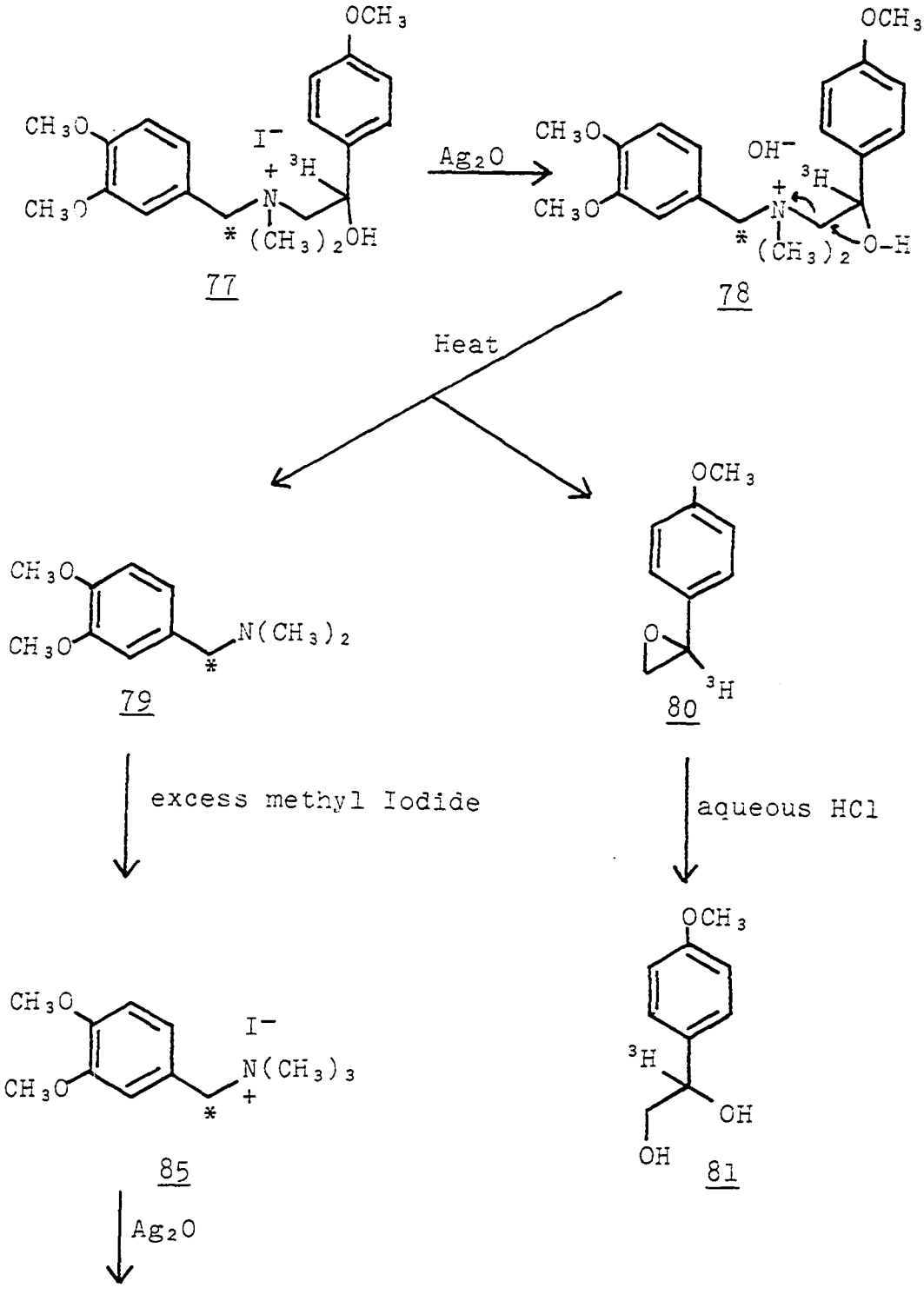


Figure 13. Degradation of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]-norbelladine hydrochloride

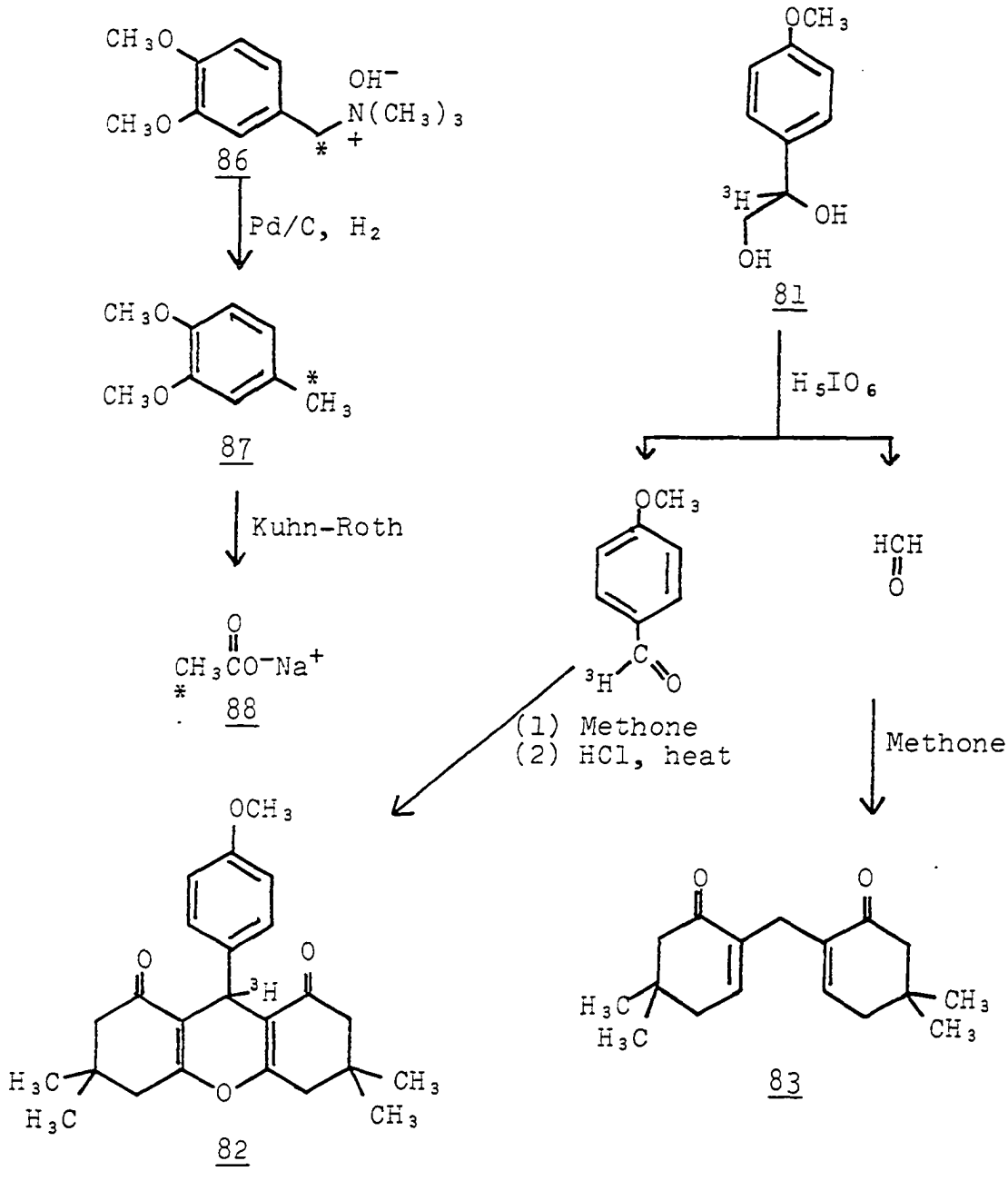


Figure 13. (Continued).

Table 3. Relative molar activities of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) and its degradation products

Product	Relative molar activity		Specific activity[a] μci/mmol	
	^{3}H	^{14}C	^{3}H	^{14}C
2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride	1.00	1.00	134	83
2-Hydroxy-[2-^{3}H, 1'-^{14}C]belladine methiodide	0.99	1.05	133	87
[2-^{3}H]1,2-Dihydroxy-2-(4-methoxyphenyl)-ethane	1.00	----	135	--
2,2'-Methylenebis(5,5-dimethyl-1,3-cyclohexanedione)	0.00	----	---	--
[9-^{3}H]Tetrahydro-9-(p-methoxyphenyl)-3,3,6,6-tetramethylxanthenedione	----	1.00	---	133.5
[1-^{14}C]N,N-Dimethylveratrylamine methiodide	----	1.05	---	87
[^{14}C-Methyl]3,4-dimethoxytoluene	----	1.06	---	88
[^{14}C-Methyl] sodium acetate	----	1.04	---	86.5

[a]Corrected for dilution factor.

between m/e 272/271 and m/e 242/241 for 2-hydroxy-O-methyl-norbelladine is the same as that for the intensity ratio between m/e 273/272 and m/e 243/242 for 2-hydroxy-O-methyl-[2-^{2}H]norbelladine, thus no deuterium was exchanged during hydrogenolysis. The major fragmentation pattern of both compounds can be summarized as in Figure 14 and in Table 4 is presented the masses and relative intensities of the major ion. It is interesting to note that the first step in the fragmentation pattern is the loss of a water molecule by cyclization to N-demethylcherylline (74). This pattern resembles chemical and biosynthetic processes (see later discussion).

Biosynthetic Investigations

Feeding experiments of doubly-labeled norbelladine-type alkaloids into Crinum powellii

The feeding experiment was carried out using Crinum powellii as the plant host. This plant was chosen because of its availability and it is known to contain the alkaloids cherylline, O-methylnorbelladine, lycorine and ambelline in isolable amounts, enough for counting to constant activity without dilution.

All radioactive precursors were recrystallized as the hydrochloride salt to constant activity before they were administered into Crinum powellii by injection into bulb.

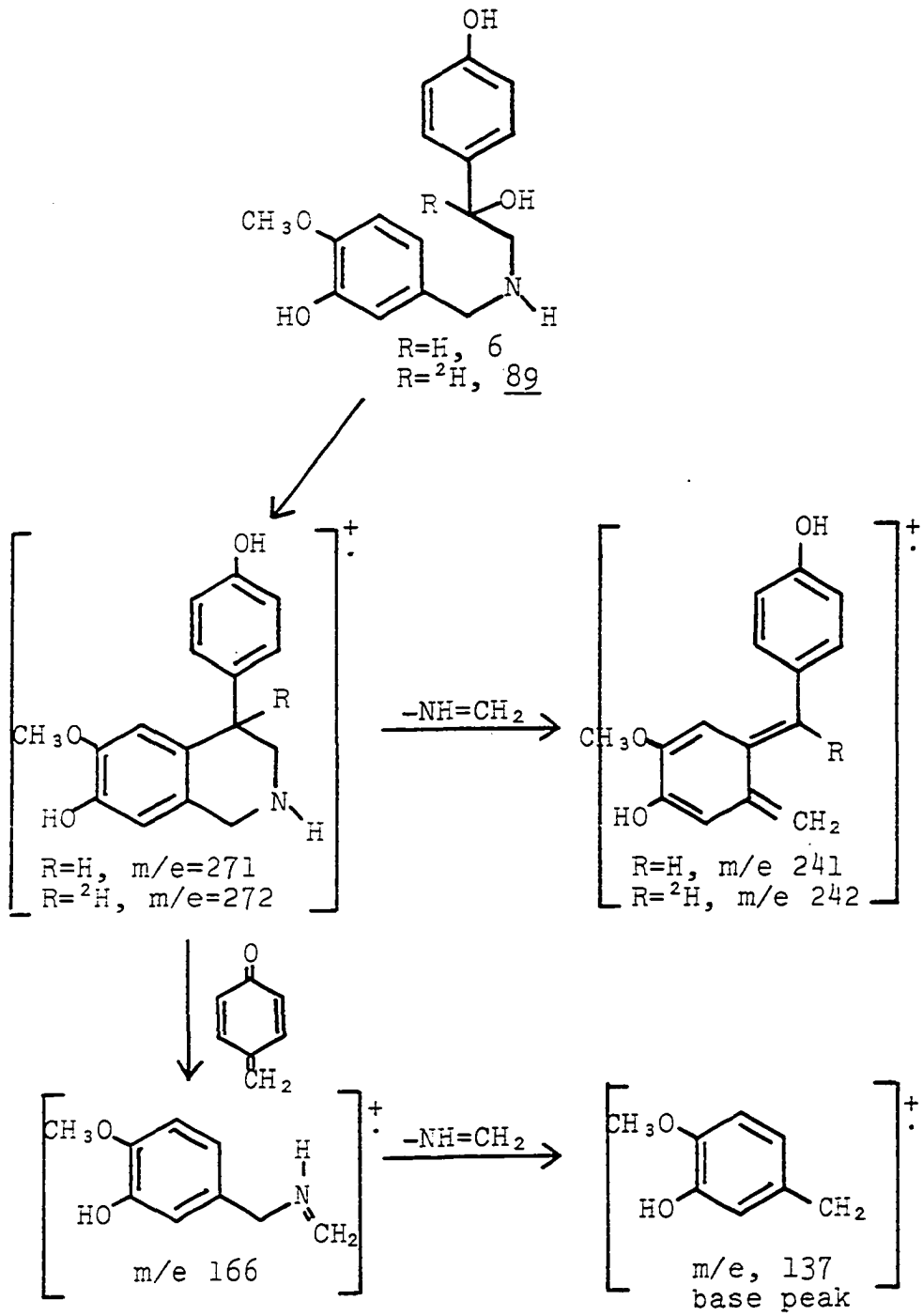


Figure 14. Mass spectral ions from 2-hydroxy-O-methylnorbelladine and 2-hydroxy-O-methyl[2-^{3}H]norbelladine

Table 4. Mass spectra of 2-hydroxy-O-methylnorbelladine (16) and 2-hydroxy-O-methyl[2-^{3}H]norbelladine (89)

2-Hydroxy-O-methylnorbelladine		2-Hydroxy-O-methyl[2-^{3}H]norbelladine	
m/e (70 eV)	Relative Intensity	m/e (70 eV)	Relative Intensity
272	3	273	1
271 (M^+-H_2O)	12	272 (M^+-H_2O)	4
270	3	271	1
242	20	243	9
241	18	242	8
225	15	226	6
212	8	212	5
211	8	211	4
210	10	210	2
166	85	166	31
153	48	153	19
152	38	152	14
138	35	138	11
137 (base peak)	100	137 (base peak)	100
136	20	136	10
122	50	122	10

The plants were allowed to grow inside a laboratory hood under artificial light. The bulbs were processed in the usual manner and the alkaloids separated and purified by preparative thin layer chromatography and crystallization.

The specific activities of radioactive precursors are given in Table 5, with the incorporations determined for alkaloids isolated from each feeding summarized in Tables 6 to 11. Per cent incorporations for ^{14}C and ^{3}H are entered separately; an intact incorporation of a precursor would be indicated by identical incorporation for each label.

Interpretation of biosynthetic results

Three week feeding of O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42) Shaffer (1) has reported that singly-labeled O-methyl[1'-^{3}H]norbelladine was converted efficiently into cherylline and lycorine. In order to prove conclusively that O-methylnorbelladine did not cleave at bonds a and/or b before conversion into the precursors of cherylline, a

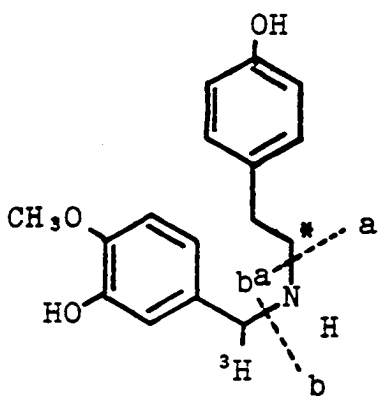


Table 5. Precursors for feeding experiments

Compound	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Isotope	$^{3}H/^{14}C$ Ratio
O-Methyl[1'-^{3}H, 1-^{14}C]-norbelladine hydrochloride (42) (Three week feeding)	7.54	39.6 12.5	298 95.3	^{3}H ^{14}C	3.12
O,N-Dimethyl[1'-^{3}H, 1-^{14}C]-norbelladine hydrochloride (43) (Three week feeding)	7.68	36.3 11.0	278 84.5	^{3}H ^{14}C	3.30
2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) (Three week feeding)	3.78	56.4 32.6	213 123	^{3}H ^{14}C	1.73
N-Demethyl[4-^{3}H, 1-^{14}C]-cherylline hydrochloride (73) (Three week feeding)	4.02	66.7 36.8	268 147.5	^{3}H ^{14}C	1.82
2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) (One day feeding)	4.56	56.4 32.6	257 149	^{3}H ^{14}C	1.73
O-Methyl[1'-^{3}H, 1-^{14}C]-norbelladine hydrochloride (42) (One day feeding)	3.70	57.5 7.4	213 27.3	^{3}H ^{14}C	7.80

Table 6. Summary of alkaloid incorporation isolated from a three week feeding of O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42)

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	^{3}H/^{14}C Ratio
Cherylline hydrochloride	65	2.80×10^{-6} 0.94×10^{-6}	1.82×10^{-4} 6.12×10^{-5}	0.061 0.064	^{3}H ^{14}C	2.96
O-Methylnorbelladine hydrochloride	6	1.64×10^{-3} 0.51×10^{-3}	9.85×10^{-3} 3.06×10^{-3}	3.30 3.32	^{3}H ^{14}C	3.20
Lycorine hydrochloride	1111	9.4×10^{-6} 2.88×10^{-6}	1.04×10^{-2} 3.20×10^{-2}	3.51 3.35	^{3}H ^{14}C	3.26
Ambelline	85	9.65×10^{-7} 3.24×10^{-7}	8.2×10^{-5} 2.76×10^{-5}	0.0276 0.0289	^{3}H ^{14}C	2.98

Table 7. Summary of alkaloid incorporation isolated from a three week feeding of O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43)

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Cherylline hydrochloride	55	2.21×10^{-6} 0.628×10^{-6}	1.22×10^{-4} 3.46×10^{-5}	0.0436 0.0410	^{3}H ^{14}C	3.5
O-Methylnorbelladine hydrochloride	5	2.77×10^{-6} 0.805×10^{-5}	1.38×10^{-4} 4.03×10^{-5}	0.0495 0.0476	^{3}H ^{14}C	3.42
Lycorine hydrochloride	1050	0	0	0	---	----
Ambelline	70	0	0	0	---	----

Table 8. Summary of alkaloid incorporation isolated from a three week feeding of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72)

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Cherylline hydrochloride	60	3.62×10^{-6} 2.10×10^{-6}	2.17×10^{-4} 1.26×10^{-4}	0.102 0.102	^{3}H ^{14}C	1.72
O-Methylnorbelladine hydrochloride	8	2.45×10^{-4} 2.17×10^{-6}	1.94×10^{-3} 1.75×10^{-5}	0.910 0.0142	^{3}H ^{14}C	1100
Lycorine hydrochloride	965	0	0	0	---	----
Ambelline	79	0	0	0	---	----

Table 9. Summary of alkaloid incorporation isolated from a three week feeding of N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride (73)

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	^{3}H/^{14}C Ratio
Cherylline hydrochloride	60	1.26×10^{-4} 0.70×10^{-4}	8.80×10^{-3} 4.2×10^{-3}	2.82 2.85	^{3}H ^{14}C	1.80
O-Methylnorbelladine hydrochloride	5	0	0	0	---	----
Lycorine hydrochloride	1400	0	0	0	---	----
Ambelline	80	0	0	0	---	----

Table 10. Summary of alkaloid incorporation isolated from a one day feeding of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72)

Alkaloid	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	$^{3}H/^{14}C$ Ratio
Cherylline hydrochloride	30	8.70×10^{-5} 5.04×10^{-5}	2.60×10^{-3} 1.51×10^{-3}	1.01 1.01	^{3}H ^{14}C	1.73
Lycorine hydrochloride	700	0	0	0	---	----
Ambelline	45	0	0	0	---	----
O-Methylnorbelladine hydrochloride[a]	25	7.33×10^{-6} 4.50×10^{-6}	--- ---	--- ---	^{3}H ^{14}C	1.64
2-Hydroxy-O-methyl-norbelladine hydrochloride[b]	15	2.57×10^{-6} 1.49×10^{-6}	--- ---	--- ---	^{3}H ^{14}C	1.71
N-Demethylcherylline hydrochloride[c]	21	1.11×10^{-3} 0.66×10^{-3}	--- ---	--- ---	^{3}H ^{14}C	1.68

[a]Isolated by dilution with 23 mg of inactive compound.

[b]Isolated by dilution with 21.4 mg of inactive compound.

[c]Isolated by dilution of 20.0 mg of inactive compound.

Table 11. Summary of alkaloid incorporation isolated from a one day feeding of O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (73)

	Quantity (mg)	Specific activity (μci/mg)	Total activity (μci)	Per cent incorporation	Isotope	^{3}H/^{14}C Ratio
Cherylline hydrochloride	72	0	0	0	---	----
O-Methylnorbelladine hydrochloride	21	2.48×10^{-3} 3.27×10^{-4}	5.20×10^{-2} 5.86×10^{-3}	24.4 25.2	^{3}H ^{14}C	7.6
Lycorine hydrochloride	1123	0	0	0	---	----
Ambelline	95	0	0	0	---	----
2-Hydroxy-O-methyl-norbelladine hydrochloride[a]	16	0	0	0	---	----
N-Demethylcherylline hydrochloride[b]	19	0	0	0	---	----

[a]Isolated by dilution with 20 mg of inactive compound.

[b]Isolated by dilution with 20 mg of inactive compound.

doubly-labeled O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride was synthesized and fed. The results showed that O-methyl [1'-^{3}H, 1-^{14}C]norbelladine hydrochloride was incorporated intact into cherylline, lycorine and ambelline, which is in accord with previous work (1,18). Also, O-methylnorbelladine was reisolated in which the ^{3}H to ^{13}C ratio remains constant when compared to that of the precursor. The per cent incorporations are not comparable to the Shaffer's results because different Crinum were used and different time periods were involved. This feeding also served as reference and control for subsequent feeding experiments.

Three week feeding of O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43) Using the technique of isotopic dilution, Barton and coworkers (20) have reported the presence of O,N-dimethylnorbelladine in the "King Alfred" Daffodil. Thus, a logical route for cherylline biosynthesis would be the N-methylation of O-methylnorbelladine to give O,N-dimethylnorbelladine, which could cyclize to give cherylline. N-Methylation has been quite well known in biological processes, including Amaryllidaceae alkaloids. O,N-Dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride was found to be incorporated intact into cherylline, though less efficiently, than O-methylnorbelladine. It also undergoes N-demethylation to O-methylnorbelladine. This is the first

conclusive proof that N-demethylation can occur in Amaryllidaceae alkaloids. One may attribute the lower incorporation of O,N-dimethylnorbelladine, when compared to O-methylnorbelladine, to the fact that O,N-dimethylnorbelladine is involved in two competing reactions, one to make cherylline and the other to undergo N-demethylation to make O-methylnorbelladine.

If O-methylnorbelladine was being formed efficiently from O,N-dimethylnorbelladine, one should expect incorporation into lycorine and ambelline. No evidence for this incorporation was detected in lycorine and ambelline, thus demonstrating that N-demethylation is not a rapid process.

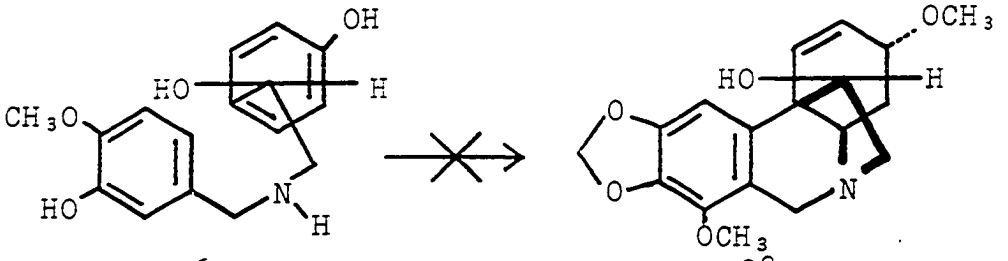
Three week feeding of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]-norbelladine hydrochloride (72) Benzylic hydroxylation is quite common in biological systems, for example, the conversion of tyramine to octopamine (41). In the Amaryllidaceae alkaloid systems, benzylic hydroxylation usually occurs at the late stage of the biosynthesis, for example, the conversion of haemanthamine (20) to heamanthidine (21). Thus, it is possible for O-methylnorbelladine to hydroxylate at the benzylic position, then cyclize to N-demethylcherylline before N-methylation to form cherylline. In Schwartz's (7) biogenetic-type synthesis of cherylline, the benzylic alcohol functionality is necessary for cyclization. 2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride

was found to be incorporated intact into cherylline. O-methylnorbelladine, as isolated was radioactive, but as a degradation product because the ^{3}H to ^{14}C ratio was not constant. Degradation of 2-hydroxy-O-methylnorbelladine apparently is a more efficient process when compared to cherylline synthesis. This could account for the observation that the per cent incorporation of 2-hydroxy-O-methyl-norbelladine was not appreciably higher compared to O-methyl-norbelladine and O,N-dimethylnorbelladine, although it involved one less step in going to cherylline.

Valuable information could have been obtained by the degradation of the isolated O-methylnorbelladine in order to pinpoint the position of ^{14}C and ^{3}H label. This could shed light on how 2-hydroxy-O-methylnorbelladine was degraded in the plant. Unfortunately, not enough compound or radio-activity was present to do the degradation.

[1, 1'-^{14}C]norbelladine (18) and crinine (32) have been found to be incorporated *in vivo* into ambelline, indicating that 11-hydroxylation may occur after the formation of the crinine ring system. The present results support this since octopamine and p-hydroxyphenylserine were shown not to be incorporated into either the C_6-C_1 or C_6-C_2 units of 6-hydroxycrinamine (29). However, it is conceivable that ambelline can be biosynthesized by another pathway. 2-Hydroxy-O-methylnorbelladine could undergo a phenyl-phenyl

coupling to give ambelline. In other words, the 2-hydroxy functionality of the precursor could provide the 11-hydroxy of ambelline. Since no incorporation was detected in ambelline, this indicated that 11-hydroxylation occurred



6 28

after the formation of the crinine ring. This feeding also showed that reduction of the precursor to O-methylnorbelladine is not an efficient process, since no radioactivity was detected in either ambelline or lycorine.

Three week feeding of N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride (73) Shaffer (1) reported that N-demethylcherylline hydrochloride was not incorporated intact into cherylline, although degraded portions of the molecule were evident in cherylline and O-methylnorbelladine. Since N-methylation is a very common process in biological systems, the Shaffer's result is difficult to reconcile with existing facts. N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride can easily be prepared from the available 2-hydroxy-O-methyl-[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride. It seemed

worthwhile to repeat the feeding experiment. The present feeding experiment showed that the precursor was incorporated intact into cherylline at an appreciably higher percentage when compared to that of O-methylnorbelladine, O,N-dimethylnorbelladine, 2-hydroxy-O-methylnorbelladine. Also, no degraded portions of N-demethylcherylline were evident in O-methylnorbelladine, lycorine and ambelline. This demonstrates that ring opening of N-demethylcherylline to O-methylnorbelladine does not occur (or is not a rapid process) in the plant. This result is more convincing than the Shaffer's result because of relatively high per cent incorporation and more reasonable results based on existing facts.

Summary of biosynthetic results from three week feeding From the three weeks feeding experiment results, two routes for the biosynthesis of cherylline can be postulated. One route starting from O-methylnorbelladine (12), hydroxylation to 2-hydroxy-O-methylnorbelladine (6), cyclization to N-demethylcherylline (74) and N-methylation to cherylline (1). The second possible route to cherylline starts with O-methylnorbelladine (12), followed by N-methylation to O,N-dimethylnorbelladine (31) and hydroxylation to 2-hydroxy-O,N-dimethylnorbelladine (33). The two proposed pathways to the biosynthesis of cherylline are illustrated in Figure 15. No feeding experiment was done

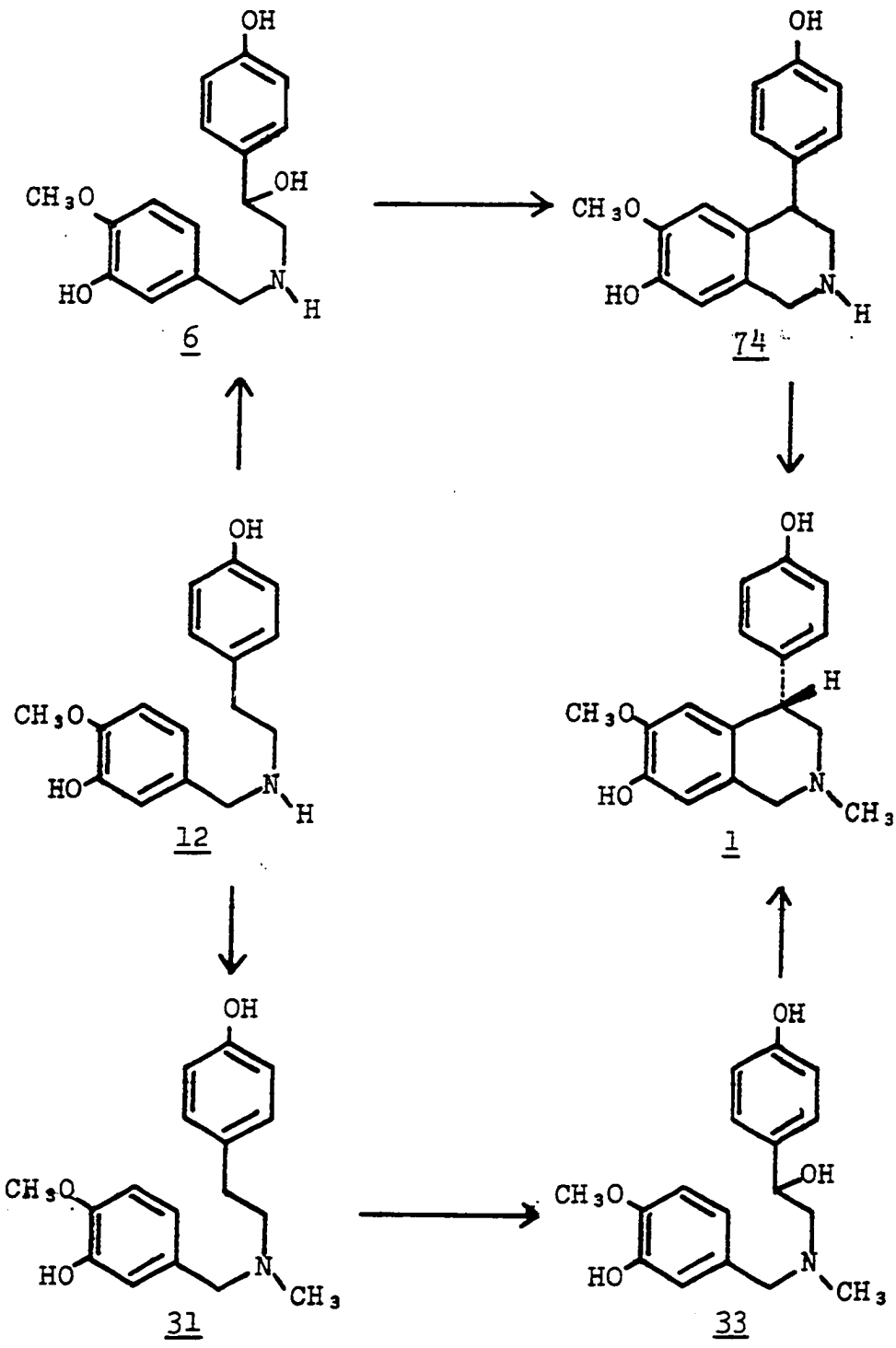


Figure 15. Proposed biosynthetic pathways for cherylline

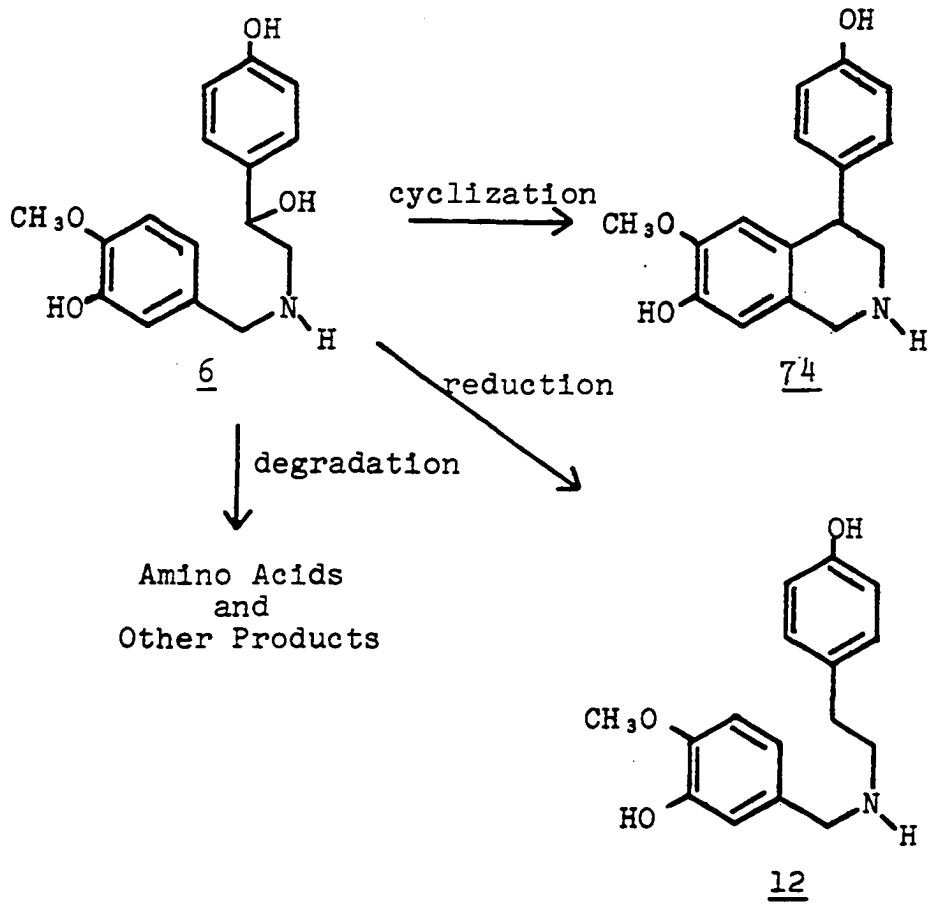
using 2-hydroxy-O,N-dimethylnorbelladine (33) because this compound, as reported by Schwartz and Scott (7), cyclized instantaneously in the presence of acid. Although, in our case, it is possible to synthesize 2-hydroxy-O,N-dimethylnorbelladine from reductive methylation of the available 2-hydroxy-O-methylnorbelladine, all the precursors were fed into the plant as the hydrochloride salt, purification of 2-hydroxy-O,N-dimethylnorbelladine would be a difficult task. Due to the ease of cyclization, it would be difficult to distinguish between chemical and enzyme-induced cyclization. No experimental evidence is available to infer which of the two possible routes is the major pathway.

One day feeding experiment of 2-hydroxy-O-methylnorbelladine hydrochloride (72) The tracer studies described so far demonstrate that the plant is capable of effecting certain well-defined chemical transformations. However, they do not prove conclusively that these processes are part of the organism's normal metabolism. It is quite possible that injection of compound into plant would induce biological reactions that normally do not occur. This is especially true when the precursors fed have not been shown to exist in the plant.

A clearer view of biosynthetic sequences, may, however, be obtained by intermediate trapping experiments. Thus,

if the hitherto unknown precursors, 2-hydroxy-O-methylnorbelladine and N-demethylcherylline can be reisolated, and the radioactivities remain intact, then one could probably say that they are intermediates in the plant metabolism processes.

A one day feeding study of 2-hydroxy-O-methylnorbelladine hydrochloride showed that most of the radioactivity in the phenolic fraction was present as N-demethylcherylline, thus cyclization is a rapid process. Since it is known that 2-hydroxy-O-methylnorbelladine did not cyclize readily in the presence of acid unless under reflux conditions, the cyclization step most probably is enzyme-induced and not a chemical process. That intact incorporation was also evident in the reisolated 2-hydroxy-O-methylnorbelladine and O-methylnorbelladine, that intact incorporation was found in O-methylnorbelladine is contradictory to the result found in the three week feeding. One may attribute this to the fact that 2-hydroxy-O-methylnorbelladine is undergoing three separate competing reactions, with degradation and cyclization steps predominating. In a three week feeding the amino acids have ample time to synthesize O-methylnorbelladine, thus masking the radioactivity of the O-methylnorbelladine obtained from the reduction step. This is the first evidence of reduction



in Amaryllidaeae alkaloid, though it is not a rapid process.

It was also interesting to note that cherylline in the one day feeding was incorporated at ten times greater efficiency than in a three week feeding. Thus, cherylline could be a very reactive metabolite, which metabolizes to other compounds in an unknown pathway.

<u>One day feeding experiment of O-methyl[1'-^{3}H, 1-^{14}C]-norbelladine hydrochloride (42)</u> A one day feeding experiment of O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride showed no incorporation into cherylline. Thus, 2-hydroxy-O-methylnorbelladine is a better precursor to cherylline when compared to O-methylnorbelladine. This

result was not evident in the three week feeding experiment. Lycorine and ambelline were also found to be inactive. 2-Hydroxy-O-methylnorbelladine and N-demethylcherylline (isolated by dilution) were also found to be inactive. The O-methylnorbelladine which was reisolated retained about 25% of the radioactivity.

SUMMARY

O-Methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride, O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride, 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride, and N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride were synthesized to study the biosynthesis of cherylline and other selected Amaryllidaceae alkaloids. Both synthetic and chemical degradation procedures for the precursors are discussed. An improved synthesis for cherylline was found.

All synthesized norbelladine-type precursors were incorporated intact into cherylline. Two routes for the biosynthesis of cherylline are postulated. The first route proceeds from O-methylnorbelladine to 2-hydroxy-O-methyl-norbelladine to N-demethylcherylline to cherylline. The second possible route to cherylline starts with O-methyl-norbelladine and proceeds through O,N-dimethylnorbelladine and 2-hydroxy-O,N-dimethylnorbelladine.

2-Hydroxy-O-methylnorbelladine was found to undergo three competing reactions: degradation to amino acids and other products, cyclization to N-demethylcherylline, and reduction to O-methylnorbelladine. 2-Hydroxy-O-methyl-norbelladine gave about ten times greater incorporation into cherylline in a one day period when compared to a three week feeding. Isotopic dilution studies of previously

unknown norbelladine-type alkaloid 2-hydroxy-O-methylnorbelladine showed that after one day most of the radioactivity in the phenolic alkaloid fraction was present intact as N-demethylcherylline. 2-Hydroxy-O-methylnorbelladine did not serve as precursor to ambelline, thus confirming that 11-hydroxylation occurred after the crinine ring was formed. It also was not a precursor of lycorine.

O-Methylnorbelladine, as expected, was incorporated into lycorine and ambelline. A one day feeding of O-methylnorbelladine showed no incorporation into cherylline, lycorine and ambelline. O,N-Dimethylnorbelladine and N-demethylcherylline did not serve as precursor for lycorine or ambelline.

The interrelationship of all biosynthetic results are discussed and related to previously reported results.

EXPERIMENTAL

Instrumentation

The infrared spectra utilized a Beckman Model IR-12 or IR-18A recording spectrometer. Samples were observed either in chloroform solution or as a potassium bromide pellet. The proton magnetic resonance spectra were run in the indicated solvent on either a Hitachi Perkin-Elmer Model R-20B or Varian Model A-60 operating at 60 MHz. Mass spectra were recorded on an Atlas CH-4 (low resolution) mass spectrometer. Melting points were observed on a Köfler hot stage apparatus and are corrected. The elemental analyses were carried out by Ilse Beetz, Microanalytical Laboratory, Kronach, West Germany.

Measurement of the Radioactivity of Compounds

All measurements of the radioactivity of the compounds were obtained with a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3002) operating at ambient temperature. The count from this instrument had a maximum error of ±3%.

Samples of high specific activity were dissolved in absolute methanol and triplicate aliquots of 1.0 ml each were counted ten times in 15 ml of toluene: POPOP, PPO [4.9 g of 2,5-diphenyloxazole (PPO) (Packard) and 0.1 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) (Packard) dissolved in sufficient dry toluene to make 1 l. of solution] and the

average value used. Samples of low specific activity were dissolved directly into 15 ml of Bray's solution (42) [60 g of naphthalene, 4 g of 2,5-diphenyloxazole (Packard), 100 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene (Packard) in 20 ml of ethylene glycol, 100 ml of methanol and sufficient dry peroxide-free dioxane to make 1 l. of solution]. Each sample was counted ten times and the average value used. ^{3}H-toluene and ^{14}C-toluene were used as internal standards by injecting 50 µl of the standard directly into the counting solution. For a doubly-labeled sample, the two channels of the scintillation counter were adjusted in such a way as to give the highest detection efficiency with complete discrimination of the tritium counts in the carbon-14 channel. The overlap of the carbon-14 counts in the tritium channel varied from 43 to 46% in Bray's solution and 20 to 22% in toluene: POPOP, PPO. The tritium counts were corrected by subtracting the overlapping carbon-14 counts. The tritium counting efficiency varied from 22 to 25% in Bray's solution and 32 to 36% in toluene: POPOP, PPO, while the carbon-14 counting efficiency varied from 43 to 46% in Bray's solution and 44 to 49% in toluene: POPOP, PPO.

Compounds were recrystallized and counted until constant activity was obtained. The per cent incorporation was calculated as 100 x total activity of isolated pure

alkaloid) divided by (total activity fed). The alkaloids were identified by their TLC migration in at least two solvent systems, and by melting point, mmp, VPC and comparison of their ir spectra with known reference spectra.

Preparative Thin Layer Chromatography

Glass plates (20 x 20 cm), coated with 0.5 mm silica gel (Merck, PF 254), were activated at least for 24 hours in the steam oven. The alkaloids were detected using UV light, and were scraped-off and soaked in either 10% hydrochloric acid or methanol overnight. To recover the alkaloids from the silica gel, the aqueous acidic slurry was made basic with ammonium hydroxide and extracted with chloroform. The methanol slurry was filtered, the solvent was removed in vacuo, and the product was redissolved in ethyl acetate or chloroform, filtered, concentrated, and weighed.

Radioactive Synthesis of O-Methyl[1'-^{3}H, 1-^{14}C]norbelladine Hydrochloride

The synthesis of O-methylnorbelladine followed essentially that of Barton and coworkers (20) with slight modifications.

O-methyl[1'-^{3}H]norbelladine hydrochloride (35)

To 173.6 mg (1 mmol) of tyramine hydrochloride (34), 152 mg (1 mmol) of isovanillin (5), and 100 mg of sodium

bicarbonate was added 12 ml of isopropyl alcohol. The mixture was stirred and refluxed for about 45 minutes under nitrogen atmosphere and then cooled in an ice bath. ^{3}H-Sodium borohydride (12.6 mg, 0.332 mmol, 100 mci, New England Nuclear Corp., Boston, Mass.) was added. The reaction mixture was stirred for 15 minutes, then 15.4 mg (0.405 mmol) of non-radioactive sodium borohydride was added. The mixture was stirred at room temperature for 2.5 hours. The isopropyl alcohol was removed *in vacuo*. The residue was dissolved in 10 ml of water, acidified with concentrated hydrochloric acid and neutralized with solid sodium carbonate. Extraction with ethyl acetate gave 221 mg of yellow oil. The oil was dissolved in a minimum amount of absolute ethanolic hydrogen chloride to give 201 mg of the hydrochloride salt, which was recrystallized from ethanol-ether to give 35: mp 203-208° (lit. (20) 205-207°).

p-Benzyloxybenzyl alcohol (37)

To a methanol solution of 35 g (0.165 mol) of p-benzyloxybenzaldehyde (36) was added slowly excess sodium borohydride, and the reaction mixture was stirred overnight. The solution was evaporated to dryness. The residue was dissolved in water, and the aqueous solution was extracted with chloroform. The chloroform solution was evaporated

to dryness and the white residue was recrystallized from hexane to obtain 30 g (86%) of 37: mp 85-86° (lit. (43) 86-87°).

p-Benzyloxybenzyl chloride (38)

A solution of 7 g (0.033 mol) of p-benzyloxybenzyl alcohol (37) in 100 ml of benzene was added slowly to a refluxing solution containing 7 ml of thionyl chloride, 20 ml of benzene and 0.1 ml of pyridine. After one hour the reaction mixture was cooled and treated with ice water. The benzene layer was then washed successively with aqueous sodium bicarbonate and water and then dried with sodium sulfate. The solution was evaporated to give 5.0 gm (65%) of 38: mp 77-78° (lit. (44) 79-80°).

[1-^{14}C]p-Benzyloxybenzyl cyanide (39)

Sodium[^{14}C]cyanide (3.2 mg, 0.065 mmol, 4 mci, New England Nuclear Corp., Boston, Mass.) was diluted with 21.3 mg (0.435 mmol) of sodium cyanide and added to 3 ml of dried dimethyl sulfoxide containing p-benzyloxybenzyl chloride (38) (116 mg, 0.5 mmol). The reaction mixture was heated for 3 hours at 100° in an oil bath, cooled, and 10 ml of water was added. The reaction mixture was extracted 4 times with 20 ml portion of ether. The ether layer was washed with sodium chloride solution, dried over sodium sulfate and

evaporated to dryness to obtain 93 mg of 39, which was used in the next step without purification.

[1-^{14}C]p-Benzyloxytyramine hydrochloride (40)

To a cold ethereal solution of lithium aluminum hydride (350 mg, 9.8 mmol) was added (350 mg, 3.3 mmol) of aluminum chloride under nitrogen atmosphere (45). To the refluxing mixed hydride solution was added dropwise a solution of 93 mg (0.417 mmol) of [1-^{14}C]p-benzyloxybenzyl cyanide (in 5 ml of absolute ether). The suspension was refluxed under nitrogen atmosphere for 1.5 hours, and cooled. The excess metal hydride was destroyed by water, and 10% aqueous sodium hydroxide. The organic layer was decanted from the aluminum salts which were washed with more ether. The combined ether extracts were washed with saturated sodium chloride solution, dried with sodium sulfate and evaporated to dryness. The residue was dissolved in a minimum amount of absolute ethanol. A few drops of anhydrous ethereal hydrochloric acid was added to give white flakes (58 mg) which were used in the next step without purification.

[1-^{14}C]Tyramine hydrochloride (34a)

[1-^{14}C]p-Benzyloxytyramine hydrochloride (40) (58 mg, 0.22 mmol) was dissolved in absolute methanol and was hydrogenolized with 10% Pd/C (50 mg). After filtration of

the reaction mixture, washing the catalyst and removal of solvent, 35 mg of compound 34a was obtained, which was used directly in the next step.

O-Methyl[1-^{14}C]norbelladine hydrochloride (41)

[1-^{14}C]Tyramine hydrochloride (34a) (35 mg, 0.206 mmol) was reductively condensed with isovanillin (35 mg, 0.201 mmol) in the same manner as that reported for O-methyl-[1'-^{3}H]norbelladine hydrochloride to obtain 30 mg of condensed product 41: mp 204-208° (lit. (20) 205-207°).

O-Methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42)

O-Methyl[1'-^{3}H]norbelladine hydrochloride (35) and O-methyl[1-^{14}C]norbelladine hydrochloride (41) were combined and recrystallized from ethanol-ether to constant activity (^{3}H - 3.96x10^{-2} mci/mg; ^{14}C - 1.25x10^{-2} mci/mg): mp 206-208° (lit. (20) 205-207°); TLC in chloroform:methanol: diethylamine (90:5:5) and ethyl acetate:methanol (70:30) showed one spot identical in R_f value with an authentic sample.

Synthesis of O,N-Dimethyl[1'-^{3}H, 1-^{14}C]norbelladine Hydrochloride

O,N-Dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43)

To a solution of 20 mg (0.64 mmol) of crude O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42) in

10 ml methanol was added 5-fold excess of 37% formaldehyde solution, followed by addition of a 10-fold excess of sodium borohydride. The reaction mixture was stirred overnight. The solution was then evaporated to dryness. The residue was dissolved in 10% hydrochloric acid and neutralized with solid sodium carbonate and extracted with chloroform. The chloroform solution was evaporated to dryness to give a white residue, which was converted into the hydrochloride salt 43 and recrystallized from ethanol-ether to constant activity (^{3}H - 3.63×10^{-2} mci/mg; ^{14}C - 1.10×10^{-2} mci/mg): mp 230° dec (lit. (46) 230° dec); TLC in chloroform:methanol:diethylamine (90:5:5) and ethyl acetate: methanol (70:30) showed one spot and identical with an authentic sample.

Degradation of O,N-Dimethyl[1'-^{3}H, 1-^{14}C]norbelladine Hydrochloride

[1'-^{3}H, 1-^{14}C]Belladine methiodide (44)

O,N-Dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43) (6.42 mg, 0.014 mmol) (^{3}H - 11.7 mci/mmol; ^{14}C - 3.56 mci/mmol) was diluted with 102 mg (0.22 mmol) of non-radioactive compound to obtain a specific activity (^{3}H - 7.01×10^{-1} mci/mmol, ^{14}C - 2.14×10^{-1} mci/mmol). This was treated with excess diazomethane in ether and let stand

for three days at room temperature. The ether solution was evaporated, the residue was dissolved in benzene, extracted with 10% aqueous sodium hydroxide solution. The benzene layer was taken to dryness. The residue was dissolved in acetone and excess methyl iodide was added to provide 74.6 mg of colorless methiodide salt 44. Recrystallization from methanol gave material with an activity of (^{3}H - $7.01x10^{-1}$ mci/mmol, ^{14}C - $2.10x10^{-1}$ mci/mmol). This was further diluted with 2.012 g (4.24 mmol) of non-radioactive belladine methiodide and recrystallized from ethanol-water to constant activity: mp 220-224° (lit. (10) 223-224°); (^{3}H - $2.43x10^{-2}$ mci/mmol; ^{14}C - $0.72x10^{-2}$ mci/mmol).

[1-^{14}C]1,2-Dibromo-2-(4-methoxyphenyl)ethane (46)

A solution of 1 g (2.1 mmol) of [1'-^{3}H, 1-^{14}C]belladine methiodide (44) (^{3}H - $2.43x10^{-2}$ mci/mmol, ^{14}C - $0.72x10^{-2}$ mci/mmol) in 20% aqueous potassium hydroxide (80 ml) was heated at 105° for 15 hours. The cooled solution was extracted with ether and the ether extract washed with water and extracted with 10% hydrochloric acid to separate the [1-^{3}H]N,N-dimethylveratrylamine (45). The ether layer was washed with water, dried with sodium sulfate, and evaporated to dryness to obtain crude [1-^{14}C]p-methoxy-styrene (46) (195 mg). This was dissolved in ether and

cooled in ice. Bromine in ether was added until the bromine was present in slight excess. This was removed with cyclohexene. The solution was concentrated. Upon cooling, a solid precipitated and was recrystallized from cyclohexane. A portion was sublimed for counting (^{3}H - $0.71x10^{-1}$ mci/mmol): mp 78-80° (lit. (47) 80-81°).

[1-^{14}C]1,2-Dihydroxy-2-(4-methoxypehnyl)ethane (48)

A solution of [1-^{14}C]1,2-dibromo-2-(4-methoxyphenyl)-ethane (47) (79 mg, 0.27 mmol) in 3 ml of dioxane was treated with 100 mg of sodium hydroxide in 3 ml of water. The two phase mixture was stirred for 2 hours, acidified just past neutrality and warmed briefly on a steam bath, diluted with water and continuously extracted with ether for 48 hours. The ether solution was evaporated to dryness to give a yellowish oil. Purification by preparative thin layer chromatography using chloroform:methanol: diethylamine (90:5:5) as solvent gave [1-^{14}C]1,2-dihydroxy-2-(4-methoxyphenyl)ethane (48). Recrystallization from benzene gave crystalline material. A portion was sublimed for counting (^{3}H - $0.71x10^{-1}$ mci/mmol): mp 81-83° (lit. (48) 81-82°).

[^{14}C-Methylene]2,2'-methylenebis(5,5-dimethyl-1,3-cyclohexanedione) (53) and tetrahydro-9-(p-methoxyphenyl)-3,3,-6,6-tetramethylxanthenedione (52)

[1-^{14}C]1,2-Dihydroxy-2-(4-methoxyphenyl)ethane (48) (17 mg, 0.101 mmol) (^{3}H - 0.71×10^{-2} mci/mmol) was diluted with 61 mg of inactive compound to obtain a specific activity (^{3}H - 1.51×10^{-2} mci/mmol). The diluted compound was dissolved in 2 ml of methanol and treated with 114 mg of periodic acid in 3 ml water, let stand for 2 hours. A small drop of piperidine was added until the solution turned slightly basic, then 300 mg of 5,5-dimethyl-1,3-cyclohexanedione (methone) was added. The reaction mixture was allowed to stand overnight at room temperature. The precipitate was washed with 50% ethanol-water solution to obtain 100 mg of brownish precipitate which was chromatographed on a silica gel column packed with hexane. Hexane:ether (95:5) was the eluting solvent. [^{14}C-Methylene]2,2'-methylenebis(5,5-dimethyl-1,3-cyclohexanedione) (53) was eluted first to give 40 mg of needle-like cyrstals. A portion was sublimed for counting (^{14}C - 1.50×10^{-2} mci/mmol): mp 190-192° (lit. (49) 191-191.5). 2,2'-(p-Methoxybenzylidene)bis[5,5-dimethyl-1,3-cyclohexanedione) (51) was eluted as a yellow oil. This was dissolved in minimum amount of ethanol, a drop of concentrated hydrochloric acid was added and the reaction

mixture was heated on a steam bath for about 5 minutes. Upon cooling and filtration, 25 mg of tetrahydro-9-(p-methoxylphenyl)-3,3,6,6-tetramethylxanthenedione (52) was obtained. A portion was sublimed for counting (non-radioactive): mp 240-242° (lit. (49) 241-243°).

[1-^{3}H]N,N-Dimethylveratrylamine hydrochloride (54)

The aqueous acidic extract (vide supra) was made alkaline with solid sodium carbonate and extracted with chloroform. The chloroform extract was then washed with water, dried with sodium sulfate and evaporated to give [1-^{3}H]N,N-dimethylveratrylamine (45) which was converted into the hydrochloride salt 54 and recrystallized from ethanol, (^{3}H - 2.56x10^{-2} mci/mmol): mp 204-206° (lit. (11) 204°).

Veratric acid (55)

[1-^{3}H]N,N-Dimethylveratrylamine hydrochloride (54) (60 mg, 0.26 mmol) was dissolved in 5 ml of water and 340 mg of potassium permanganate in 10 ml of water was added dropwise. Sodium carbonate was added to make the solution basic. The reaction mixture was heated for one hour on a steam bath. Sulfur dioxide was bubbled through the solution until it became clear. The solution was made acidic with sulfuric acid, extracted with ether (4 x 15 ml). The ether extract was evaporated to give

40 mg of crude veratric acid (55) which was recrystallized from water. A portion was sublimed for counting (non-radioactive): mp 180-182° (lit. (50) 181°).

Synthesis of 2-Hydroxy-O-methylnorbelladine

Guaiacol carbonate (57)

To 20 g (0.16 mol) of guaiacol (56) in 60 g of pyridine was added 16 g (0.17 mol) of ethyl chloroformate with cooling. The reaction mixture was let stand for 24 hours in the cold, then poured into a 5% hydrochloric acid solution and extracted with ether. The ether layer was washed with 10% sodium hydroxide. The ether layer was evaporated to dryness to obtain 16 g (58%) of oil: bp 262-265 (atm) (lit. (51) 265° (atm)); ir ($CHCl_3$) 1760 cm^{-1} (C=O), nmr ($CDCl_3$) δ 1.3 (t, 3, $C\underline{H}_3$), 3.7 (s, 3, $O\underline{CH}_3$), 4.2 (q, 2, $C\underline{H}_2$), and 6.8-7.8 ppm (m, 4, aromatic).

5-Bromoguaiacol carbonate (58)

To 15 g (0.0765 mol) of guaiacol carbonate (57) in 50 ml of chloroform was added 20 g (0.12 mol) of bromine dissolved in 50 ml of chloroform. The reaction mixture was let stand for 2 hours. It was washed with water. The chloroform solution was dried with sodium sulfate and evaporated to dryness to give 15 g (75%) of oil. A portion was sublimed for mp 48-49°; ir ($CHCl_3$) 1780 (C=O),

1500 and 1260 cm^{-1}, nmr ($CDCl_3$) δ 1.3 (t, 3, $C\underline{H}_3$), 3.8 (s, 3, $OC\underline{H}_3$), 4.3 (q, 2, $C\underline{H}_2$), and 6.7-7.4 ppm (m, 3, aromatic).

Anal. Calcd. for $C_{10}H_{11}O_4Br$: C, 43.64; H, 4.00; Br, 29.09. Found: C, 43.58; H, 4.08; Br, 29.05.

5-Bromoguaiacol (59)

To 15 g (0.05 mol) of 5-bromoguaiacol carbonate (58) in 100 ml of methanol was added 40 g (1 mol) of sodium hydroxide dissolved in a small amount of water with cooling. The reaction mixture was refluxed for 30 minutes, neutralized with concentrated hydrochloric acid, extracted with chloroform. The chloroform extract was dried with sodium sulfate and evaporated to dryness. An oil was obtained which crystallized out from hexane, yield 7.9g (79%): mp 68-69° (lit. (52) 65°); ir ($CHCl_3$) 1600, 1500, 1290 and 1260 cm^{-1}, nmr ($CDCl_3$) δ 3.8 (s, 3, $OC\underline{H}_3$), 5.1 (s, 3, $OC\underline{H}_2Ph$), and 6.7-7.4 ppm (m, 8, aromatic).

2-Benzyloxy-4-bromoanisole (60)

To 1.37 g (5 mmol) of 5-bromoguaiacol (59) in 40 ml of absolute methanol was added 0.65 g (5 mmol) of potassium carbonate and 0.64 g (5 mmol) of benzyl chloride. The reaction mixture was refluxed overnight. Chloroform (50 ml) was added and the organic layer was extracted with 10% sodium hydroxide, dried with sodium sulfate and evaporated to dryness. Recrystallization from hexane yielded 10 g

(90%) of white needles: mp 110-111°; ir ($CHCl_3$) 1470, 1240 and 1120 cm^{-1}, nmr ($CDCl_3$) δ 3.8 (s, 3, OCH_3), 5.1 (s, 3, OCH_2Ph), and 6.6-7.4 ppm (m, 8, aromatic).

Anal. Calcd. for $C_{14}H_{13}O_2Br$: C, 57.33; H, 4.45; Br, 27.05. Found: C, 57.52; H, 4.54; Br, 27.16.

3-Benzyloxy-4-methoxybenzoic acid (61)

To 0.12 g (5 mmol) of magnesium in 10 ml of dried tetrahydrofuran was added 1.46 g (5 mmol) of 2-benzyloxy-4-bromoanisole (60). A small crystal of iodine was added and the reaction was initiated using a heat gun, after which it was refluxed under nitrogen atmosphere for 1 hour. Carbon dioxide gas generated from barium carbonate and sulfuric acid according to the method of Dauben and coworkers (35) was trapped into the cold Grignard solution. After carbonation, the solution was hydrolyzed with 6N sulfuric acid. The reaction mixture was extracted with ether. The ether layer in turn was extracted with 10% sodium hydroxide. The aqueous basic layer was neutralized with concentrated hydrochloric acid and extracted with ether. The ether layer was evaporated to dryness to give a brown residue which crystallized from ethanol to yield 0.8 g (62%) of product 61: mp 178-180° (lit. (53) 177°); ir ($CHCl_3$) 1700 (C=O) 1600, 1670, 1550, 1320 and 1370 cm^{-1}, nmr ($CDCl_3$) δ 3.9 (s, 3, OCH_3), 5.1 (s, 2, OCH_2Ph), and 6.8-7.8 ppm (m, 8, aromatic).

3-Benzyloxy-4-methoxybenzyl alcohol (62)

Lithium aluminum hydride (76 mg, 2 mmol) was suspended in 30 ml of dried tetrahydrofuran and 500 mg (2 mmol) of 3-benzyloxy-4-methoxybenzoic acid (61) in 10 ml of tetrahydrofuran was added dropwise. The reaction mixture was refluxed overnight. The excess hydride was destroyed. The reaction mixture was extracted with ether. The ether extract was dried with sodium sulfate. The solution was evaporated to dryness to give 244 mg (50%) of 62: mp 76° (hexane); ir 3550 (OH), 1500, 1250, 1130 and 1020 cm^{-1}, nmr ($CDCl_3$) δ 3.8 (s, 3, OCH_3), 4.4 (s, 2, CH_2OH), 5.1 (s, 2, CH_2Ph), and 6.8-7.5 ppm (m, 8, aromatic).

Anal. Calcd. for $C_{15}H_{16}O_3$: C, 73.75; H, 6.63. Found: C, 73.73; H, 6.55.

O-Benzylisovanillin (63)

A solution of 245 mg (1 mmol) of 3-benzyloxy-4-methoxybenzyl alcohol (62) in 40 ml of chloroform was stirred at room temperature with 1.0 g of manganese dioxide overnight. The reaction mixture was filtered through Celite and the Celite was washed with chloroform. The chloroform solution was evaporated to dryness to give 245 mg (99%) of 63: mp 60-61° (lit. (54) 60-61°); ir ($CHCl_3$) 1680 (C=O), 1600, 1440, 1280 and 1030 cm^{-1}, nmr ($CDCl_3$) δ 3.9 (s, 3, OCH_3), 5.1 (s, 2, OCH_2Ph), 6.7-7.5 (m, 8, aromatic), and 9.8 ppm (s, 1, CHO).

p-Benzyloxy-ω-bromoacetophenone (68)

To 45.2 g (0.2 mol) of p-benzyloxyacetophenone (67) in 600 ml of ethanol was added five drops of concentrated hydrochloric acid. Bromine (64 g, 0.4 mol) in 100 ml of methanol was then added slowly. The mixture was stirred for 3 hours. A solid precipitated, was filtered and recrystallized from ethanol to yield 60 g (99%) of product 68: mp 83-84° (lit. (55) 83-84°); ir ($CHCl_3$) 1680 (C=O), 1600, 1260 and 1180 cm^{-1}, nmr ($CDCl_3$) δ 4.4 (s, 2, $C\underline{H}_2Br$), 5.1 (s, 2, $OC\underline{H}_2Ph$), 6.9 and 7.9 (AA'BB', 4, $\underline{J}$ = 8 Hz, aromatic), and 7.4 ppm (s, 5, $OCH_2\underline{Ph}$).

p-Benzyloxy-ω-aminoacetophenone hydrochloride and hydrobromide (69)

A solution of 45.7 g (0.15 mol) of p-benzyloxy-ω-bromoacetophenone (68) in 100 ml of chloroform was added to a solution of 22 g (0.16 mol) of hexamethylenetetramine in 100 ml of chloroform. The reaction mixture was refrigerated overnight. The precipitate was filtered and washed with chloroform to yield 25 g of hydrochloride and hydrobromide salt. The mixed salt was added to a solution of 27 ml of concentrated hydrochloric acid and 54 ml of water. After stirring for 1 hour, 80 ml of ethanol was added and the stirring was continued for 1 additional hour at room temperature. The undissolved material (which was a mixture of p-benzyloxy-ω-aminoacetophenone hydrochloride and hydrobromide) was

filtered and recrystallized from ethanol to yield 13 g of white powder 69: mp 228-230° (lit. (55) 228-230°); ir (KBr) 1640 (C=O), 1560, 1470, 1220 and 1140 cm^{-1}, nmr (DMSO-d_6) δ 4.5 (s, 2, $C\underline{H}_2NH_2$), 5.2 (s, 2, $OC\underline{H}_2Ph$), 7.1 and 8.0 (AA'BB', 4, $\underline{J}$ = 10 Hz, aromatic), 7.35 (s, 5, $OCH_2\underline{Ph}$), and 8.55 ppm (broad, 2, $N\underline{H}_2$, exchangeable with D_2O).

O-Benzyloctapamine (64)

p-Benzyloxy-ω-aminoacetophenone mixed salt (69) (276 mg, 1 mmol) was dissolved in 30 ml of water by warming. Sodium borohydride (38 mg, 1 mmol) was added slowly with stirring. The reaction mixture was allowed to stand overnight. The reaction mixture was extracted with chloroform. The chloroform was dried with sodium sulfate and evaporated to dryness to give 193 mg of compound 64: mp 104-105° (lit. (7) 101-103°); ir (KBr) 1780, 1880, 1340, 1170 and 960 cm^{-1}, nmr ($CDCl_3$) δ 2.2 (s, 2, $N\underline{H}_2$, exchangeable with D_2O), 2.7 (s, 2, $C\underline{H}_2N$), 4.5 (t, 1, $\underline{J}$ = 6 Hz, $C\underline{H}OH$), 5.0 (s, 2, $OC\underline{H}_2Ph$), δ 6.90 and 7.25 (AA'BB', 4, $\underline{J}$ = 8 Hz, aromatic), and 7.35 ppm (s, 5, $OCH_2\underline{Ph}$).

2-Hydroxy-O-methylnorbelladine hydrochloride (66)

A mixture of 242 mg (1 mmol) of O-benzyloctopamine hydrochloride (64), 243 mg (1 mmol) of O-benzylsovanillin (63) and 100 mg of sodium bicarbonate in 20 ml of absolute ethanol was stirred at room temperature for 15 minutes.

Anhydrous benzene (2 ml) was added and the mixture was distilled thru a short Vigreux column until 4 ml of distillate was collected. The reaction mixture was cooled to room temperature, 150 mg of sodium borohydride was added slowly and the resulting mixture was stirred at room temperature overnight. Most of the solvent was evaporated under reduced pressure. The residue was dissolved in 4 ml of water, acidified to pH 8 by addition of concentrated hydrochloric acid and brought to about pH 7 by addition of 5 ml concentrated pH 7 buffer solution. The solution was extracted with ethyl acetate:methanol (95:5). The organic layer was washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and the solvent was evaporated to afford a yellow oil. The yellow oil was dissolved in absolute ethanol and a few drops of ethereal hydrochloric acid solution was added. The white precipitate was filtered to yield 237 mg (47%) of 65. Compound 65 was dissolved in ethanol, added to a suspension of 100 mg of 10% Pd/C in ethanol. The reaction mixture was hydrogenolized. The catalyst was filtered, washed with ethanol and the filtrate and wash were evaporated to give 76 mg (50%) of the hydrochloride 66, which was recrystallized from ethanol-ether: mp 188-190°; free base (recrystallized from ethyl acetate-MeOH) mp 146-148° (lit. (7) 145-148°); ir (KBr) 1500, 1420, 1280

and 1260 cm^{-1}, nmr (DMSO-d_6) δ 2.9 (m, 2, NCH_2), 3.74 (s, 3, OCH_3), 4.0 (s, 2, $PhCH_2N$), 4.9 (t, 1, J = 8 Hz, PhCHO) and 6.56-7.23 ppm (m, 7, aromatic).

Synthesis of 2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]-norbelladine Hydrochloride

All synthetic procedures described in these sections were described in detail in previous sections.

2-Hydroxy-O-methyl[2-^{3}H]norbelladine hydrochloride (71a)

p-Benzyloxy-ω-aminoacetophenone mixed salt (69) (276 mg, 1 mmol) was reduced using 15 mg (0.394 mmol) of ^{3}H-sodium borohydride (100 mci, New England Nuclear Corp., Boston, Mass.) and 23 mg (0.606 mmol) of sodium borohydride to give [2-^{3}H]O-benzyloctopamine hydrochloride (64a), which was then reductively condensed with O-benzylisovanillin (63) and hydrogenolized to give 92 mg of 2-hydroxy-O-methyl[2-^{3}H]norbelladine hydrochloride (71a).

2-Hydroxy-O-methyl[1'-^{14}C]norbelladine hydrochloride (66a)

The Grignard reagent prepared from 803 mg (2.75 mmol) of 2-benzyloxy-4-bromoanisole (60) and 66 mg (2.75 mmol) of magnesium was treated with $^{14}CO_2$ [168 mg (0.85 mmol) of $Ba^{14}CO_3$, 50 mci, New England Nuclear Corp., Boston, Mass. and 325 mg (1.65 mmol) of inactive $BaCO_3$] to give [^{14}C-carboxyl]3-benzyloxy-4-methoxybenzoic acid (61a). The [^{14}C-carboxyl]3-benzyloxy-4-methoxybenzoic acid (61a) was

reduced to [^{14}C-benzylalcohol]3-benzyloxy-4-methoxybenzyl-alcohol (62a), oxidized to the [^{14}C-carbonyl]p-benzyloxy-isovanillin (63a), and reductively condensed with O-benzyloxyoctopamine hydrochloride (64) and hydrogenolized to give 132 mg of 2-hydroxy-O-methyl[1'-^{14}C]norbelladine hydrochloride (66a).

2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72)

O-Methyl[2-^{3}H]norbelladine hydrochloride (71a) and 2-hydroxy[1'-^{14}C]norbelladine hydrochloride (66a) were combined and recrystallized to constant activity from methanol-ether (^{3}H - 56.4 mci/mg, ^{14}C - 32.6 mci/mg): mp 188-190°; TLC in ethyl acetate:methanol (80:20) and chloroform:methanol:diethylamine (80:15:5) showed one spot and identical in R_f value with an authentic sample.

Synthesis of N-Demethyl[4-^{3}H, 1'-^{14}C]-cherylline Hydrochloride

N-Demethyl[4-^{3}H, 1'-^{14}C]cherylline hydrochloride (73)

A solution of 45 mg (0.14 mmol) of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) (^{3}H - 17.6 mci/mmol, ^{14}C - 10.1 mci/mmol) in 30 ml of 10% hydrochloric acid was refluxed for 5 hours, then cooled. The acidic aqueous solution was extracted with chloroform,

treated with solid sodium carbonate until a pH 8-9 was obtained. The solution was extracted with 3 x 50 ml ethyl acetate. The organic layer was dried with sodium sulfate, evaporated to dryness to obtain 17 mg of white powder, converted into its hydrochloride salt and recrystallized from ethanol-ether to constant activity (^{3}H - 16.8 mci/mmol, ^{14}C - 9.85 mci/mmol): mp 244-247° (lit. (1) 228-230°); TLC in ethyl acetate:methanol (70:30); chloroform:methanol: diethylamine (85:10:5) and ethyl acetate:methanol: diethylamine (70:29:1) showed one spot and identical in R_f value with authentic sample.

In a separate experiment with an inactive sample, N-demethylcherylline hydrochloride (74): mp 244-247° (lit. (1) 228-230°); ir (nujol) 1600, 1520, 1390, 1270 and 1220 cm^{-1}, nmr (methanol-d_4) δ 3.5 (m, 2, NCH_2), 3.6 (s, 3, OCH_3), 4.4 (m, 2 , $PhCH_2N$ and methine proton at C-4), 6.4 (s, 2, aromatic), 6.8 and 6.95 ppm (AA'BB', 4, J = 9 Hz, aromatic); mass spectrum (70 eV) m/e 271 (M^+).

Anal. Calcd. for $C_{16}H_{18}NO_3Cl$: C, 67.98; H, 5.85; N, 4.55. Found: C, 67.88; H, 5.87; N, 4.50.

Degradation of 2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine Hydrochloride

[2-^{3}H, 1'-^{14}C]2-Hydroxybelladine methiodide (77)

2-Hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) (1.46 g, 4.55 mmol) (^{3}H - 1.34×10^{-1} mci/mmol, ^{14}C - 0.82×10^{-1} mci/mmol) was converted into [2-^{3}H, 1'-^{14}C]-2-hydroxybelladine methiodide (77) (^{3}H - 1.34×10^{-1} mci/mmol, ^{14}C - 0.82×10^{-1} mci/mmol) by treatment with excess diazomethane, followed by reaction with excess methyl iodide. [2-^{3}H, 1'-^{14}C]2-Hydroxybelladine methiodide (77) (565 mg, 1.19 mmol) was diluted with 600 mg (1.27 mmol) of inactive compound and recrystallized from water to constant activity (^{3}H - 6.41×10^{-2} mci/mmol, ^{14}C - 4.10×10^{-2} mci/mmol): mp 188-189°.

In a separate non-radioactive synthesis of 2-hydroxybelladine methiodide the following properties were observed: mp 188-189°; ir (KBr) 1610, 1530, 1270, 1160 and 1030 cm^{-1}; nmr of free methiodide salt ($CDCl_3$) δ 2.3 (s, 3, NCH_3), 2.5 (m, 2, NCH_2), 3.5 and 3.7 (AB Pattern, 2, J = 12 Hz, $PhCH_2N$), 3.75 (s, 3, OCH_3), 3.90 (s, 6, OCH_3), 4.60 (m, 1, PhCH-O) and 6.7-7.3 ppm (m, 7, aromatic).

Anal. Calcd. for $C_{20}H_{28}NO_4I$: C, 50.74; H, 5.90; N, 2.95. Found: C, 50.86; H, 5.91; N, 2.90.

[2-^{3}H]1,2-dihydroxy-2-(4-methoxyphenyl)ethane (81)

[2-^{3}H, 1'-^{14}C] 2-Hydroxybelladine (77) (500 mg, 1.05 mmol) (^{3}H - 6.41x10^{-2} mci/mmol, ^{14}C - 4.10x10^{-2} mci/mmol) was treated with freshly prepared excess silver oxide (prepared from 2 g of silver nitrate and sodium hydroxide), stirred for 10 minutes. The reaction mixture was filtered thru Celite. The water was removed *in vacuo* to obtain 388 mg of 78. The methohydroxide 78 was pyrolyzed under vacuum (5 mm) at 100° for an hour. 10% Hydrochloric acid (30 ml) was added, and the reaction mixture heated on a steam bath for 15 minutes. The aqueous acidic solution was continuously extracted with ether for 48 hours. The aqueous acidic solution gave [1-^{14}C] N,N-dimethylveratrylamine (79) (see later section). The ether fraction gave [2-^{3}H]1,2-dihydroxy-2-(4-methoxyphenyl)ethane (81), recrystallized from hexane. A portion was sublimed for counting (^{3}H - 6.47x10^{-2} mci/mmol): mp 81-83° (lit. (48) 81-82°).

2,2'-Methylenebis(5,5-dimethyl-1,3-cyclohexanedione (83), and [9-^{3}H]tetrahydro-9-(p-methoxyphenyl)-3,3,6,6-tetramethylxanthenedione (82)

[2-^{3}H]1,2-Dihydroxy-2-(4-methoxyphenyl)ethane (81) (140 mg) was converted into 83 (non-radioactive) and 82 (^{3}H - 6.41x10^{-2} mci/mmol) according to procedure described in the previous section.

[1-^{14}C]N,N-Dimethylveratrylamine methiodide (85)

The aqueous acidic fraction was neutralized with solid sodium carbonate, extracted with chloroform to obtain 168 mg of [1-^{14}C]N,N-dimethylveratrylamine (79), which was converted into its methiodide salt 85 using excess methyl iodide: mp 180-182° (lit. (56) 175-176°) (^{14}C - 4.33×10^{-2} mci/mmol).

[^{14}C-Methyl]3,4-dimethoxytoluene (87)

[1-^{14}C]N,N-Dimethylveratrylamine methiodide (85) (101.4 mg, 0.3 mmol) (^{14}C - 4.33×10^{-2} mci/mmol) was diluted with 337.9 mg (1 mmol) of inactive compound to obtain a specific activity of (^{14}C - 1.00×10^{-2} mci/mmol). It was then converted into [1-^{14}C]N,N-dimethylveratrylamine methohydroxide (86) using freshly prepared silver oxide. [1-^{14}C]N,N-Dimethylveratrylamine methohydroxide (86) in water was hydrogenated at room temperature and atmospheric pressure using 10% Pd/C. The suspension was filtered, washed with methanol. The combined aqueous and alcohol wash were made acidic using concentrated hydrochloric acid, and extracted with ether and dried with sodium sulfate. The organic layer was evaporated to dryness to obtain 122 mg of crude [^{14}C-methyl]3,4-dimethoxytoluene (87). It was purified by preparative thin layer chromatography on silica gel using ethyl acetate:methanol:

diethylamine (70:29:1) as solvent to give 77 mg of pure [^{14}C-methyl]3,4-dimethoxytoluene (87), R_f identical with an authentic sample (^{14}C - 9.9x10^{-3} μci/mmol).

Kuhn-Roth oxidation (57) of [^{14}C-methyl]3,4-dimethoxytoluene (87)

To a 50 ml 3-necked flask was added 70 mg (0.46 mmol) of [^{14}C-methyl]3,4-dimethoxytoluene (87), 5 ml of cooled oxidation mixture (20 ml of 5.0 N chromic acid and 5 ml of concentrated sulfuric acid). The reaction mixture was refluxed in an oil bath for 1.5 hours under a nitrogen atmosphere. Water (15 ml) was added, acetic acid was removed by steam distillation by continuous addition of water. The distillate was collected (50 ml). Sodium hydroxide (0.09766 N) (3.8 ml) was needed for titration (phenolphthalein indicator). The [^{14}C-methyl] sodium acetate (88) (30 mg) obtained was recrystallized from ethanol to give a specific activity (^{14}C - 9.75x10^{-3} μci/mmol).

Crinum Powellii Feeding Experiment

Administration of labeled precursors to the plant

The labeled precursors used in the three week feeding experiments were O-methyl[1'-^{3}H, 1-^{14}C]norbelladine (42) (^{3}H - 298 μci, ^{14}C - 95.3 μci); O,N-dimethyl[1'-^{3}H, 1-^{14}C]-norbelladine (43) (^{3}H - 278 μci, ^{14}C - 84.5 μci); 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine (72) (^{3}H - 213 μci,

^{14}C - 123 μci); N-demethyl[4-^{3}H, 1-^{14}C]cherylline (73) (^{3}H - 268 μci, ^{14}C - 123 μci). All of the above mentioned doubly-labeled compounds were purified and fed as the hydrochloride salt. Each compound was dissolved in 0.5 ml to 1 ml of water (pH 6) and introduced into the bulb using a fine hypodermic needle. The plants were grown in a fume hood under artificial light for a period of three weeks. The bulbs were harvested and processed. The plants were obtained from International Growers Exchange, Inc., Farmington, Michigan.

Each isolation was carried out in the same manner and in the interest of brevity, only the extraction and isolation of the alkaloids derived from O-methly[1'-^{3}H, 1-^{14}C]-norbelladine hydrochloride are described below.

The bulbs (1.565 Kg) were macerated in a Waring Blendor with ethanol (2.5 l.), left to stand overnight and filtered. The solid was extracted two additional times with 95% ethanol (2 x 1 l.). The combined ethanol extracts were evaporated to about 1 l. under reduced pressure, saturated with tartaric acid and filtered. The aqueous solution was extracted with benzene (3 x 200 ml) to remove neutral substances (3.107 g) (^{3}H - 0.494%; ^{14}C - 0.0014% incorporation). Extraction with chloroform gave the crude chloroform soluble tartrates (7.51 g) (^{3}H - 0.925%,

^{14}C - 0.184% incorporation). The aqueous solution was then basicified to pH 8-9 with ammonium hydroxide and extracted with chloroform (5 x 100 ml) until the extract failed to give a positive alkaloid test with silicotungstic acid. The basic chloroform extract upon evaporation to dryness gave the main crude alkaloidal fraction (2.730 g) (^{3}H - 34.8%, ^{14}C - 29.2% incorporation). The residue was dissolved in chloroform and let stand overnight. The chloroform solution was filtered to give 1.476 g of crude lycorine (^{3}H - 18.1%, ^{14}C - 11.8% incorporation). The lycorine was purified and recrystallized (ethanol-water) as its hydrochloride salt, 1.111 g: mp 214-215° (lit. (8) 215-216°) (^{3}H - 3.51%; ^{14}C - 3.35% incorporation). The filtrate was then evaporated to dryness to obtain 1.264 g of crude chloroform insoluble "bases" (^{3}H - 16.7%, ^{14}C - 17.4% incorporation).

The chloroform insoluble "bases" (1.246 g) were dissolved in 40 ml of chloroform, extracted with 0.5 N sodium hydroxide (3 x 25 ml). The basic aqueous solution was extracted with 25 ml chloroform and combined with the original chloroform solution. The combined chloroform extracts were evaporated to dryness to obtain 847 mg of crude non-phenolic alkaloids (^{3}H - 1.76%, ^{14}C - 1.68% incorporation). The non-phenolic alkaloids (847 mg) were chromatographed on a basic alumina column (30 g) packed

with chloroform. Elution with chloroform gave crude ambelline in the first four fractions (15 ml each collection). The crude ambelline was purified by preparative thin layer chromatography using chloroform:methanol:diethylamine (90:5:5) as solvent to give 85 mg of pure product (recrystallized from ethanol): mp 258-260° (lit. (8) mp 260-261°) (^{3}H - 0.0276%, ^{14}C - 0.0289% incorporation).

The basic aqueous solution was acidified to pH 3 using concentrated hydrochloric acid and extracted with 25 ml of chloroform which was discarded. The acidic aqueous solution was basicified to pH 8-9 with ammonium hydroxide and then extracted (5 x 25 ml) with chloroform. The combined chloroform extracts were evaporated to dryness to give 185 mg of crude phenolic alkaloids (^{3}H - 11%, ^{14}C - 10.9% incorporation). The crude phenolic alkaloids (185 mg) were separated by preparative-scale thin layer chromatography using ethyl acetate:methanol (70:30) as solvent. Four bands were sectionalized and alkaloids recovered from the silica gel. The band at $R_f \approx 0.6$ was purified by preparative-scale thin layer chromatography using chloroform:methanol:diethylamine (90:5:5) as solvent to give cherylline which was recrystallized as its hydrochloride salt from ethanol-ether, 60 mg: mp 236-237° (lit. (3) 238-239°) (^{3}H - 0.061%, ^{14}C - 0.064% incorporation). The band at $R_f \approx 0.5$ was separated by a second preparative-scale

thin layer chromatography using chloroform:methanol:diethylamine (85:10:5) as solvent to give 5 mg of cherylline hydrochloride (combined with the cherylline hydrochloride isolated from band at $R_f \approx 0.6$) and O-methylnorbelladine which was recrystallized as its hydrochloride salt from ethanol-ether, 6 mg: mp 204-205° (lit. (20) 205-207°) (^3H - 3.2%, ^{14}C - 3.51% incorporation).

The labeled precursors used in the one day feeding experiments were 2-hydroxy-O-methyl[2-^3H, 1'-^{14}C]norbelladine hydrochloride (72) (^3H - 257 μci, ^{14}C - 149 μci) and O-methyl[1'-^3H, 1-^{14}C]norbelladine hydrochloride (42) (^3H - 213 μci, ^{14}C - 27.3 μci). The precursors were fed and processed in the same manner as that for a three week feeding experiment, except that the band at $R_f \approx 0.5$ from the phenolic fraction, in the case of the 2-hydroxy-O-methyl-[2-^3H, 1'-^{14}C]norbelladine hydrochloride feeding, was diluted with 23 mg of O-methylnorbelladine, 21.4 mg of 2-hydroxy-O-methylnorbelladine hydrochloride and 20 mg of N-demethylcherylline. The diluted mixture was separated by preparative-scale thin layer chromatography using chloroform:methanol:diethylamine (85:10:5) as solvent to give 25 mg of O-methylnorbelladine hydrochloride ($R_f \approx 0.5$), 21 mg of N-demethylcherylline hydrochloride ($R_f \approx 0.4$) and 15 mg of 2-hydroxy-O-methylnorbelladine hydrochloride ($R_f \approx 0.3$).

The data from the Crinum Powellii isolations are presented in Tables 12 to 17.

Table 12. Alkaloids isolated from a three week feeding O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42) into Crinum powelliii

Alkaloid[a]	Weight of alkaloid (mg)	Per cent alkaloid in extract	Per cent alkaloid in bulb
Cherylline	65	2.40	0.0042
O-Methylnor-belladine	6	0.22	0.0004
Lycorine	1111	40.60	0.0710
Ambelline	85	3.10	0.0054

Weight of wet bulbs	1.565 Kg
Per cent incorporation into crude extract	^{3}H - 34.8%, ^{14}C - 29.2%
Weight of crude extract	2.730 g

[a]Alkaloids were isolated and purified as the hydrochloride salt except ambelline.

Table 13. Alkaloids isolated from a three week feeding O,N-dimethyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (43) into Crinum powellii

Alkaloid[a]	Weight of alkaloid (mg)	Per cent alkaloid in extract	Per cent alkaloid in bulb
Cherylline	55	2.30	0.0040
O-Methylnor-belladine	5	0.21	0.0004
Lycorine	1050	43.50	0.0770
Ambelline	70	2.90	0.0052

Weight of wet bulbs	1.36 Kg
Per cent incorporation into crude extract	^{3}H - 37%, ^{14}C - 34%
Weight of crude extract	2.41 gm

[a]Alkaloids were isolated and purified as the hydrochloride salt except ambelline.

Table 14. Alkaloids isolated from a three week feeding of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) into Crinum powellii

Alkaloid[a]	Weight of alkaloid (mg)	Per cent alkaloid in extract	Per cent alkaloid in bulb
Cherylline	60	2.80	0.0046
O-Methylnor-belladine	8	0.37	0.0006
Lycorine	965	45.40	0.0740
Ambelline	79	3.70	0.0060

Weight of wet bulbs	1.3 Kg
Per cent incorporation into crude extract	--- ---
Weight of crude extract	2.134 g

[a]Alkaloids were isolated and purified as the hydrochloride salt except ambelline.

Table 15. Alkaloids isolated from a three week feeding of N-demethyl[4-^{3}H, 1-^{14}C]cherylline hydrochloride (73) into Crinum powellii

Alkaloid[a]	Weight of alkaloid (mg)	Per cent alkaloid in extract	Per cent alkaloid in bulb
Cherylline	60	1.60	0.0045
O-Methylnor-belladine	5	0.14	0.0004
Lycorine	1400	38.00	0.1000
Ambelline	80	2.20	0.0060

Weight of wet bulbs	1.35 Kg
Per cent incorporation into crude extract	^{3}H - 34.8%, ^{14}C - 33%
Weight of crude extract	3.712 g

[a]Alkaloids were isolated and purified as the hydrochloride salt except ambelline.

Table 16. Alkaloids isolated from a one day feeding of 2-hydroxy-O-methyl[2-^{3}H, 1'-^{14}C]norbelladine hydrochloride (72) into Crinum powellii

Alkaloid[a]	Weight of alkaloid (mg)	Per cent alkaloid in extract	Per cent alkaloid in bulb
Cherylline	30	2.72	0.0042
Lycorine	700	63.65	0.0975
Ambelline	45	4.1	0.0062

Weight of wet bulbs	717 g
Per cent incorporation into crude extract	^{3}H - 29%, ^{14}C - 26.3%
Weight of crude extract	1.1 g

[a]Alkaloids were isolated and purified as the hydrochloride salt except ambelline.

Table 17. Alkaloids isolated from a one day feeding of O-methyl[1'-^{3}H, 1-^{14}C]norbelladine hydrochloride (42) into Crinum powellii

Alkaloid[a]	Weight of alkaloid (mg)	Per cent alkaloid in extract	Per cent alkaloid in bulb
Cherylline	72	3.06	0.0040
O-Methylnor-belladine	1123	47.80	0.0625
Lycorine	21	0.94	0.0017
Ambelline	95	4.05	0.0023

Weight of wet bulbs	1.800 Kg
Per cent incorporation into crude extract	^{3}H - 41.2%, ^{14}C - 42.7%
Weight of crude extract	2.347 g

[a]Alkaloids were isolated and purified as the hydrochloride salt except ambelline.

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