1967

The incorporation of 14C-labeled [beta]-phenylethylamine derivatives and 3H-vittatine into Amaryllidaceae alkaloids

Allen Irwin Feinstein
Iowa State University

FEINSTEIN, Allen Irwin, 1940-

THE INCORPORATION OF ^{14}C-LABELED
β-PHENYLETHYLAMINE DERIVATIVES AND
^{3}H-VITTATINE INTO AMARYLLIDACEAE ALKALOIDS.

Iowa State University of Science and Technology, Ph.D., 1967
Chemistry, organic

University Microfilms, Inc., Ann Arbor, Michigan

THE INCORPORATION OF ^{14}C-LABELED β-PHENYLETHYLAMINE DERIVATIVES AND ^{3}H-VITTATINE INTO AMARYLLIDACEAE ALKALOIDS

by

Allen Irwin Feinstein

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

Major Subject: Organic Chemistry

Approved:

Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
Head of Major Department

Signature was redacted for privacy.
Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1967

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

INTRODUCTION

Many investigations have been carried out in the last five years to determine the biosynthetic pathways leading to the Amaryllidaceae alkaloids. Relatively little consideration has been given to the study of *in vivo* rearrangements, alkaloid interconversions, and the exploration of alternate biosynthetic routes. This thesis is concerned with these three aspects of biosynthesis.

The alkaloids haemanthamine and crinamine have been shown to undergo *in vitro* rearrangements to compounds possessing the montanine ring system. It is known that haemanthamine is not a precursor of the montanine-type alkaloids from tracer studies. The nature of the appropriately substituted haemanthamine-type precursor which could be converted to the montanine nucleus in the plant was a problem that required further consideration. Part of this thesis is concerned with the biosynthesis of montanine with particular emphasis on the nature of the precursors involved and the mechanism of their rearrangement to the montanine nucleus.

Biosynthetic studies with *Nerine bowdenii* have suggested that alkaloids possessing the (+)-crinine absolute configuration are not derived from (-)-crinine, but this has never been fully established. There existed the possibility that certain biosynthetic intermediates possessing enantiomeric ring systems were being interconverted in the plant *via* a symmetrical

precursor. The study of this phenomenon was carried out in order to gain a better understanding of alkaloid interconversions and to learn more about the stage at which the absolute configuration of the crinine-type alkaloids is determined.

It is well known that the C_6-C_2 units of the Amaryllidaceae alkaloids are derived from the precursors tyrosine and tyramine. Recent studies with the Berberine alkaloids have shown that a hydroxylated C_6-C_2 unit can utilize a different biosynthetic pathway than its non-hydroxylated counterpart and become specifically incorporated into a more highly oxygenated alkaloid. There existed the possibility that a hydroxylated C_6-C_2 unit could be more efficiently incorporated into some of the more highly oxygenated Amaryllidaceae alkaloids. This possibility was examined in order to determine whether the same biosynthetic pathways hold for this alkaloid family as well.

HISTORICAL

Ring Systems

Plants of the Amaryllidaceae family produce many different alkaloids (well over one hundred are known)[1], all of which can be structurally divided into seven ring systems (Figure 1).

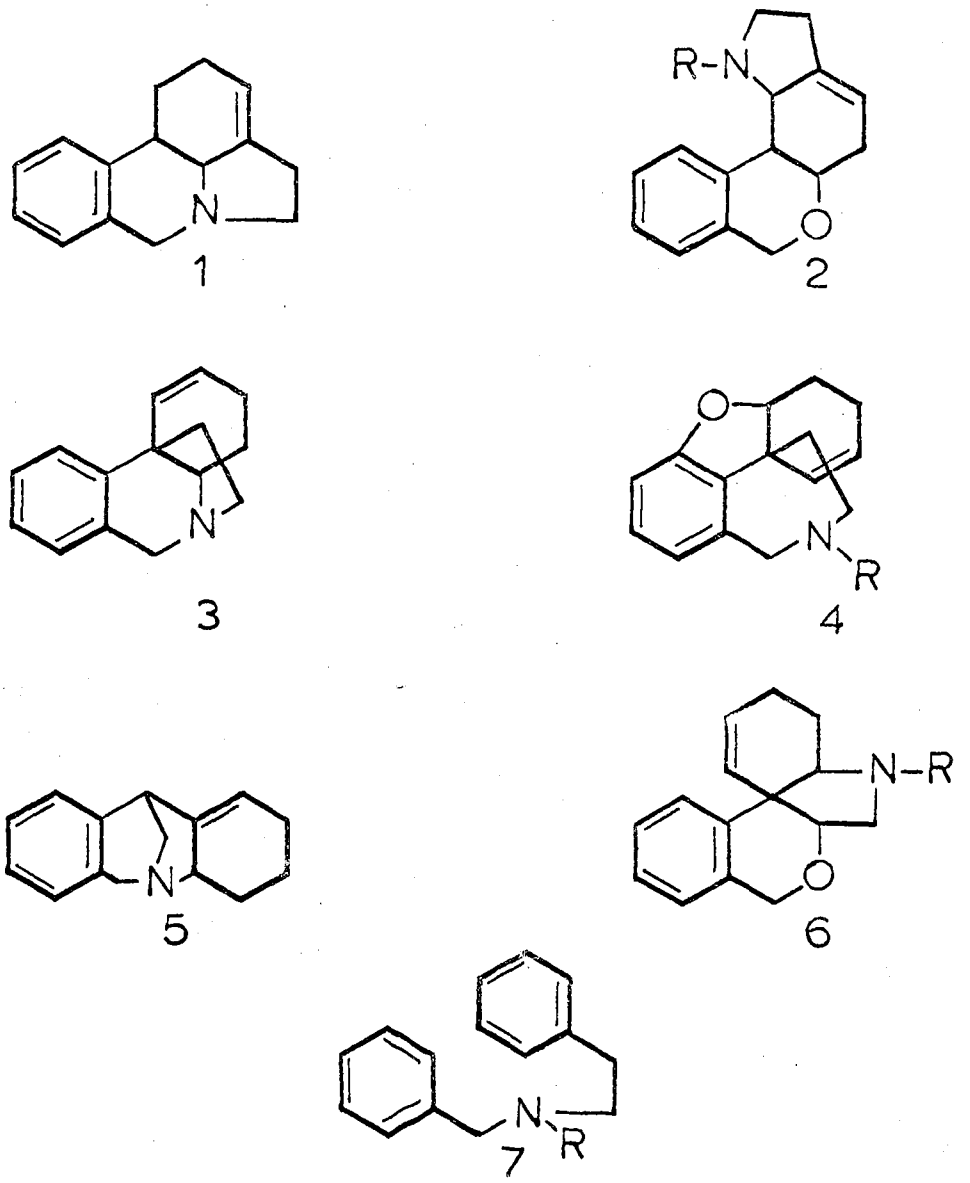


Figure 1. Amaryllidaceae ring systems

For convenience, each ring system has been given the name of the most common alkaloid of that type. The seven ring systems are lycorine (1), lycorenine (2), crinine (3), galanthamine (4), montanine (5), tazettine (6) and belladine (7). Each ring system contains an aromatic C_6-C_1 and a hydroaromatic C_6-C_2 unit shown by the heavy lines in each nucleus. Belladine has an aromatic rather than a hydroaromatic C_6-C_2 unit.

The large number of alkaloids are a result of the variations in oxygen substitution within the ring systems. The aromatic ring may be di- or tri-oxygenated and the hydroaromatic ring may be mono- or di-oxygenated. The various substitution patterns are shown in Figure 2.

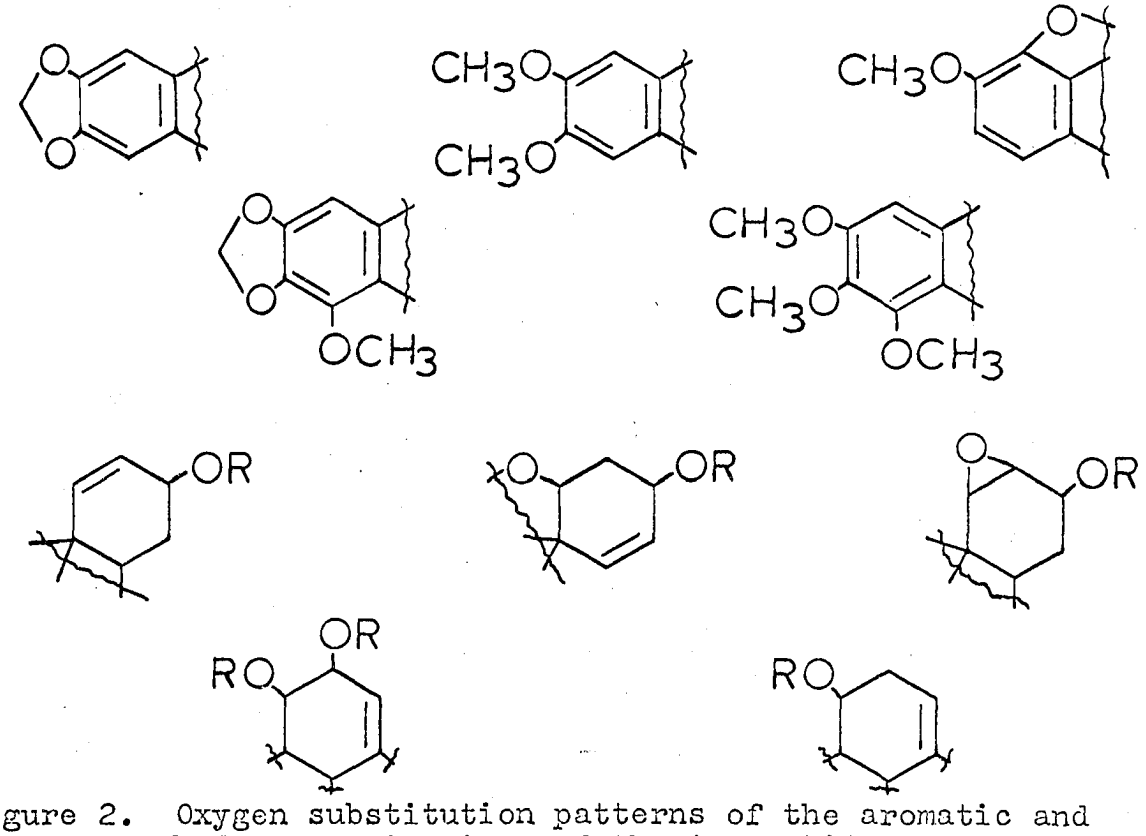
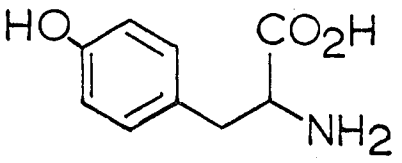


Figure 2. Oxygen substitution patterns of the aromatic and hydroaromatic rings of the Amaryllidaceae alkaloids

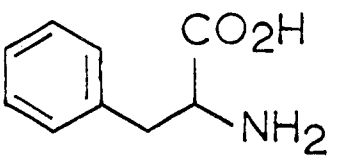
The most common substitution pattern is a di-oxygenated aromatic ring and a mono-oxygenated hydroaromatic ring. Additional variations in the oxygen substitution pattern result from the occurrence of oxygen substituents at the benzylic carbon atom of the C_6-C_1 units and at C_1 of the C_6-C_2 units.

Biosynthesis of the Amaryllidaceae Alkaloids

Biosynthetic investigations of the Amaryllidaceae alkaloids began in 1960 with the use of radioactive tracers. Early tracer studies dealt with the determination of the amino acid precursors of the alkaloids. The investigations, summarized in Table 1, show that tyrosine (8) is a precursor of the hydroaromatic C_6-C_2 unit and phenylalanine (9) is the aromatic C_6-C_1 unit precursor. These incorporations were shown to be highly specific. The tyrosine was never incorporated into the aromatic C_6-C_1 unit and the phenylalanine was shown to be incorporated only into the aromatic C_6-C_1 unit.



8



9

There have been two pathways[2] proposed for the incorporation of phenylalanine into the C_6-C_1 unit of the Amaryllidaceae alkaloids: (a) phenylalanine ⟶ phenylserine ⟶ benzaldehyde ⟶ protocatechuic aldehyde and (b) phenylalanine ⟶ trans-cinnamic acid (10) ⟶ caffeic acid (11) ⟶ protocatechuic aldehyde (13). The data in Table 2, shows negligible incorporation of phenylserine and benzaldehyde compared with protocatechuic aldehyde and trans-cinnamic acid. The data suggests that phenylalanine is incorporated into the C_6-C_1 unit by path (b) as shown in Figure 3.

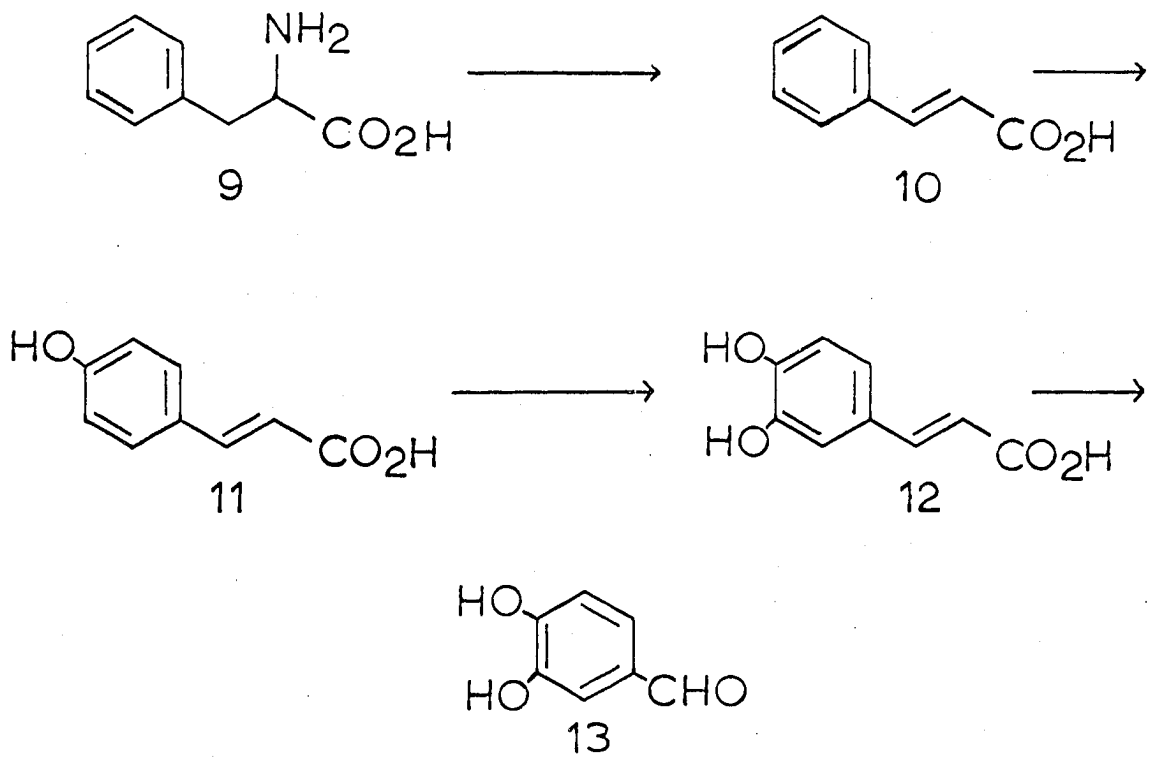


Figure 3. Incorporation of phenylalanine into the C_6-C_1 unit of the Amaryllidaceae alkaloids via trans-cinnamic acid

Table 1. Incorporation of amino acids into Amaryllidaceae alkaloids

Precursor	Plant	Alkaloid	% Inc.	Ref.
DL-2-^{14}C-Tyrosine	*Narcissus* "Twink"	Haemanthamine	0.08	3,4
		Norpluviine	0.15	3,5
		Caranine	0.08	3
		Lycorine	0.23	3,5
	Narcissus "King Alfred"	Galanthamine	0.013	6
	Narcissus "Texas"	Haemanthamine	0.13	6
		Lycorine	0.44	6
	Narcissus "Deanna Durbin"	Lycorine[a]	-----	7
DL-3-^{14}C-Tyrosine	*Sprekelia formossisima*	Haemanthamine[a]	-----	8
		Haemanthidine	0.20	8
		Tazettine	0.16	8
	Haemanthus natalensis	Haemanthamine	0.97	9
		Haemanthidine	0.19	9
		6-Hydroxycrinamine	1.18	9
	Nerine bowdenii	Lycorine	0.11	10
		Belladine	0.82	10
DL -2-^{14}C-Phenylalanine	*Narcissus incomparibilis*	Lycorine	0.00	11

[a]Percentage of incorporation was either not reported or quoted in figures that could not be converted to per cent incorporation. In all cases, appreciable incorporations were evident.

Table 1. (Continued)

Precursor	Plant	Alkaloid	% Inc.	Ref.
DL-3-^{14}C-Phenylalanine	*Narcissus incomparibilis*	Lycorine	0.18	11
	Nerine bowdenii	Belladine	0.42	10
		Lycorine	0.095	10
	Narcissus "Deanna Durbin"	Lycorine[a]	-----	7
		Pluviine[a]	-----	7
		Haemanthamine[a]	-----	7
		Galanthine[a]	-----	7

Table 2. Incorporation of possible C_6-C_1 unit precursors into Amaryllidaceae alkaloids

Precursor	Plant	Alkaloid	% inc.	Ref.
DL-3-^{14}C-Phenylserine	Narcissus pseudonarcissus	Haemanthamine[a]	-----	2,12
	Narcissus incomparabilis	Lycorine	0.00	12
7-^{14}C-Benzaldehyde	Narcissus pseudonarcissus	Haemanthamine	0.00	2
	Sprekelia formossisima	Tazettine	0.005	13
p-Hydroxy-7-^{14}C-benzaldehyde	Narcissus pseudonarcissus	Haemanthamine	0.00	2,12
Trans-3-^{14}C-cinnamic acid	Narcissus pseudonarcissus	Haemanthamine[a]	-----	2,12
Sodium 3-^{14}C-cinnamate	Nerine bowdenii	Lycorine	0.02	13
3-^{14}C-p-Hydroxycinnamic acid	Narcissus pseudonarcissus	Haemanthamine[a]	-----	2,14
	Nerine bowdenii	Lycorine	3.1	15
3-^{14}C-Caffeic acid	Narcissus incomparabilis	Haemanthamine[a]	-----	14,16
		Lycorine[a]	-----	14,16
^{3}H-Protocatechuic aldehyde	Narcissus incomparabilis	Lycorine	0.23	17,18
7-^{14}C-Protocatechuic aldehyde	Narcissus incomparabilis	Haemanthamine[a]	-----	16
		Lycorine[a]		
7-^{14}C-Protocatechuic acid	Narcissus "Deanna Durbin"	Lycorine	0.002	3
		Norpluviine	0.001	3

[a]Percentage of incorporation was either not reported or quoted in figures that could not be converted to per cent incorporation. In all cases, appreciable incorporations were evident.

The incorporation of tyrosine (Table 1) and tyramine (24)[3,19] into the Amaryllidaceae alkaloids suggests that tyrosine is decarboxylated to tyramine which then serves as the C_6-C_2 unit.

Barton and Cohen[20] proposed the theory that many natural products originate from the ortho-ortho, ortho-para or para-para oxidative coupling of phenol radicals. They postulated that the Amaryllidaceae alkaloids originate from the phenyl-phenyl oxidative coupling of the precursor norbelladine (14a). At the time that they postulated this precursor, the alkaloid belladine (14b) was unknown. The subsequent isolation of belladine[21] from Nerine bowdenii and Amaryllis belladonna lent support to their hypothesis. Barton and Cohen derived[20] a biogenetic scheme for the formation of lycorine (16), caranine (15) and galanthamine (18) (Figures 4 and 5). In vitro chemical verification for their hypothesis was obtained when galanthamine was formed in a synthesis from the norbelladine derivative 17 by manganese dioxide oxidation followed by lithium aluminum hydride reduction[22].

The biosynthesis of tazettine (21) via phenyl-phenyl oxidative coupling proved difficult to explain. However, recent studies[23] have shown (Figure 6) that haemanthamine (19) and haemanthidine (20) are precursors of tazettine in Sprekelia formosissima. Tritium-labeled haemanthamine afforded radioactive haemanthidine and tazettine three days after feeding. Tritiated haemanthidine formed radioactive

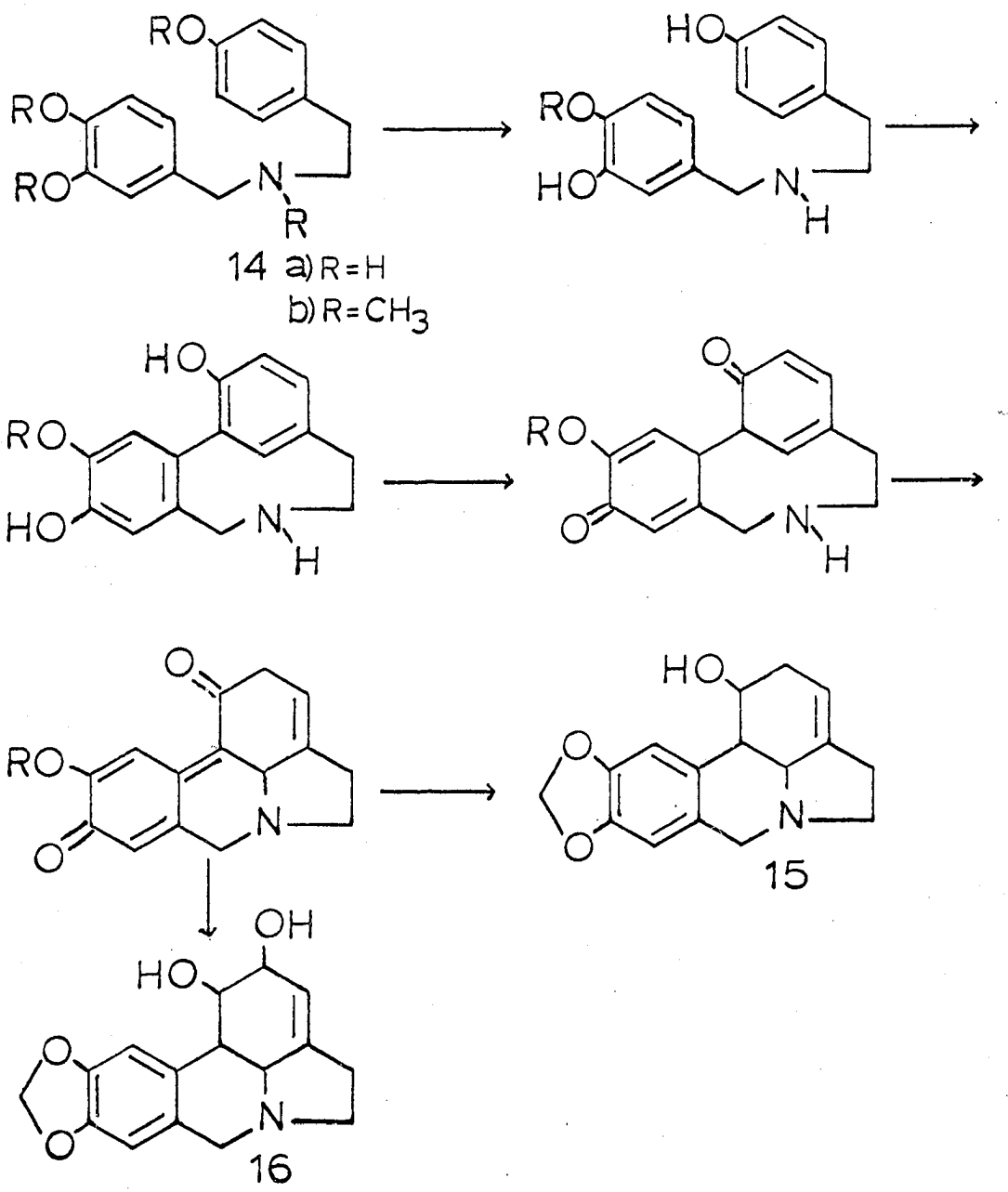


Figure 4. Biogenesis of lycorine and caranine (Barton and Cohen)

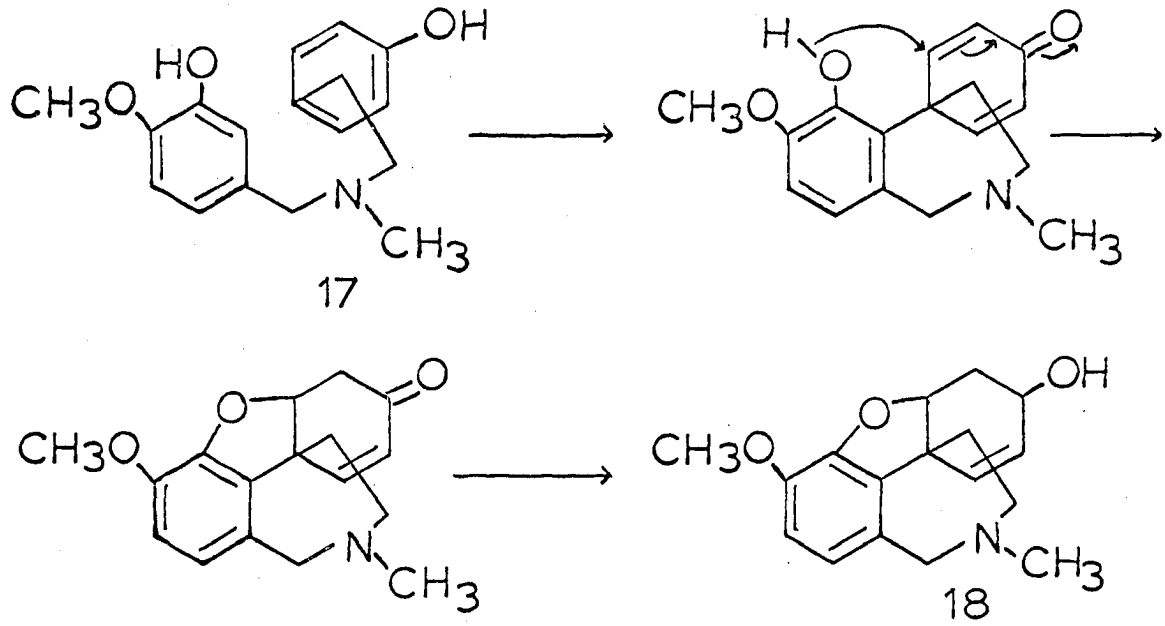


Figure 5. Biogenesis of galanthamine (Barton and Cohen)

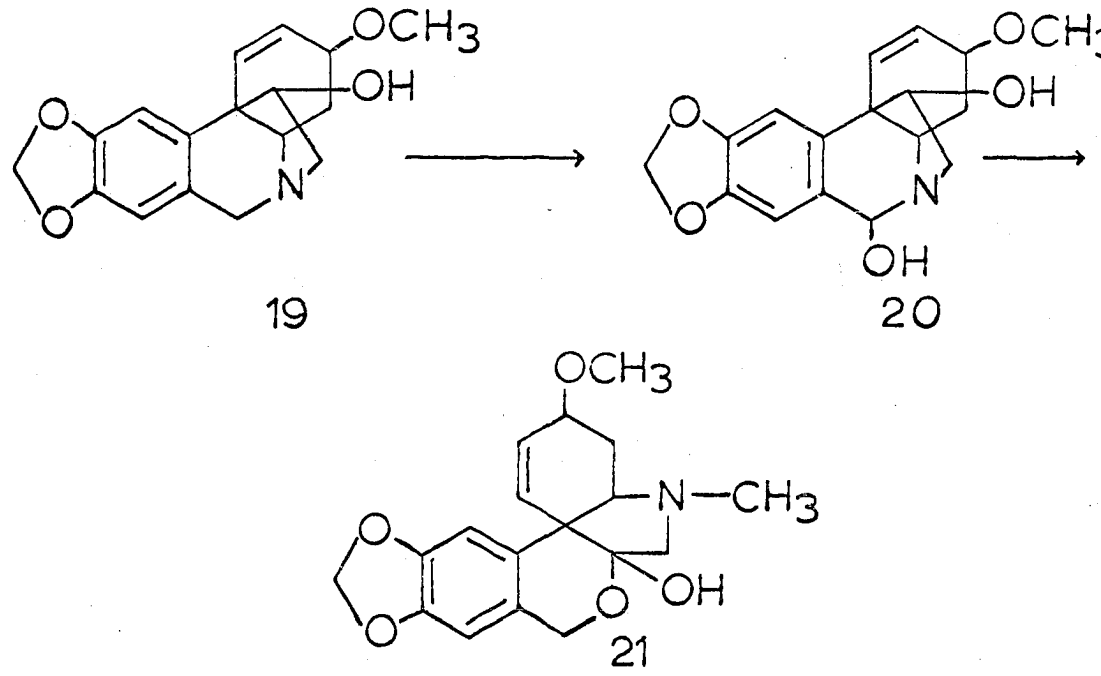
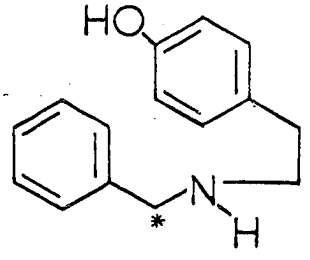


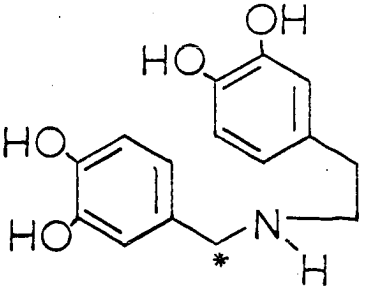
Figure 6 Conversion of haemanthamine to tazettine <u>in vivo</u> (Fales and Wildman)

tazettine but the haemanthamine was found to be inactive. Tritiated tazettine was not converted in significant quantities to either haemanthamine or haemanthidine.

Further biosynthetic studies, summarized in Table 3, have shown double-and triple-labeled norbelladine derivatives to be incorporated intact into the Amaryllidaceae alkaloids. The incorporation data and chemical degradations of the radioactive alkaloids support the theory of phenyl-phenyl oxidative coupling of a norbelladine-type precursor. Studies carried out by Wildman and Battersby[13] have shown negligible incorporation of 1-^{14}C-bisdeoxynorbelladine (22) and of 1'-^{14}C-hydroxynorbelladine (23) into haemanthamine, tazettine and lycorine type alkaloids (Table 3). These data suggest that the aromatic ring in the C_6-C_1 unit must be di-oxygenated and the aromatic ring in the C_6-C_2 unit must be mono-oxygenated prior to the phenyl-phenyl oxidative coupling step. The combined biosynthetic pathway for the incorporation of phenylalanine and tyrosine into norbelladine is given in Figure 7.



22



23

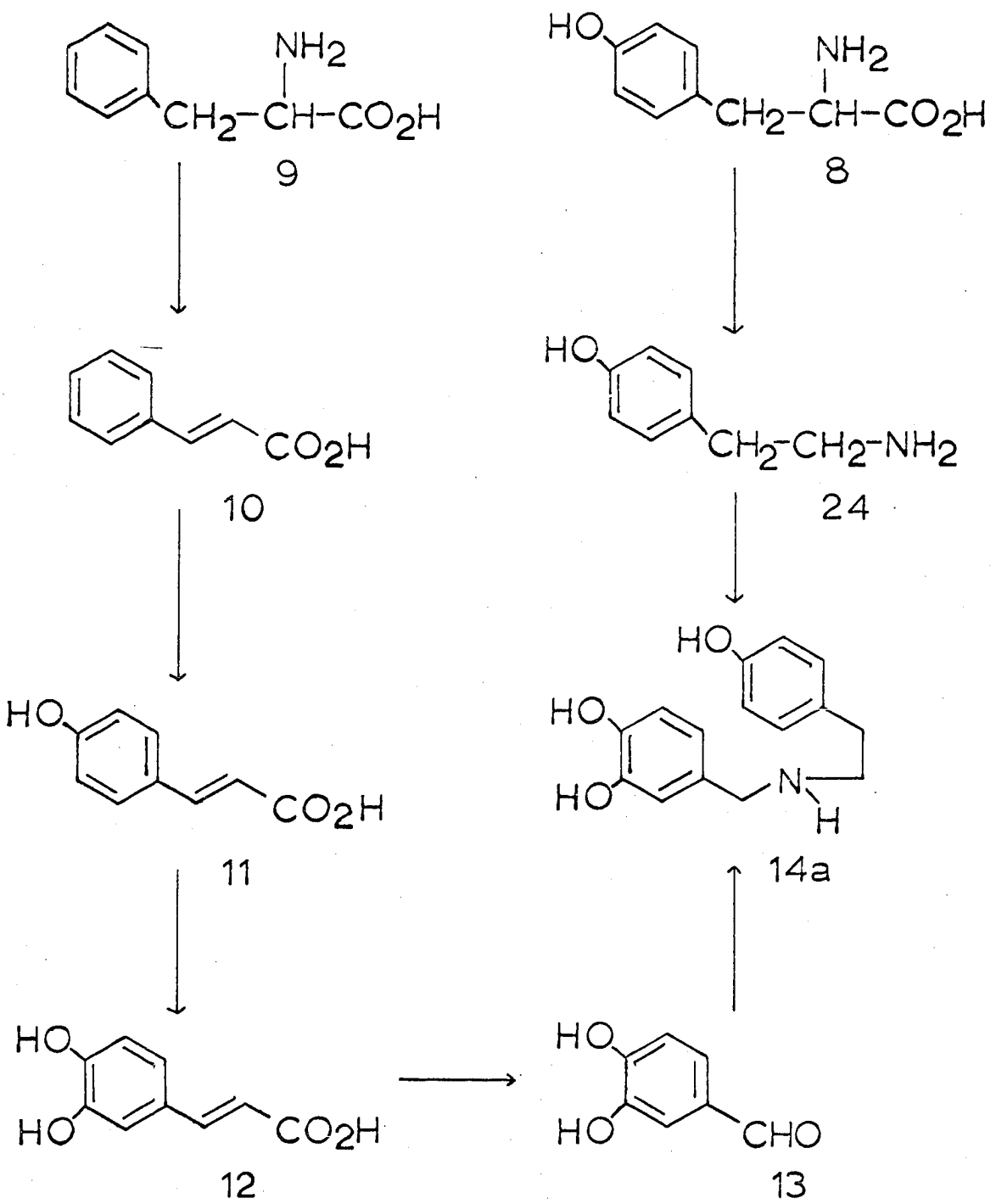


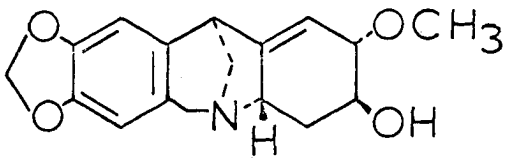
Figure 7. Incorporation of phenylalanine and tyrosine into norbelladine

Table 3. Incorporation of norbelladine and norbelladine derivatives into Amaryllidaceae alkaloids

Precursor	Plant	Alkaloid	% inc.	Ref.
1-^{14}C-Norbelladine	Narcissus "Twink"	Lycorine	0.24	24
		Norpluviine	0.74	24
		Haemanthamine	0.15	4
1-1'-^{14}C-Norbelladine	Nerine bowdenii	Lycorine	0.07	25
		Crinamine	0.0009	25
		Belladine	2.64	25
O-^{14}C-Methyl-N-^{14}C-methyl-1-^{14}C-norbelladine	Narcissus "King Alfred"	Galanthamine	0.018	26
		Galanthine	0.00	26
		Haemanthamine	0.00	26
1'-^{14}C-Bisdeoxynorbelladine	Narcissus "Twink"	Tazettine	0.00	13
		Haemanthamine	0.00	13
1'-^{14}C-Hydroxynorbelladine	Narcissus "Twink"	Lycorine	0.0007	13
		Norpluviine	0.0017	13

Chemistry and Biosynthesis of Montanine

The structure of montanine (25), a major alkaloid of Rhodophiala bifida[27] and various Haemanthus species[28], was determined by W. C. Wildman and co-workers by degradative and spectroscopic methods[29]. The absolute configuration of the montanine-type alkaloids was derived from the known



25

structures of haemanthamine (19) and crinamine (27)[29]. Mesylation of haemanthamine and treatment with aqueous alkali afforded a sulfur-free isomer of haemanthamine. The physical and spectral data indicated that the haemanthamine ring system had undergone a rearrangement to give a compound similar to, but not identical with, montanine (25) or coccinine (31). Chemical and spectral characterization established this isomer to be isohaemanthamine (26). When crinamine was treated with mesyl chloride and pyridine under the same conditions, two rearranged products (28 and 29) resulted. These differed only in the configuration of the hydroxyl group, since manganese dioxide oxidation of each gave the same methoxy ketone (30). These rearrangement reactions are illustrated in Figure 8.

The mesylate group appeared essential for the rearrangement since haemanthamine and crinamine formed normal O-acetyl derivatives in the presence of pyridine and acetic anhydride[28,30].

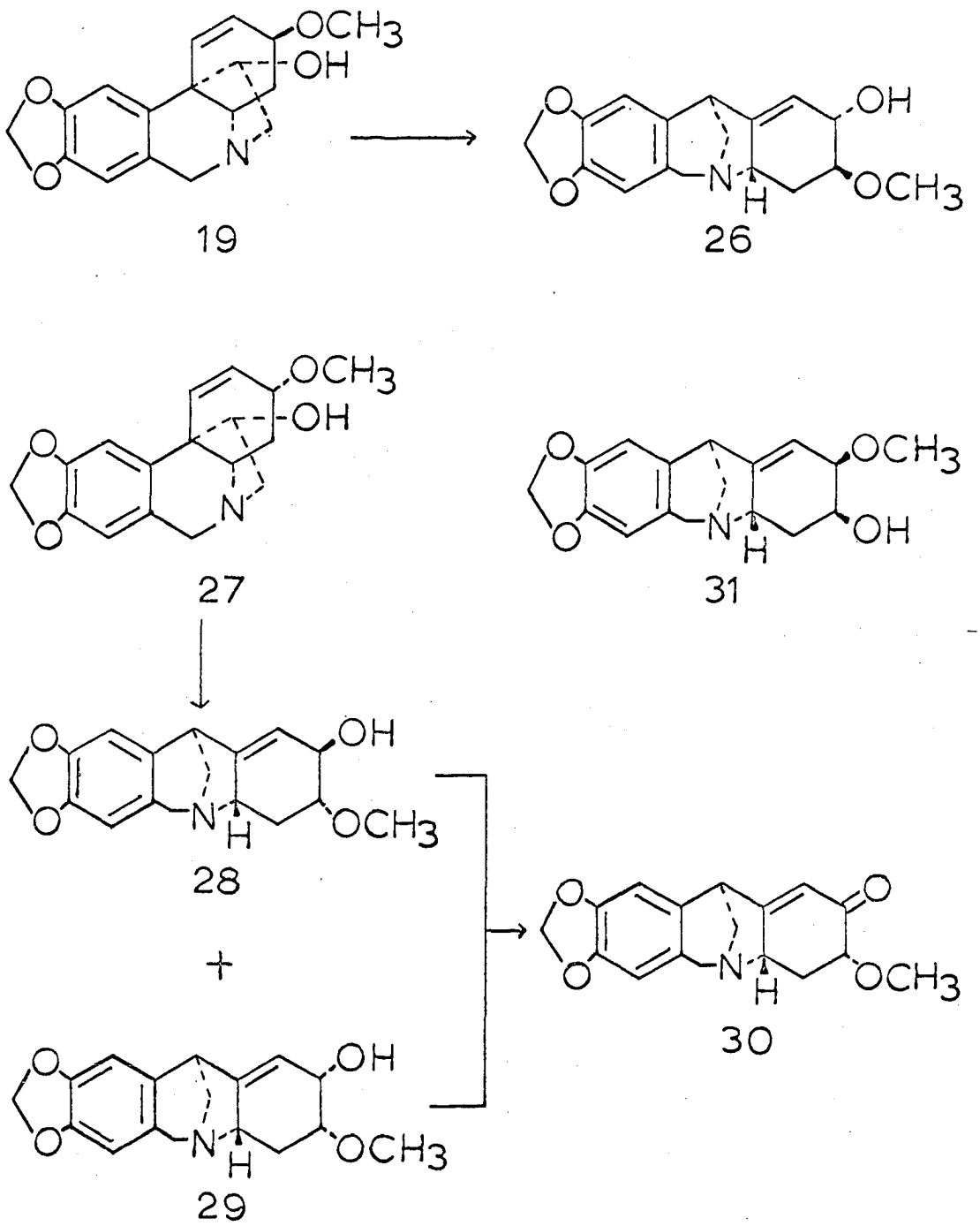
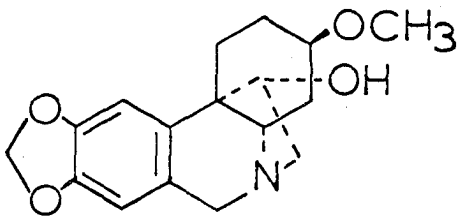
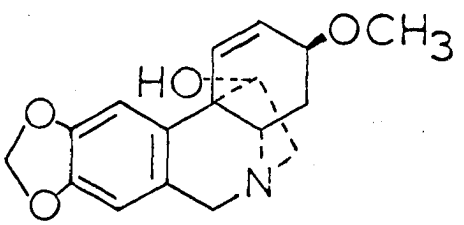


Figure 8. Rearrangements to montanine ring system

The rearrangement did not occur with dihydrohaemanthamine (32) or epihaemanthamine (33). The presence of a double bond and a specific configuration of the hydroxyl group appeared to be a prerequisite for the rearrangement.



32



33

A proposed mechanism[29] for the rearrangement involved the formation of a normal mesylate in the first step. Inspection of structure 34 indicates that the aryl group is ideally situated for nucleophilic displacement of the mesylate. The migration of the aryl group is reasonable because it is a relatively rich source of electrons at a stereochemical position which is trans-antiparallel to the departing mesylate group. The rearrangement is thought to be completed by attack of base at C_2 and migration of the double bond (Figure 9). The rearrangement of 34 has also been considered to proceed through $aryl_{1-3}$ participation[31] which would lead to an intermediate aronium ion (36). Attack of base at C_2 would then lead to 35.

The mesylate of haemanthamine (34, R=OCH_3, R_1=H) underwent attack by base on the side away from the pseudoaxial methoxyl group giving a product with a trans relationship of the functional groups at C_2 and C_3. In the mesylate of

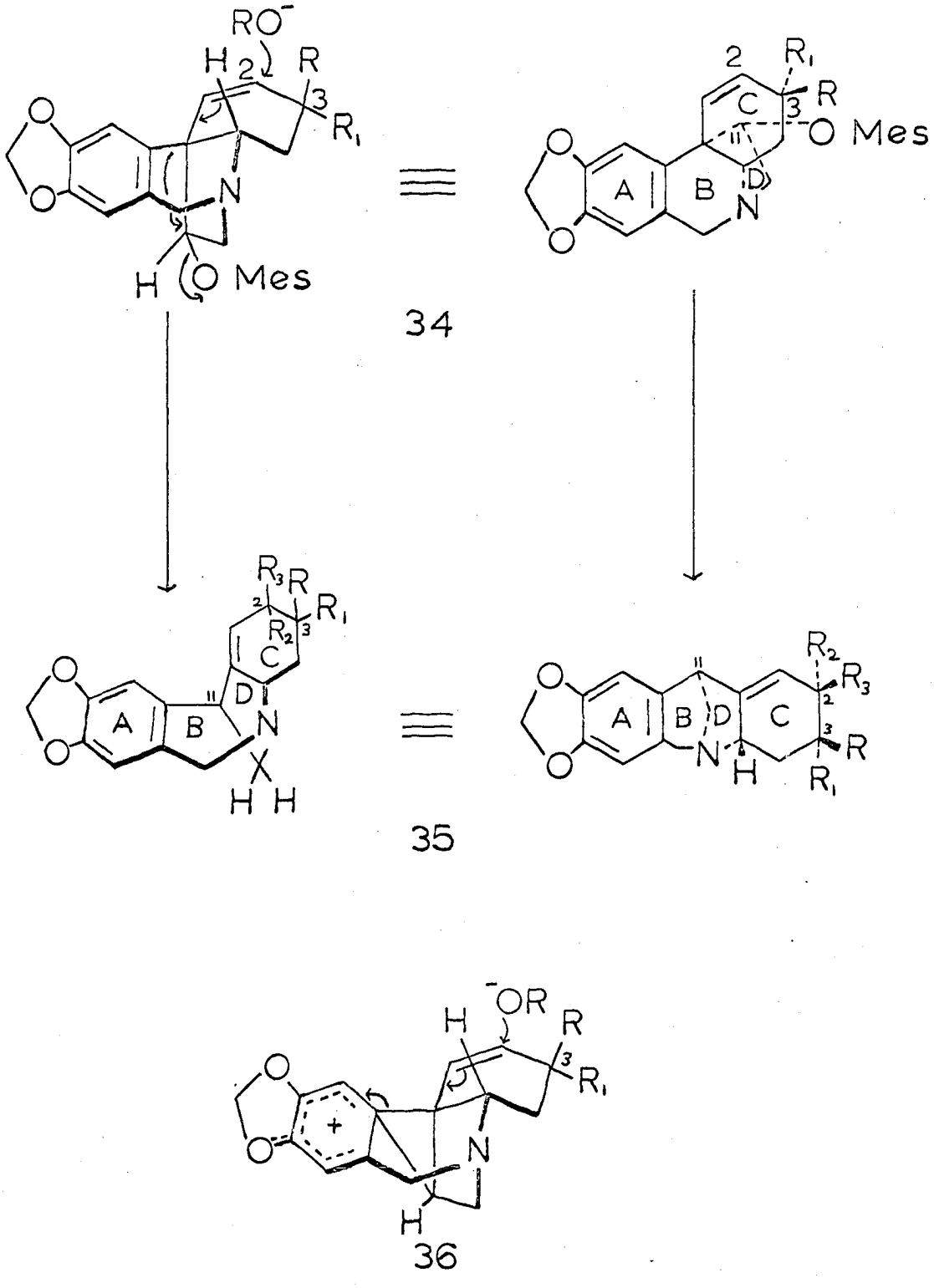
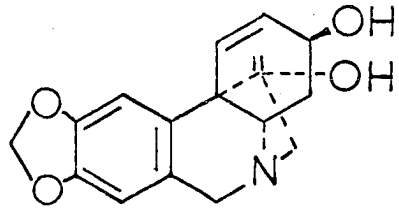


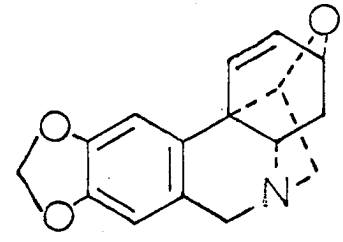
Figure 9. Rearrangement mechanism to montanine ring system

crinamine (34, R=H, R_1=OCH_3), the methoxyl group has a pseudo-equatorial configuration allowing attack of base on both sides of the molecule and two isomers (28 and 29) were formed.

The alkaloid 11-hydroxyvittatine (37) was recently isolated from P. maritimum[32] and Rhodophiala bifida[33]. During the chemical characterization of this base, it was observed that the 0,0-diacetyl derivative was converted to apohaemanthamine (38) upon mild treatment with mineral acid[33]. The hydroxyl group at C_{11} was assigned the configuration

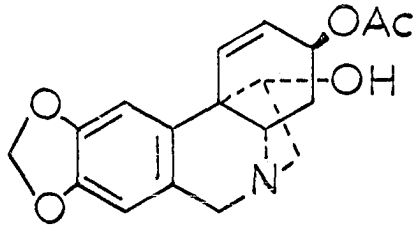


37

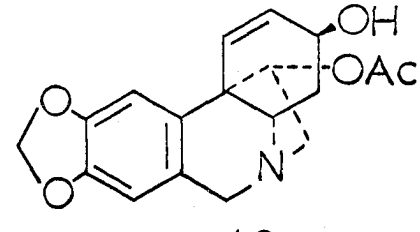


38

depicted in 37 because epihaemanthamine (33) gave very poor yields of 38 with acid[34]. Mild hydrolysis of the 0,0-diacetyl derivative of 37 furnished a separable mixture of acetates (39 and 40). Mesylation of 39 followed by treatment with methanolic sodium methoxide gave a good yield of montanine (25).



39



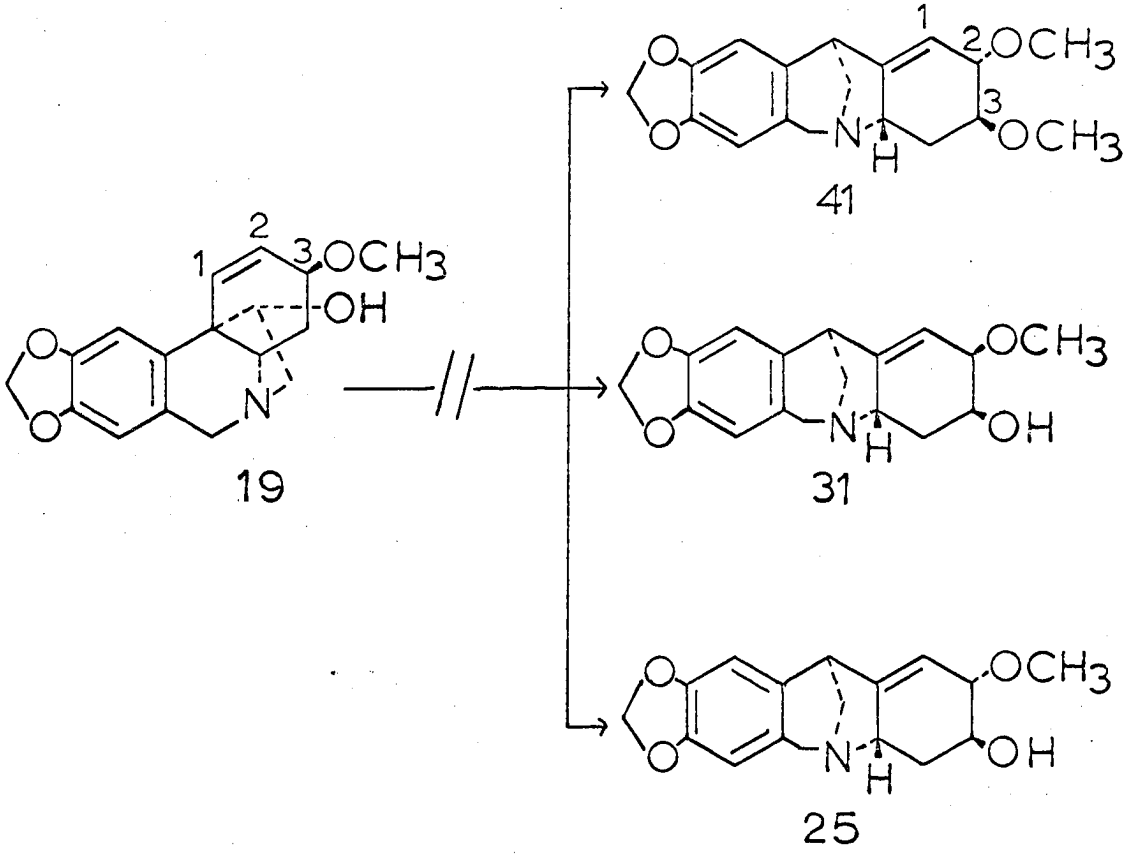
40

The *in vitro* rearrangements of 11-hydroxyvittatine and haemanthamine to the montanine nucleus stimulated research concerning the biosynthesis of the montanine-type alkaloids. Early tracer work dealt with amino acid feedings along with a study of the biosynthetic relationship between haemanthamine and the montanine-type alkaloids. Radioactively-labeled tyrosine and phenylalanine were fed to blooming *Haemanthus coccineus* and the phenylalanine was found to be incorporated into coccinine (31)[35]. The incorporation of tyrosine was too low to indicate significant incorporation. Because of the low activity of the alkaloids isolated from these feeding experiments, no degradations were carried out. In another experiment[36] tyrosine and phenylalanine were fed to *Rhodophiala bifida* and were shown to be incorporated into montanine (Table 4). The montanine isolated from this feeding was degraded and the precursors were shown to be incorporated intact. Although montanine is derived from the same C_6-C_1 and C_6-C_2 units as haemanthamine, the montanine nucleus cannot be formed directly by phenyl-phenyl oxidative coupling of a norbelladine-type precursor. Since the mesylate of haemanthamine was found to rearrange in dilute alkali to the montanine ring system, it seemed possible that montanine-type alkaloids were synthesized in the plant by a similar type of rearrangement. To examine this hypothesis, ar-tritiated haemanthamine (19) was prepared and fed to blooming *Haemanthus coccineus*[35]. This plant is

Table 4. Incorporation of amino acids into montanine-type alkaloids

Precursor	Plant	Alkaloid	Specific Activity dpm/mM	% inc.	Ref.
DL-3-^{14}C-Phenylalanine	Haemanthus coccineus	Coccinine	1.11×10^5	0.03	35
	Rhodophiala bifida	Montanine	1.24×10^6	0.023	36
		Haemanthamine	6.17×10^6	0.012	36
DL-3-^{14}C-Tyrosine	Rhodophiala bifida	Montanine	9.10×10^4	0.0015	36
		Haemanthamine	42.9×10^4	0.0011	36

known to contain the alkaloids manthine (41), montanine, coccinine and haemanthamine. The plants were processed after one month and no alkaloid containing the montanine nucleus was found to be radioactive. This feeding experiment suggested that haemanthamine is not a precursor of the montanine-type alkaloids. The methoxyl group at C_3 of haemanthamine would appear to be a likely point of interference in the <u>in vivo</u> rearrangement, since montanine and coccinine have a free hydroxyl group at the equivalent position. If haemanthamine was to rearrange to these alkaloids it would have to undergo a demethylation step either before or after rearrangement.

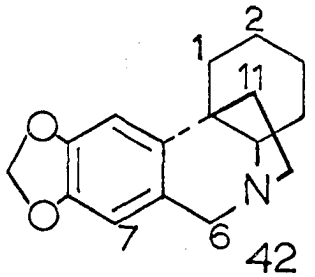


Demothylations of this type have never been observed in the biosynthesis of the Amaryllidaceae alkaloids. Manthine, which contains methoxyl groups at C_2 and C_3, was also found to be devoid of radioactivity. This finding seems to indicate that the oxygen function at the C_3 position of manthine is being methylated after the rearrangement step.

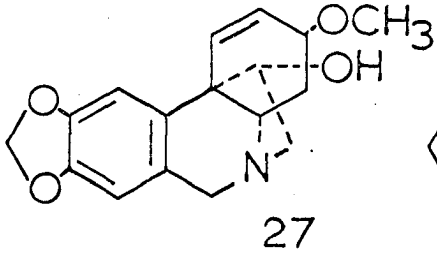
Hydroxylated C_6-C_2 Units as Possible Precursors to Amaryllidaceae Alkaloids

Late stage hydroxylations

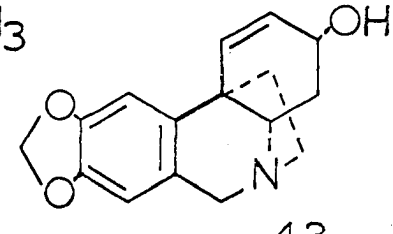
Approximately eleven alkaloids isolated from Nerine bowdenii are based on the (-)-crinane (42) ring system[37]. They differ only in the level of oxidation at C_1, C_2, C_6, C_7 and C_{11}. Crinamine (27) and (+)-epicrinine (43) are also present in N. bowdenii but possess ring systems which are enantiomeric with that of (-)-crinine (44). If the C_3-hydroxyl of (+)-epi-



42

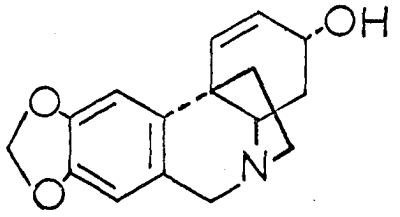


27

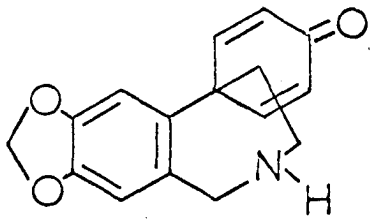


43

crinine and crinine (44) could be converted to a ketone in the plant, the enantiomers might be interconvertible via the symmetrical dienone (45). To examine this possibility, ar-tritiated crinine was fed to N. bowdenii[38]. The plants were processed after three weeks and all alkaloids derived from the

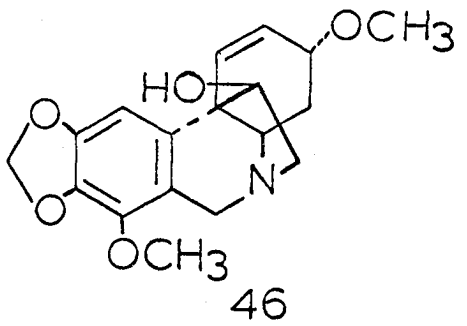


44



45

(-)-crinine nucleus were found to be radioactive. Neither crinamine nor (+)-epicrinine was radioactive. These results eliminated the possibility that crinine can revert to 45. One of the radioactive alkaloids isolated in this experiment was ambelline (46). This finding indicated that C_{11}-hydroxylation can occur after phenyl-phenyl oxidative coupling takes place.

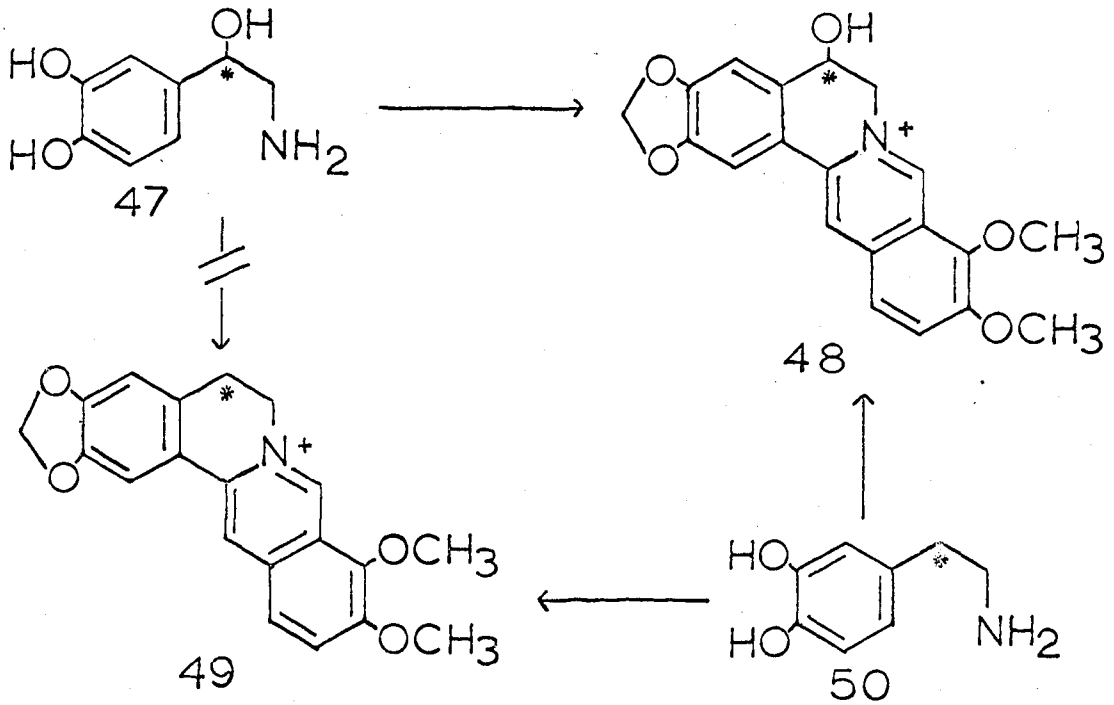


46

Early stage hydroxylations

Berberastine (48) was isolated from the extracts of Hydrastis canadensis[39]. This alkaloid differs from berberine (49) by an additional hydroxyl group located at a site which, in the biosynthesis of berberine, is derived from the benzylic

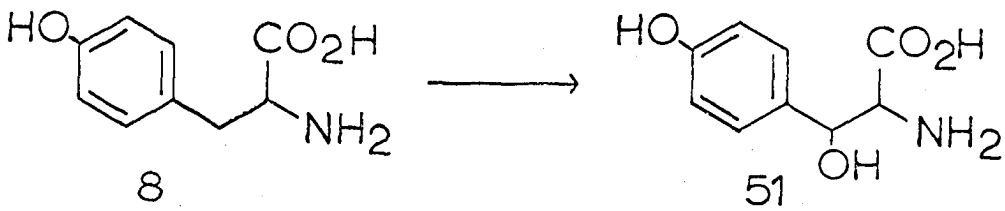
carbon atom of 3,4-dihydroxyphenylethylamine (50)[40]. When either 1-^{14}C-dopamine or DL-2-^{14}C-noradrenaline (47) was fed to plants of H. canadensis, berberastine of high specific activity was obtained[41]. Berberine derived from noradrenaline-fed plants was found to be almost devoid of radioactivity.



The noradrenaline-derived berberastine was degraded and the radioactive carbon was shown to be confined to the predicted site. The specific activity of dopamine-derived berberastine was found to be substantially higher than that of the berberine isolated from the same experiment. This finding indicated that berberine was not a precursor of berberastine. Noradrenaline has been found in a number of plants[42] and a dopamine-β-hydroxylase preparation has been obtained from a plant source[43]. These

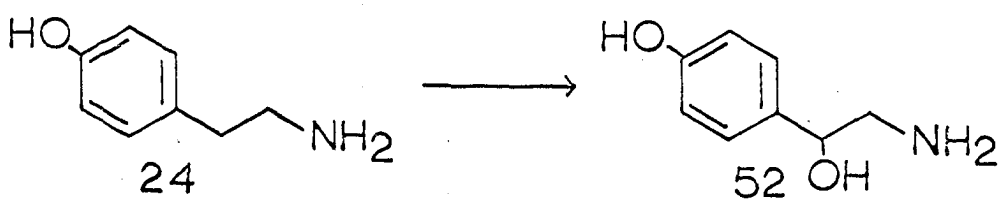
results showed that a β-phenylethylamine derivative was undergoing β-hydroxylation and then followed a different biosynthetic path than its non-hydroxylated counterpart.

The β-hydroxylation of tyrosine and tyramine has been demonstrated in living systems[44-46]. In Sorghum vulgare tyrosine was shown to be converted to p-hydroxyphenylserine (51) by radioactive labeling experiments[44].



8 51

The enzyme, 3,4-dihydroxyphenylethylamine-β-oxidase, was found not to be specific, but was able to catalyze the oxidation of tyramine to octopamine (52)[45]. This β-oxidation process was found to be very rapid in beef adrenal medulla. This oxidation was also observed to take place in mice hearts[46]. Octopamine was first extracted from the salivary glands of the octopus[47]. It was later isolated from human and animal urine[48]. Recently, octopamine was isolated from lemon leaves[49]. This was the first time that this amine was obtained from a plant source.



24 52

Metabolism of β-Phenylethanolamines

The most definitive studies on the metabolism of β-phenylethanolamines have been carried out with adrenaline (53) and noradrenaline (47)[50,51]. In man, both substances are rapidly metabolized by two mechanisms, O-methylation and oxidative deamination[50]. The liver is the major site for these transformations. The principal metabolites of adrenaline and noradrenaline found in urine are 3-methoxy-4-hydroxymandelic acid (60), 3-methoxyepinephrine (55) and 3-methoxy-4-hydroxyphenylglycol (59). The corresponding catechols, 3,4-dihydroxymandelic acid (57), 3,4-dihydroxyphenylglycol (56) and adrenaline are minor excretion products. The human metabolism of adrenaline and noradrenaline is illustrated in Figure 10.

The metabolism of these hormones in the guinea pig was found to differ markedly from that in man. Tracer studies have shown[51] that adrenaline and noradrenaline are oxidized to adrenalone (61) and noradrenalone (62) respectively. There is no interconversion of the adrenaline derivatives with their noradrenaline counterparts. Both adrenaline and noradrenaline undergo oxidative deamination to 3,4-dihydroxyphenylglyoxal (63). The glyoxal is then fragmented to protocatechuic aldehyde (13) which in turn is oxidized to protocatechuic acid (64). The metabolism scheme is summarized in Figure 11.

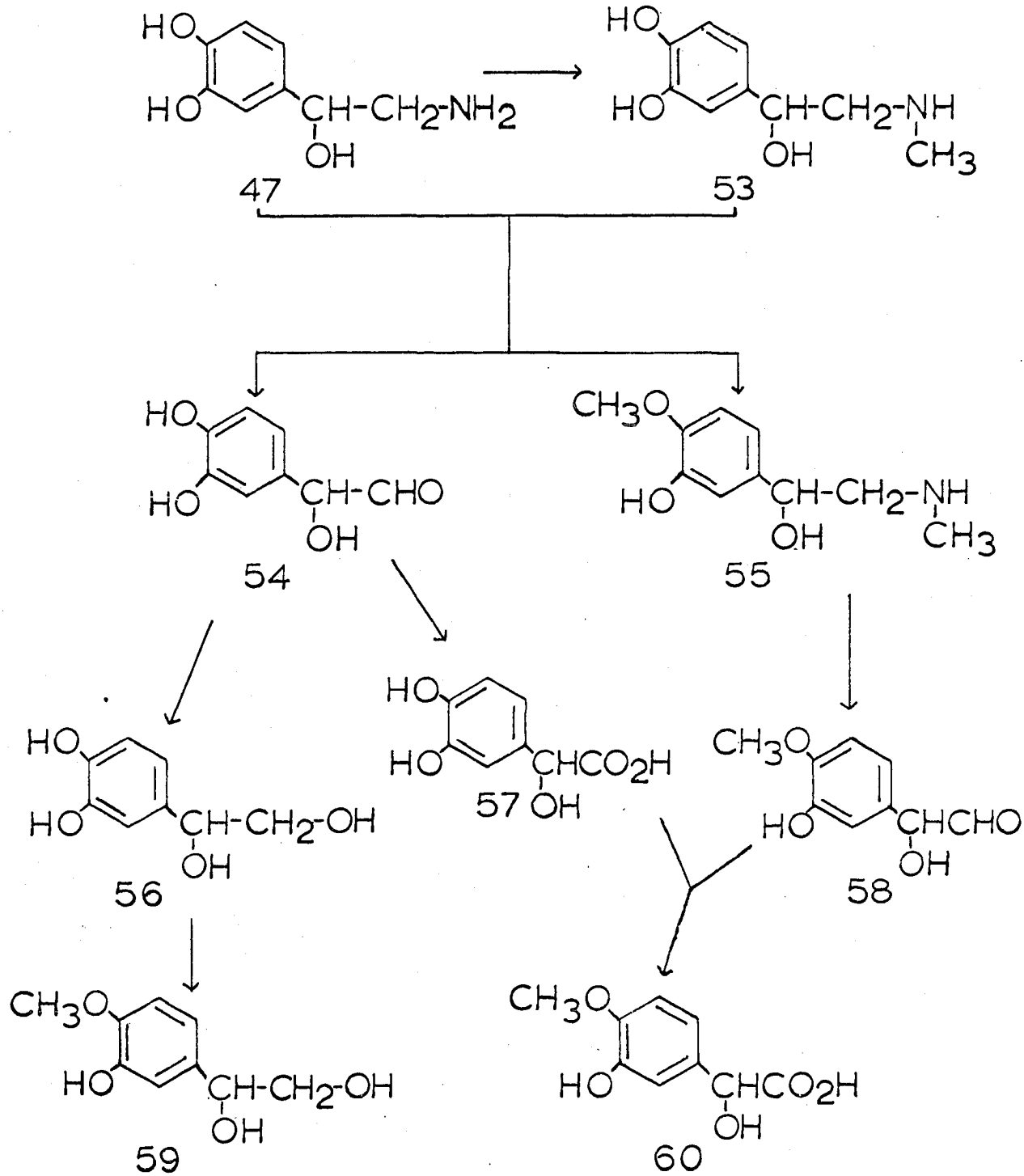


Figure 10. Metabolism of adrenaline and noradrenaline in man

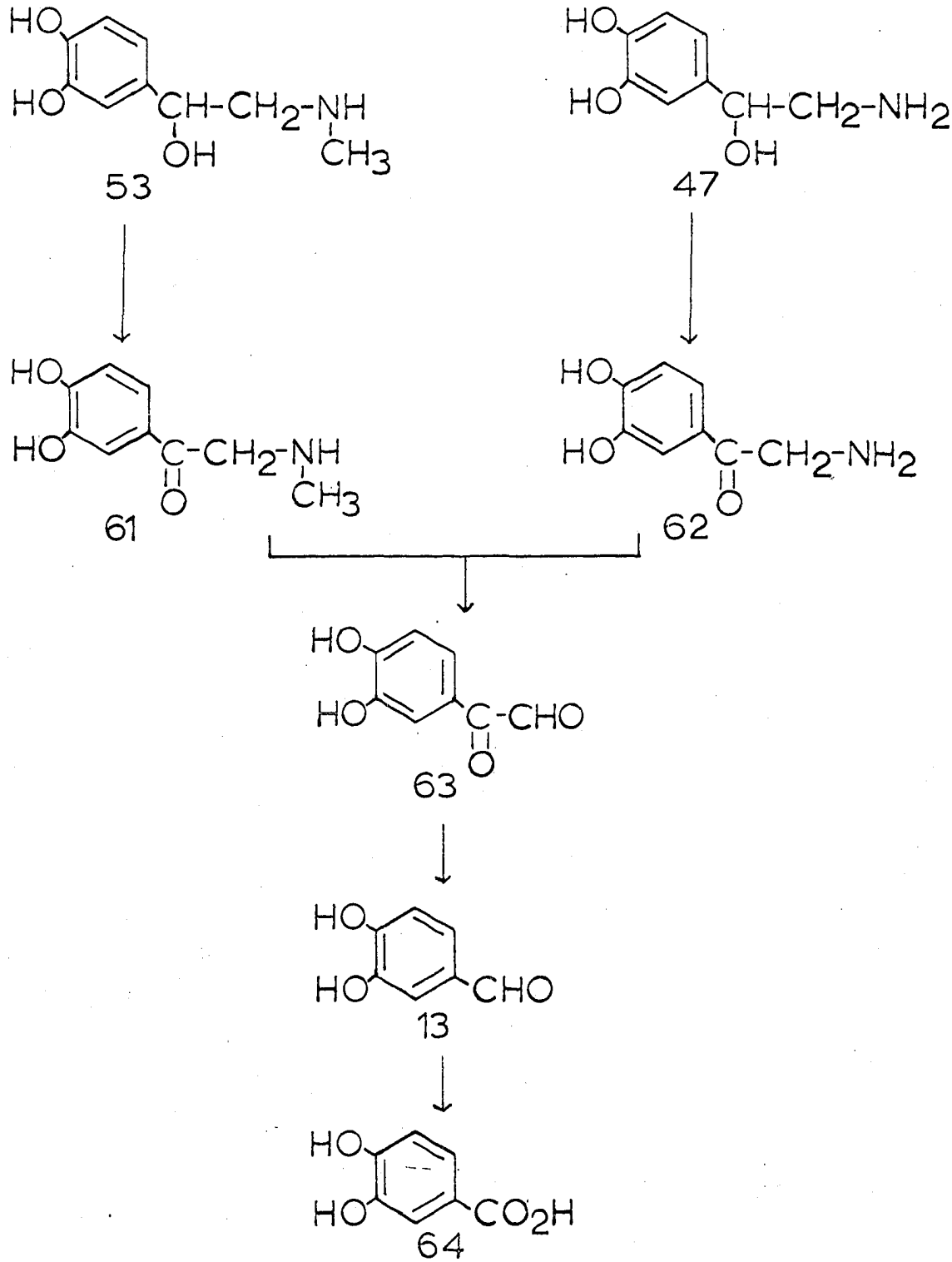


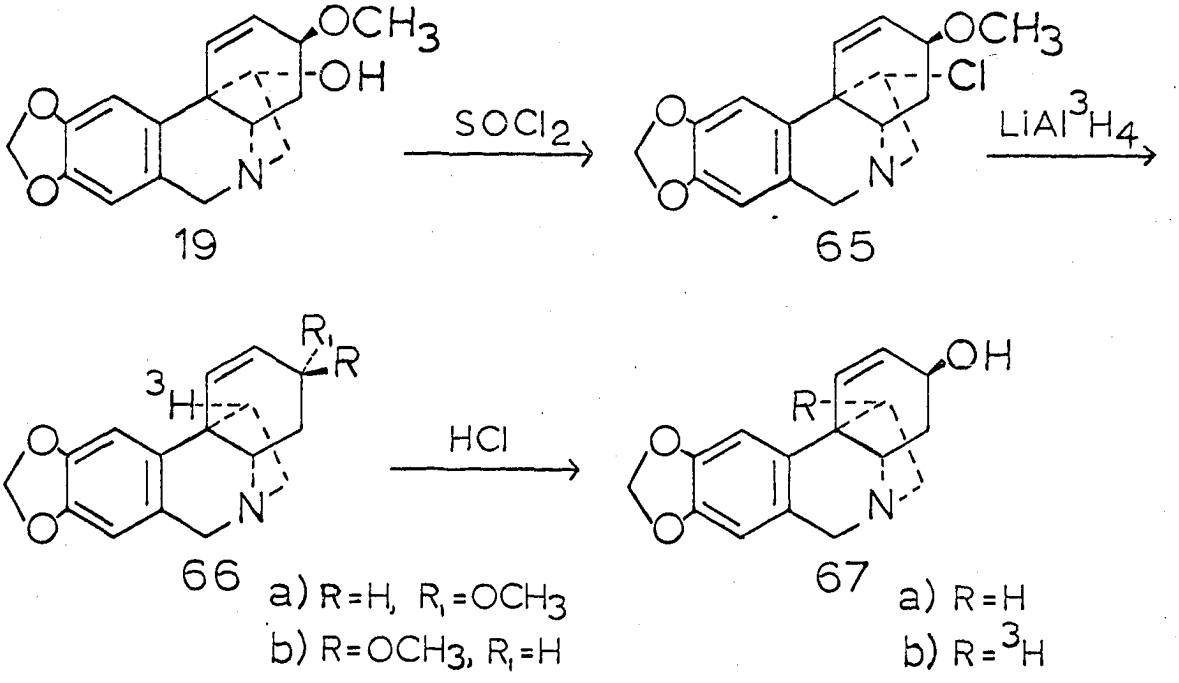
Figure 11. Metabolsim of adrenaline and noradrenaline in the guinea pig

RESULTS AND DISCUSSION

The *in vitro* studies on the rearrangement of haemanthamine-type alkaloids to alkaloids containing the montanine nucleus led to the hypothesis that a similar rearrangement might also occur in the plant[35]. The failure of the conversion of haemanthamine to manthine or montanine in *H. coccineus*[35] and the production of haemanthamine from phenylalanine and tyrosine-fed plants[36] possessing a higher specific activity than montanine, suggests that haemanthamine and montanine are derived from a common precursor. The object of this investigation is to determine the biosynthetic pathway by which the montanine nucleus is formed and to establish the relationship between the biosynthesis of haemanthamine and montanine.

To carry out this biosynthetic study, suitable radioactively-labeled precursors for montanine had to be found. All previous research in this area suggested that 11-hydroxyvittatine was a likely precursor to the montanine-type alkaloids. This choice was influenced by two recent observations: (a) 11-hydroxyvittatine was isolated from a plant that contains the alkaloids vittatine (67a), haemanthamine and montanine[33], and (b) montanine was formed from the *in vitro* rearrangement of the mesylate of 11-hydroxyvittatine[33]. A precursor in the form of ^{14}C or ^{3}H-11-hydroxyvittatine was needed, but because of the scarcity of 11-hydroxyvittatine, a method was sought to make a ^{3}H-derivative of vittatine. In the

course of this study, an attempt was made to replace the C_{11}-hydroxyl group of crinamine with hydrogen. Although the mesylate of crinamine was found to rearrange to the montanine ring system in the presence of base[29], it was hoped that the reaction of lithium aluminum hydride on the C_{11}-chloride would give rise to some unrearranged product and furnish 66a. The reaction could then be repeated on haemanthamine using lithium aluminum tritide followed by cleavage of the methoxyl group[52] to give tritium-labeled vittatine (67b).



The exploratory work was carried out with crinamine due to the limited supply of haemanthamine that was available. Crinamine was allowed to react with thionyl chloride and the resulting product was reduced without purification, with lithium aluminum hydride. Gas phase chromatographic analysis of the

reduced product showed one major product which accounted for more than 90% of the product mixture. The infrared spectrum of the purified reaction product indicated the absence of a hydroxyl function and contained two bands at 1330 and 1370 cm^{-1} which are characteristic of the montanine-type alkaloids. The nmr spectrum was not in accord with that known for the vittatine nucleus and allowed the postulation of desoxyisocrinamine (68) for the structure of the dehydroxylated crinamine derivative.

The structure of the reaction product was based on a careful analysis and comparison of the nmr and mass spectral data of the compound with that of montanine. The nmr spectra of montanine and desoxyisocrinamine are shown in Figure 12. The striking similarity between the two spectra suggested a common ring system for both compounds. A significant feature of the nmr spectrum of 68 is the presence of only one olefinic proton resonance. The loss of an olefinic hydrogen and a hydroxyl group from C_2 in the conversion of crinamine to 68 is characteristic of the rearrangement of crinamine to isocrinamine.

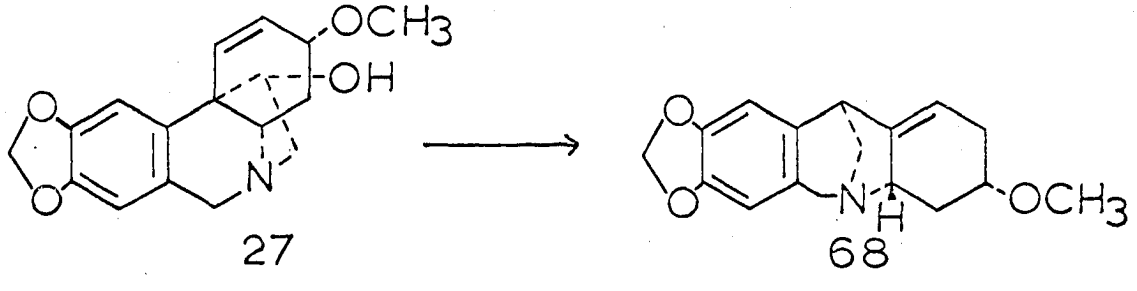
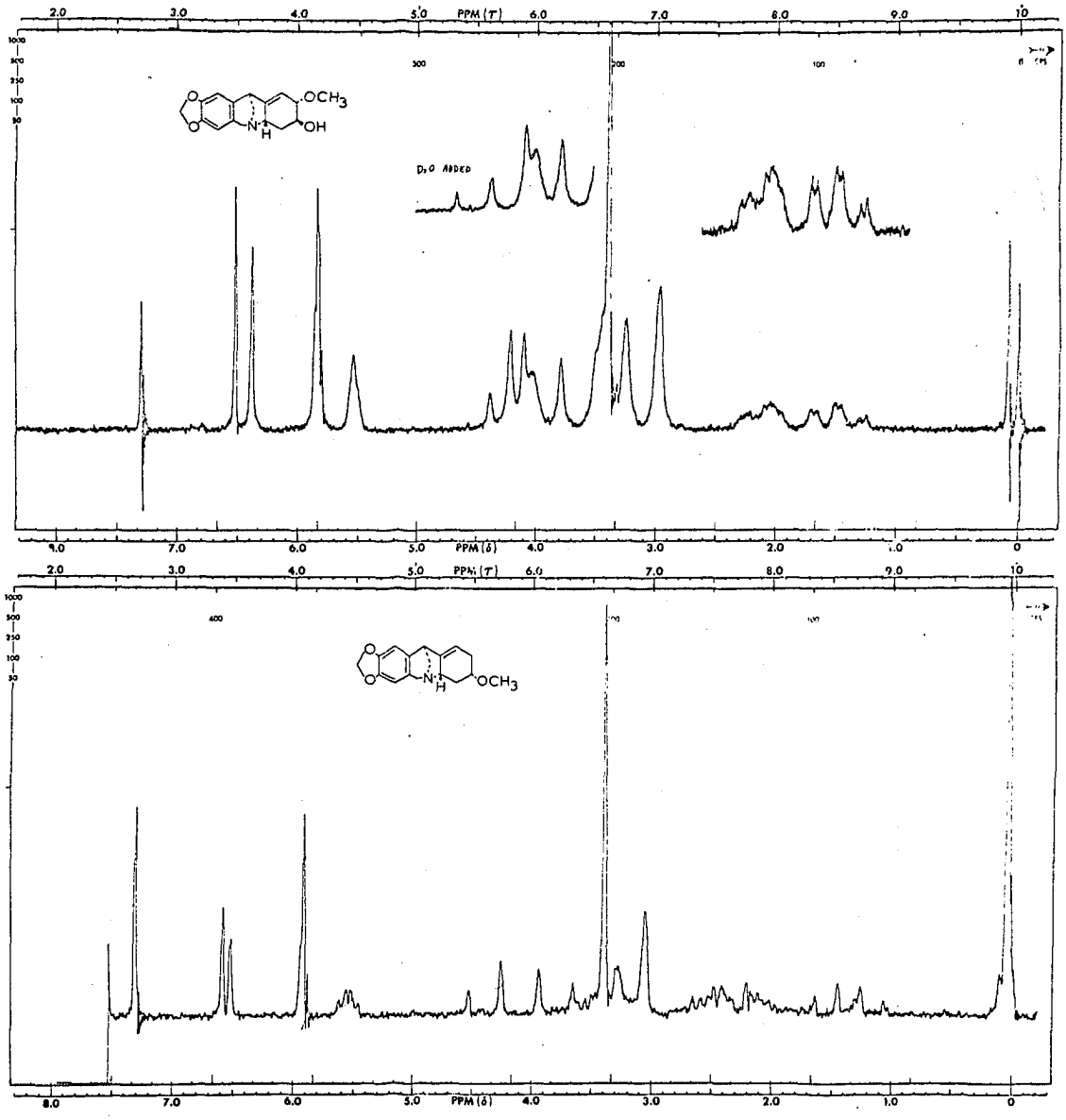


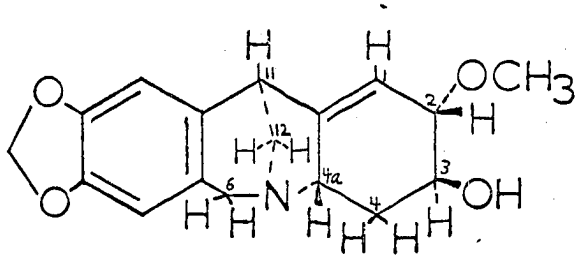
Figure 12. Nuclear magnetic resonance spectra

top - montanine (25)

bottom - desoxyisocrinamine (68)


2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9.0
10
OCH3
OH
N
H
D2O ADDED
9.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0
OCH3
8.0

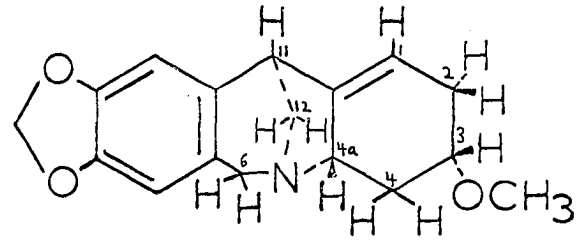
In order to establish the structure of desoxyisocrinamine, a complete analysis of the nmr spectrum of montanine was undertaken. The two singlets in the spectrum of montanine at 6.51 and 6.43 ppm correspond to the two aromatic protons. The singlet at 5.55 ppm is assigned to the olefinic proton resonance. The two protons of the methylenedioxy group appear as a singlet at 5.83 ppm. The peaks between 4.40 and 3.50 ppm were assigned with the aid of spin-spin decoupling experiments. The singlet at 4.21 ppm disappeared when D_2O was added to the sample tube and was therefore assigned to the proton of the hydroxyl group. Decoupling studies showed that the peaks at 4.40, 4.11 and 3.80 ppm are part of an AB pattern centered at 3.95 ppm (J=17 cps). The AB pattern is assigned to the benzylic protons on C_6. The integral of the broad peak centered at 3.35 ppm corresponds to the resonance of five protons. This peak is assigned to the methoxyl proton resonances and the resonance of the protons at C_2 and C_{4a}. This assignment is based on a spin decoupling study which showed the olefinic



25

proton at 5.55 ppm to be coupled to a proton at 3.42 ppm. This effect is attributed to the vicinal coupling between the olefinic proton and the proton at C_2 plus a small contribution arising from the allylic coupling of the proton at C_{4a}. The two broad singlets at 3.25 and 2.98 ppm are assigned to the benzylic proton at C_{11} and the two bridge protons at C_{12} respectively. An examination of the integral of the spectrum reveals the presence of two protons which appear as a complex multiplet in the region between 1.25 and 2.40 ppm. This multiplet is assigned to the resonances of the protons at C_4. Decoupling experiments showed that the protons at C_4 are coupled to the proton which appears as a multiplet at 4.04 ppm and to a proton located at 3.40 ppm. The multiplet at 4.04 ppm which is superimposed on the AB pattern, is assigned to the hydrogen adjacent to the hydroxyl group at C_3. The proton at 3.40 ppm that is coupled to the C_4 protons is assigned to the hydrogen at C_{4a}.

The assignment of the protons in the nmr spectrum of desoxyisocrinamine is based on the analysis of the montanine spectrum. The two singlets in the spectrum of desoxyisocrinamine at 6.60 and 6.51 ppm are assigned to the aromatic proton resonances. The quartet centered at 5.55 ppm represents the olefinic proton at C_1 and the singlet at 5.91 ppm corresponds to the methylenedioxy group protons. The four peaks between 3.68 and 4.52 ppm make up a well-defined AB pattern and are assigned to the benzylic protons at C_6. The singlet at 3.40



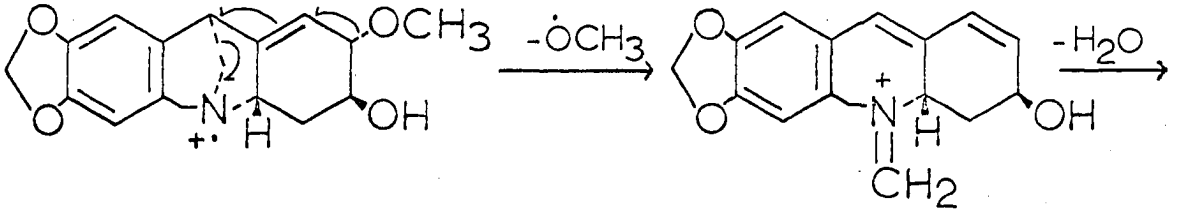
68

ppm is assigned to the methoxyl group protons. The remaining assignable peaks are the broad singlets at 3.28 and 3.05 ppm which are assigned to the benzylic proton at C_{11} and the bridge protons at C_{12} respectively. The complex multiplet in the region between 1.01 and 2.85 ppm contains four protons representing the protons on C_2 and C_4. When the protons of the methoxyl group and those assigned to C_{11} and C_{12} were subtracted, the integration showed that the region between 3.00 and 3.60 ppm represented two protons. The multiplet in this region is assigned to the protons at C_3 and C_{4a}.

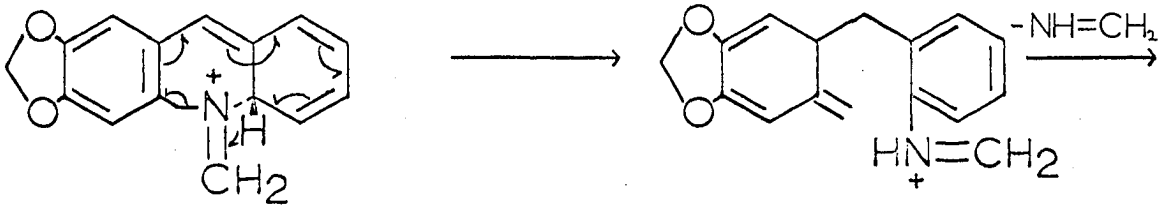
The splitting patterns of this spectrum are more complex than those of the montanine spectrum. This increase in complexity is attributed to the two methylene protons at C_2 of desoxyisocrinamine. If the assigned structure is correct, then the protons at C_2 and C_1 should constitute an ABX system and afford a four line splitting pattern for the olefinic proton. The olefinic proton in the desoxyisocrinamine spectrum is split into a quartet as predicted whereas the olefinic proton of

montanine is represented by a broad singlet. The nmr results are therefore consistent with the proposed structure.

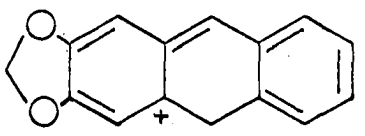
The analysis of the mass spectra of montanine and desoxyisocrinamine (Figure 13) offered another means of comparing the two compounds. A recent study[53] of the fragmentation pattern of montanine showed the base peak in the spectrum to be the molecular ion at m/e 301. A less abundant ion at mass 286 (M-15) was assigned to the loss of a methyl radical from the methoxyl group. The intense peak at m/e 270 (M-31) was postulated to be derived from the loss of the allylic methoxyl group according to the scheme 69 ⟶ 70. A metastable ion at mass



69 70, m/e 270



71, m/e 252 72

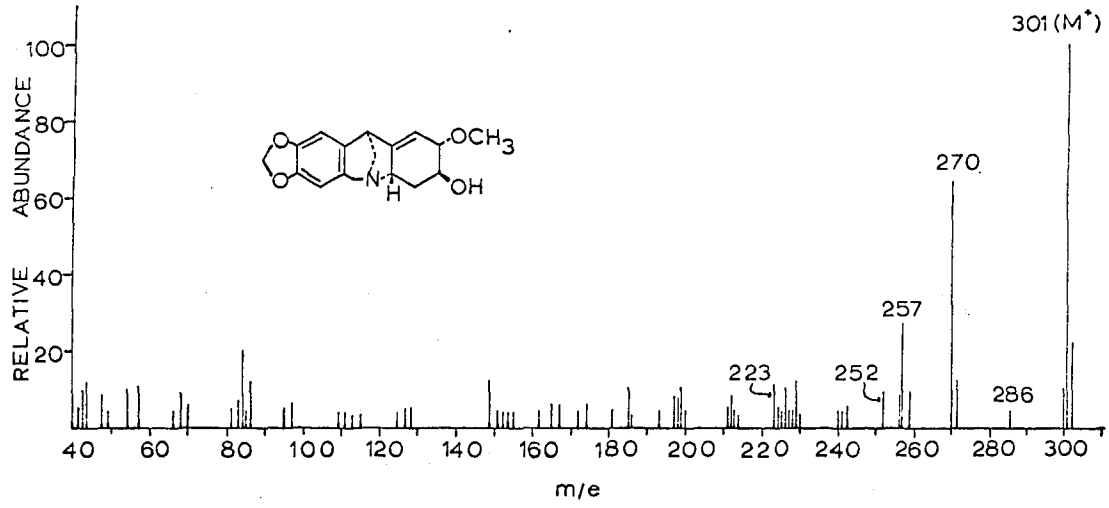


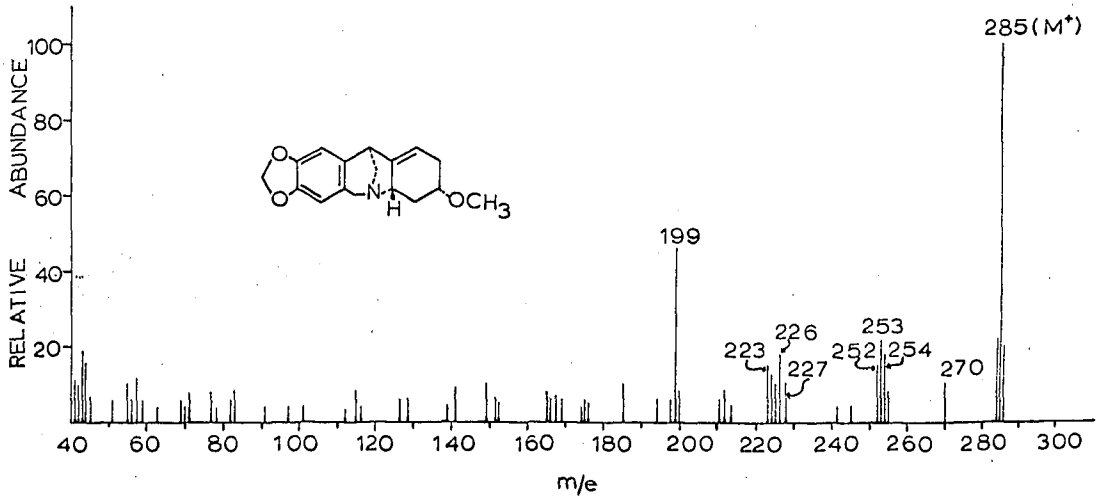
73, m/e 223

Figure 13. Mass spectra

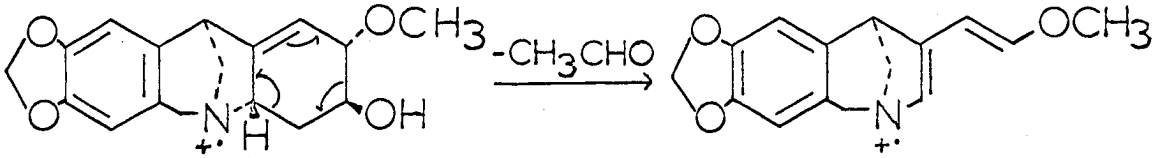
top - montanine (25)

bottom - desoxyisocrinamine (68)


301(M+)
270
257
223
252
286
RELATIVE ABUNDANCE
m/e
OCH3
OH
N
H


285(M+)
199
226
223
227
253
252
254
270
RELATIVE ABUNDANCE
m/e
OCH3
N
H

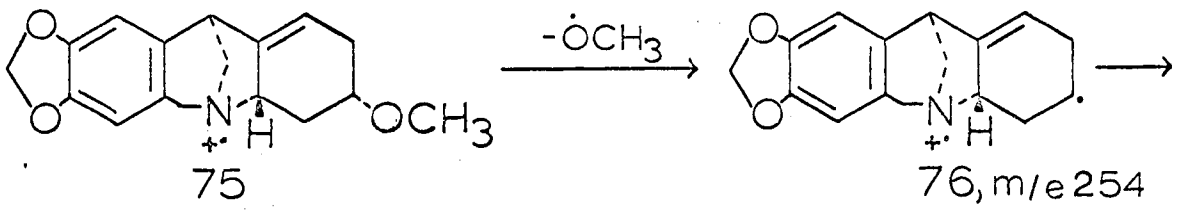
235.5 ($252.5^2/270=235.3$) in the spectrum of montanine confirmed the loss of water from the species 70. This process was visualized as a 1,2-elimination with the formation of 71 (m/e 252). Further decomposition of the species 71 by the elimination of 29 mass units ($CH_2=NH$) was proposed according to the scheme 71 ⟶ 72 ⟶ 73 (m/e 223). A peak of substantial abundance at m/e 257 (M-44) in the spectrum is derived from a retro-Diels-Alder fragmentation of ring C according to 69 ⟶ 74 (m/e 257). A metastable ion at mass 219.5 ($257^2/301=219.4$) established that the ion at m/e 257 arose from a single stage decomposition of the molecular ion of montanine.



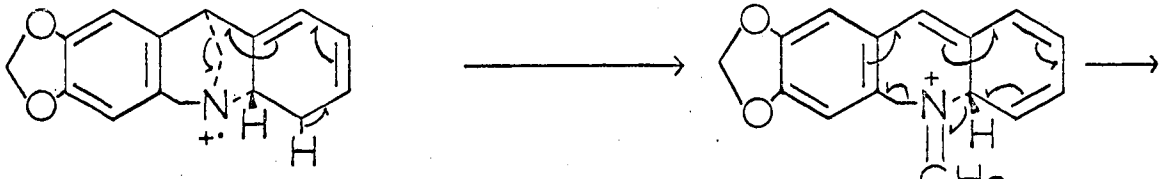
69 74, m/e 257

The most prominent ion in the spectrum of desoxyisocrinamine is again the molecular ion. The small ion peak at m/e 270 (M-15) is attributed to the loss of the methyl radical from the methoxyl group. The peak at m/e 254 (M-31) represents the loss of the methoxyl radical to give the species 76 (m/e 254). This fragmentation is substantiated by the observation of a metastable ion at mass 226.5 ($254^2/285=226.3$). The fragment 76 may then lose a hydrogen atom to give fragment 77 corresponding to the peak at m/e 253 (M-32). The peak at m/e 223 can be visualized as arising from the aromatization of ring

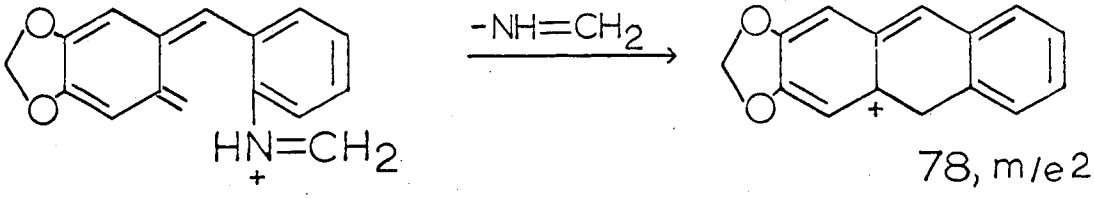
C of 77 followed by the elimination of 29 mass units (CH_2=NH) to give the conjugated ion 78.



75 76, m/e 254

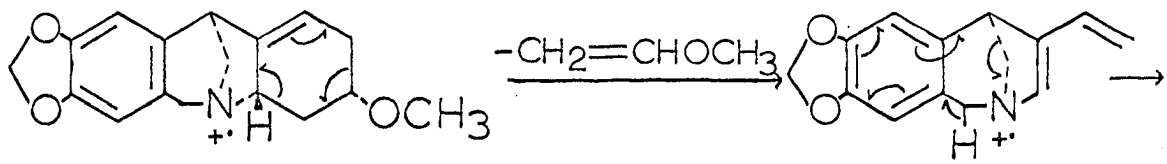


77, m/e 253

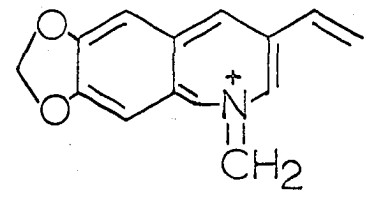


78, m/e 223

The molecular ion loses 58 mass units by undergoing a retro-Diels-Alder fragmentation of ring C to give an ion of mass 227 which is represented by 79. This loss occurs <u>via</u> a one-step decomposition of the molecular ion (metastable ion at mass 180.7 ($227^2/285$=180.6)). The presence of a peak at m/e 226 in the spectrum of desoxyisocrinamine can be interpreted

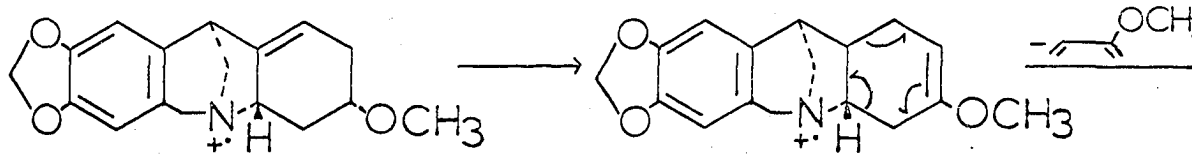


75 79, m/e 227

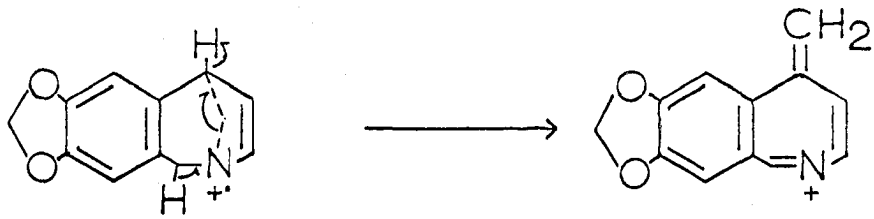


80, m/e 226

mechanistically by the loss of a hydrogen atom from 79 to form the more conjugated species 80. A peak of substantial abundance at m/e 199 corresponds to the loss of 86 mass units and can be rationalized by the mechanistic scheme 75 ⟶ 81 ⟶ 82 ⟶ 83.



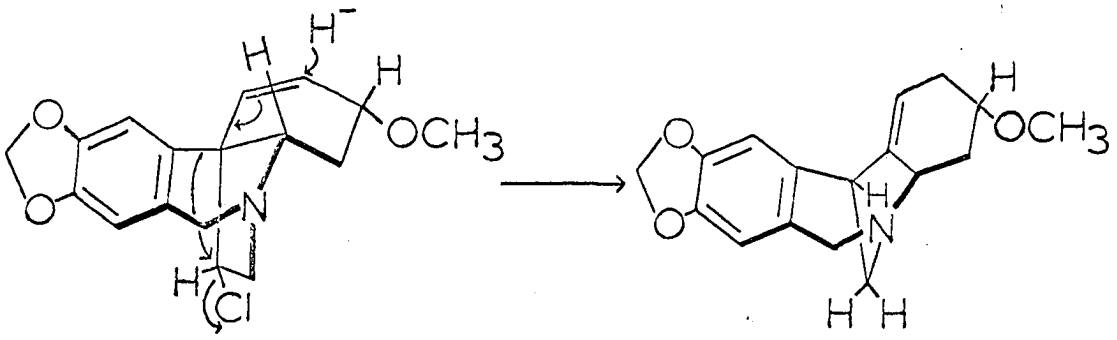
75 81



82 83, m/e 199

Certain similarities are observed in the fragmentation patterns of montanine and desoxyisocrinamine. Both compounds display peaks at M-31 (attributed to the loss of a methoxyl radical) and m/e 223. The two compounds also undergo similar retro-Diels-Alder fragmentations in a single stage decomposition from their respective molecular ions. An important difference between the mass spectra of these bases is the substantial abundance of the ion at m/e 199 in the spectrum of desoxyisocrinamine. The low abundance of this ion in the montanine spectrum can be attributed to the additional oxygen function at C_2 which appears to have a significant effect on the fragmentation pattern of this molecule.

The nmr and mass spectral data indicate that the crinamine ring system has undergone a rearrangement to the montanine nucleus. The fact that no unrearranged product was obtained in the reaction of lithium aluminum hydride with the chlorine derivative of crinamine attests to the facility of the rearrangement. A possible mechanism for this rearrangement involves the attack of hydride at C_2 followed by the migration of the double bond and subsequent displacement of the chloride ion by the aryl group. This mechanism is illustrated by the conversion of 84 $\longrightarrow$ 68.



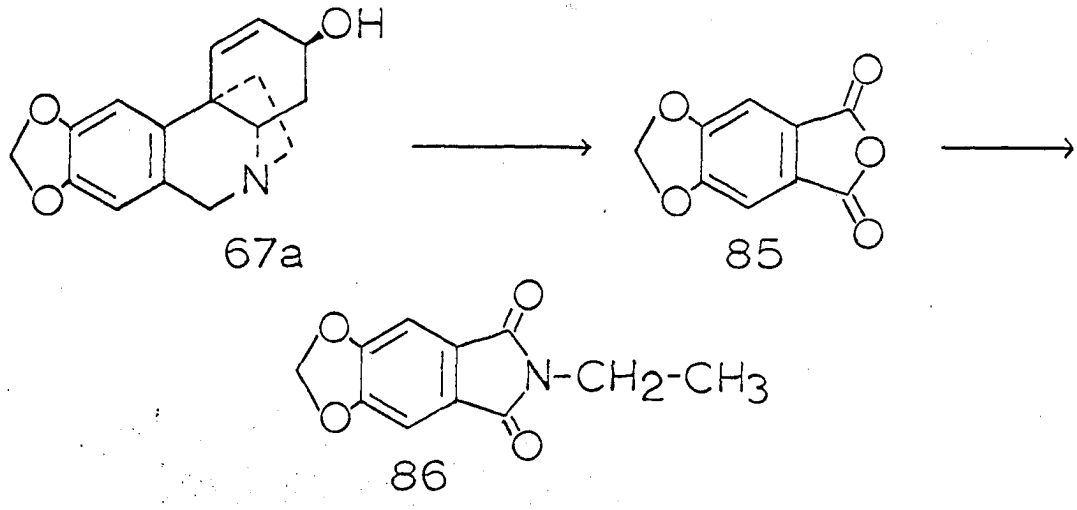
84 68

^{3}H-Vittatine Feeding to Rhodophiala bifida

Several methods are known for introducing tritium atoms into organic molecules via chemical exchange techniques[54-56]. The Wilzbach technique[54] has been successfully used to prepare tritium-labeled steroids[55]. The tritium label is introduced by exposing the compound to tritium gas of high specific activity for long periods of time. Another successful technique, used primarily on aromatic compounds, involves the acid catalyzed exchange of aromatic hydrogen atoms for tritium atoms[23,56]. The latter method was tried in a second attempt to aquire tritium-labeled vittatine.

A small amount of vittatine was converted to O-acetyl-vittatine and subjected to acid catalyzed exchange with tritiated acetic acid (The tritium exchange work was carried out by New England Nuclear Corp., Boston, Mass.). The radioactive O-acetylvittatine was hydrolyzed and diluted with cold vittatine. In order to show that the radioactivity was confined to the aromatic ring, the vittatine was oxidized to hydrastic

anhydride (85) with potassium permanganate. The hydrastic anhydride was converted to N-ethylhydrastimide (86) which was shown to account for all the activity of the labeled vittatine.



The feeding experiment was carried out using Rhodophiala bifida as the plant host. This plant was chosen because it contains montanine as the major alkaloid[27]. Other alkaloids present in Rhodophiala bifida include haemanthamine, vittatine and 11-hydroxyvittatine. However, in a small scale feeding, haemanthamine is present in isolatable quantities while vittatine and 11-hydroxyvittatine are only found in trace amounts. The objective of the feeding experiment was to learn whether vittatine is a precursor of montanine and to obtain some data regarding the relative incorporation of vittatine into haemanthamine and montanine. This data would be helpful in determining whether haemanthamine and montanine have a common biosynthetic precursor.

Ar-tritiated vittatine was administered to Rhodophiala

bifida by injection into the bulb at pH6. The plants were allowed to grow for three weeks in a greenhouse. The bulbs were processed in the usual manner and the alkaloids were separated and purified by column chromatography and crystallization. The data obtained from the feeding experiment are summarized in Table 5.

Table 5. Feeding experiment with Rhodophiala bifida as plant host

Precursor	Alkaloid	Activity (dpm/mM)	Dilution[a]	% inc.
^{3}H-Vittatine[b]	Haemanthamine	35.2×10^{6}	2.80×10^{3}	0.20
	Montanine	4.21×10^{6}	23.4×10^{3}	0.057

[a]Dilution = specific activity of precursor ÷ specific activity of alkaloid.

[b]The total activity of ^{3}H-vittatine was 1.64 mc.

Each of the isolated alkaloids was oxidized with potassium permanganate, and the hydrastic anhydride was converted to its N-ethylimide with ethylamine. The activity of the imide relative to the corresponding alkaloid is given in the last column of Table 6, and illustrates that all of the tritium resides in the aromatic A ring and has not been scrambled during the experiment.

Table 6. Degradation of alkaloids isolated from the ^{3}H-vittatine feeding experiment

Alkaloid	Activity (dpm/mM)	N-ethyl-hydrastimide activity (dpm/mM)	Activity of imide relative to alkaloid
Haemanthamine	1.46×10^5	1.39×10^5	0.96
Montanine	1.31×10^5	1.27×10^5	0.97

The data from the feeding experiment show that vittatine is a precursor of haemanthamine and montanine in Rhodophiala bifida. Since it has already been shown that haemanthamine is not a precursor of montanine[35], the above results indicate that haemanthamine and montanine have a common precursor. This precursor is probably 11-hydroxyvittatine. The specific activity of vittatine-derived haemanthamine is substantially higher than that of the montanine isolated from the same experiment. This finding seems to indicate that the proposed intermediate (11-hydroxyvittatine) is converted to haemanthamine at a faster rate than to the montanine nucleus by rearrangement. This is a reasonable assumption because the formation of haemanthamine only requires the methylation of the hydroxyl function at C_3 of 11-hydroxyvittatine while the formation of montanine involves a rearrangement of the vittatine ring system plus the methylation of the oxygen function at C_2. The biosynthetic scheme of haemanthamine and montanine is illustrated in Figure 14.

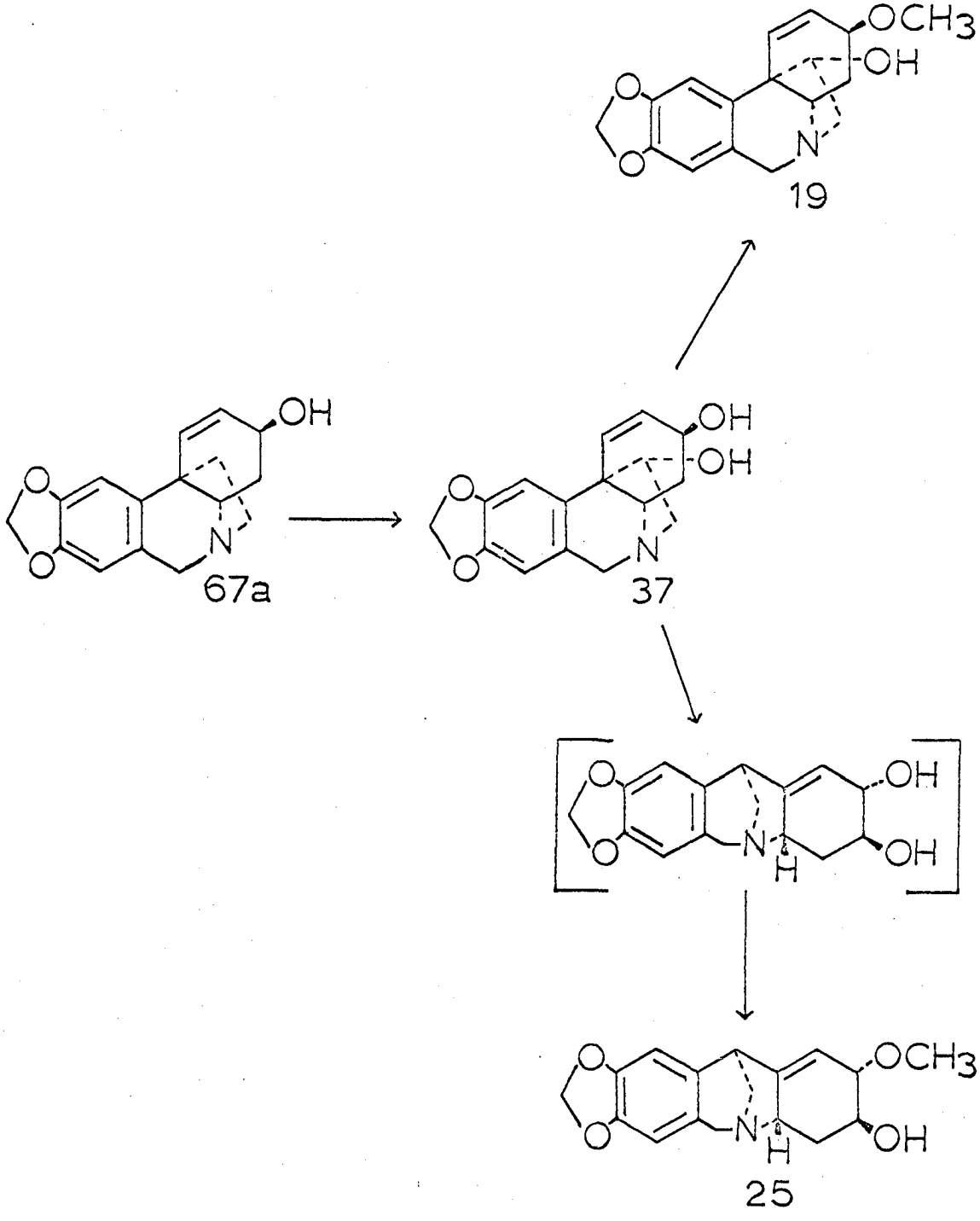
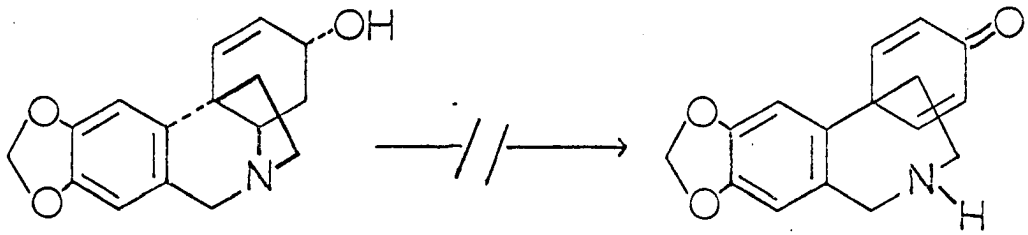


Figure 14. Biosynthetic conversion of vittatine to haemanthamine and montanine

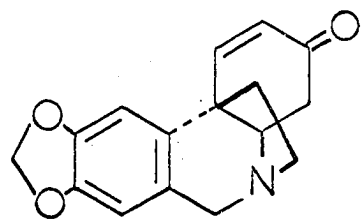
^{3}H-Oxovittatine Feeding to Nerine bowdenii

Recent biosynthetic studies[38] have shown that the alkaloid crinine does not revert to the symmetrical dienone 45 in N. bowdenii. It was thought that, if an oxidative process can occur in the plant to convert the C_3-hydroxyl of either crinine or (+)-epicrinine to a ketone, the enantiomers should be

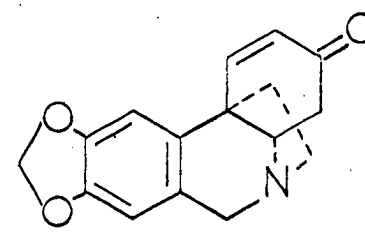


44 45

interconvertible via 45. This hypothesis assumes that if crinine is oxidized to oxocrinine 87, the ketone will readily undergo β-elimination to form 45. This is not a valid assumption because the oxocrinine can be formed and reduced on the surface of an enzyme before it has a chance to β-eliminate. Since the crinine feeding to N. bowdenii did not show any evidence for the oxidative formation of oxocrinine, it is still not known whether the crinine and vittatine alkaloids are interconvertible via the symmetrical dienone 45.



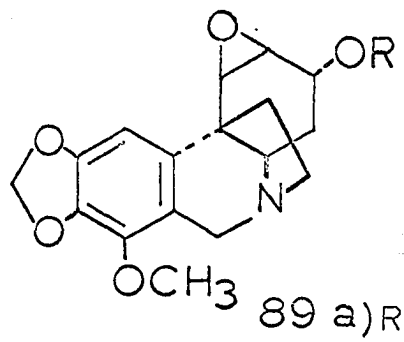
87



88

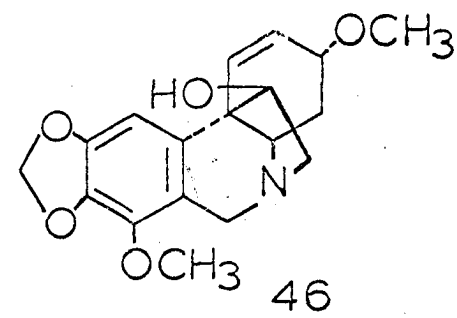
A more profitable approach to this problem would be to feed either radioactive oxocrinine or oxovittatine (88) to a plant which produces both crinine and vittatine-type alkaloids and determine the extent of the incorporation of the ketonic precursor. If the labeled oxo-compound is converted to 45 via β-elimination, then the alkaloids possessing the crinine and vittatine steriochemistry will be radioactive, but if the oxo-compound is being preferably reduced to the alcohol, then only those alkaloids possessing the same absolute configuration as the precursor will be radioactive.

To study this phenomenon, oxovittatine was the preferred precursor, since a supply of ar-tritiated vittatine was readily available. The tritium was shown by degradative methods to be confined to the aromatic ring. A small amount of ^{3}H-vittatine was oxidized to ^{3}H-oxovittatine with manganese dioxide. The ^{3}H-oxovittatine was fed to N. bowdenii and the plants were allowed to grow for three weeks in a greenhouse. The bulbs were processed and the alkaloids were isolated in the usual manner. It was found that all alkaloids derived from the (-)-crinine nucleus were inactive. These included undulatine (89a), crinamidine (89b) crinine and ambelline (46). As expected, lycorine and belladine were inactive. Both crinamine and (+)-epicrinine were isolated by dilution and found to be radioactive. The crinamine was oxidized to hydrastic anhydride which was then converted to its N-ethylimide with



89 a) R = CH_3

b) R = H



46

ethylamine. The activity of the imide relative to the crinamine showed the tritium to be confined to the aromatic A ring. The data of the feeding experiment are summarized in Table 7.

Table 7. Feeding experiment with Nerine bowdenii as plant host

Precursor	Alkaloid	Activity[a] (dpm/mM)	Dilution	% inc.
^{3}H-Oxovittatine[b]	Crinamine	4.64×10^6	3.76×10^3	0.06
	(+)-Epicrinine	6.59×10^6	2.63×10^3	0.09

[a]Each alkaloid was isolated by dilution with 70 mg. of cold compound.

[b]The total activity of the ^{3}H-oxovittatine was 0.88 mc.

The results of this biosynthetic investigation show that in N. bowdenii, oxovittatine is reduced to (+)-epicrinine and is not undergoing a β-elimination to the symmetrical dienone 45. It appears that the phenyl-phenyl oxidative coupling step is occurring on the surface of an enzyme which exhibits a high degree of specificity. It is at this stage in the biosynthetic

pathway that the absolute configuration of the crinine-type alkaloids is determined. The results of this investigation are schematically represented in Figure 15.

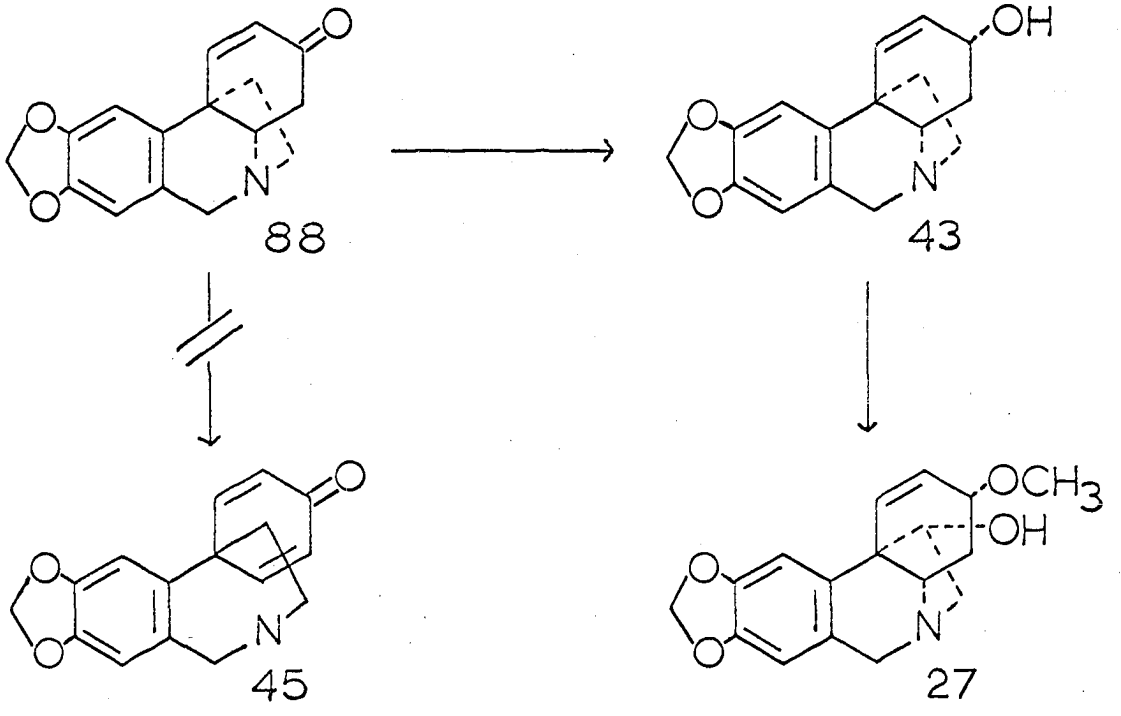


Figure 15. Biosynthetic conversion of oxovittatine to (+)-epicrinine and crinamine

^{14}C-Labeled β-Phenylethylamine Feedings to *Crinum erubescens*

Some crinine-type alkaloids possess hydroxyl functions at the C_{11} position. These include crinamine, 6-hydroxycrinamine, haemanthamine, haemanthidine, 11-hydroxyvittatine and ambelline. The conversion of crinine to ambelline in *N. bowdenii*[38] showed that the hydroxyl function at C_{11} can be introduced after the phenyl-phenyl oxidative coupling step. There exists the

possibility of an alternate biosynthetic pathway for the C_{11}-hydroxylated alkaloids, as in the biosynthesis of berberastine in which a hydroxylated C_6-C_2 unit followed a different biosynthetic path than its non-hydroxylated counterpart[41]. (See historical section)

A hydroxylated C_6-C_2 unit such as octopamine could conceivably react with protocatechuic aldehyde to form the hydroxylated norbelladine derivative (90). This phenolic amine could then be converted to the enantiomeric ketones 91 and 92 possessing the crinine nucleus, via phenyl-phenyl oxidative coupling. The proposed biosynthetic scheme is illustrated in Figure 16.

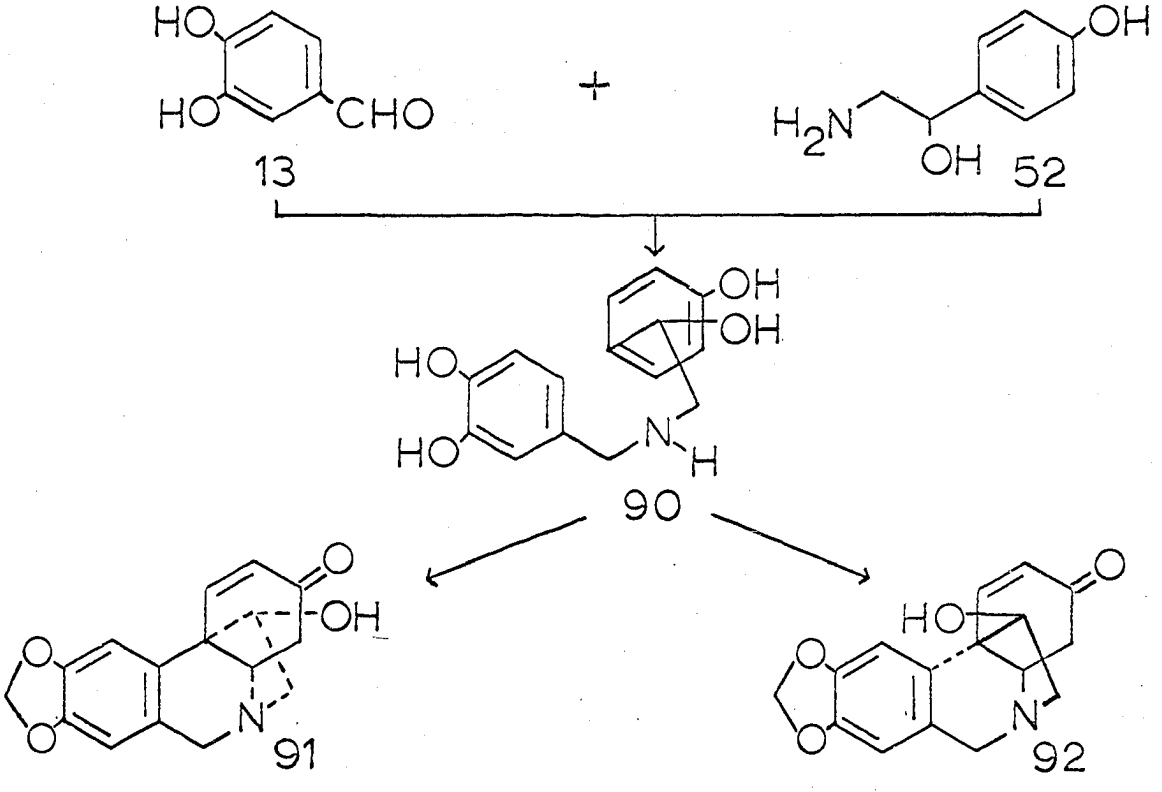


Figure 16. Alternate biosynthetic pathway for C_{11}-hydroxylated alkaloids

A controlled feeding experiment was designed to determine whether the C_{11}-hydroxylated alkaloids were derived from hydroxylated C_6-C_2 unit precursors. Octopamine and p-hydroxyphenylserine were selected as possible hydroxylated precursors for this study. These phenylethanolamines are the β-hydroxylated derivatives of tyrosine and tyramine which serve as the precursors of the C_6-C_2 units of the Amaryllidaceae alkaloids. (The β-hydroxylation of tyrosine and tyramine has been observed in living system[44-46]). Radioactive tyrosine and tyramine served to act as a control in the feeding experiment.

Except for octopamine, all of the radioactive compounds were commercially available. A synthetic route was devised which afforded octopamine tartrate (97) in an overall yield of 21%. The radioactive label was introduced by allowing sodium cyanide-^{14}C to react with p-hydroxybenzaldehyde (93) to form the cyanohydrin (94) in the first step of the synthesis. The cyanohydrin was then converted to the di-tetrahydropyranyl ether (95) with dihydropyran[57]. Lithium aluminum hydride reduction of 95 afforded the di-tetrahydropyranyl ether of octopamine (96). The ether was hydrolyzed with aqueous tartaric acid and the resulting octopamine was separated and purified on an ion exchange column. The pure radioactive octopamine was converted to its crystalline tartrate salt (97) which was recrystallized to constant activity. The synthesis of octopamine tartrate is illustrated in Figure 17.

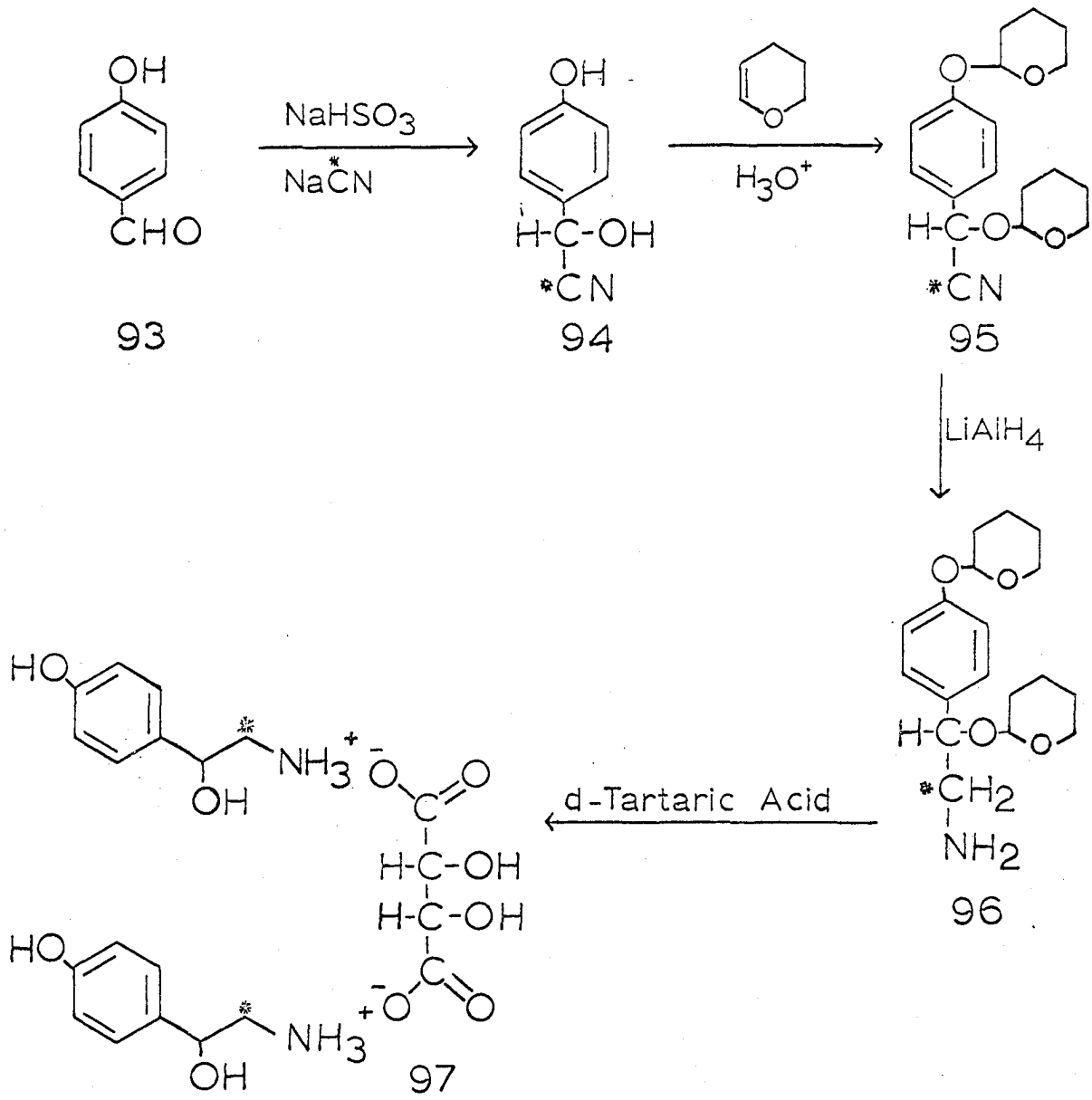
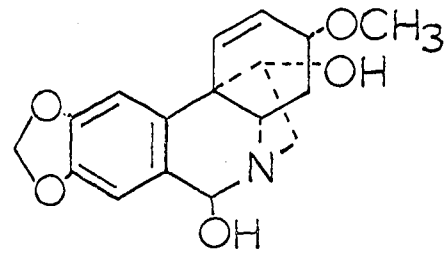


Figure 17. Synthesis of DL-1-^{14}C-octopamine tartrate

Crinum erubescens was chosen for this feeding experiment because it contains lycorine, crinamine, and 6-hydroxycrinamine (98) as the major alkaloids[58]. If a hydroxylated C_6-C_2 unit is a precursor to the C_{11}-hydroxylated alkaloids, then octopamine and/or p-hydroxyphenylserine would have a higher per cent



98

incorporation into crinamine and 6-hydroxycrinamine than would tyrosine and tyramine. The hydroxylated precursors would also have a much lower incorporation into lycorine than would their non-hydroxylated counterparts. The possibility also exists that the hydroxylated phenylethanolamine derivatives can undergo reduction to tyrosine and tyramine. If this should occur, then the alkaloids derived from the tyramine and tyrosine-fed plants would exhibit a higher per cent incorporation than those derived from the phenylethanolamine feedings.

The activity of the compounds used in this feeding experiment is given in Table 8. The labeled compounds were fed to Crinum erubescens and the plants were allowed to grow in a greenhouse for three weeks. The plants were then processed and the alkaloids isolated and purified in the usual manner. The data from the feeding experiment are presented in Tables 9-12. In Crinum erubescens the levels of incorporation of the radioactive tracers, DL-3-^{14}C-tyrosine and DL-2-^{14}C-p-hydroxyphenylserine were comparable. However, 1-^{14}C-tyramine exhibited a very high level of incorporation while DL-1-^{14}C-

Table 8. Radioactive compounds fed to Crinum erubescens

Compound	Total activity fed (mc)	Specific activity (mc/mM)
DL-3-^{14}C-Tyrosine	100	6.71
1-^{14}C-Tyramine[a]	100	4.71
DL-2-^{14}C-p-Hydroxy-phenylserine	100	9.00
DL-1-^{14}C-Octopamine[b]	42	1.49

[a]The tyramine was fed as the hydrobromide salt.

[b]The octopamine was fed as the tartrate salt.

octopamine had a relatively low incorporation into the three alkaloids. The alkaloids had to be degraded in order to determine the mode of incorporation of the precursor. Because of previous biosynthetic experiments with DL-3-^{14}C-tyrosine, this substance was used as a reference; its incorporation into the C_6-C_2 unit of many Amaryllidaceae alkaloids is well established (Table 1). Therefore, no degradations were carried out on the alkaloids derived from the DL-3-^{14}C-tyrosine-fed bulb.

Degradation of 6-Hydroxycrinamine Derived From the 1-^{14}C-Tyramine Feeding

Tyramine was shown to be incorporated intact into the C_6-C_2 unit of lycorine[3,19]. Since tyramine had never been shown to be incorporated into the crinine-type alkaloids, it was necessary to establish the mode of incorporation of this precursor into either crinamine or 6-hydroxycrinamine. The

Table 9. DL-3-^{14}C-tyrosine feeding to Crinum erubescens

Alkaloid	Weight isolated	Specific activity (dpm/mM)	Dilution	Per cent incorporation
Lycorine	0.372 g.	8.50×10^5	1.77×10^4	0.51
Crinamine	0.027 g.	4.32×10^6	3.46×10^3	0.17
6-hydroxycrinamine	0.440 g.	1.43×10^5	1.05×10^5	0.09

Table 10. 1-^{14}C-tyramine feeding to Crinum erubescens

Alkaloid	Weight isolated	Specific activity (dpm/mM)	Dilution	Per cent incorporation
Lycorine	0.571 g.	1.86×10^6	5.63×10^3	1.60
Crinamine	0.140 g.	6.35×10^6	1.65×10^3	1.30
6-hydroxycrinamine	0.513 g.	3.21×10^5	3.27×10^4	0.23

Table 11. DL-2-^{14}C-p-hydroxyphenylserine feeding to Crinum erubescens

Alkaloid	Weight isolated	Specific activity (dpm/mM)	Dilution	Per cent incorporation
Lycorine	0.536 g.	3.31×10^5	6.04×10^4	0.28
Crinamine	0.071 g.	2.37×10^6	8.49×10^3	0.25
6-hydroxycrinamine	0.380 g.	2.14×10^5	9.27×10^4	0.12

Table 12. DL-1-^{14}C-octopamine feeding to Crinum erubescens

Alkaloid	Weight isolated	Specific activity (dpm/mM)	Dilution	Per cent incorporation
Lycorine	0.660 g.	1.88×10^4	1.75×10^5	0.046
Crinamine	0.162 g.	3.15×10^4	1.06×10^5	0.018
6-hydroxycrinamine	0.586 g.	4.15×10^3	7.84×10^5	0.008

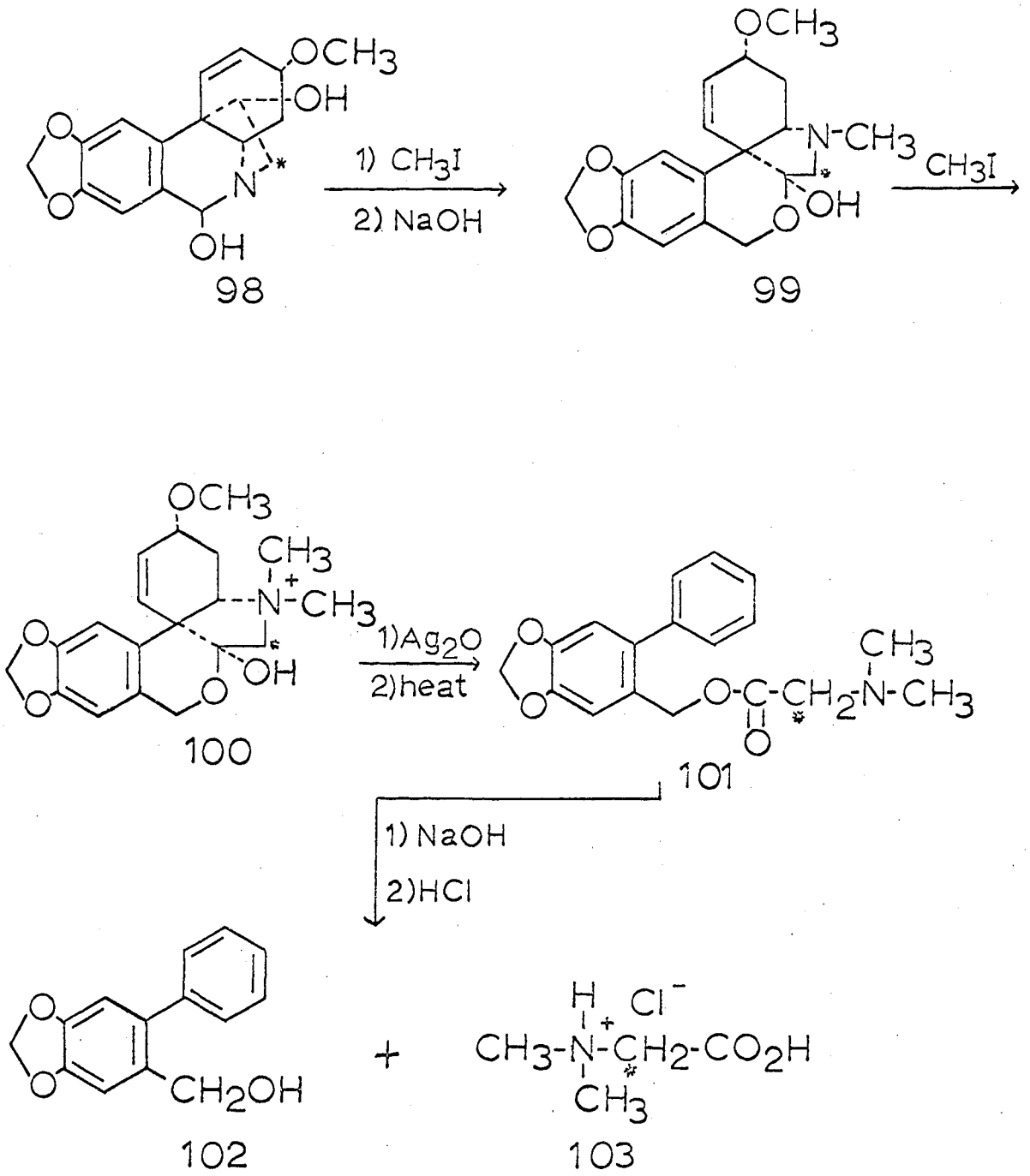


Figure 18. Degradation of 6-hydroxycrinamine

6-hydroxycrinamine was preferably degraded because of the relatively large amount isolated from the feeding experiment. On the basis of earlier experiments, it would be expected that the 6-hydroxycrinamine isolated from the 1-^{14}C-tyramine feeding would be labeled at the C_{12} position.

The 6-hydroxycrinamine was converted to criwelline (99)[58,59] which was then degraded according to the procedure[60] given in Figure 18. If the 6-hydroxycrinamine was labeled at the C_{12} position, the radioactivity would appear in the criwelline methine (101) at the position indicated by the asterisk. Basic hydrolysis of 101 gave 6-phenylpiperonyl alcohol (102) and dimethylglycine, which was isolated as the hydrochloride (103). The dimethylglycine hydrochloride was oxidized with lead tetraacetate[61] to obtain the carbonyl group as carbon dioxide and the methylene group as formaldehyde dimedone adduct (104) (Figure 19).

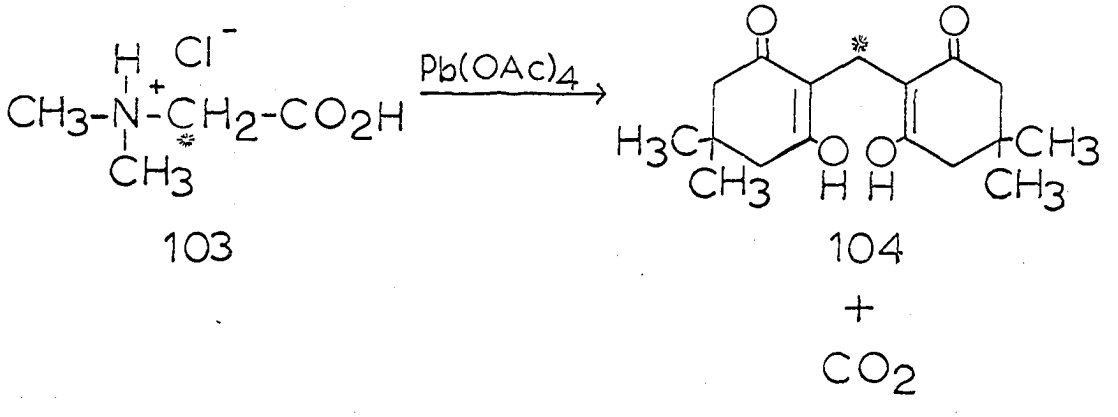


Figure 19. Lead tetraacetate oxidation of dimethylglycine hydrochloride

The radioactivity was found to be confined to the formaldehyde dimedone adduct as would be expected for the intact incorporation of 1-^{14}C-tyramine into 6-hydroxycrinamine. The results of the degradation are summarized in Table 13.

Table 13. Degradation of 6-hydroxycrinamine isolated from the 1-^{14}C-tyramine feeding experiment

Compound	Yield	Amt. used next rctn.	Rel. act.
6-Hydroxycrinamine		230 mg.	1.00
Criwelline	240 mg.	210 mg.	1.01
Criwelline methiodide	212 mg.	200 mg.	0.95
6-Phenylpiperonyl alcohol	93 mg.		0.00
Dimethylglycine hydrochloride	30 mg.	28 mg.	0.97
Formaldehyde dimedone adduct from dimethylglycine hydrochloride	6 mg.		0.98
Carbon dioxide from dimethylglycine hydrochloride[a]	-------		0.00

[a]The carbon dioxide was trapped in Hyamine hydroxide 10X (Packard) and toluene-POPOP scintillation solution.

6-Hydroxycrinamine Isolated From the DL-2-^{14}C-p-hydroxyphenylserine Feeding Experiment

The 6-hydroxycrinamine derived from the DL-2-^{14}C-p-hydroxyphenylserine feeding was degraded to 6-phenylpiperonyl alcohol and dimethylglycine hydrochloride. If the precursor was incorporated intact, the dimethylglycine hydrochloride would be expected to contain all of the activity. However, the

dimethylglycine hydrochloride was found to be inactive while the 6-phenylpiperonyl alcohol moiety accounted for 53% of the total activity. During the conversion of criwelline methiodide to criwelline methine (Figure 18) a methoxyl group is lost which could contain the remaining 47% of the activity. In order to substantiate this, a methoxyl determination was carried out on 6-hydroxycrinamine using the Zeisel method (Figure 20). The methoxyl group was cleaved with hydroiodic acid and the resulting methyl iodide was isolated as the

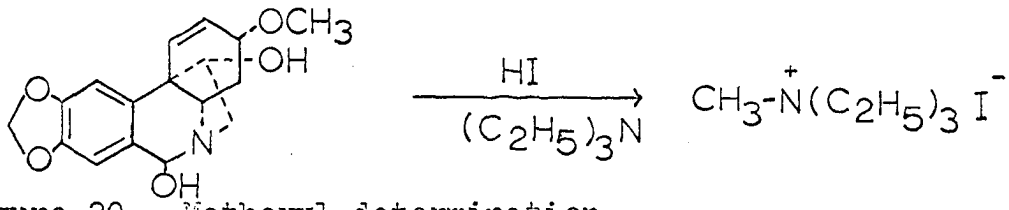


Figure 20. Methoxyl determination

methiodide salt of triethylamine. The methiodide salt was found to contain 49% of the total activity. The radioactivity of the 6-phenylpiperonyl alcohol moiety was assumed to be confined to the methylenedioxy group since it is also derived from a one carbon fragment[62]. The activity of the methylenedioxy group was determined by acid hydrolysis to formaldehyde[63] (Figure 21), which was isolated as the dimedone adduct. The methylenedioxy group was shown to have 51% of the total radioactivity of the 6-hydroxycrinamine. The results of the degradation are summarized in Table 14. The one carbon unit in lycorine was also shown to be derived from DL-2-^{14}C-p-hydroxyphenylserine. The lycorine isolated from this feeding

was degraded and all the activity was found to be confined to the methylenedioxy group.

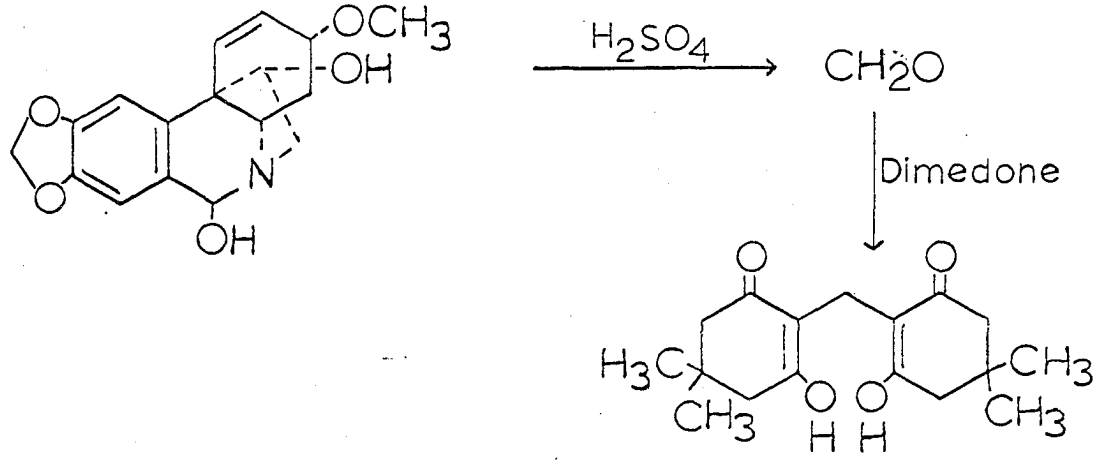


Figure 21. Methylenedioxy determination

Table 14. Degradation of 6-hydroxycrinamine isolated from the DL-2-^{14}C-p-hydroxyphenylserine feeding experiment

Compound	Yield	Amt. used next rctn.	Rel. act.
6-Hydroxycrinamine		450 mg.	1.00
Criwelline	395 mg.	390 mg.	0.98
Criwelline methiodide	530 mg.	525 mg.	0.98
6-Phenylpiperonyl alcohol	260 mg.		0.53
Dimethylglycine hydrochloride	100 mg.		0.00
Methyltriethyl ammonium iodide	34 mg.[a]		0.49
Formaldehyde dimedone adduct from methylenedioxy determination	12 mg.[b]		0.51

[a]Derived from 55 mg. of 6-hydroxycrinamine.

[b]Derived from 100 mg. of 6-hydroxycrinamine.

Lycorine and 6-Hydroxycrinamine Isolated From the DL-1-^{14}C-Octopamine Feeding Experiment

Since DL-2-^{14}C-p-hydroxyphenylserine was found to be a precursor of one carbon units in lycorine and 6-hydroxycrinamine, it was conceivable that the structurally related octopamine could also have a comparable mode of incorporation. Methylenedioxy and methoxyl determinations were carried out on both the lycorine and 6-hydroxycrinamine isolated from the DL-1-^{14}C-octopamine-fed Crinum erubescens. The results of these degradations are presented in Table 15.

Table 15. Degradation of 6-hydroxycrinamine and lycorine isolated from the DL-1-^{14}C-octopamine feeding experiment

Alkaloid	% of total activity	
	Methylenedioxy group	Methoxyl group
6-Hydroxycrinamine	47	51
Lycorine	96	-

It is clear from these experiments that both p-hydroxyphenylserine and octopamine serve solely as precursors of C_1 units such as methoxyl and methylenedioxy groups. The relative activities of the methylenedioxy and methoxyl groups of the 6-hydroxycrinamine derived from the DL-2-^{14}C-p-hydroxyphenylserine and DL-1-^{14}C-octopamine feedings are comparable to those obtained for tazettine derived from 3-^{14}C-serine-fed plants[61]. In each case, the activity ratio of the methylenedioxy and methoxyl groups were 1:1. A priori, one would have expected

the carbon atom of the methoxyl group at C_3 to have a relatively higher activity than the methylene carbon of the methylenedioxy group since the methoxyl carbon probably is introduced at a later stage in the biosynthetic pathway. Biosynthetic studies[62] have shown that the methylene carbon of the methylenedioxy group originates from an O-methyl group which is formed before the phenyl-phenyl oxidative coupling step. The conversion of vittatine to haemanthamine in Rhodophiala bifida indicates that the methoxyl group at C_3 is formed after the phenyl-phenyl oxidative coupling step and possibly after the hydroxylation at the C_{11} position.

In order to explain the 1:1 activity ratio of the methylenedioxy and methoxyl groups of tazettine and 6-hydroxycrinamine, one could postulate that the phenyl-phenyl oxidative coupling step and the methylation of the hydroxyl function at C_3 are very rapid processes so that no C_3 hydroxylated intermediates are ever present in appreciable quantities. This postulation is supported by the observation that there are no major alkaloids in Crinum erubescens or Sprekelia formosissima (plants in which 3-^{14}C-serine was incorporated into tazettine[61]) possessing hydroxyl functions at the C_3 position[58,64]. Additional feeding experiments should be carried out in which either radioactive serine or formate is fed to plants which contain appreciable quantities of alkaloids with C_3 hydroxyl functions. A comparison should then be made of the activity ratio of the methylenedioxy and methoxyl groups of the alkaloids

isolated, with the results described above. A study of this type could provide some useful information concerning the relative rates at which various biosynthetic interconversions take place. Nerine bowdenii would be a plant to consider since it contains isolable quantities of crinamine and (+)-epicrinine.

The feeding experiments performed with Crinum erubescens showed that β-phenylethanolamine derivatives such as octopamine and p-hydroxyphenylserine can undergo a transformation to one carbon fragments which then become the source of C_1 units such as the methoxyl and methylenedioxy groups in the alkaloids. The fact that both precursors have the same mode of incorporation into the methoxyl and methylenedioxy groups of the alkaloids studied, suggests that p-hydroxyphenylserine is being converted to octopamine via a decarboxylation. This transformation is analogous to the conversion of tyrosine to tyramine which has been proposed on the basis of earlier feeding experiments[3,19]. A possible biosynthetic pathway for the fragmentation of octopamine can be envisaged as involving the oxidation of the benzylic hydroxyl function in the first step to give the ketone (105). This ketone can then undergo oxidative deamination[50] to form the glyoxal (106). Enzymatic fragmentation of the glyoxal can then occur with the subsequent transfer of the formyl group to tetrahydrofolic acid[65]. The transfer of a C_1 unit from formylated tetrahydrofolic acid to methylating agents such as methionine is well known[65]. It has been shown that

L-methyl-^{14}C-methionine is a source of the C_1 unit of the methylenedioxy and methoxyl groups of the lycorine-type alkaloids[13]. The proposed biosynthetic pathway for p-hydroxyphenylserine and octopamine is illustrated in Figure 22. More than one biosynthetic scheme can be proposed for octopamine and the one presented in Figure 22 should only be considered as a tentative rationalization. However, this scheme is preferred because it is analogous to the results obtained for the metabolism of adrenaline and noradrenaline[51] (see historical section).

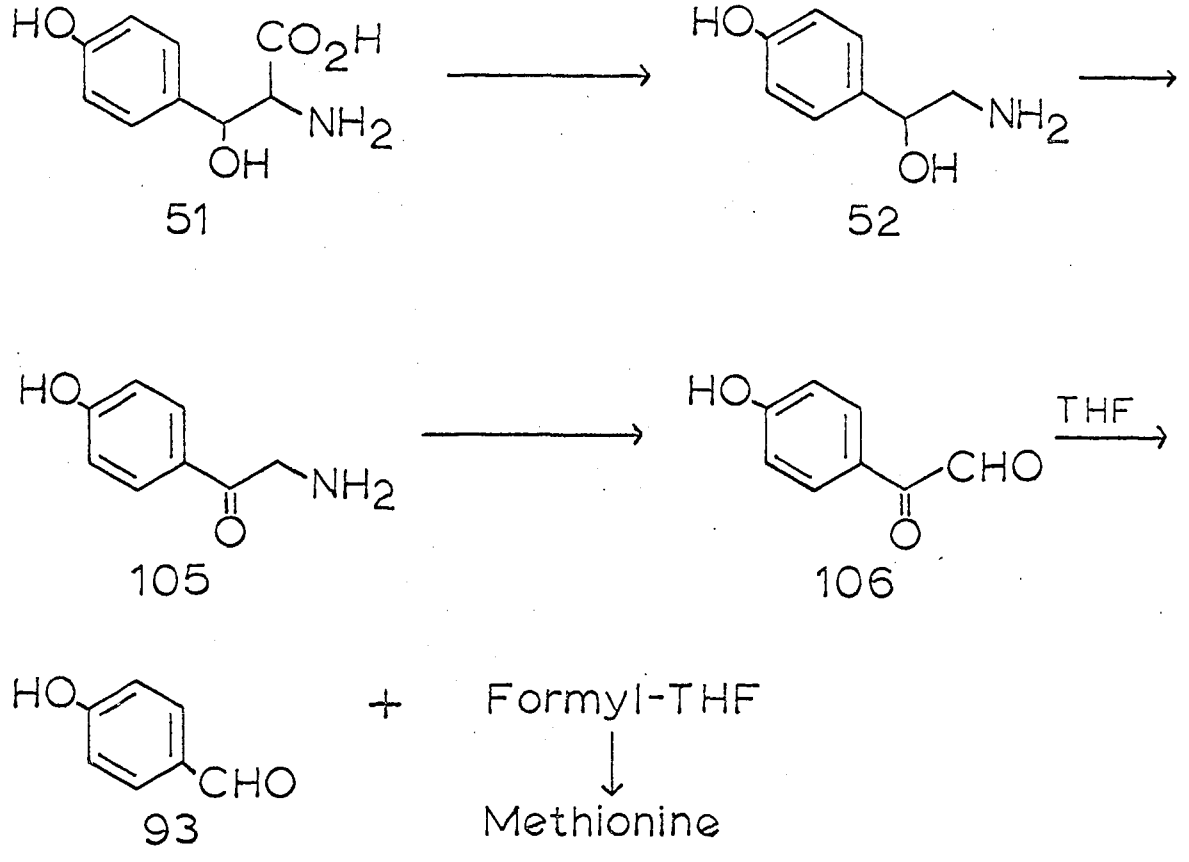


Figure 22. Proposed biosynthetic pathway for p-hydroxyphenylserine and octopamine (THF=tetrahydrofolic acid)

It is theoretically possible for the aldehyde (93), which results from the fragmentation of the glyoxal, to undergo further oxidation to protocatechiuc aldehyde and thereby become incorporated into the C_6-C_1 unit of the Amaryllidaceae alkaloids. Additional experiments are needed using DL-2-^{14}C-octopamine in order to determine the biosynthetic fate of the proposed C_6-C_1 fragment derived from octopamine.

SUMMARY

The Amaryllidaceae alkaloid vittatine was selected as a likely precursor to the montanine-type alkaloids and the approaches used to introduce a tritium label into the vittatine nucleus are discussed. In the course of this study crinamine was found to rearrange to desoxyisocrinamine which was shown to be a new compound possessing the montanine ring system. An interpretation of this rearrangement as well as spectral data are presented.

It has been shown that the alkaloid vittatine is a precursor to haemanthamine and montanine. The data presented in this study suggest that both haemanthamine and montanine are derived from a common intermediate which is postulated to be 11-hydroxyvittatine. Evidence is presented that oxovittatine is incorporated only into alkaloids of the same absolute configuration and is not undergoing a β-elimination to a symmetrical dienone prior to incorporation into the alkaloids.

Octopamine and p-hydroxyphenylserine serve as precursors of the methoxyl and methylenedioxy groups of 6-hydroxycrinamine and the methylenedioxy group of lycorine. These precursors are shown not to be incorporated into either the C_6-C_1, or C_6-C_2 units of lycorine or 6-hydroxycrinamine while tyramine was shown to be incorporated intact into the C_6-C_2 unit of 6-hydroxycrinamine. Biogenetic routes are presented which are in accord with these experimental findings.

EXPERIMENTAL

Source of Plant Materials and Radioactive Precursors

The Nerine bowdenii bulbs were obtained from the Walter Marx Gardens, Boring, Oregon. The Rhodophiala bifida and the Crinum erubescens bulbs were supplied by Robert D. Goedert, Jacksonville, Florida.

The ^{3}H-vittatine used in the Rhodophiala bifida feeding was labeled by the New England Nuclear Corp., Boston, Mass. The DL-1-^{14}C-octopamine was synthesized using sodium ^{14}C-cyanide which was secured from Tracerlab Inc., Waltham, Mass. The DL-2-^{14}C-p-hydroxyphenylserine was obtained from Calbiochem, Los Angeles, California. All other radioactive precursors (Table 8) were obtained from New England Nuclear Corp.

Measurement of the Radioactivities of the Compounds

Activities were measured by scintillation counting in solution. The radioactive sample (1-5 mg.) was counted in 15 ml. of Bray's scintillation solution [60 g. of naphthalene, 4 g. of 1,4-bis-2-(5-phenyloxazolyl)-benzene (Packard) and 100 mg. of 2,5-diphenyloxazole (Packard) in 20 ml. of 1,2-ethanediol, 100 ml. of methanol and sufficient dry, peroxide-free dioxane to make 1 l. of solution]. The efficiency of this method is generally 80-85% for carbon-14 and 13-17% for tritium counting and it was determined for each sample by means of an internal standard of ^{14}C or ^{3}H-toluene. All measurements of the radioactivities of the compounds were obtained with a

Packard Tri-Carb Liquid Scintillation Spectrometer System (model 3002). The counts obtained from this instrument had a maximum error of ±3 per cent.

Purity of Precursors and Isolated Alkaloids

The DL-1-^{14}C-octopamine tartrate and the tritium-labeled vittatine and oxovittatine were purified by recrystallization until constant activity was achieved. The phenolic precursors (Table 8) were chromatographed on Whatman No. 1 paper with the solvent system n-butanol-acetic acid-water (4:1:1) and autoradiographs were taken with x-ray film. The phenols were detected by spraying with diazotized p-nitroaniline. The radioactive area and the "phenolic spot" coincided, and generally no radiochemical impurities were present.

The isolated alkaloids were purified by recrystallization, as the bases and as salts, until constant activity was achieved. Dilution with pure inactive alkaloid and further recrystallization established radiochemical purity, as shown by the fact that no activity was lost in the course of the degradations described in this section. The incorporations of activity were calculated as (100 x total activity of isolated alkaloid) divided by (total activity of precursor fed). For this purpose, the final constant activity of the alkaloid per mg. was used and this was multiplied by the quantity of alkaloid isolated (in mg.) which was of good chemical purity.

Administration of Labeled Precursors to the Plants

An aqueous solution (pH 6) of the precursor (usually 0.5-1.0 ml.) was introduced directly into the bulb with a fine hypodermic needle, 3-5 days later a second injection (0.5-1.0 ml.) was made, and the plants were grown in pots in a greenhouse at Iowa State University and harvested after 3 weeks.

Conversion of Crinamine to Desoxyisocrinamine

A solution of 148 mg. of crinamine in 15 ml. of thionyl chloride was refluxed for 1.5 hours. The solvent was removed under reduced pressure, and the resulting residue was dissolved in 10 ml. of dry tetrahydrofuran. The tetrahydrofuran solution was added dropwise to a suspension of 284 mg. of lithium aluminum hydride in 40 ml. of dry tetrahydrofuran. After the addition was completed, the reaction mixture was refluxed for 5 hours. The excess hydride was decomposed by the addition of water. The tetrahydrofuran was decanted. The precipitate was washed with chloroform, and the washings were combined with the tetrahydrofuran. Evaporation of the dried solvents left 150 mg. of crude product which was chromatographed over alumina. Elution with ethyl acetate in benzene (1:10) afforded 82 mg. of white crystals which were shown to be pure by gas phase and thin layer chromatography. The compound was recrystallized from ether, m.p. 143-144°. The infrared spectrum showed the absence of hydroxyl absorption and exhibited bands at 1330 and 1370 cm^{-1} which are characteristic of the montanine nucleus.

The molecular weight by mass spectrometry was 285.

Anal. Calcd. for $C_{17}H_{19}NO_3$: C,71.56; H,6.71; N,4.91. Found: C,71.52; H,6.75; N,4.94.

Conversion of Vittatine to O-Acetylvittatine

A solution of 100 mg. of vittatine in 20 ml. of pyridine and 1 ml. of acetic anhydride was stirred overnight at room temperature. The pyridine was removed under reduced pressure and the resulting residue dissolved in chloroform. The chloroform solution was washed with a solution of sodium carbonate and then dried with magnesium sulfate. The chloroform was removed under reduced pressure to give 100 mg. of crude O-acetylvittatine. Recrystallization from benzene-Skelly-A afforded 60 mg. of pure O-acetylvittatine, m.p. 145-146° (reported for enantiomer[1], m.p. 143-145°). The infrared spectrum was superimposable with that of an authentic sample.

Tritium-Labeled Vittatine

Tritiation was conducted by combining 50 mg. of O-acetylvittatine with 0.2 ml. of glacial acetic acid containing 10 curies of tritiated water, and 25 mg. of prereduced platinum catalyst and heating the mixture at 100° overnight. The catalyst was removed and the radioactive sample was divided into seven aliquots (100 mc./7 mg.) in acetic acid (New England Nuclear Corp., Boston, Mass.). One of these aliquots was purified in the following manner. The acetic acid was removed

under reduced pressure and the resulting residue was dissolved in 15 ml. of methanol and 1 ml. of 50% sodium hydroxide. The basic solution was allowed to stand at room temperature for two days. The methanol was then removed under reduced pressure and the residue was dissolved in 50 ml. of water. The aqueous solution was diluted with 35 mg. of inactive vittatine and extracted with chloroform. The chloroform solution was washed repeatedly with water in order to remove the remaining labile tritium. Concentration of the dried chloroform extract gave 30 mg. of crude tritiated vittatine. The vittatine was recrystallized from acetone to constant activity to give 10 mg., m.p. 210-211° (reported[1] m.p. 211-212°), 9.89 x 10^{10} dpm/mM.

Rhodophiala bifida Feeding Experiment

Administration of labeled precursor

The ^{3}H-vittatine (1.64 mc., 10 mg.) was dissolved in 0.5 ml. of water (pH 6) and introduced directly into the Rhodophiala bifida bulbs (20 bulbs) with a fine hypodermic needle. The radioactive residue in the vial was dissolved in 0.5 ml. of water and injected into six additional bulbs 3 days later. The plants were grown in pots in a greenhouse at Iowa State University for a period of 3 weeks after which the bulbs were harvested and the alkaloids isolated in the usual manner.

Extraction and separation of alkaloids from Rhodophiala bifida bulbs

The twenty-six bulbs (437 g.) were macerated with 3 l. of 95% ethanol in a Waring Blendor. The mixture was filtered and the filter cake was allowed to stand overnight in 1 l. of 95% ethanol. The solid material was separated by filtration and the filtrates were combined and concentrated under reduced pressure. The resulting crude residue (5.3 g.) was acidified with 2N hydrochloric acid and filtered through a sintered glass funnel to remove the acid-insoluble material. The insoluble material was heated with 2N hydrochloric acid and refiltered. The filtrates were combined and extracted five times with benzene to remove neutral and acidic material. The benzene extract gave a negative alkaloid test with silicotungstic acid and was discarded.

The aqueous acidic solution was made basic (pH 10) with ammonium hydroxide and extracted several times with chloroform. The aqueous solution was then adjusted to pH 12 by the addition of 10% sodium hydroxide and extracted three times with 20% ethanol in chloroform solution. The extracts were combined and concentrated under reduced pressure to give 2.0 g. of crude basic material. The basic residue was dissolved in a minimum amount of acetone. To the acetone solution was added ten drops of 70% perchloric acid followed by enough ether to cause the solution to become turbid. The solution was allowed to stand at 0° for two hours in order to insure complete precipitation

of montanine perchlorate. The crystalline perchlorate salt of montanine (109 mg.) was removed by filtration and recrystallized from acetone to give 100 mg., m.p. 249-250°. The filtrate was dissolved in water; made basic with ammonium hydroxide, and extracted with chloroform. Evaporation of the chloroform gave 1.0 g. of a crude residue which exhibited an activity of 1.61×10^6 dpm/mg.

A benzene solution of the crude residue was chromatographed on alumina packed in benzene. Elution with 25% chloroform in benzene afforded fractions from which 62 mg. of haemanthamine, m.p. 199-200°, was obtained by crystallization from acetone. Elution with 50-75% chloroform in benzene gave fractions rich in montanine from which 91 mg. of montanine perchlorate, m.p. 250-251°, was obtained in the usual manner. Further elution with chloroform and 1-10% methanol-chloroform solutions gave no characterizable products.

The haemanthamine and montanine perchlorate were recrystallized to constant activity to give a total of 62 mg. of haemanthamine, m.p. 199-200° (reported[1] 200-201), 3.52×10^7 dpm/mM and 200 mg. of montanine perchlorate, m.p. 250-251° (reported[27] m.p. 248-251°), 4.21×10^6 dpm/mM.

Degradation of Tritiated Vittatine, Haemanthamine, and Montanine

Permanganate oxidation of vittatine

Vittatine (800 mg., 7.29×10^7 dpm/mM) was dissolved in 20 ml. of dilute hydrochloric acid. The solution was made basic (pH 8) with sodium carbonate. This solution was stirred while a solution of 4.8 g. of potassium permanganate in 200 ml. of water was added dropwise over a period of 30 minutes. The reaction mixture was allowed to stir for 24 hours at room temperature. No permanganate remained at this point. The manganese dioxide was dissolved by the addition of sulfur dioxide. The clear yellow solution was acidified with dilute sulfuric acid and extracted with five portions of ethyl acetate. The ethyl acetate extract was dried over magnesium sulfate, filtered and evaporated to leave 113 mg. of yellow oil.

The original aqueous solution from the oxidation was continuously extracted with ether for 48 hours. The ether extract was dried over magnesium sulfate, filtered and evaporated to leave 24 mg. of yellow oil. The two extracts were combined (137 mg.) and partitioned between dilute sodium bicarbonate solution and ethyl acetate. The bicarbonate extract was washed once with ether, acidified with dilute sulfuric acid, and the solution continuously extracted with ether overnight. The ether extract was dried over magnesium sulfate, filtered, and concentrated to leave 86 mg. of acidic material which was sublimed at 120° (0.01 mm.). The crystals were separated from

the oily material to give 6 mg., m.p. 159-170° (reported[29] 159-176°), of crude hydrastic anhydride. The crude hydrastic anhydride was triturated with several drops of 70% ethylamine solution in a sublimation tube. The excess ethylamine and water were evaporated under reduced pressure and the residue was sublimed at 120° (0.01 mm.). The crystals were recrystallized from ethanol to give 2 mg., m.p. 170-171°, of N-ethyl hydrastimide (7.01 x 10^7 dpm/mM). The mixed melting point with authentic N-ethyl hydrastimide (m.p. 169-170°) was 169-170°.

Oxidation of haemanthamine and montanine isolated from the ^{3}H-vittatine feeding to Rhodophiala bifida

Both haemanthamine and montanine were oxidized according to the procedure described above for the permanganate oxidation of vittatine. The results of these oxidations are summarized in Table 16.

Table 16. Permanganate oxidation of alkaloids isolated from ^{3}H-vittatine feeding with Rhodophiala bifida as plant host

Alkaloid	Activity (dpm/mM)	Yield of N-ethyl hydrastimide	Imide activity (dpm/mM)	Activity of imide relative to alkaloid
Haemanthamine[a]	1.46 x 10^5	50 mg.	1.39 x 10^5	0.96
Montanine[a]	1.31 x 10^5	25 mg.	1.27 x 10^5	0.97

[a]The oxidation was carried out on 1.5 g. of alkaloid.

Conversion of ^{3}H-Vittatine to ^{3}H-Oxovittatine

To a stirred solution of 57 mg. of ^{3}H-vittatine (1.73×10^{10} dpm/mM) in 20 ml. of chloroform was added 350 mg. of manganese dioxide[66]. The reaction mixture was stirred at room temperature for 8 hours and then filtered to remove the manganese dioxide. The oxide was washed four times with warm ethanolic chloroform. The combined filtrates were concentrated under reduced pressure to give a colorless oil that was crystallized from ether-chloroform. Recrystallization from ether-chloroform afforded pure oxovittatine, 40 mg., m.p. 185-187° (reported for enantiomer[52] m.p. 184-186°), 1.75×10^{10} dpm/mM.

Nerine bowdenii Feeding Experiment

Administration of labeled precursor

The ^{3}H-oxovittatine (0.88 mc., 30 mg.) was dissolved in 0.5 ml. of water (pH 6) and introduced directly into the Nerine bowdenii bulbs (8 bulbs) with a fine hypodermic needle. The radioactive sample tube was washed with 0.5 ml. of water and the water was injected into the same 8 bulbs one week later. The plants were grown in a greenhouse at Iowa State University for a period of 3 weeks after which the bulbs were harvested and the alkaloids isolated in the usual manner.

Extraction and separation of alkaloids from Nerine bowdenii bulbs

The fresh bulbs (463 g.) were ground three times in a Waring Blendor with 2 l. of 95% ethanol. The mixture was filtered and the filter cake was allowed to stand overnight in 1 l. of 95% ethanol. The solid material was separated by filtration and the filtrates were combined and concentrated under reduced pressure to 100 ml. This concentrate was diluted with two volumes of water, acidified with 2N hydrochloric acid, and filtered through Celite. The Celite cake was washed with 100 ml. of 2N hydrochloric acid and the filtrates were added to the original aqueous filtrate. The aqueous solution was extracted with 300 ml. of benzene in order to remove neutral and acidic material. The benzene extract gave a negative alkaloid test with silicotungstic acid and was discarded. The solution was then extracted with 1.5 l. of chloroform. The chloroform extract was concentrated under reduced pressure to give 600 mg. of alkaloids forming chloroform-soluble hydrochlorides. The aqueous solution was basified with ammonium hydroxide to pH 10 and extracted several times with chloroform. Concentration of this chloroform extract gave 50 mg. of lycorine which precipitated and was removed by filtration. Further concentration of the chloroform solution gave 600 mg. of crude alkaloids forming chloroform-insoluble hydrochlorides.

Alkaloids forming chloroform-insoluble hydrochlorides

A chloroform solution containing approximately 600 mg. of alkaloids was chromatographed on Florisil packed in chloroform. Elution with 1% methanol in chloroform eluted a large fraction (140 mg.) of a mixture of ambelline, crinamine, and a trace of buphanisine. Elution with 5% methanol in chloroform afforded a mixture (150 mg.) of crinamidine, crinine, and (+)- epi-crinine. Subsequent fractions gave no characterizable alkaloids.

The ambelline containing mixture (140 mg.) was rechromatographed on Florisil packed in chloroform. Elution with 1% methanol in chloroform gave fractions from which 62 mg. of ambelline, m.p. 258-260° (reported[30] m.p. 260-261°), was obtained by crystallization from ethyl acetate. Further elution with this solvent gave fractions from which 15 mg. of crinamidine, m.p. 232-234° (reported[37] m.p. 229-230°), was obtained by crystallization from chloroform-acetone.

The crinine containing mixture (150 mg.) was rechromatographed on Florisil packed in chloroform. Elution with 2% methanol in chloroform gave fractions from which 10 mg. of crinamidine was obtained by crystallization from chloroform-acetone. Further elution with 5% methanol in chloroform gave fractions from which 61 mg. of crinine, m.p. 205-207° (reported[30] m.p. 209-210°), was obtained after crystallization from acetone.

The ambelline filtrate was diluted with 70 mg. of inactive crinamine. The crinamine was recovered and recrystallized from acetone to afford radioactive crinamine, m.p. 199-200° (reported[30] m.p. 198-199°), 4.64 x 10^6 dpm/mM. The crinine filtrate was diluted in a similar manner with 70 mg. of inactive (+)-epicrinine. Recrystallization from chloroform-acetone afforded radioactive (+)-epicrinine, m.p. 208-209° (reported[37] m.p. 207-209°), 6.59 x 10^6 dpm/mM.

Alkaloids forming chloroform-soluble hydrochlorides

The extract containing 600 mg. of chloroform-soluble hydrochloride salts was dissolved in water and basified to pH 10 with ammonium hydroxide. The aqueous solution was extracted several times with chloroform and the chloroform extract was concentrated under reduced pressure to leave 500 mg. of crude alkaloids. A benzene solution of the crude alkaloids forming chloroform-soluble hydrochlorides (500 mg.) was chromotographed on Florisil packed in benzene. Elution with 10% ethyl acetate in benzene gave fractions containing belladine. Further elution with 50% ethyl acetate in benzene afforded a mixture of belladine and undulatine. Continued elution with ethyl acetate gave fractions containing undulatine which was crystallized from ethanol-water to give 60 mg., m.p. 151-152° (reported[37] m.p. 149-150°). Further elution with increasing percentages of methanol in ethyl acetate gave no additional characterizable alkaloids. The undulatine was separated from the undulatine-

belladine mixture by crystallization and the total amount of undulatine was combined and recrystallized to give 110 mg., m.p. 151-152°. The belladine was dissolved in methanol and the solution was saturated with gaseous hydrogen chloride. Ether was added to the point of permanent turbidity and the flask was seeded with belladine hydrochloride. The resulting precipitate was filtered and recrystallized from methanol to give 128 mg. of belladine hydrochloride, m.p. 193-194° (reported[37] m.p. 190-192°). The data from this isolation are tabulated in Table 17.

Table 17. Tabulated results of the Nerine bowdenii isolation

Alkaloid	Amount isolated	Activity (dpm/mM)	Yield, %
Lycorine	50 mg.	↑	0.011
Ambelline	62 mg.	|	0.013
Crinamidine	25 mg.	|	0.0054
Crinine	61 mg.	inactive	0.013
Belladine[a]	128 mg.	|	0.028
Undulatine	110 mg.	↓	0.024
Crinamine[b]	-------	4.64×10^6	-----
(+)-Epicrinine[b]	-------	6.59×10^6	-----

[a]Isolated as the hydrochloride salt.

[b]Isolated by dilution with 70 mg. of inactive alkaloid.

Oxidation of Crinamine isolated from the ^{3}H-oxovittatine feeding to Nerine bowdenii

The crinamine isolated by dilution from the ^{3}H-oxovittatine feeding to Nerine bowdenii was further diluted to give 800 mg., 7.53 x 10^{4} dpm/mM. The crinamine was then oxidized according to the procedure described in this section for the permanganate oxidation of vittatine. The crude hydrastic anhydride (17 mg.) derived from this oxidation was converted to N-ethyl hydrastimide which was recrystallized from ethanol to give 8 mg., m.p. 168-169°, 8.02 x 10^{4} dpm/mM. The activity of the N-ethyl hydrastimide relative to the crinamine was 1.06.

Synthesis of DL-1-^{14}C-Octopamine

Conversion of p-hydroxybenzaldehyde to 4-hydroxymandelonitrile

The p-hydroxybenzaldehyde (170 mg.) was dissolved in 1.7 ml. of 10% sodium bisulfite solution at 0°. The sodium ^{14}C-cyanide (2 mc., 8.4 mg.) was diluted with 76 mg. of inactive sodium cyanide and dissolved in 0.8 ml. of water. The aqueous cyanide solution was added dropwise to the stirred bisulfite solution at 0°. The solution was allowed to stir for one hour at 0°. After this time, 2 ml. of ether was added and the reaction mixture was allowed to stir for an additional 25 minutes. The reaction mixture was extracted with ether and the ether extract was washed twice with 20 ml. of 10% sodium bisulfite and once with 20 ml. of saturated sodium chloride solution. The ether extract was then dried with sodium sulfate

and concentrated to give an oily residue. This residue was dried under vacuum in the dark for five hours to give 128 mg. of 4-hydroxymandelonitrile, m.p. 100-101° (reported[67] m.p. 99-102°).

Di-tetrahydropyranyl ether of 4-hydroxymandelonitrile

The dry 4-hydroxymandelonitrile (128 mg.) was dissolved in 2.5 ml. of dihydropyran. To this solution was added two drops of concentrated hydrochloric acid and the solution was allowed to stand at room temperature for 24 hours. The reaction mixture was mixed with 60 ml. of ether and shaken vigorously with 20 ml. of 10% sodium hydroxide to insure removal of all traces of acid and any phenolic compounds present. The ether solution was washed with a saturated sodium chloride solution and dried over sodium sulfate. The ether and excess dihydropyran were removed under reduced pressure to leave 500 mg. of an oily mixture of the di-tetrahydropyranyl ether of 4-hydroxymandelonitrile and decomposition products of dihydropyran. The infrared spectrum of the mixture showed the absence of hydroxyl absorption. No further purification was carried out and the dry oily mixture was used directly in the next reaction.

Reduction of the di-tetrahydropyranyl ether of 4-hydroxymandelonitrile

The mixture containing the di-tetrahydropyranyl ether of 4-hydroxymandelonitrile (500 mg.) was dissolved in 10 ml. of ether and added dropwise under nitrogen to a stirred suspension

of 76 mg. of lithium aluminum hydride in 40 ml. of ether. After the addition was completed, the reaction mixture was allowed to stir for 20 minutes at room temperature. The lithium aluminum hydride was decomposed with dilute sodium hydroxide and the reaction mixture was filtered. The filter cake was washed with ether and the combined ether filtrates were dried with sodium sulfate and concentrated to leave 300 mg. of a mixture of the di-tetrahydropyranyl ether of octopamine and decomposition products from dihydropyran. The infrared spectrum of the mixture showed bands at 3480 and 3550 cm^{-1} corresponding to the asymmetric and symmetric stretching of the primary amino group.

Conversion of the di-tetrahydropyranyl ether of octopamine to octopamine tartrate

The mixture containing the di-tetrahydropyranyl ether of octopamine (300 mg.) was dissolved in a solution of 57 mg. of d-tartaric acid in 8 ml. of water and allowed to stand at room temperature for 3.5 hours. The aqueous solution was chromotographed on a cation-exchange column (Mallinckrodt, Amberlite CG-120, 100-200 mesh) which was prepared in the following manner. The resin (1 g.) was allowed to stand in water for one hour. The water was decanted and the resin was packed in a one cm. diameter column in water. The resin was washed with 50 ml. of dilute sodium hydroxide solution, 50 ml. of water and 100 ml. of 2N hydrochloric acid. A final wash was made with water until the aqueous eluate was neutral.

After the addition of the aqueous tartrate solution, the column was eluted with 50 ml. of water in order to remove the tartaric acid and other impurities. Elution with 4N ammonium hydroxide eluted fractions containing octopamine. The ammoniacal fractions were combined and concentrated under reduced pressure in a nitrogen atmosphere to leave a brown oily residue. The residue was dissolved in 4 ml. of absolute ethanol and added to a solution of 37 mg. of d-tartaric acid in 1 ml. of absolute ethanol. The tartrate salt of octopamine precipitated and was recrystallized to constant activity from ethanol-water to give 70 mg., (21% overall yield) m.p. 195-197°, (reported[68] m.p. 195-200°) 3.31×10^9 dpm/mM. An elemental analysis was carried out on an inactive sample synthesized according to the procedure described in this section.

Anal. Calcd. for $C_{20}H_{28}N_2O_{10}$: C,52.62; H,6.19; N,6.14. Found: C,52.40; H,6.24; N,6.13.

Crinum erubescens Feeding Experiment

Administration of labeled precursors

The labeled compounds used in this feeding experiment were DL-3-^{14}C-tyrosine (0.1 mc.), 1-^{14}C-tyramine (0.1 mc.), DL-1-^{14}C-octopamine (0.042 mc.), and DL-2-^{14}C-p-hydroxyphenylserine (0.1 mc.). Each compound was dissolved in 0.5 ml. of water (pH 6) and introduced into one Crinum erubescens bulb with a fine hypodermic needle. The plants were grown in a greenhouse at Iowa State University for a period of 3 weeks after which

the bulbs were harvested.

Extraction and separation of alkaloids from Crinum erubescens bulbs

Each isolation was carried out in exactly the same manner and in the interest of brevity, only the extraction and isolation of the alkaloids derived from the DL-3-^{14}C-tyrosine feeding are described below.

The bulb (305 g.) was ground three times in a Waring Blendor with 2 l. of 95% ethanol. The mixture was filtered and the filter cake was allowed to stand overnight in 1 l. of 95% ethanol. The mixture was filtered and the filtrates were combined and concentrated under reduced pressure to approximately 100 ml. This concentrate was diluted with 200 ml. water, acidified with 2N hydrochloric acid, and filtered through Celite. The Celite cake was washed with 100 ml. of 2N hydrochloric acid and the filtrates were added to the original aqueous filtrate. The aqueous solution was extracted with 200 ml. of benzene in order to remove neutral and acidic material. The benzene extract gave a negative alkaloid test with silicotungstic acid and was discarded. The aqueous solution was then basified with 10% sodium hydroxide to pH 12 and extracted with 20% ethanol in chloroform solution until the extract failed to give a positive alkaloid test with silicotungstic acid. The ethanolic chloroform extract was concentrated under reduced pressure to give 372 mg. of lycorine which precipitated and was removed by filtration. The lycorine was

dissolved in methanol and the solution was saturated with gaseous hydrogen chloride. Ether was added to the point of permanent turbidity and the flask was seeded with lycorine hydrochloride. The resulting precipitate was removed by filtration and recrystallized from methanol to give 400 mg. of lycorine hydrochloride, m.p. 214-215° (reported[1] m.p. 212-214°), 8.50×10^5 dpm/mM. Further concentration of the ethanolic chloroform solution gave 1.39 g. of crude alkaloids.

The crude alkaloids (1.39 g.) were chromatographed on Florisil packed in chloroform. Elution with chloroform and 1% methanol in chloroform gave fractions from which 27 mg. of crinamine, m.p. 199-200°, 4.32×10^6 dpm/mM, was obtained by crystallization from acetone. Further elution with 2-4% methanol in chloroform gave fractions from which 440 mg. of 6-hydroxycrinamine, m.p. 210-211° (reported[58] m.p. 210°), 1.43×10^5 dpm/mM, was obtained by crystallization from chloroform-acetone. The data from the Crinum erubescens isolations are presented in Tables 18-21.

Table 18. Alkaloids isolated from the DL-3-^{14}C-tyrosine feeding to Crinum erubescens

Alkaloid	Weight of alkaloid	Per cent alkaloid in extract[a,b]	Per cent alkaloid in bulb[c]
Lycorine	372 mg.	27	0.12
Crinamine	27 mg.	2	0.009
6-hydroxycrinamine	440 mg.	32	0.14

[a]Weight of bulb..... 305 g.

[b]Per cent incorporation into extract..... 1.9%

[c]Weight of extract..... 1.39 g.

Table 19. Alkaloids isolated from the 1-^{14}C-tyramine feeding to Crinum erubescens

Alkaloid	Weight of alkaloid	Per cent alkaloid in extract[a,b]	Per cent alkaloid in bulb[c]
Lycorine	571 mg.	29	0.13
Crinamine	140 mg.	7	0.03
6-hydroxycrinamine	513 mg.	26	0.12

[a]Weight of bulb..... 441 g.

[b]Per cent incorporation into extract..... 10.4%

[c]Weight of extract..... 1.97 g.

Table 20. Alkaloids isolated from the DL-2-^{14}C-p-hydroxyphenylserine feeding to Crinum erubescens

Alkaloid	Weight of alkaloid	Per cent alkaloid in extract[a,b]	Per cent alkaloid in bulb[c]
Lycorine	536 mg.	34	0.17
Crinamine	71 mg.	5	0.02
6-hydroxycrinamine	380 mg.	24	0.12

[a]Weight of bulb..... 317 g.

[b]Per cent incorporation into extract..... 1.13%

[c]Weight of extract..... 1.56 g.

Table 21. Alkaloids isolated from the DL-1-^{14}C-octopamine feeding to Crinum erubescens

Alkaloid	Weight of alkaloid	per cent alkaloid in extract[a,b]	Per cent alkaloid in bulb[c]
Lycorine	660 mg.	34	0.18
Crinamine	162 mg.	8	0.044
6-hydroxycrinamine	586 mg.	30	0.16

[a]Weight of bulb..... 366 g.

[b]Per cent incorporation into extract..... 0.24%

[c]Weight of extract..... 1.96 g.

Degradation of 6-hydroxycrinamine derived from the 1-^{14}C-tyramine feeding to Crinum erubescens

The 6-hydroxycrinamine (110 mg., 3.21 x 10^5 dpm/mM) from the 1-^{14}C-tyramine feeding experiment was diluted with inactive 6-hydroxycrinamine (170 mg.) and recrystallized from chloroform-acetone to give 6-hydroxycrinamine having an activity of 1.27 x 10^5 dpm/mM.

Conversion of 6-hydroxycrinamine to criwelline The 6-hydroxycrinamine (230 mg.) was dissolved in 40 ml. of acetone and 3 ml. of methyl iodide was added. After the solution was allowed to stand at room temperature for 5 hours, the solvents were removed under reduced pressure to give the methiodide salt of 6-hydroxycrinamine. The methiodide salt was dissolved in 20 ml. of water and the solution was made basic (pH 12) with 5 ml. of 30% sodium hydroxide and allowed to stand at room temperature for 2 hours. The aqueous solution was extracted with chloroform and the chloroform extract was concentrated under reduced pressure to leave 240 mg. of criwelline. The criwelline was recrystallized from acetone to give 220 mg., m.p. 205-206° (reported[1] m.p. 205-206°), 1.29 x 10^5 dpm/mM.

Criwelline methiodide To a solution of 210 mg. of criwelline in 25 ml. of acetone was added 2 ml. of methyl iodide, and the solution was allowed to reflux for 2 hours. The solvents were removed under reduced pressure to leave 212 mg. of criwelline methiodide. The methiodide salt was recrystallized from acetone-ether to give white crystals, m.p. 201-

202°, 1.21 x 10^5 dpm/mM.

Dimethylglycine hydrochloride and 6-phenylpiperonyl alcohol To an aqueous solution of 200 mg. of criwelline methiodide was added an excess of silver oxide prepared from 350 mg. of silver nitrate. The mixture was allowed to stir for one hour after which the slurry was filtered. The filtrate was concentrated under reduced pressure and the resulting residue was pyrolyzed for one hour on a steam bath under aspirator vacuum to give 140 mg. of criwelline methine, which was not crystalline. The material was identified by its infrared spectrum which was identical with an authentic sample.

To a methanolic solution (25 ml.) of the methine was added 5 ml. of 3N sodium hydroxide. The reaction mixture was allowed to reflux for one hour and then concentrated to 5 ml. The concentrate was mixed with 25 ml. of water and extracted with ether. Evaporation of the ether gave a residue which crystallized from ether to give 93 mg. of 6-phenylpiperonyl alcohol, m.p. 101-102° (reported[61] m.p. 98-99°). The alcohol was found to be non-radioactive.

The aqueous solution remaining after the ether extraction, was acidified with dilute hydrochloric acid and concentrated under reduced pressure to give a mixture of sodium chloride and dimethylglycine hydrochloride. The dimethylglcyine hydrochloride was separated from the sodium chloride by sublimation at 135° (0.01 mm.). Resublimation of the dimethylglycine hydrochloride gave white crystals (30 mg.), m.p. 184-185°

(reported[61] m.p. 184-185°), 1.23 x 10^5 dpm/mM.

Lead tetraacetate oxidation of dimethylglycine hydrochloride To 25 mg. of dimethylglycine hydrochloride in a three necked, round-bottomed flask was added 10 ml. of 1% lead tetraacetate-glacial acetic acid solution. The flask was fitted with a nitrogen outlet that led to a trap containing a saturated dimedone solution in water and a second trap containing 1 ml. of Hyamine hydroxide 10X (Packard) and 10 ml. of toluene POPOP scintillation solution (Figure 23). The flask

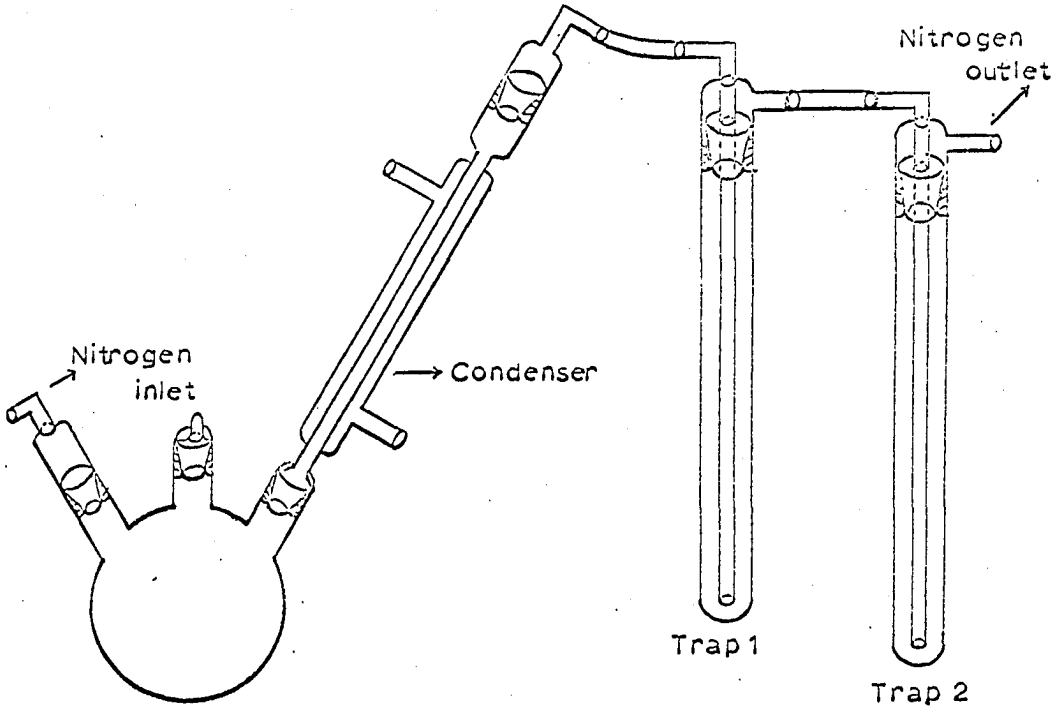


Figure 23. Apparatus for lead tetraacetate oxidation of dimethylglycine hydrochloride (61, p. 86)

was heated on a steam bath for 30 minutes with a nitrogen flow. The formaldehyde dimedone adduct was separated from the dimedone solution by filtration. The adduct was recrystallized from methanol to give 6 mg., m.p. 195-196° (reported[61] m.p. 192-193°), 1.25 x 10^5 dpm/mM. The scintillation solution containing the carbon dioxide was found to be inactive.

Degradation of 6-hydroxycrinamine derived from the DL-2-^{14}C-p-hydroxyphenylserine feeding to Crinum erubescens

The 6-hydroxycrinamine (100 mg., 2.20 x 10^5 dpm/mM) from the DL-2-^{14}C-p-hydroxyphenylserine feeding experiment was diluted with inactive 6-hydroxycrinamine (350 mg.) and recrystallized from chloroform-acetone to give 450 mg., 4.88 x 10^4 dpm/mM. The 6-hydroxycrinamine was degraded to 6-phenylpiperonyl alcohol and dimethylglycine hydrochloride according to the procedure previously described for the degradation of 6-hydroxycrinamine derived from the 1-^{14}C-tyramine feeding. The 6-phenylpiperonyl alcohol was found to have an activity of 2.58 x 10^4 dpm/mM, while the dimethylglycine hydrochloride was found to be inactive.

Methylenedioxy determination To 100 mg. of 6-hydroxycrinamine (1.71 x 10^4 dpm/mM) and 300 mg. of dimedone was added 7 ml. of sulfuric acid (6 ml. of sulfuric acid in 10 ml. of water). The reaction mixture was heated on a steam bath for 18 hours and added to 25 ml. of water. The aqueous solution was heated in order to dissolve the excess dimedone. The hot solution was filtered and the precipitate was recrystallized

from methanol to give 12 mg., of formaldehyde dimedone adduct, m.p. 193-194°, 8.67 x 10^3 dpm/mM.

Methoxyl determination The methoxyl determination was conducted in the apparatus shown in Figure 24. A solution of 0.5 ml. of 5% cadmium sulfate and 0.5 ml. of 5% sodium thiosulfate was placed in the iodine trap B. A solution of 5 ml. of 5% triethylamine in absolute ethanol was placed in trap C to trap the methyl iodide generated in the reaction as methyltriethyl ammonium iodide. Trap C was emersed in a dry ice-chloroform bath.

To 55 mg. of 6-hydroxycrinamine and 500 mg. of phenol in flask A was added 2 ml. of 47% hydroidodic acid. Flask A was then connected to the nitrogen source and heated at 125-132° in a Wood's metal bath for 45 minutes. Trap C was then detached at point D (rubber joint) and stored under nitrogen overnight. The ethanol-triethylamine solution was concentrated under reduced pressure to leave a residue which crystallized from methanol-ether to give 34 mg. of methyltriethyl ammonium iodide, m.p. 295-297°, identical with an authentic sample (8.38 x 10^3 dpm/mM).

Methylenedioxy determination of lycorine hydrochloride derived from the DL-2-^{14}C-p-hydroxyphenylserine feeding to Crinum erubescens

The lycorine hydrochloride (100 mg., 2.07 x 10^4 dpm/mM) was degraded according to the procedure described for the methylenedioxy determination of the 6-hydroxycrinamine derived from

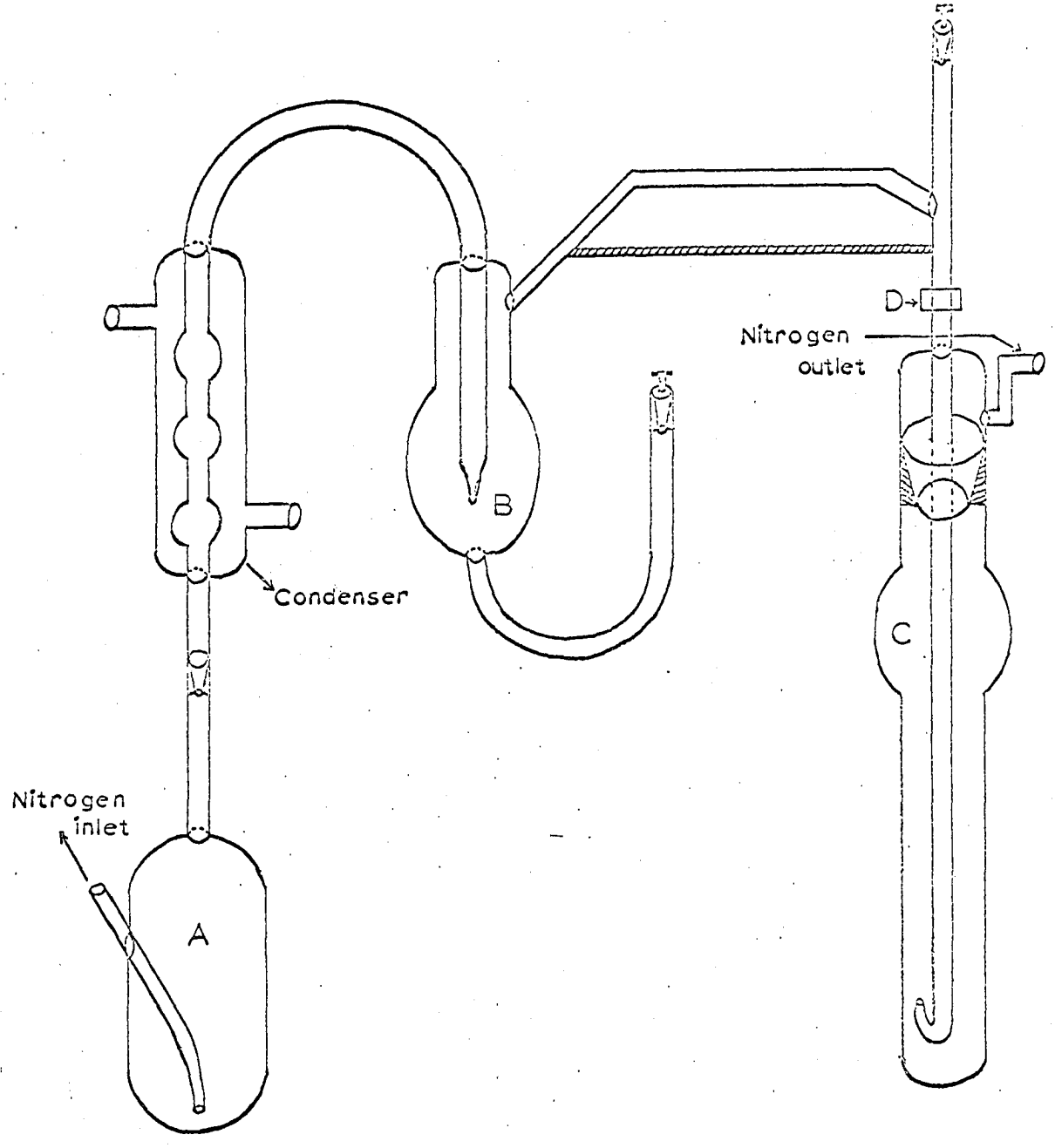


Figure 24. Apparatus for methoxyl determination (61, p. 89)

the DL-2-^{14}C-p-hydroxyphenylserine feeding. The formaldehyde dimedone adduct (34 mg., m.p. 195-196°) obtained in the degradation was found to have an activity of 2.16 x 10^4 dpm/mM.

Methoxyl determination of 6-hydroxycrinamine derived from the DL-1-^{14}C-octopamine feeding to Crinum erubescens

The 6-hydroxycrinamine (40 mg., 3.84 x 10^3 dpm/mM) was degraded according to the procedure described for the methoxyl determination carried out on the 6-hydroxycrinamine which was isolated from the DL-2-^{14}C-p-hydroxyphenylserine feeding. The methyltriethyl ammonium iodide (27 mg., m.p. 295-297°) isolated in the degradation, was found to have an activity of 1.94 x 10^3 dpm/mM.

Methylenedioxy determination of 6-hydroxycrinamine and lycorine hydrochloride derived from the DL-1-^{14}C-octopamine feeding to Crinum erubescens

The 6-hydroxycrinamine (80 mg., 3.84 x 10^3 dpm/mM) and lycorine hydrochloride (64 mg., 1.05 x 10^4 dpm/mM) were degraded according to the procedure previously described for the alkaloids derived from the DL-2-^{14}C-p-hydroxyphenylserine feeding. The formaldehyde dimedone adduct (7 mg., m.p. 195-196°) derived from the 6-hydroxycrinamine exhibited an activity of 1.81 x 10^3 dpm/mM, while the adduct obtained from the lycorine hydrochloride degradation (18 mg., m.p. 195-196°) had an activity of 1.01 x 10^4 dpm/mM.

Instrumentation

The nuclear magnetic resonance spectra were run on a Varian A-60 spectrometer operating at 60 Mc.p.s. The spin decoupling experiments were performed with a Varian HR-60 spectrometer by a modification of the method of Johnson[69]. All mass spectra were determined with an Atlas CH-4 mass spectrometer using the TO-4 ion source (70 e.v.).

Melting points were taken on a Kofler microscope hot stage and are corrected. The infrared spectra were obtained on the Perkin-Elmer Model 21 and the Beckman IR 12 spectrophotometers. All gas phase analyses were done on a Chromalab Model A-110 gas phase chromatograph using a 12 ft. glass column containing 1% Silicone GE SE-30 on Gas-Chrom Q (Applied Science Laboratories). The elemental analyses were carried out by Ilse Beetz Micro-analytical Laboratory, Kronach, West Germany.

LITERATURE CITED

1. W. C. Wildman, Alkaloids of the Amaryllidaceae, in F. H. F. Manske, ed., The Alkaloids, Vol. 6, pp. 289-413, Academic Press, Inc., New York, New York, 1961.

2. R. J. Suhadolnik, A. G. Fischer and J. Zulalian, Biochem. Biophys. Res. Comm., 11, 208 (1963).

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

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

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

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

7. R. J. Suhadolnik and A. G. Fischer, American Chemical Society Abstract of Papers, 140, 39Q (1961).

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

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

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

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

12. J. Zulalian, A. G. Fischer and R. J. Suhadolnik, American Chemical Society Abstract of Papers, 144, 30L (1963).

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

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

15. R. J. Suhadolnik, Lloydia, 27, 315 (1964).

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

17. R. J. Suhadolnik, A. G. Fischer and J. Zulalian, J. Am. Chem. Soc., 84, 4128 (1962).

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

19. A. G. Fischer and R. J. Suhadolnik, Fed. Proc., 39, 399 (1962).

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

21. E. W. Warnhoff, Chem. and Ind., 1385 (1957).

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

23. H. M. Fales and W. C. Wildman, J. Am. Chem. Soc., 86, 294 (1964).

24. A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales and W. C. Wildman, Proc. Chem. Soc., 243 (1961).

25. W. C. Wildman, H. M. Fales, R. J. Highet, S. W. Breuer and A. R. Battersby, Proc. Chem. Soc., 180 (1962).

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

27. C. L. Brown, The Structure of Pancracine, unpublished Ph.D. thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1966.

28. W. C. Wildman and C. J. Kaufman, J. Am. Chem. Soc., 77, 1248 (1955).

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

30. L. H. Mason, E. R. Puschett and W. C. Wildman, J. Am. Chem. Soc., 77, 1253 (1955).

31. R. Heck and S. Winstein, J. Am. Chem. Soc., 79, 3105 (1957).

32. F. Sandberg and K. H. Michel, Lloydia, 26, 78 (1963).

33. K. H. Michel, Isolation and Structure of 11-Hydroxyvittatine, unpublished report, Dr. W. C. Wildman, Department of Chemistry, Iowa State University of Science and Technology, Ames, Iowa, 1966.

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

35. J. Keller, ^{3}H-Haemanthamine Feeding to H. coccineus, unpublished report, Dr. W. C. Wildman, Department of Chemistry, Iowa State University of Science and Technology, Ames, Iowa, 1963.

36. Y. Yamamoto, Biosynthesis of Montanine, unpublished report, Dr. W. C. Wildman, Department of Chemistry, Iowa State University of Science and Technology, Ames, Iowa, 1967.

37. R. E. Lyle, E. A. Kielar, J. R. Crowder and W. C. Wildman, J. Am. Chem. Soc., 82, 2620 (1960).

38. D. Archer, ^{3}H-Crinine Feeding to N. bowdenii, unpublished report, Dr. W. C. Wildman, Department of Chemistry, Iowa State University of Science and Technology, Ames, Iowa, 1964.

39. M. M. Nijland, Pharm. Wukblad., 98, 301 (1963).

40. I. Monkovic and I. D. Spenser, Proc. Chem. Soc., 223 (1964).

41. I. Monkovic and I. D. Spenser, J. Am. Chem. Soc., 87, 1137 (1965).

42. S. Udenfriend, W. Lovenberg and A. Sjoerdsma, Arch. Biochem. Biophys., 85, 487 (1959).

43. W. J. Smith and N. Kirshner, J. Biol. Chem., 237, 1890 (1962).

44. J. E. Gander, Fed. Proc., 18, 232 (1959).

45. J. J. Pisano, C. R. Creveling and S. Udenfriend, Biochim. et Biophys. Acta, 43, 566 (1960).

46. Arvid Carlsson and Bertil Waldeck, Acta Pharmacol. Toxicol., 20, 371 (1963).

47. V. Erspamer, Nature 169, 375 (1952).

48. Y. Kakimoto and M. D. Armstrong, J. Biol. Chem., 237, 422 (1962).

49. I. Stewart and T. A. Wheaton, Science, 145, 60 (1964).

50. A. White, P. Handler and E. L. Smith, Principles of Biochemistry, 3rd ed., McGraw-Hill Book Co., Inc., New York, New York, 1964.

51. R. Imaizumi, H. Yoshida and T. Kita, Japan. J. Pharmacol., 8, 9 (1959).

52. W. C. Wildman, J. Am. Chem. Soc., 80, 2567 (1958).

53. A. M. Duffield, R. T. Aplin, H. Budzikiewicz, Carl Djerassi, C. F. Murphy and W. C. Wildman, J. Am Chem. Soc., 87, 4902 (1965).

54. K. E. Wilzbach, J. Am. Chem. Soc., 79, 1013 (1957).

55. R. E. Peterson, Atomlight (New England Nuclear Corp., Boston, Mass.), 24, 2 (1962).

56. L. Cheong, M. A. Rich and M. Eidinoff, J. Biol. Chem., 235, 1441 (1960).

57. William E. Parham and E. L. Anderson, J. Am. Chem. Soc., 70, 4187 (1948).

58. H. M. Fales, D. H. S. Horn and W. C. Wildman, Chem. Ind. (London), 1415 (1959).

59. C. F. Murphy and W. C. Wildman, Tetrahedron Letters, 51, 3863 (1964).

60. W. I. Taylor, S. Uyeo and H. Yajima, J. Chem. Soc., 2962 (1955).

61. J. A. Miller, Biosynthetic Studies on Tazettine and Ambelline, unpublished Ph.D. thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1966.

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

63. R. N. Gupta and I. D. Spenser, Canad. J. Chem., 43, 133 (1965).

64. J. J. Willaman and B. G. Shubert, U.S. Dept. Agr. Tech. Bull., 1234 (1964).

65. Josse C. Rabinowitz, Folic Acid, in P.D. Boxer, H. Lardy and K. Myrback, eds., The Enzymes, Vol. 2, pp. 185-252, 2nd ed., Academic Press, Inc., New York, New York, 1960.

66. J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. H. Evans, B. A. Hems, A. A. Jansen and T. Walker, J. Chem. Soc., 1094 (1952).

67. K. Ladenburg, K. Folkers and R. T. Major, J. Am. Chem. Soc., 58, 1292 (1936).

68. T. Kappe and M. D. Armstrong, J. Med. Chem., 7, 569 (1964).

69. L. F. Johnson, Proton-Proton Spin Decoupling Using the Varian V-3521 Integration, Varian Associates, Palo Alto, California, 1962.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. W. C. Wildman, for his guidance, encouragement and patience during the course of this investigation. The author would also like to acknowledge Dr. T. H. Kinstle, for giving freely of his time in discussing mass spectral data.

The author thanks his parents for their encouragement throughout the course of his education. He especially wishes to thank his wife, Millie, for the many sacrifices she has made and expresses his sincere gratitude for her patience and understanding during his years in graduate school and for her meticulous typing of this thesis.

These studies were supported by a grant to Iowa State University from the United States Public Health Service (Grant HE 7503) and a one-year (September, 1965 to September, 1966) fellowship granted by the Shell Companies Foundation.