Iowa State University
Digital Repository

Retrospective Theses and Dissertations

Iowa State University Capstones, Theses and Dissertations

1974

Hemi acetal aldehyde ammonia-interconversions and structures in Amaryllidaceae alkaloids

Christopher Paul Christenson
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Organic Chemistry Commons

Recommended Citation

Christenson, Christopher Paul, "Hemi acetal aldehyde ammonia-interconversions and structures in Amaryllidaceae alkaloids " (1974). *Retrospective Theses and Dissertations*. 5134.
https://lib.dr.iastate.edu/rtd/5134

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

www.manaraa.com

INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms
300 North Zeeb Road
Ann Arbor, Michigan 48106

75-10,469

CHRISTENSON, Christopher Paul, 1947-
HEMI ACETAL ALDEHYDE AMMONIA- INTERCONVERSIONS
AND STRUCTURES IN AMARYLLIDACEAE ALKALOIDS.

Iowa State University, Ph.D., 1974
Chemistry, organic

Xerox University Microfilms, Ann Arbor, Michigan 48106

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED.

Hemi acetal aldehyde ammonia- interconversions and structures in Amaryllidaceae alkaloids

by

Christopher Paul Christenson

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

Department: Chemistry
Major: Organic Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1974

TABLE OF CONTENTS

Page

PREFACE iii

HISTORICAL 1

- Biosynthesis and Late-Stage Hydroxylations 1
- The Symmetry and Optical Activity of 2,3-Dimethoxy-6,12-methano-9,10,methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[c,f]azocine 18
- Structure and Optical Activity of the Aromatic Chromophore 19

RESULTS AND DISCUSSION 39

- The Stereochemistry of the C-2 Hydroxylation in Pluviine 39
- Chemical Hydroxylation of C-7 in Lycorine 63
- X-ray Structure and CD Spectra of 2,3-Dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[*c*,*f*]azocine(17,31) 88
- Structure and Optical Activity in the Amaryllidaceae Alkaloids 93

EXPERIMENTAL 109

- Methods and Procedures 109

SUMMARY 117

LITERATURE CITED 118

ACKNOWLEDGEMENTS 122

APPENDIX 123

- Crystal Structure of 2,3-Dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[c,f]azocine done with Dr. J. Clardy and Dr. S. Porter 123

PREFACE

The Amaryllidaceae alkaloids have been studied since 1877 and, as new instrumentation and separation techniques have been developed, the research in this plant family has moved to utilize these methods. In recent years only a few biosynthetic and stereochemical problems remained to be resolved.

The C-2 hydroxylation of the lycorine-type alkaloids has been interpreted by various groups as proceeding either with retention or with inversion of configuration.

The optical activity of the aromatic chromophore has been the subject of much study and several rules have been proposed to explain the ^1L_a and ^1L_b Cotton effects of the alkaloids. However, none of these rules consistently predict the absolute configuration of all the Amaryllidaceae alkaloids. Closely related to this was the structure and optical activity of 2,3-dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[*c*,*f*]azocine(17, **31**).

Finally, the intermediacy of 7-hydroxylycorine has been suggested in the biosynthesis of hippeastrine although this compound has not been observed.

This thesis was undertaken in an attempt to resolve these questions.

HISTORICAL

Biosynthesis and Late-Stage Hydroxylations

The major biosynthetic pathways of the Amaryllidaceae alkaloids have been extensively explored (1), and the roles of phenylalanine (2,3,4,5,6), tyrosine (7,8,9,10), and the phenyl-phenyl oxidative coupling mechanism of Barton and Cohen (11) have been investigated in detail.

Recently, the focus of the research in this area turned to the stereochemistry of the late-stage hydroxylations at the benzylic, the allylic carbon atoms as well as the two carbon bridge which is common to most of the alkaloids. These investigations not only more clearly elucidated the mechanism of hydroxylation, but also provided insight into the mechanism of the rearrangements among the ring systems. Before discussing the late-stage oxidations, a brief account of these rearrangements *in vivo* and *in vitro* is presented.

The biosynthesis of lycorine and lycorenine-type alkaloids is as intertwined as their names would imply. O-methylnorbelladine (1) (2) and norpluviine (2) (4) were shown to be the precursors to both of these systems (Figure 1).

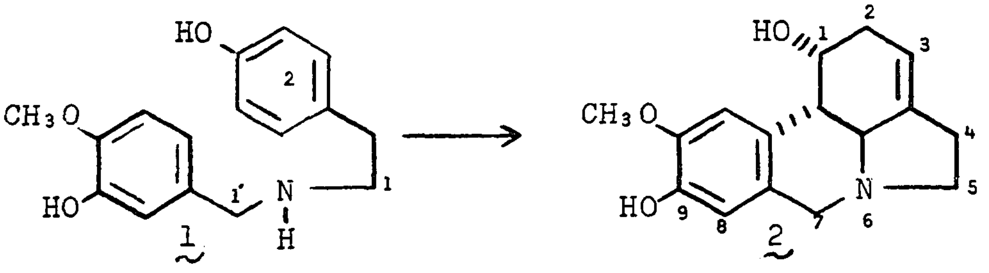


Figure 1. Conversion of norbelladine to norpluviine

Further transformations of norpluviine were reported by Harken (12), and by Wildman and Heimer (13) as shown in Figure 2. Harken fed [8-^{3}H]norpluviine (2) to *Narcissus* "King Alfred" and isolated lycorenine (3), pluviine (4), methylpseudolycorine (5), galanthine (6), and narcissidine (7), each containing tritium. However, when [8-^{3}H]pluviine (4) was fed to *Narcissus poeticus*, only galanthine (6), methylpseudolycorine (5), narcissidine (7), and lycorine (8) were found to contain the tritium label. The lycorenine isolated in this feeding was found to be inactive. This suggested that pluviine was not an important precursor to lycorenine and that a free hydroxyl group at C-9 seems to facilitate lycorenine formation. The incorporation of [8-^{3}H]pluviine into lycorine but not into lycorenine indicated that the conversion of the dimethoxy group to a methylenedioxy ring is not a simple demethylation to norpluvine. If this were true, the lycorenine isolated should have incorporated the label.

Wildman and Heimer have shown that caranine (9) is a precursor to lycorine (8) and hippeastrine (10). It was not possible to determine whether the C-7 oxidation in this system occurred before or after the C-2 hydroxylation. The isolation of radioactive hippeastrine is not in agreement with the findings of Harken and Wildman.

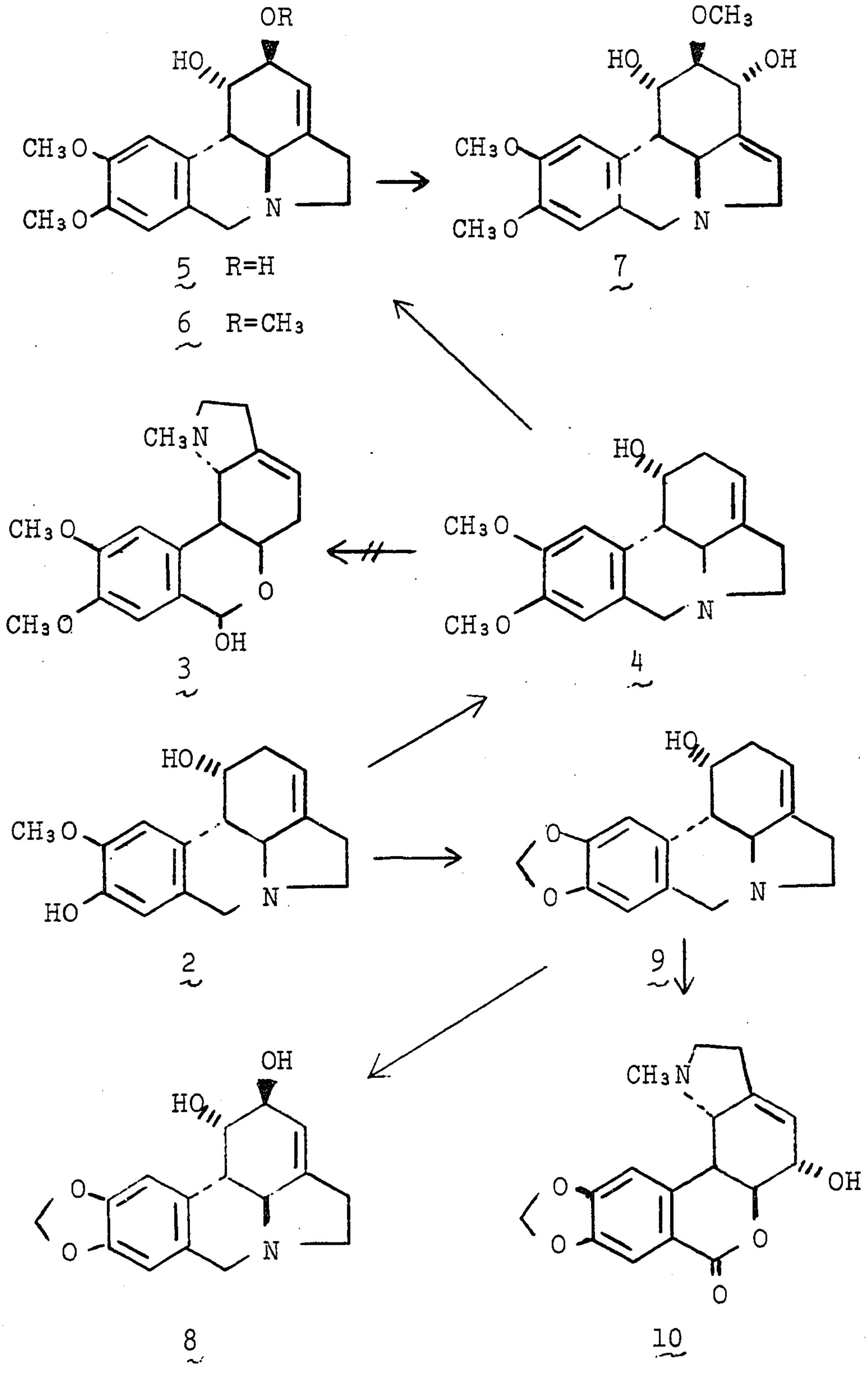

5 R=H
6 R=CH3
7
3
4
2
9
8
10

Figure 2. Biosynthetic pathways from norpluviine (2)

The interconversion of the lycorenine and lycorine ring systems has also been achieved by chemical methods. Boit and Ehmke showed that hippeastrine (10) was converted to lycorine β-methiodide (11) by a lithium aluminum hydride reduction followed by treatment with *p*-toluenesulfonyl chloride, then by iodide ion (Figure 3) (14). With the known stereochemistry of lycorine (8) this conversion established the stereochemistry of hippeastrine. Similar reactions have shown the relationship of homolycorine (12) to pluviine (4) (Figure 4) (15). In the reverse direction dihydrocaranine

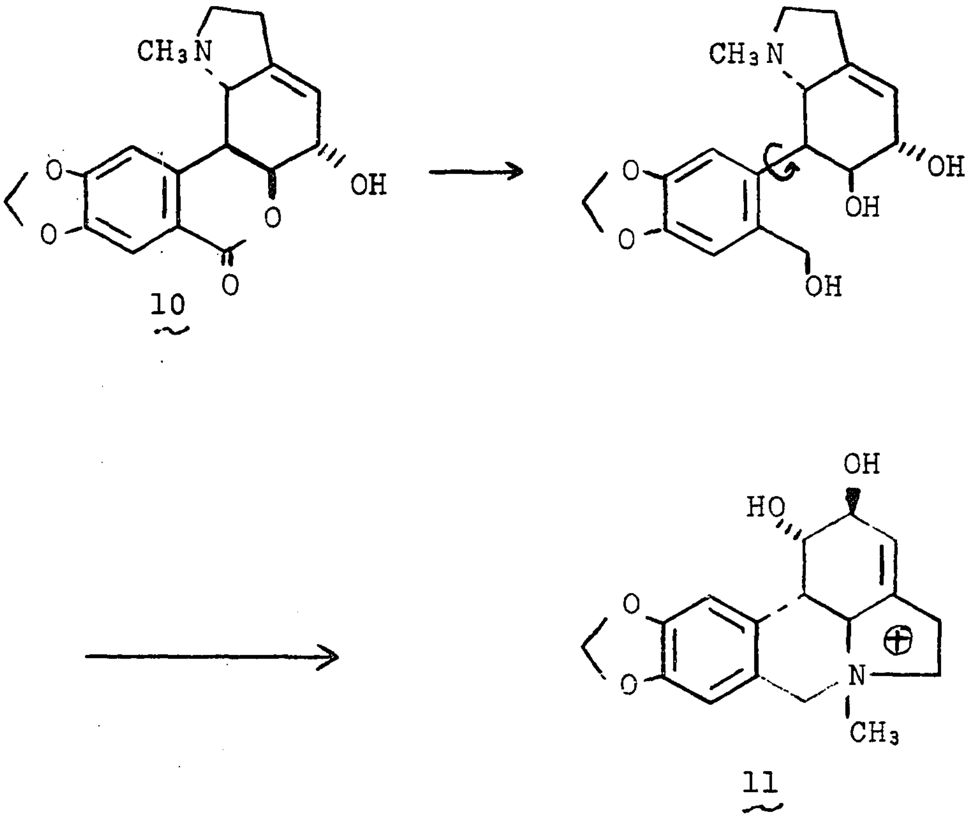
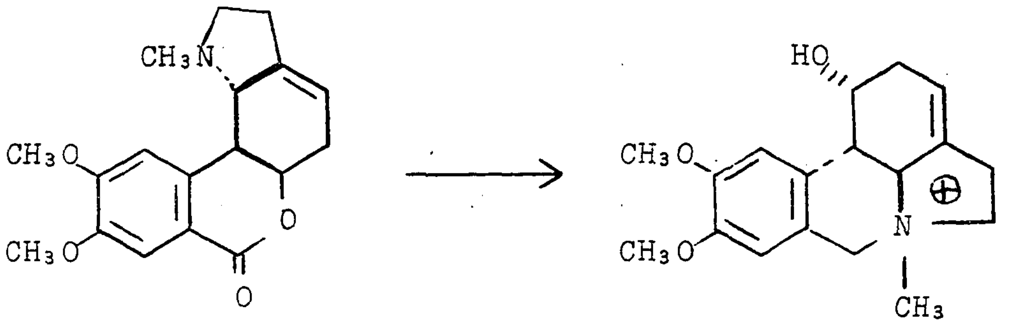


Figure 3. Conversion of hippeastrine to lycorine β-methiodide


CH3N
CH3O
CH3O
O
O
HO
CH3O
CH3O
N
⊕
CH3

12

Figure 4. Rearrangement of homolycorine to pluviine methiodide

(13) was converted to α-deoxydihydrooduline by Mizukami (16) via a cyanogen bromide cleavage of the nitrogen C-7 bond to give a benzyl bromide. The halogen was displaced by the C-1 alcohol to produce the rearranged product (14). Lithium aluminum hydride reduction and methylation produced α-deoxydihydrooduline (15) (Figure 5).

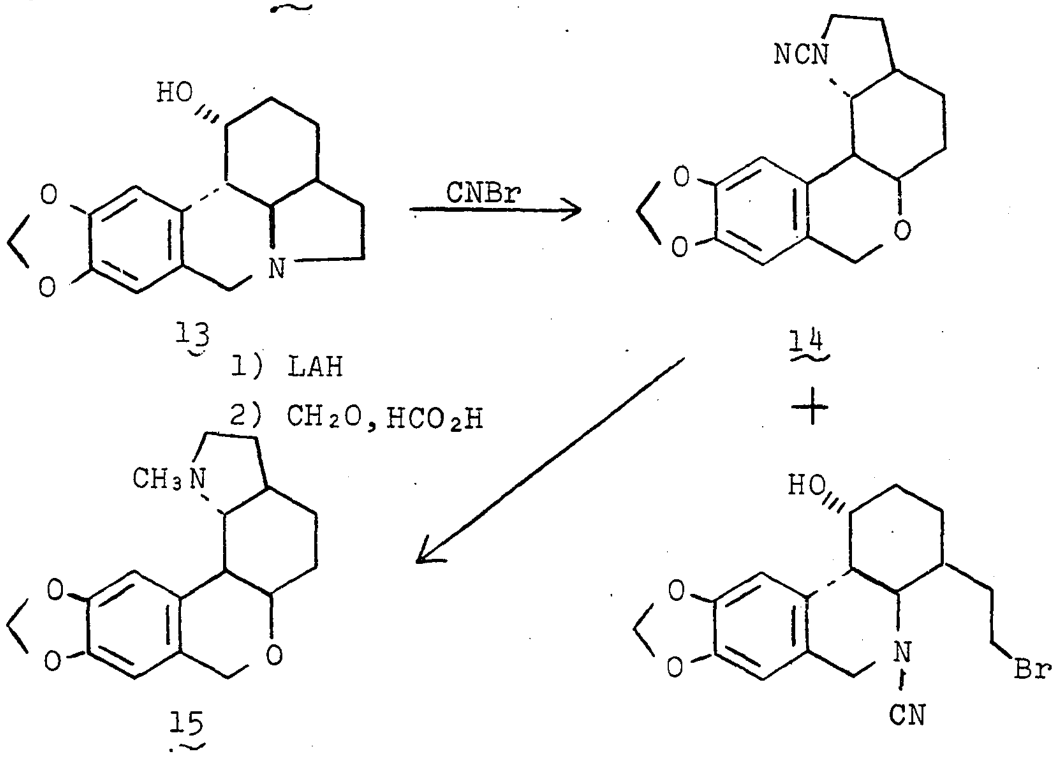

HO
O
O
N
13
CNBr
NCN
O
O
O
14
1) LAH
2) CH2O, HCO2H
+
CH3N
O
O
O
15
HO
O
O
N
CN
Br

Figure 5. Conversion of dihydrocaranine to α-deoxydihydrooduline

The conversion of the 11-hydroxylated derivatives of the crinane nucleus to montanine-type derivatives by Inubushi and co-workers (17) illustrated the chemical rearrangements which ultimately led to assignment of the structure of montanine, coccinine, manthine and pancracine. The key reactions consisted of treating haemanthamine (15) with mesyl chloride and hydrolyzing the crude reaction product with aqueous alkali to yield a sulfur free compound (16) (Figure 6).

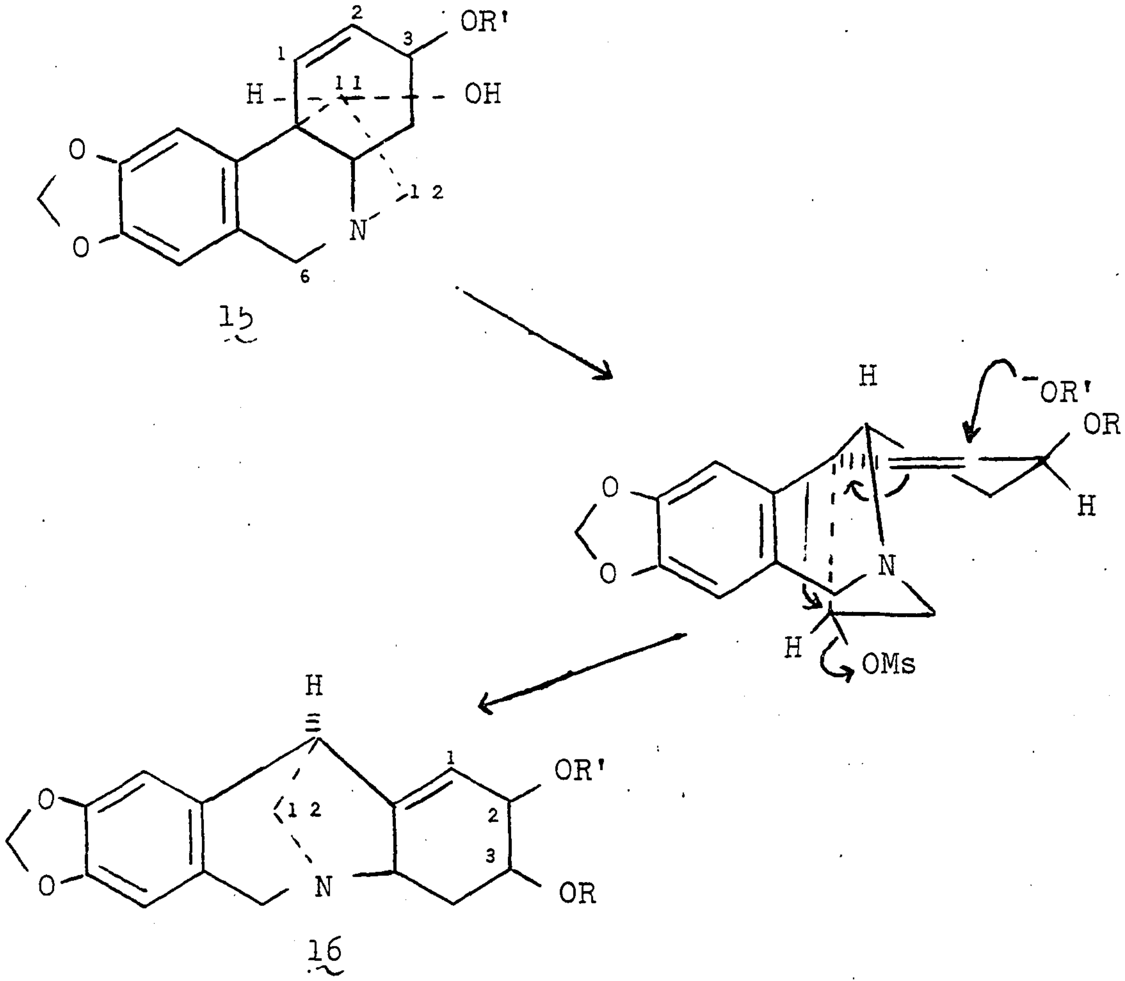


Figure 6. Rearrangement of haemanthine-type to montanine-type ring system

The synthesis of montanine had indicated its relationship to the 11-hydroxylated crinane nucleus. Since 11-hydroxyvittatine (17) was known to be a minor alkaloid in *Rhodophiala bifida* (18), Feinstein (19) investigated the biosynthesis of haemanthamine (15) and montanine (18) by feeding ^{3}H-vittatine (19). The conversion of vittatine into montanine and haemanthamine from tracer studies supported the biosynthesis of montanine *via* 11-hydroxyvittatine (Figure 7).

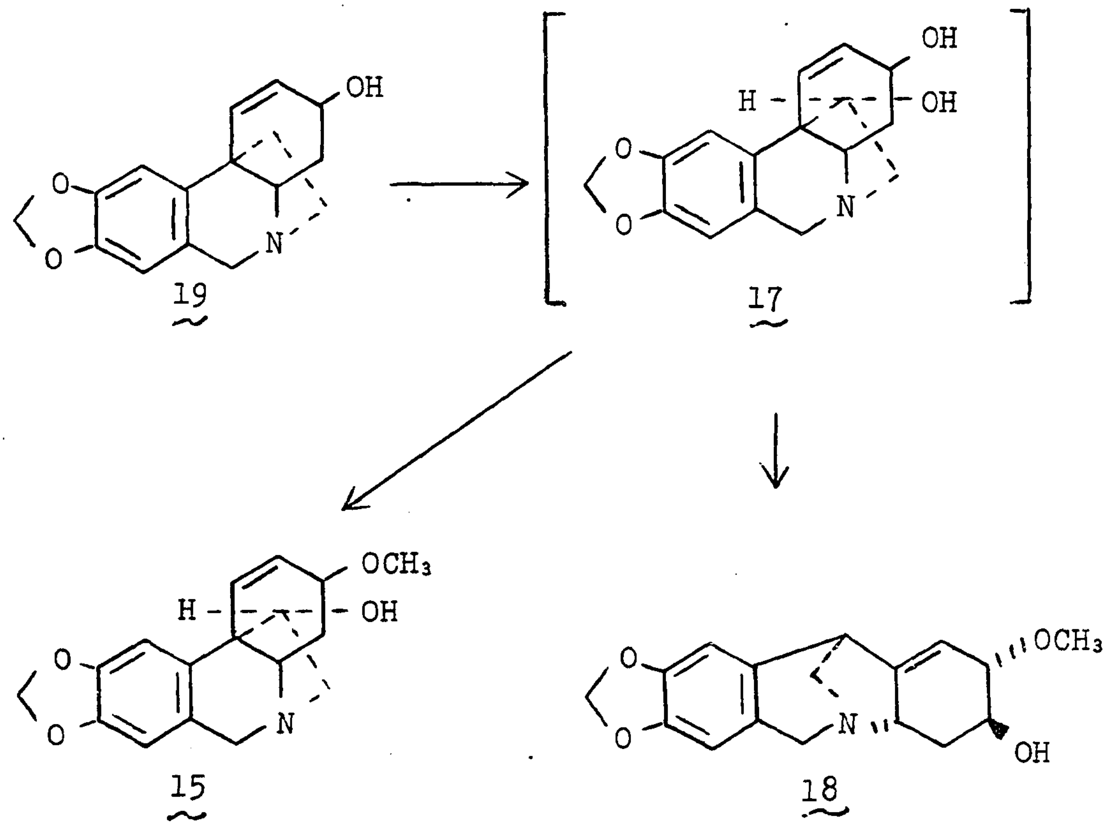


Figure 7. Biosynthesis of haemanthamine and montanine

The rearrangement of haemanthidine (20) to pretazettine (21) was investigated by Brown (20) and by Bailey (21). The N-methylation of haemanthidine was shown to cause a rearrangement to pretazettine as shown in Figure 8. This rearrangement was also shown to convert 6-hydroxycrinamine to precriwelline. In stronger base the C-3 epimers of 20 are converted to tazettine and criwelline by an internal Cannizzaro reaction.

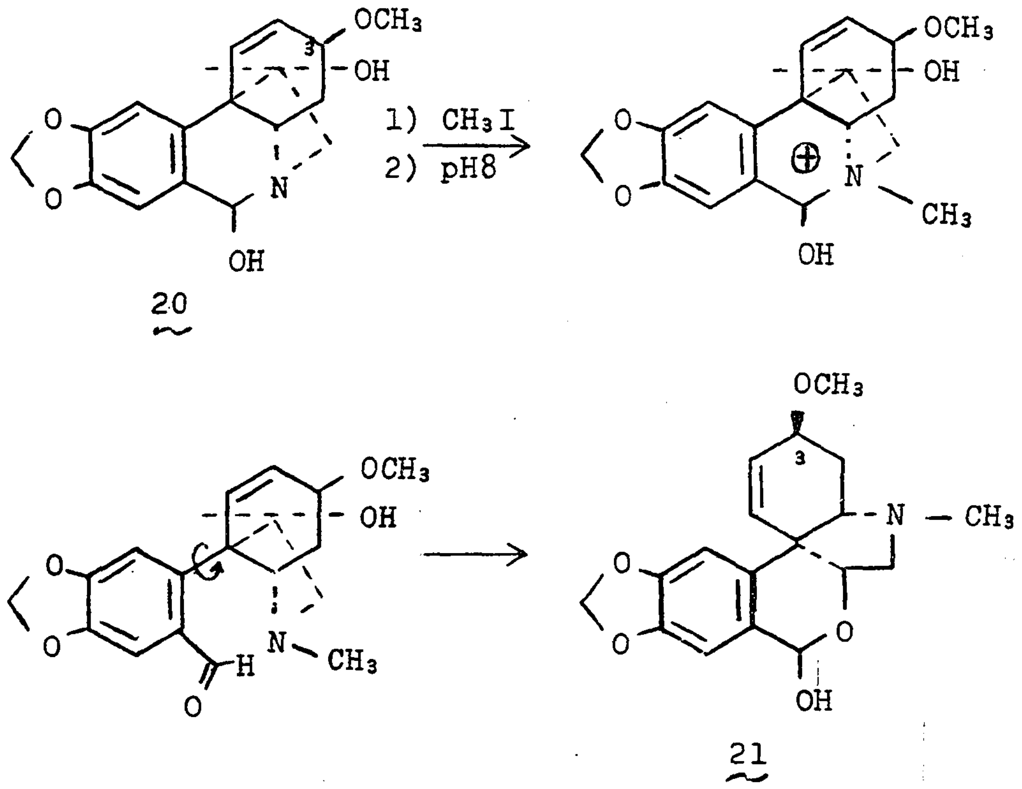

OCH3
3
OH
O
O
N
OH
20
1) CH3I
2) pH8
OCH3
OH
O
O
⊕
N
CH3
OH
OCH3
OH
O
O
H
O
N
CH3
OCH3
3
N
CH3
O
O
O
OH
21

Figure 8. Formation of pretazettine-type nucleus

The stereochemical requirements for late-stage hydroxylations have been studied in some detail for all these rearrangements. However, the results seem to be conflicting in some cases.

Fuganti and Mazza have shown that [7-^{3}H]protocatechuic aldehyde (**22**) was incorporated into norpluvine (**2**) and haemanthamine (**15**) without loss of label *via* O-methyl norbellidine (**1**) (22). The norpluvine and haemanthamine isolated in this experiment were mixed with norpluvine and with haemanthamine produced by feeding O-methyl [1-^{14}C]-norbelladine to a Narcissus species and refed. The doubly labeled compounds were incorporated into lycorenine (**3**) and haemanthidine (**20**) without loss of either label. This required that the hydrogen added in the formation of norbelladine is the hydrogen lost in the hydroxylation. O-Methyl[1'R-^{3}H,1-^{14}C]norbelladine was then fed to a *Narcissus* species and incorporated into lycorenine with complete loss of the pro-R hydrogen (Figure 9) (23).

Fuganti's results seem to be in conflict with the recent work by Virnig (24). O-Methyl[1'R-^{3}H,1-^{14}C]-norbelladine was fed to *Crinum erubescensce* and *Nerine bowdenii* and was incorporated intact into 6-hydroxycrinamine (**23**) and 6-hydroxybuphanidrine (**24**) with no loss of tritium label (Figure 10). The optical purity of the norbelladine was known, and the percentage of tritium incorporated required that only the pro-R hydrogen was incorporated into 6-hydroxycrinamine and 6-hydroxybuphanidrine. The results of Fuganti and Virnig may only appear to be in conflict since the stereochemical change at C-3 may require a

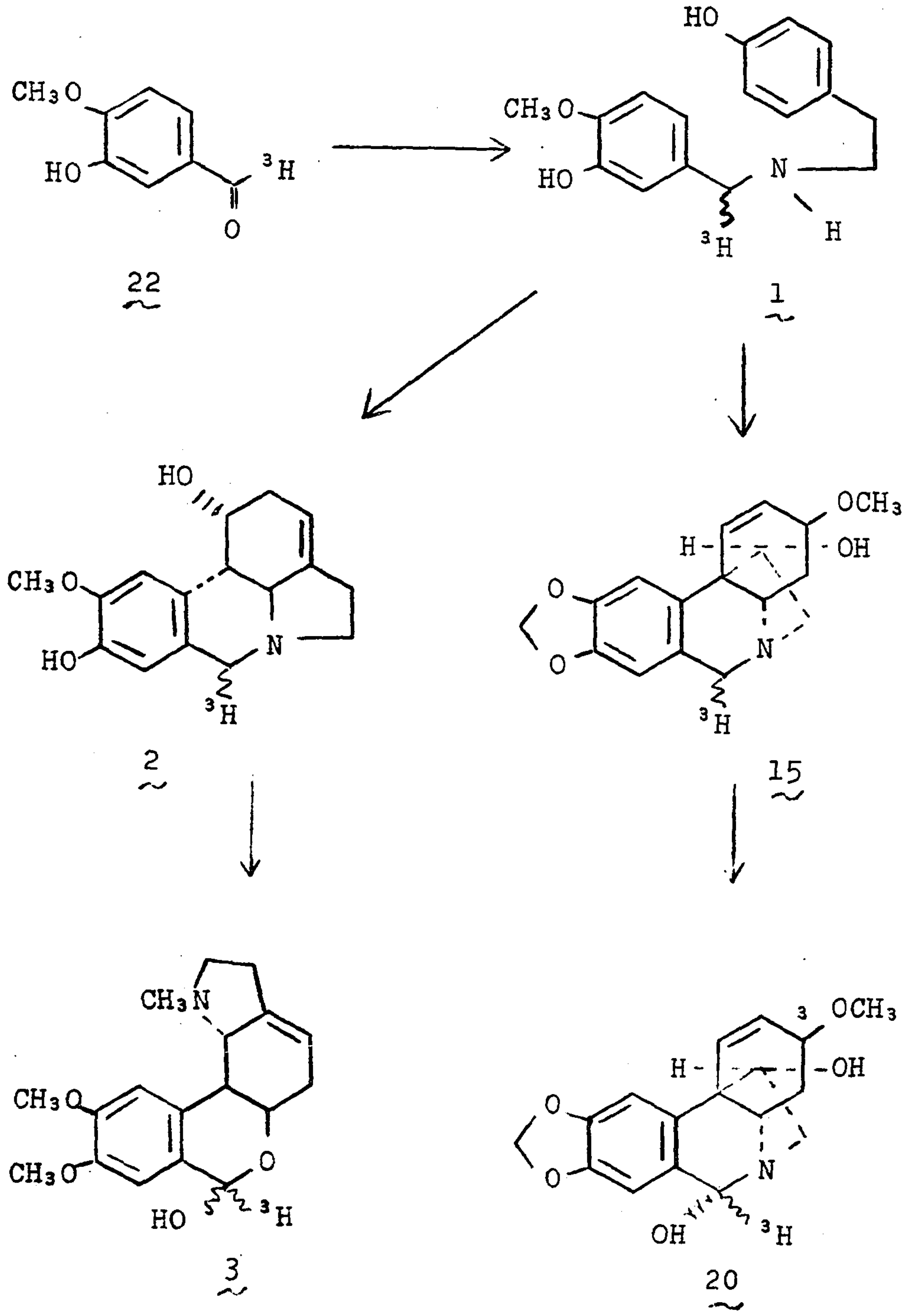


Figure 9. Conversion of protocatechuic aldehyde to norpluvine and haemanthamine

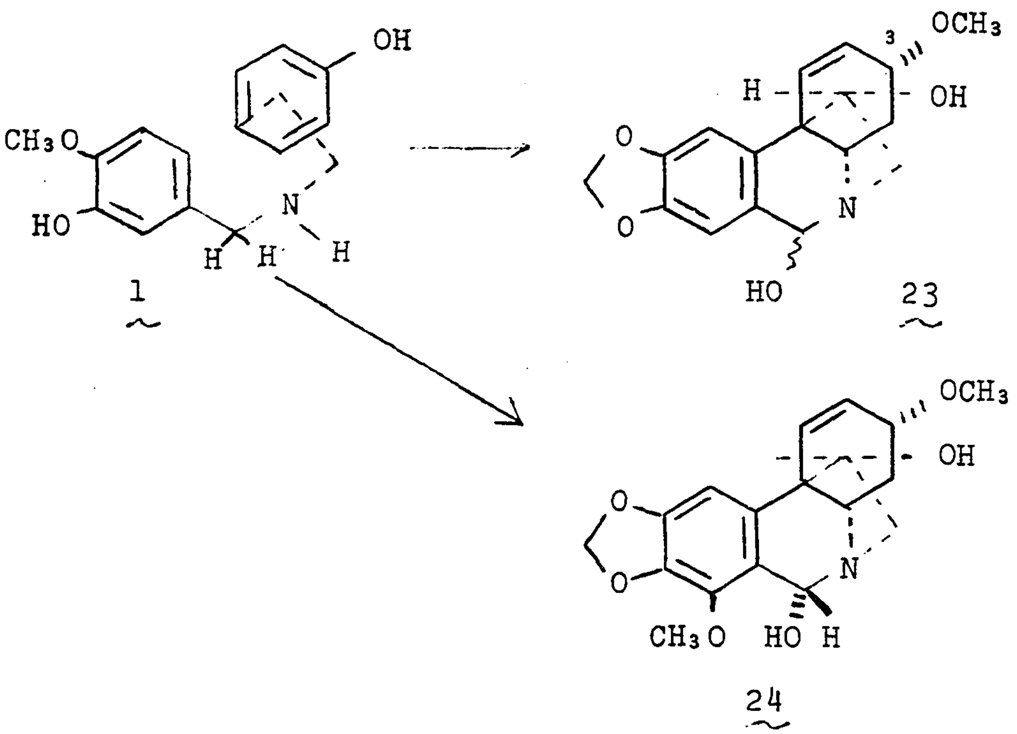


Figure 10. Conversion of norbelladine to 6-hydroxycrinamine and 6-hydroxybuphanidrine

different enzyme for the hydroxylation at C-6 or the molecule may be bound to the same enzyme in a different manner.

Using nonstereospecifically labeled O-methyl[1-^{14}C,2-^{3}H]-norbelladine (1), Battersby and co-workers (25) showed (Figure 11) that fifty percent of the tritium at C-11 was lost in the biosynthesis of haemanthamine (15). To probe the mechanism of the hydroxylation at the C-11 position in haemanthamine, Battersby prepared the appropriate stereospecifically double labeled O-methylnorbelladines (25). The feeding experiments with pro-R and pro-S-O-methyl[2-^{3}H]norbelladines

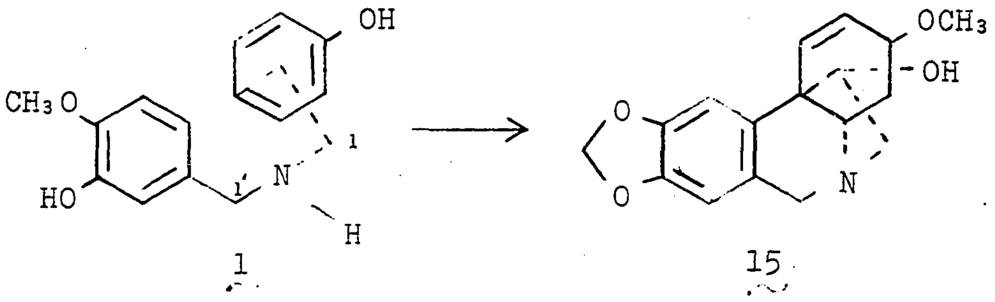
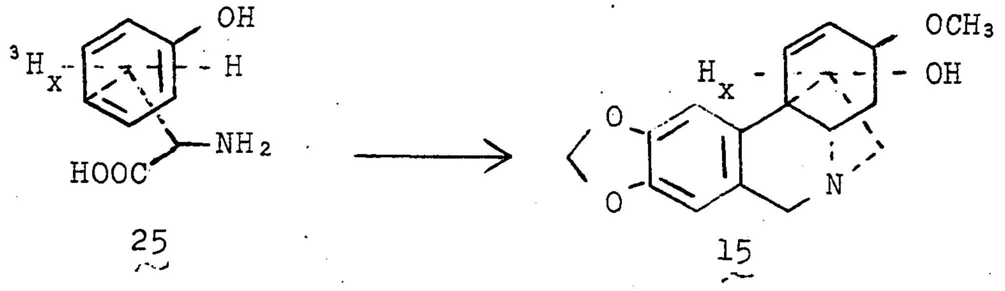


Figure 11. Biosynthesis of haemanthamine

showed that the hydroxylation at C-11 in the biosynthesis of haemanthamine proceeded with retention of configuration, that is with loss of the pro-R hydrogen.

Kirby and Michael have also studied the C-11 hydroxylation in the biosynthesis of haemanthamine by feeding the appropriately labeled tyrosine (26,27). They fed (Figure 12) DL-[β-R-^{3}H,α-^{14}C]tyrosine (25) and DL-[β-S-^{3}H, α-^{14}C]tyrosine (26) and found that only the pro-S hydrogen was incorporated into haemanthamine (15).



25 X = pro-R ⟶ H

26 X = pro-S ⟶ ^{3}H

Figure 12. Biosynthesis of haemanthamine from tyrosine

Fuganti and co-workers observed some novel results in their studies of the biosynthesis of montanine (28). In contrast to the predicted results, Fuganti observed incorporation of the pro-R hydrogen at C-11 in the biosynthesis of montanine. In this work doubly labeled O-methylnorbelladine was fed to *Haemanthus coccineus* and isolated montanine with retention of the pro-R hydrogen only. Since Feinstein showed that montanine is derived from vittatine (19), Fuganti's work may be summerized as shown in Figure 13.

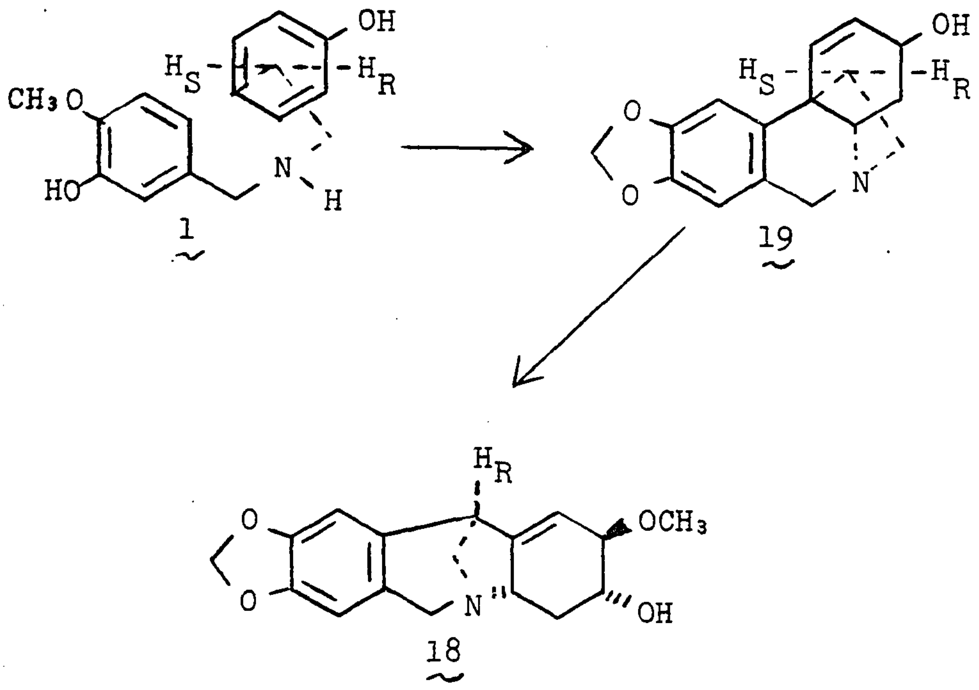
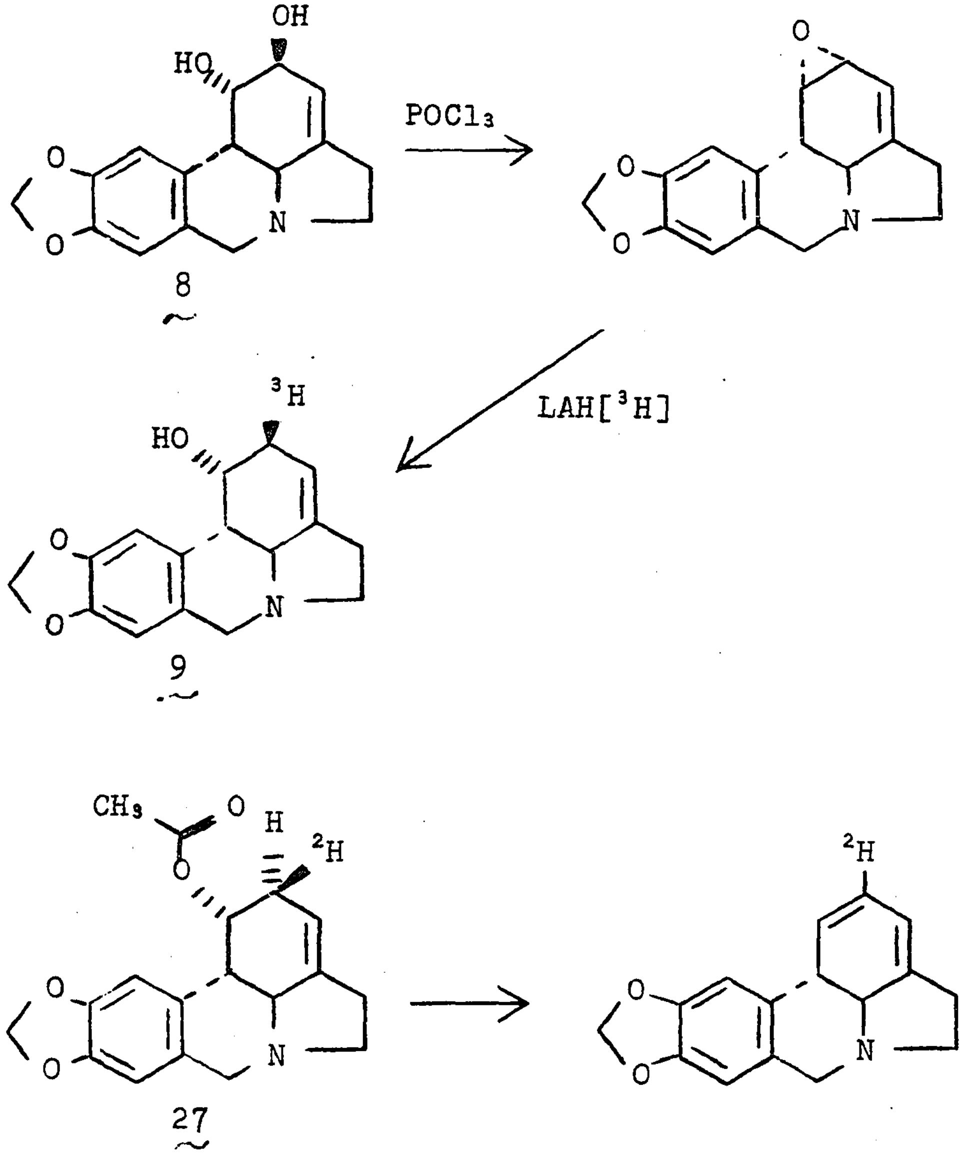


Figure 13. Biosynthesis of montanine

This work required that 11-hydroxyvittatine (17) is not the precursor of montanine unless 11-hydroxyvittatine is produced by a pathway different from the one observed by Battersby and Kirby.

There is a more definitive disagreement about the stereochemistry of the C-2 hydroxylation in lycorine than any other hydroxylation. Wildman and Heimer prepared [2β-^{2}H]caranine as shown in Figure 14 (13). The stereochemistry of this label



OH
HO
POCl$_3$
O
8
LAH[^{3}H]
^{3}H
HO
9
CH$_3$
O
O
H
^{2}H
27
^{2}H
N

Figure 14. Preparation of [2β-^{2}H]caranine

was demonstrated *via* the mass spectral loss of acetic acid in the corresponding [2β-^{2}H]acetylcaranine (27). This singly labeled caranine (27) was fed to *Zephyranthes candida* and the lycorine isolated contained the tritium label in the 2-position. As indicated earlier, hippeastrine (10) was also investigated and found to incorporate caranine when [2β-^{3}H]-caranine was fed to *Hymenocallis americana* (Figure 15) (29).

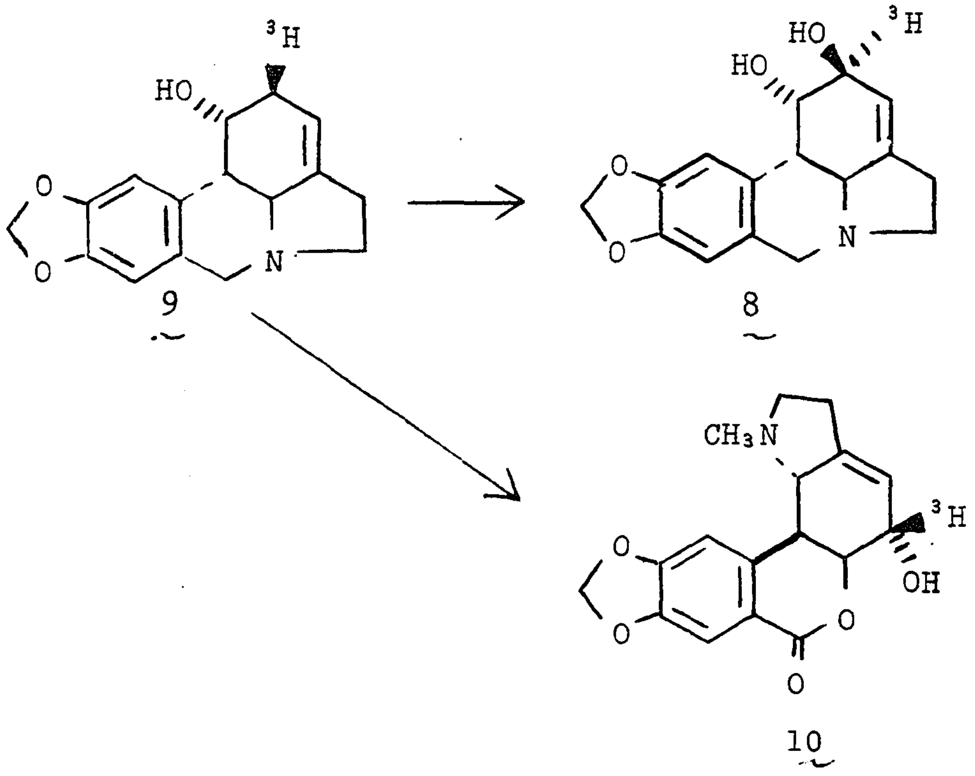


Figure 15. Biosynthesis of hippeastrine and lycorine

This showed that caranine is a precursor to lycorine and hippeastrine and indicated that the hydroxylation proceeded with inversion in both cases.

This was confirmed by Bruce and Kirby (30,31) when they fed DL-[3,5-^{3}H,α-^{14}C]tyrosine to "Twink" and "Deanna Durbin" daffodils. The tyrosine was incorporated into norpluvine (2) and lycorine (8). After N-methylation, the tritium was shown to be in the 2β-position of norpluvine methiodide via the stereospecific 1,4-elimination of the quaternary nitrogen (Figure 16). Since norpluvine has been shown to be a precursor to lycorine, it followed that the C-2 position was oxidized by an inversion mechanism.

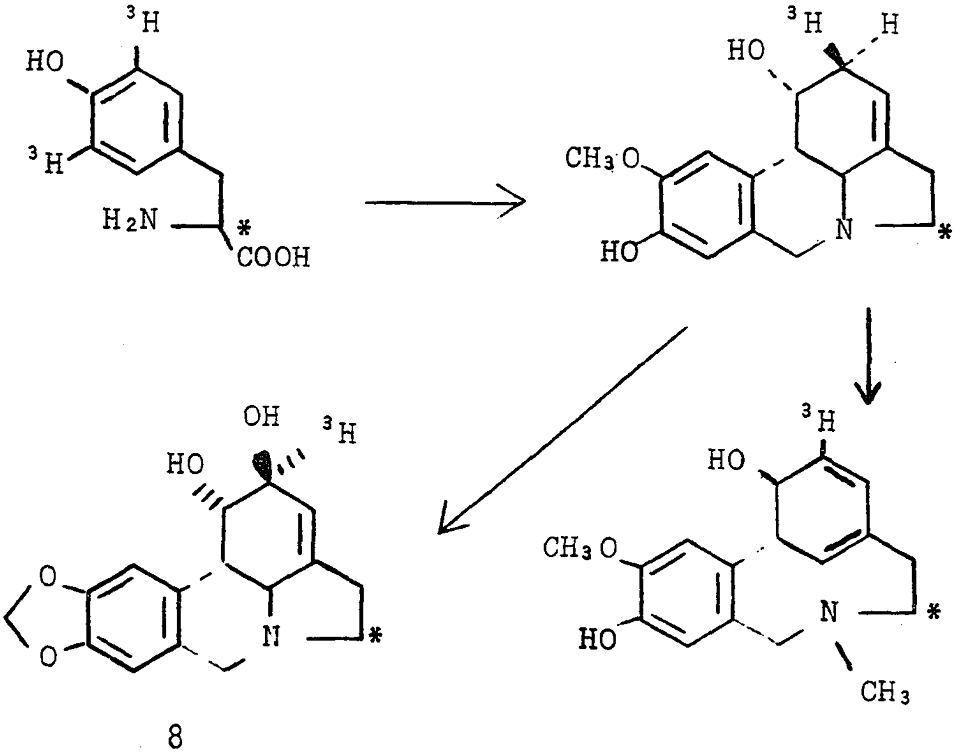


Figure 16. The biosynthesis of lycorine from tyrosine

In contrast, Fuganti and Mazza (32) fed [2α-^{3}H,5-^{14}C]-caranine (27) and [2β-^{3}H,5-^{14}C]norpluviine (2) to Clivia miniata. Their results suggested, that in Clivia miniata, the C-2 hydroxylation of lycorine occurs with retention of configuration (Figure 17).

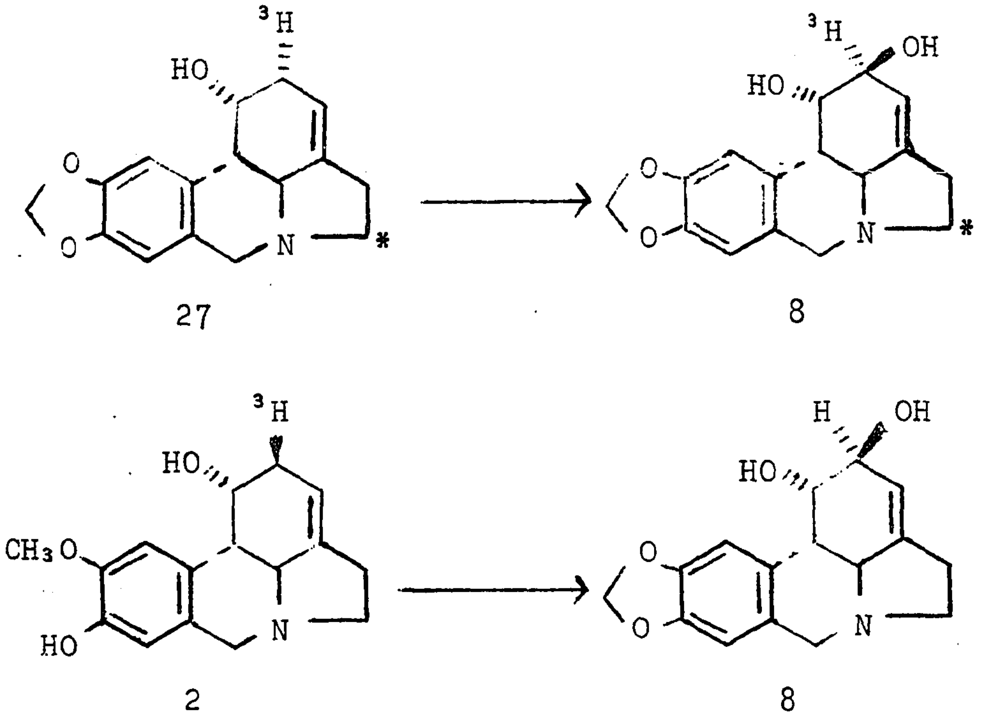


Figure 17. C-2 hydroxylation of lycorine with retention

The hydroxylation mechanism in the Amaryllidaceae alkaloids is currently clouded by these apparently conflicting results. The resolution of this experimental evidence will be considered in the next section.

The Symmetry and Optical Activity of 2,3-Dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[*c*,*f*]azocine

The degradation of montanine (18) by an Oppenauer oxidation afforded dehydrococcinine (29). Dehydrococcinine formed an O,N-dimethyl derivative (30) in low yield on reaction with diazomethane. In an attempt to improve the yield, N-methylation with formaldehyde and formic acid followed by treatment with diazomethane was tried. This reaction produced the pentacyclic azocine (31) instead (Figure 18) (33).

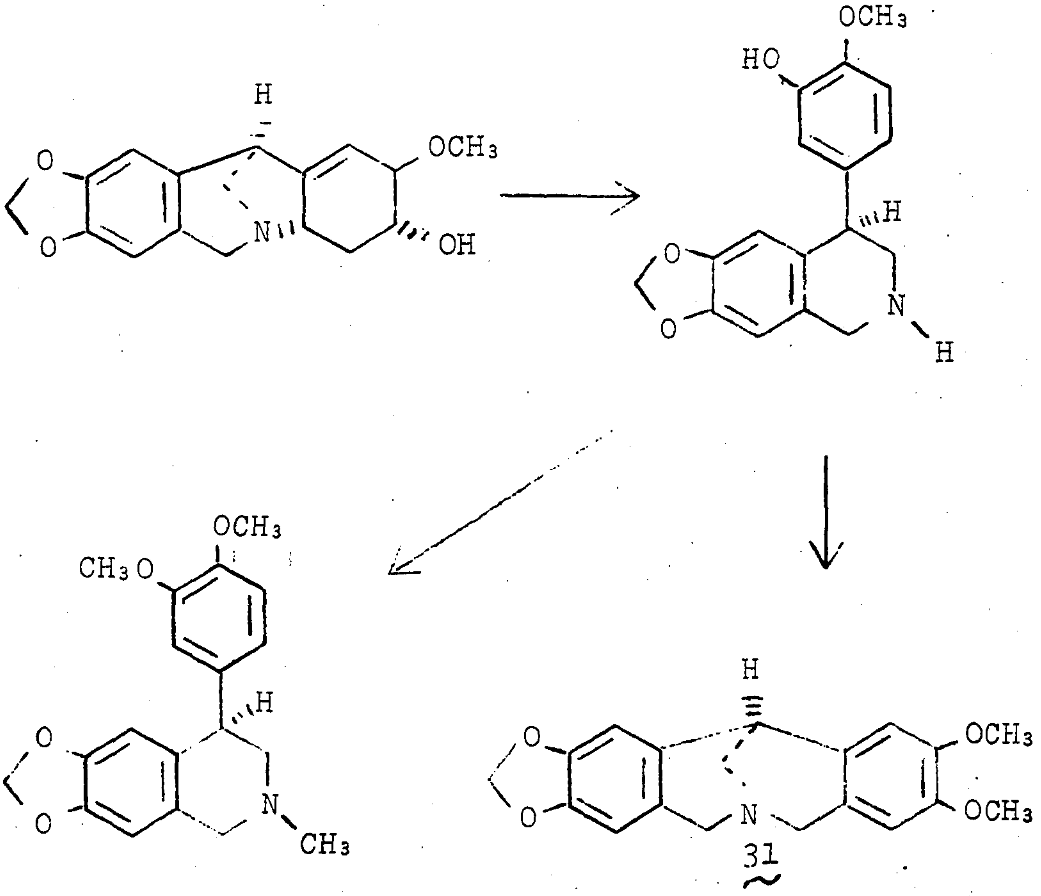


Figure 18. Synthesis of azocine

Although the structure was supported by synthesis and spectral data, the structure of this compound seemed inconsistent with the large rotation ($[\alpha]_D$+54°) observed (34, 35).

Structure and Optical Activity of the Aromatic Chromophore

Interest in the optical activity of the aromatic chromophore has been extensive in the last few years. DeAngelis and Wildman in the first systematic work on the aromatic chromophore (36,37) recorded and correlated the CD spectra of most of the Amaryllidaceae alkaloids and a number of other aromatic natural products. Although this early work was hampered by a lack of reference compounds of known absolute configuration, it marked the first major step in the elucidation of the optical activity of the aromatic chromophore. Reasoning that, since the optical activity was due to the dissymmetry of the molecule, and the most important asymmetry was the closest to the chromophore, they centered their work on the axis to the asymmetric carbon adjacent to the ring through the benzene ring (Figure 19). Following the

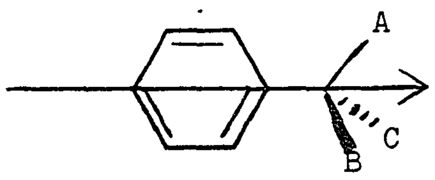


Figure 19. The axis of DeAngelis and Wildman

earlier work on the carbonyl octant rule (38), a Cartesian coordinate system was constructed. In order to determine the signs of the sectors they examined the two pertinent alkaloids (galanthamine and morphine) as shown in Figure 20. The need for two rules was required by the normally antipodal relationship between the ^1L_a (240 nm) absorption and the ^1L_b (285 nm) band. One of the problems which they studied was the dichotomous CD spectra of powellane (32). This was a

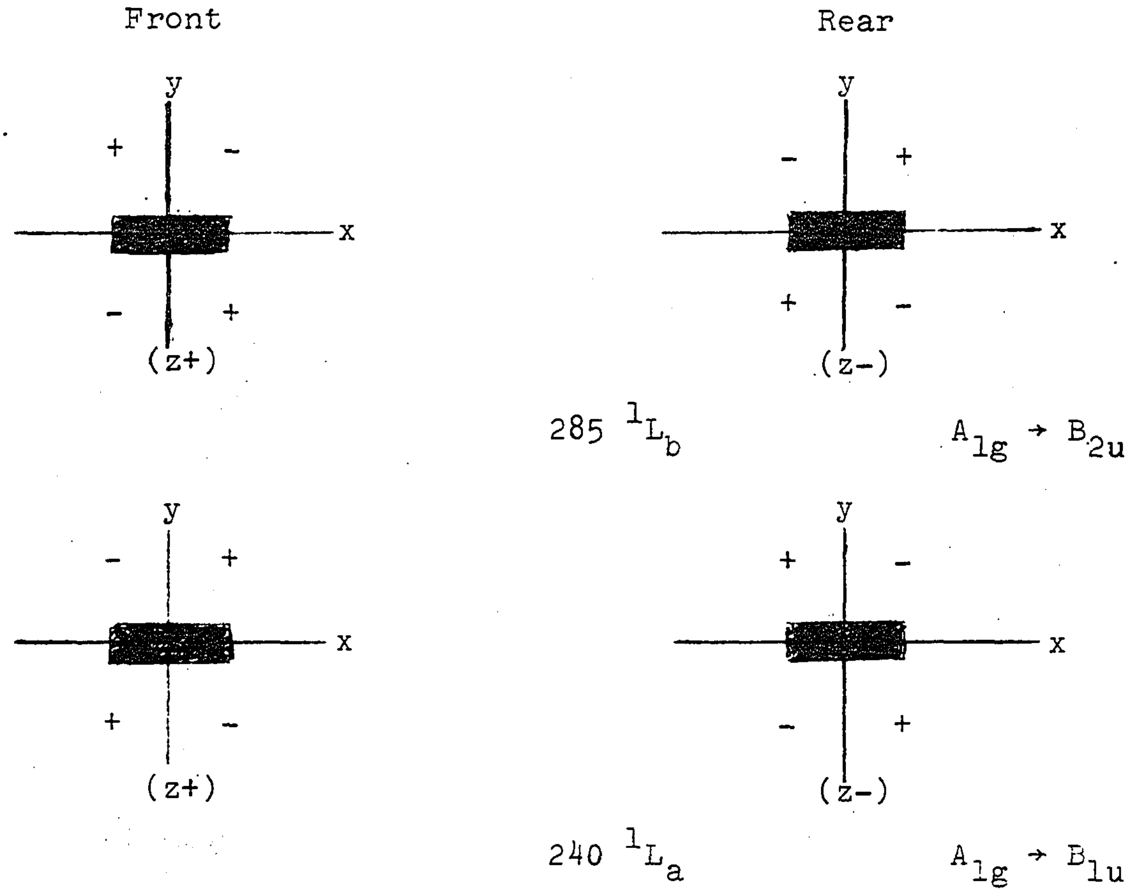
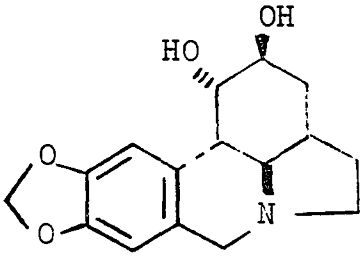

Front
Rear
y
x
+
-
(z+)
(z-)
285 ^1L_b
$A_{1g} \rightarrow B_{2u}$
240 ^1L_a
$A_{1g} \rightarrow B_{1u}$

Figure 20. Octant rule for aromatic chromophore

problem since the absolute stereochemistry of powellane was known to be the same as crinane (33). This was further complicated by the spectra of powelline (34) and crinine (35) and dihydropowelline (36) and dihydrocrinine (37) (Figures 21,22,23,24,25,26). Clearly, the ^1L_b band was anomalous. They showed that the change in the sign of the Cotton effect (^1L_b) for methylenedioxymethoxy alkaloids on reduction of the double bond was general and that there were other compounds with comparable ^1L_b Cotton effects. Wildman and DeAngelis concluded that this was a result of interaction between the double bond and the aromatic ring. The inconsistency of the ^1L_b band led them to suggest that the ^1L_a band was preferable to the ^1L_b band for predicting absolute configuration.

Kuriyama and co-workers (39) independently studied the optical activity of lycorine basing their work on the recently completed X-ray structure of dihydrolycorine hydrobromide (38) (40,41). They chose to examine the symmetry



38

Figure 21. ORD and CD of powellane (36)

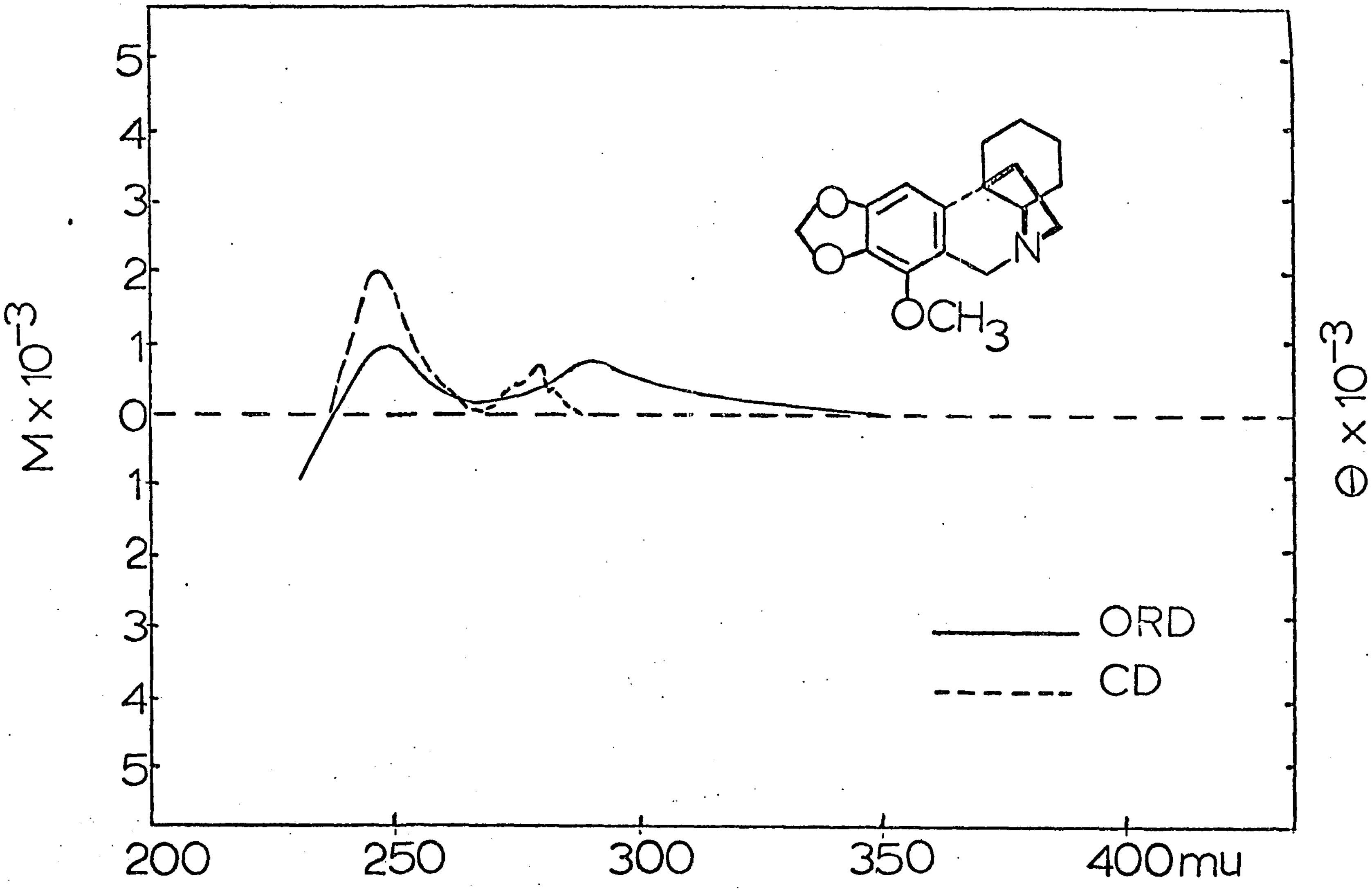

5
4
3
2
1
0
1
2
3
4
5
M × 10⁻³
Θ × 10⁻³
200
250
300
350
400 mu
ORD
CD
N
OCH3

Figure 22. ORD and CD of crinane (36)

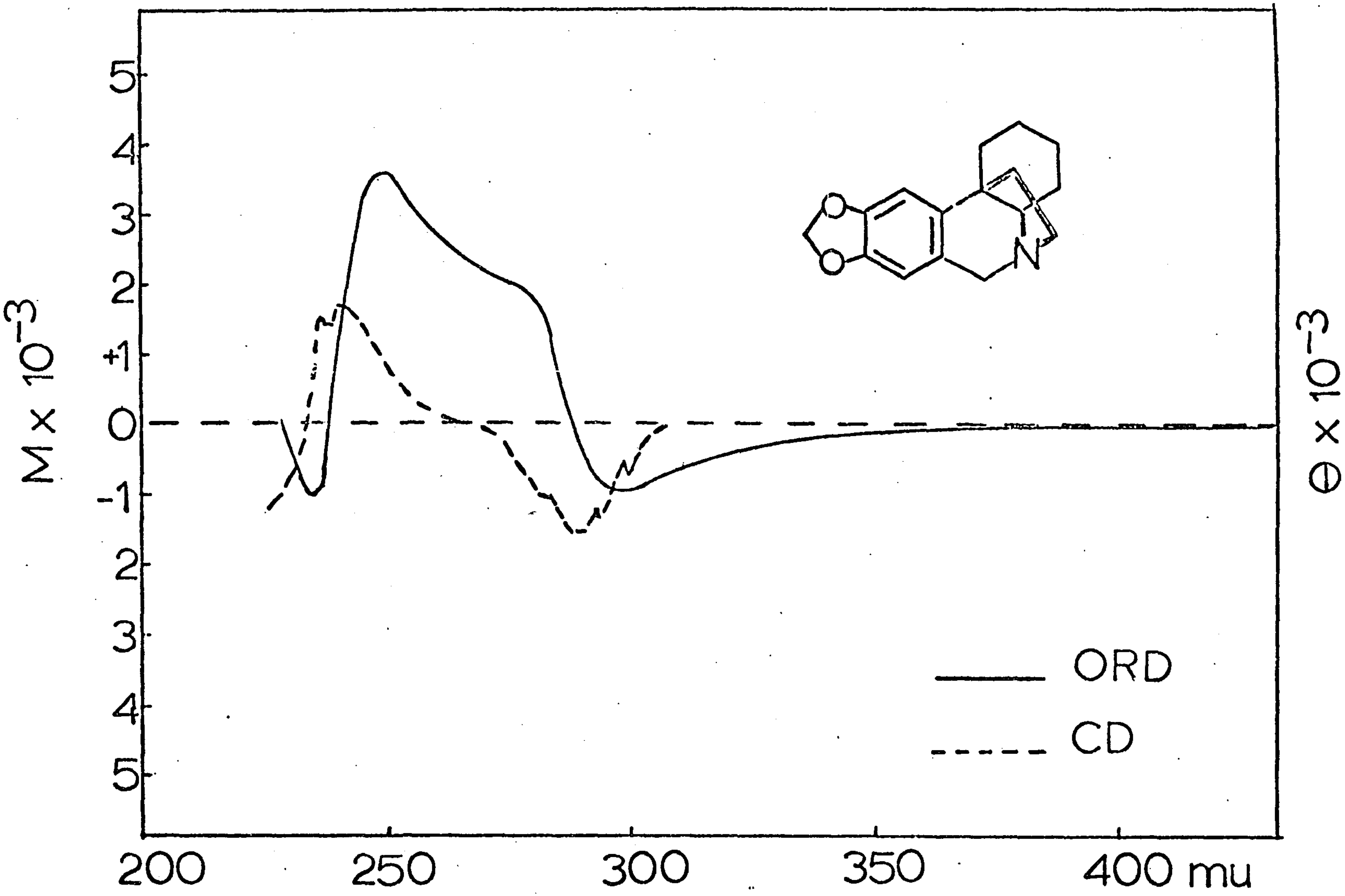

M × 10⁻³
5
4
3
2
+1
0
-1
2
3
4
5
Θ × 10⁻³
200
250
300
350
400 mu
N
ORD
CD

Figure 23. ORD and CD of powelline (36)

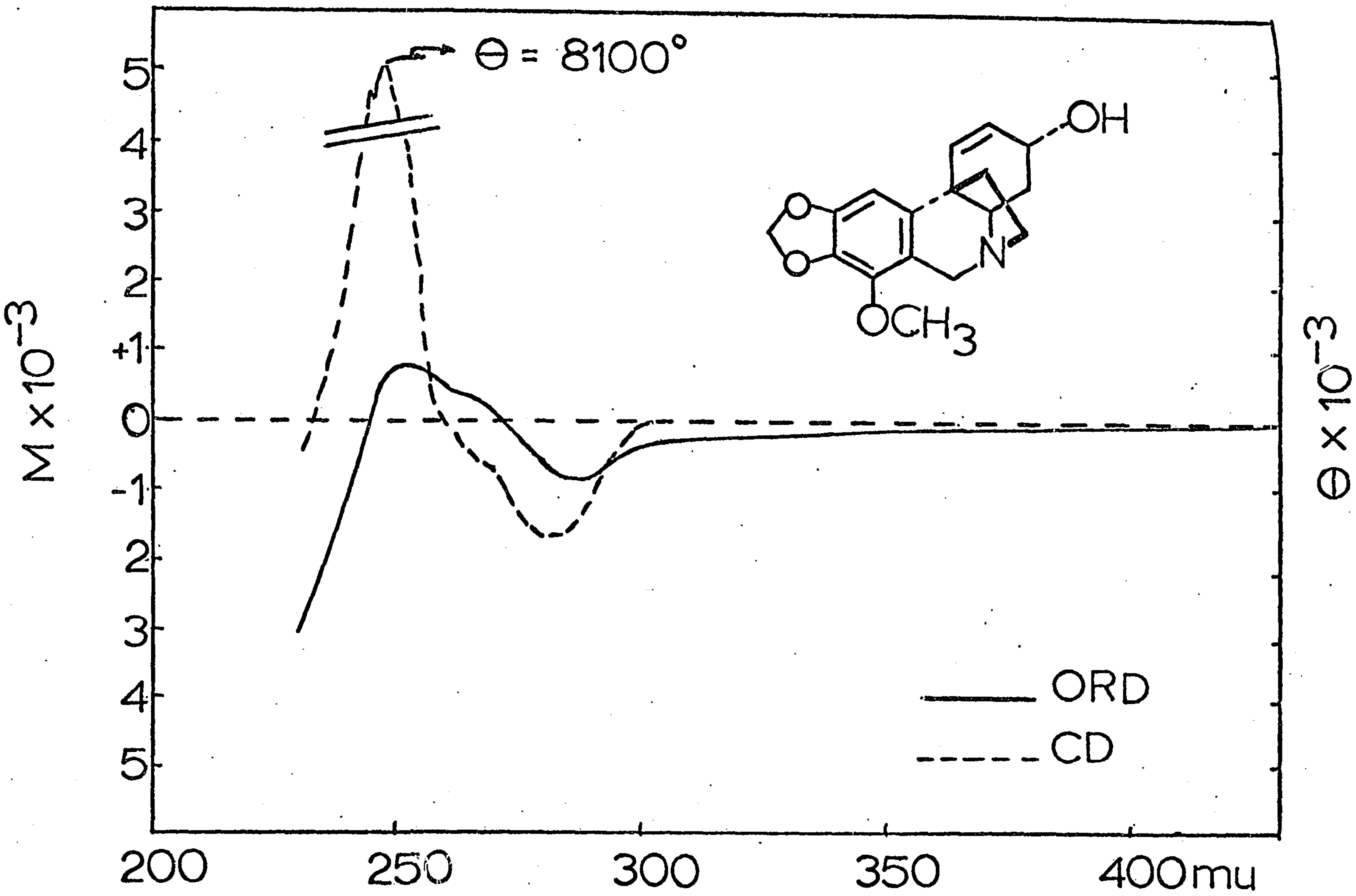

Θ = 8100°
OH
N
OCH3
M × 10^-3
Θ × 10^-3
5
4
3
2
+1
0
-1
2
3
4
5
200
250
300
350
400mu
ORD
CD

Figure 24. ORD and CD of crinine (36)

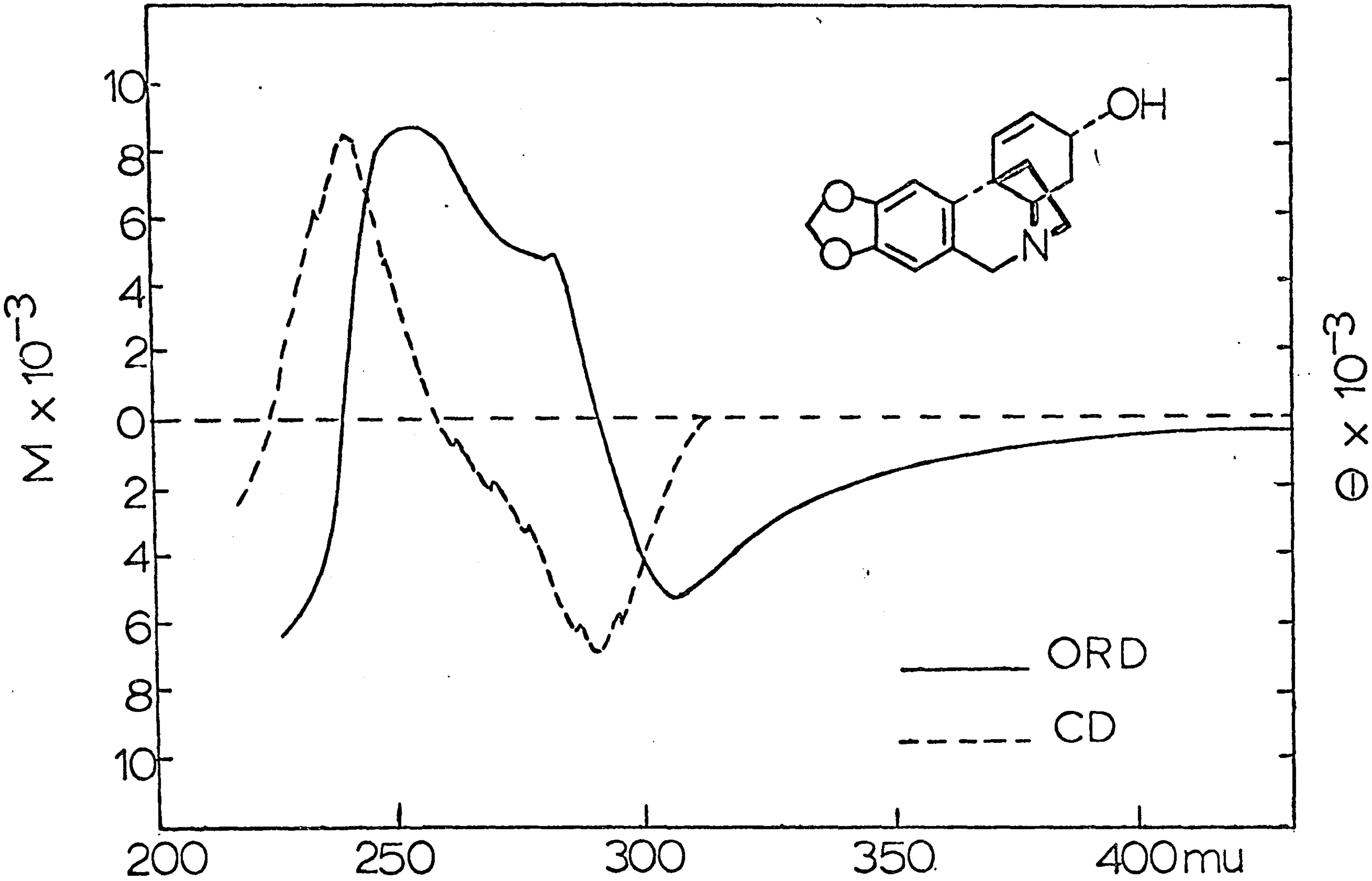

OH
N
ORD
CD
M × 10^{-3}
Θ × 10^{-3}
10
8
6
4
2
0
2
4
6
8
10
200
250
300
350
400 mu

Figure 25. ORD and CD of dihydropowelline (36)

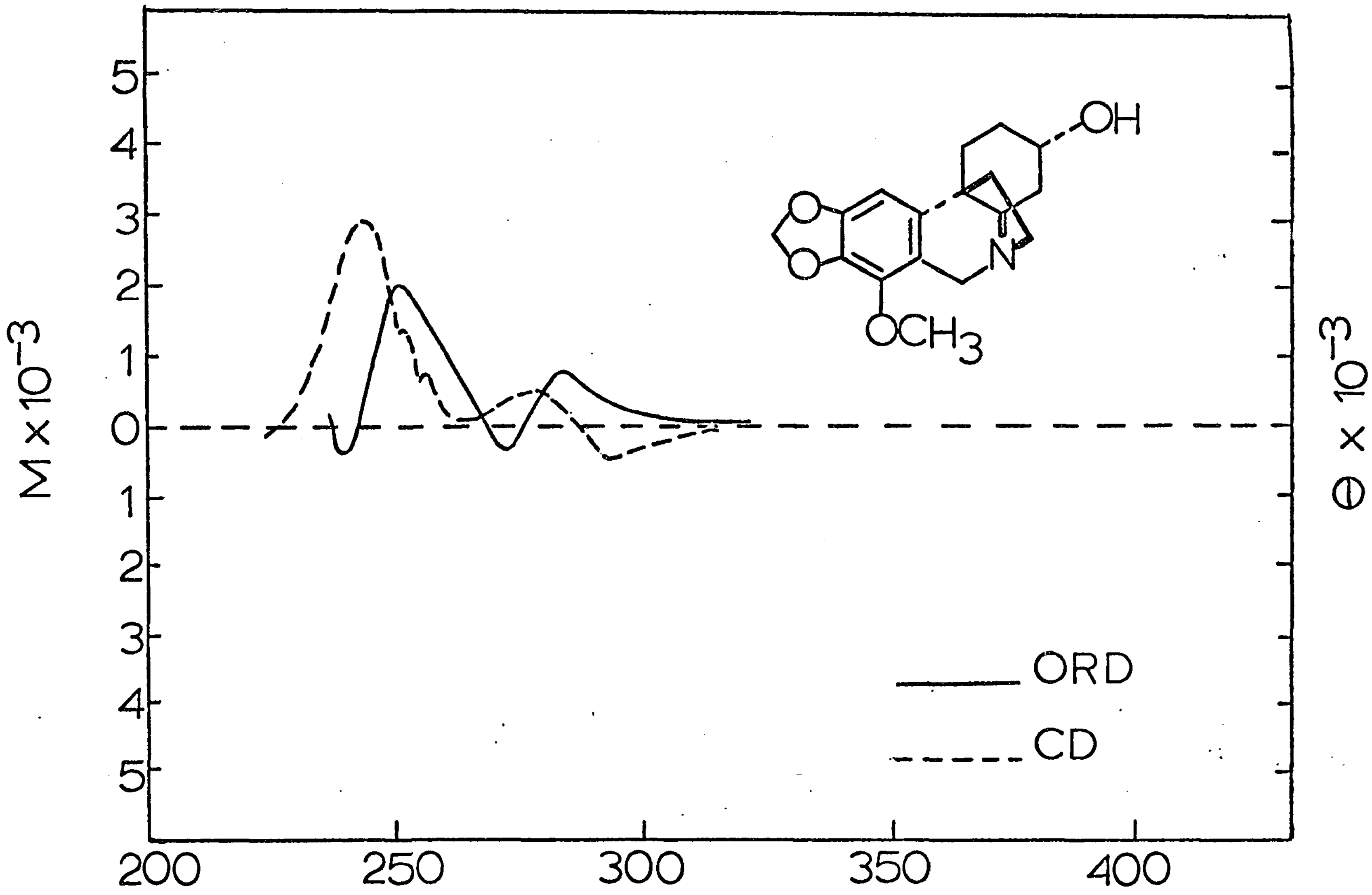
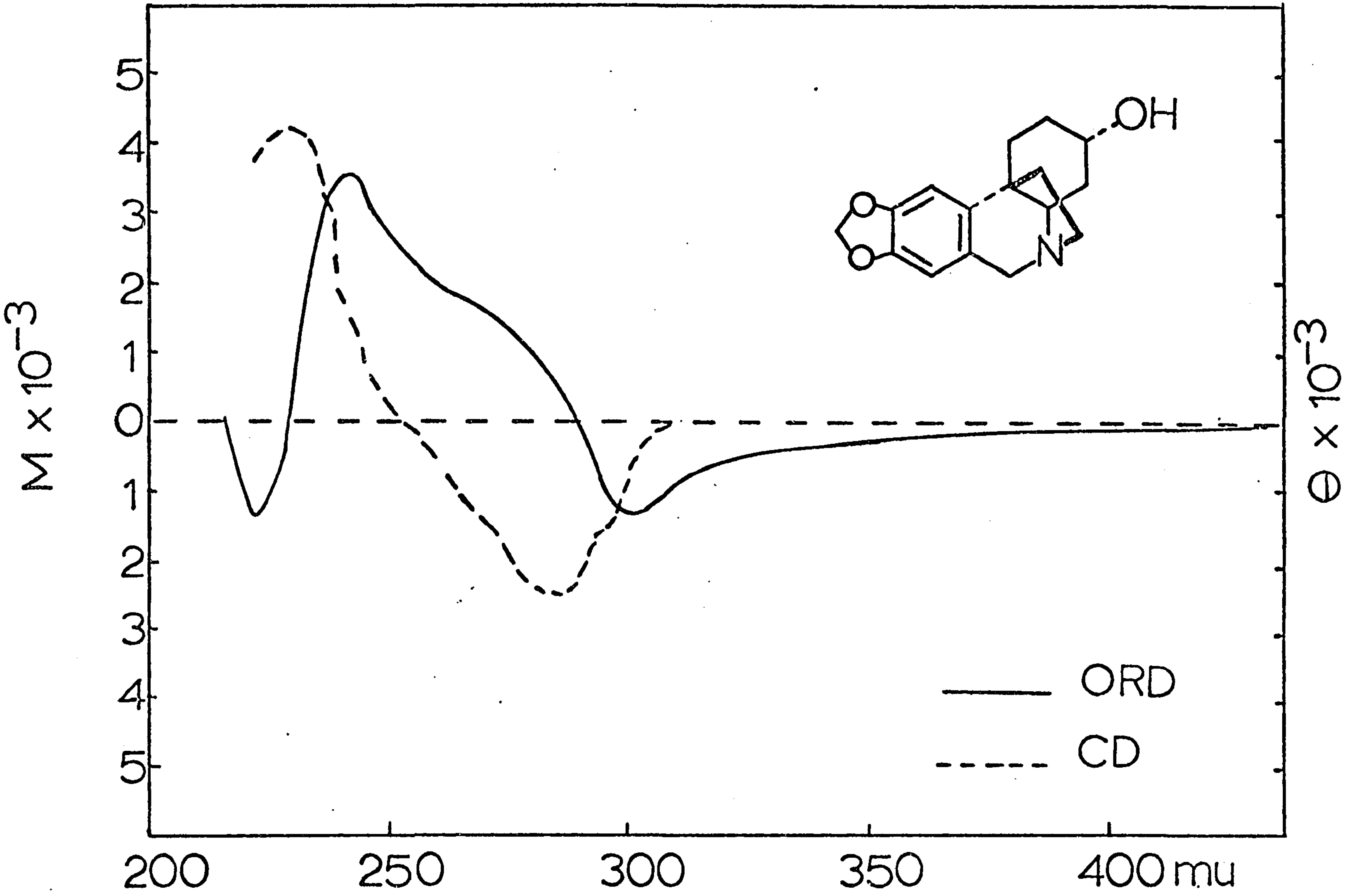

M × 10^{-3}
Θ × 10^{-3}
5
4
3
2
1
0
1
2
3
4
5
200
250
300
350
400
OH
OCH$_3$
N
ORD
CD

Figure 26. ORD and CD of dihydrocrinine (36)


M × 10⁻³
Θ × 10⁻³
5
4
3
2
1
0
1
2
3
4
5
200
250
300
350
400 mu
OH
N
ORD
CD

axis of the dioxybenzene chromophore, and based their work on the ^1L_b absorption. Their rule works well for those alkaloids whose ^1L_a and ^1L_b Cotton effects are antipodal. It fails to explain the crinine-powelline anomaly observed by DeAngelis and Wildman. The symmetry axis was chosen to be the Z-axis and the signs of the sectors were determined to be as illustrated in Figure 27.

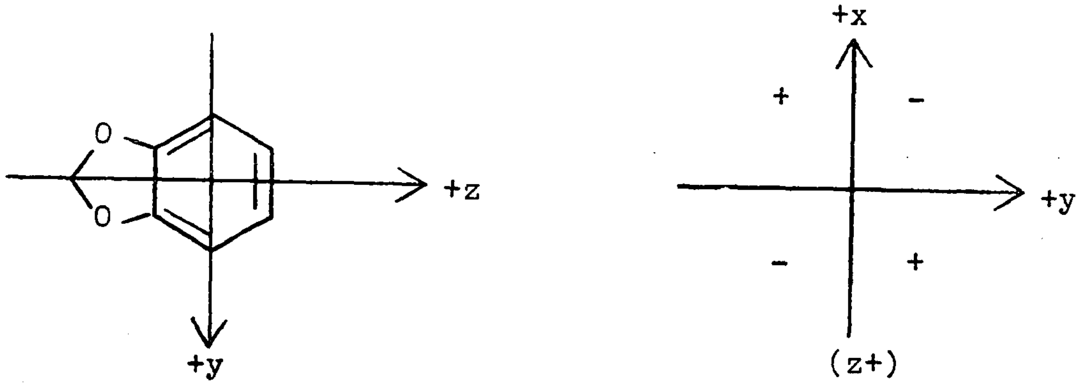


Figure 27. Sector rule of Kuriyama and co-workers

The theoretical basis for sector rules in general was investigated by Schellman (42,43). His work required that the local symmetry axis of the chromophore be used as the basis of the sector rule and that the atoms in the sectors should contribute in a manner proportional to the distance from the nodal planes, the distance from the chromophore, and the sign and magnitude of the charge.

Snatzke and co-workers (44,45) have recently published the results of their research on the chirooptical properties of the benzene chromophore which is based on two assumptions:

1) That the chiral sphere which is nearest to the chromophore determines in general the sign of the Cotton effect, and

2) Sector rules are built up from the nodal planes of the corresponding polarization diagram of the respective transitions ($^1B_{2u}$=^1L_b and $^1B_{1u}$=^1L_a), the plane of the ring and additional planes if the local symmetry of the compounds investigated requires them.

They then defined three chiral spheres (for a tetralin or a tetrahydroisoquinoline):

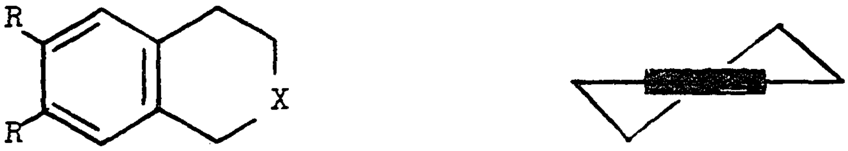
1) the chromophore itself (in this case achiral),

2) the cyclohexene(piperidine) ring,

3) those groups attached to the second sphere.

For achiral chromophores, they suggested that the second sphere determines the sign and largely the magnitude of the Cotton effect, and that the third sphere is only important when the second is achiral, although, the third sphere can be expected to influence the magnitude of the Cotton effect. From these considerations Snatzke and co-workers developed a two part rule:

1) The chirality of the second sphere will determine the sign of the Cotton effect as shown in Figure 28;



X=CR' or NR'
R=H or OR"

Chirality of second sphere leading to a negative ^1L_b Cotton effect

Figure 28. Chirality of the second sphere

2) The third sphere contribution is determined by sector rules (Figure 29).

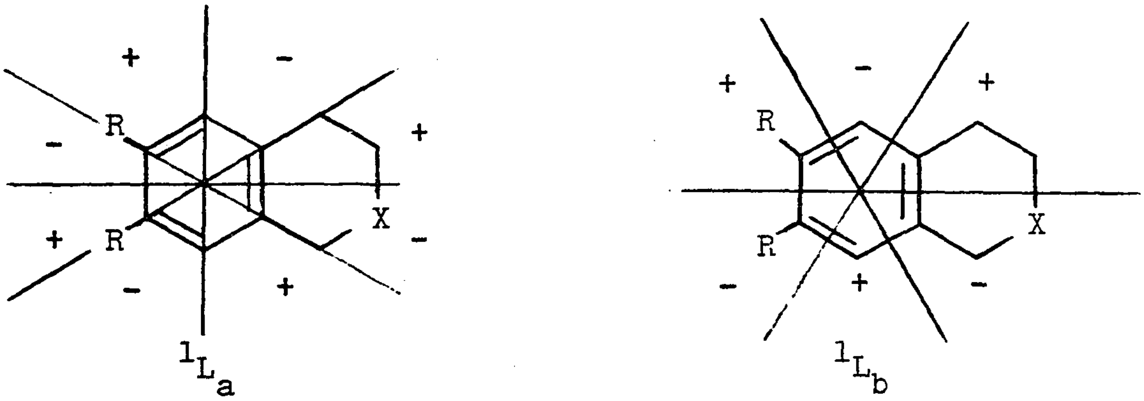
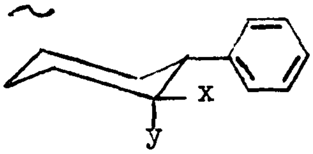


Figure 29. Chirality of the third sphere

This rule also worked well for those compounds where the Cotton effects were antipodal.

Verbit and Price have recently reported the results of their study of the circular dichroism of 1-substituted 2-phenylcyclohexanes (39) (46). Their results suggested a



39

sector rule for the ^1L_a band like that of DeAngelis and Wildman but with opposite signs (Figure 30). They reported that there is no apparent correlation of the sign of the ^1L_b band with the absolute configuration of the 1-substituted 2-phenylcyclohexanes.

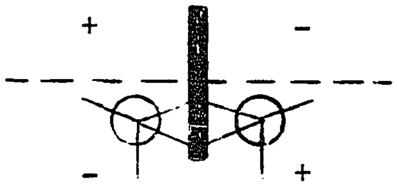


Figure 30. Sector rule of Verbit and Price

Smith and Willis have proposed that α- and β-phenylalkylamine hydrochlorides follow a sector rule (Figure 31) (47), the reverse of that proposed by Kuriyama and co-workers and by Snatzke and co-workers. Brewster and Prudence in their work in spiroindanes suggested that the conflict

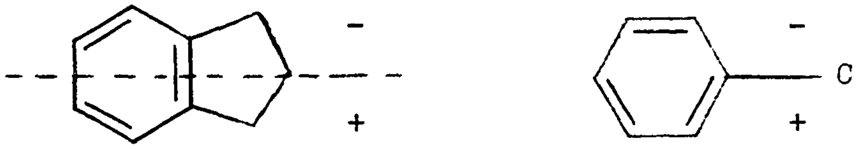


Figure 31. Sector rule of Smith and Willis. Sector rules for the ^1L_b aromatic Cotton effect related to the C_2 axis of the ring. The sign of only the upper sector is shown.

between the sector rules may be due to a change in the direction of the transition moment (48). They proposed a sector rule keyed to the transition moment (Figure 32).

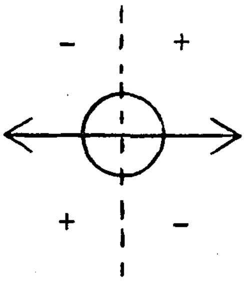


Figure 32. General octant rule for aromatic transitions. Signs of the upper octants shown.

The problem was to now find a way to resolve the differences in these rules so that the problems indicated would be eliminated.

RESULTS AND DISCUSSION

The Stereochemistry of the C-2 Hydroxylation in Pluviine

In reviewing the work on the C-2 hydroxylation in lycorine, it seemed that another doubly labeled precursor should be prepared and fed so that the work of Wildman and Harken and of Bruce and Kirby could be reconciled with the contradictory work of Fuganti and Mazza. The desired molecule would have a stereospecific tritium label in the 2-position and a ^{14}C label at some other position in the molecule. The work of Harken, and of Wildman and Heimer both suggested that pluviine (4) might be a suitable molecule, since the ^{14}C label could be introduced *via* a diazomethane reaction with norpluviine, and the tritium label could be introduced into methylpseudolycorine (5) in a manner similar to that used by Wildman and Heimer (13) (Figure 33).

One of the central problems was to devise a method of preparing methylpseudolycorine (5) from lycorine so that enough of this minor alkaloid would be available for the synthesis of the labeled material. This was accomplished by cleaving the methylenedioxy ring in lycorine (8) with boron tribromide (49) and methylation with diazomethane (Figure 34).

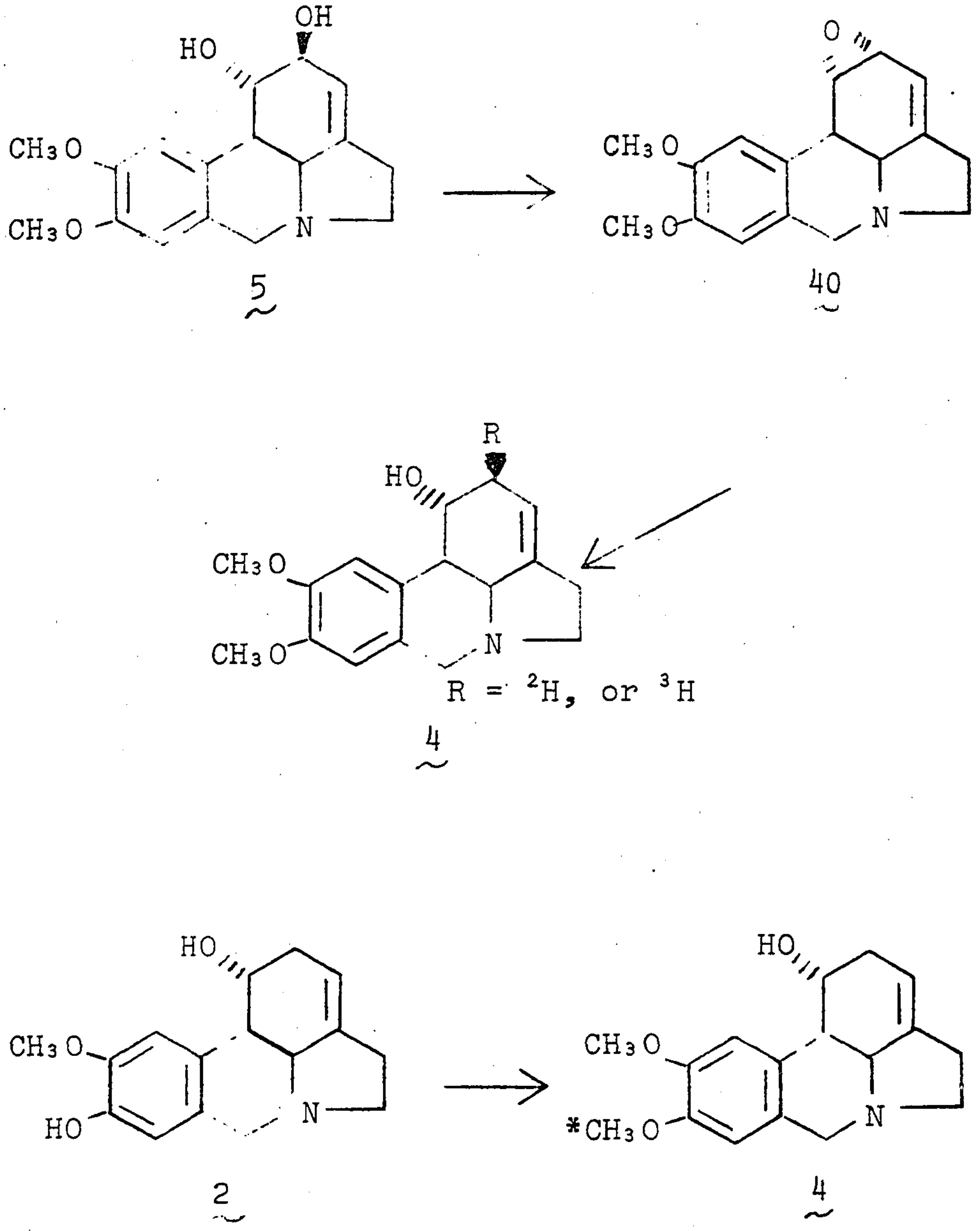

HO
OH
CH_3O
CH_3O
N
5
O
CH_3O
CH_3O
N
40
R
HO
CH_3O
CH_3O
N
R = ^{2}H, or ^{3}H
4
HO
CH_3O
HO
N
2
HO
CH_3O
*CH_3O
N
4

Figure 33. Preparation of [9-O-methoxy-^{14}C,2β-^{3}H]pluviine

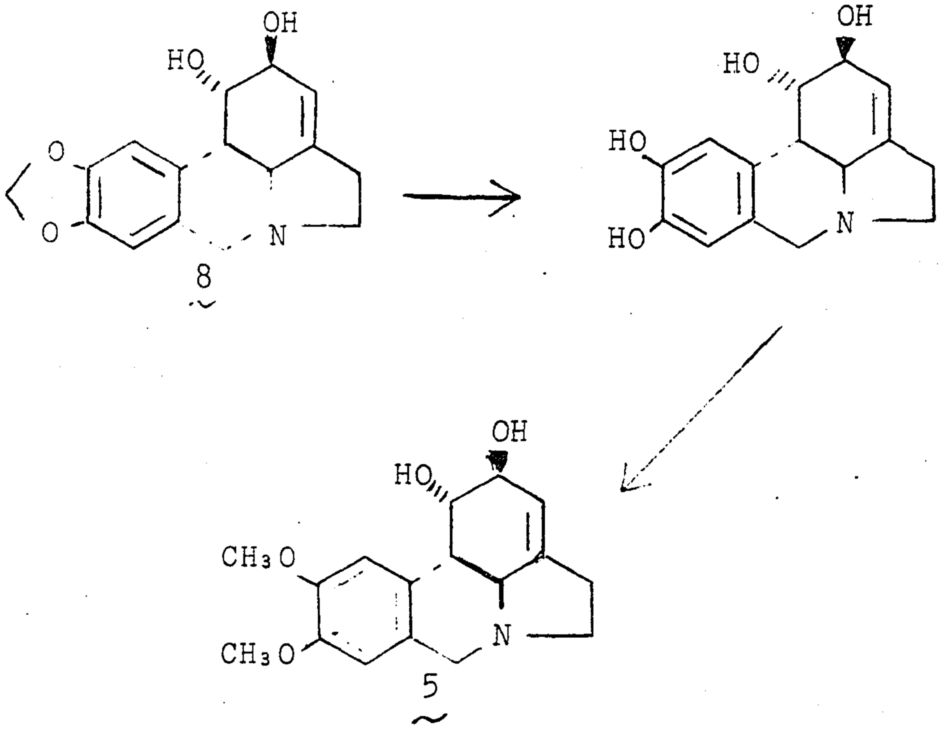


Figure 34. Preparation of methylpseudolycorine

The introduction of the tritium label was patterned after the work on lycorine by Wildman and Heimer. The epoxide (40) in this case was unstable and could not be characterized completely (Figure 35,36). The material was contaminated with anhydromethylpseudolycorine (41) (Figure 37,38). The NMR spectra of the epoxide was in good agreement with lycorine α-epoxide (Figure 39,40) prepared by Wildman and Heimer (13) if the peaks due to anhydromethylpseudolycorine were subtracted. The infrared spectra was also in

Figure 35. NMR spectrum of methylpseudolycorine α-epoxide

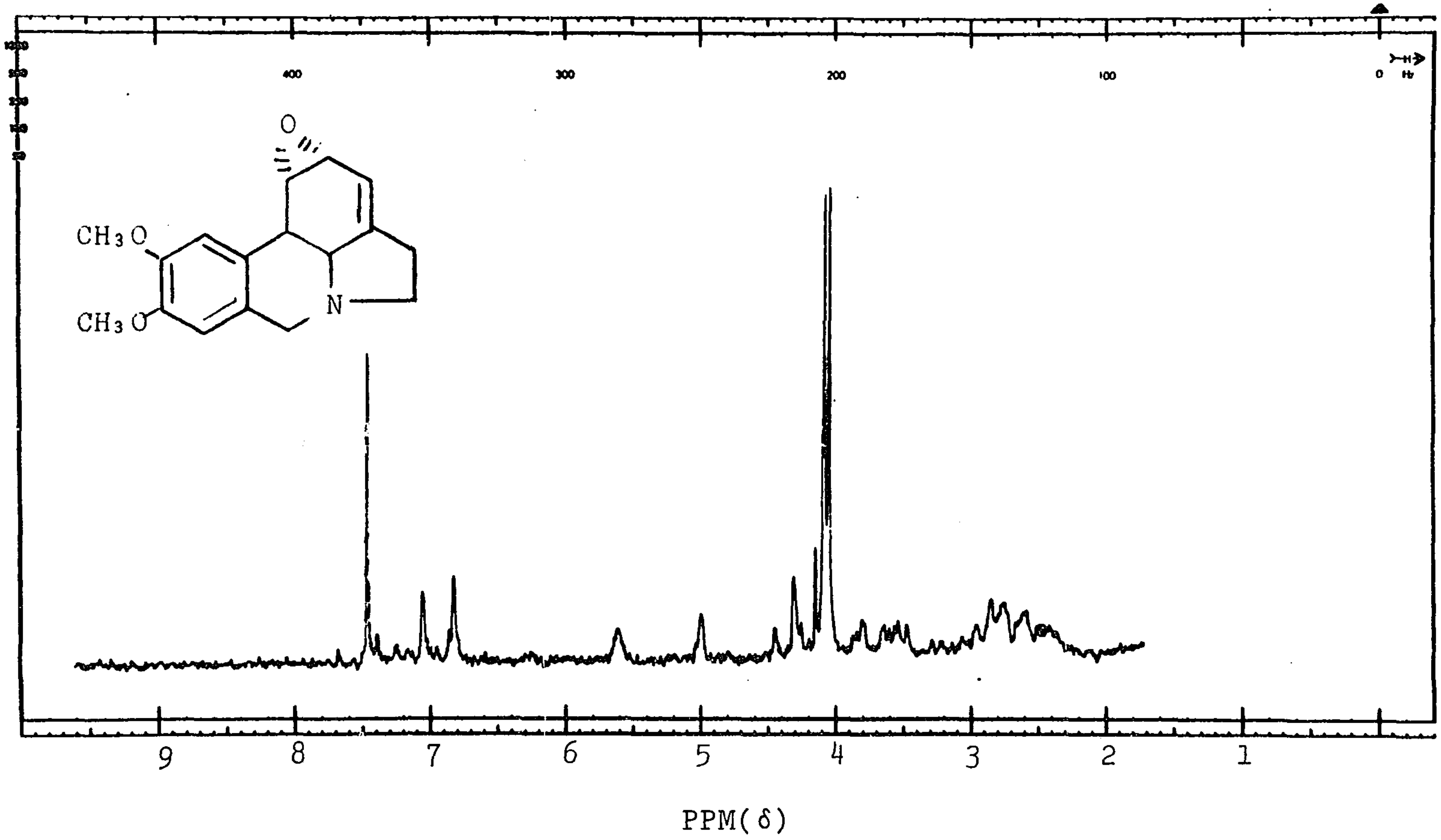

400
300
200
100
0 Hz
O
CH₃O
CH₃O
N
9
8
7
6
5
4
3
2
1
PPM(δ)

Figure 36. IR spectrum of methylpseudolycorine α-epoxide

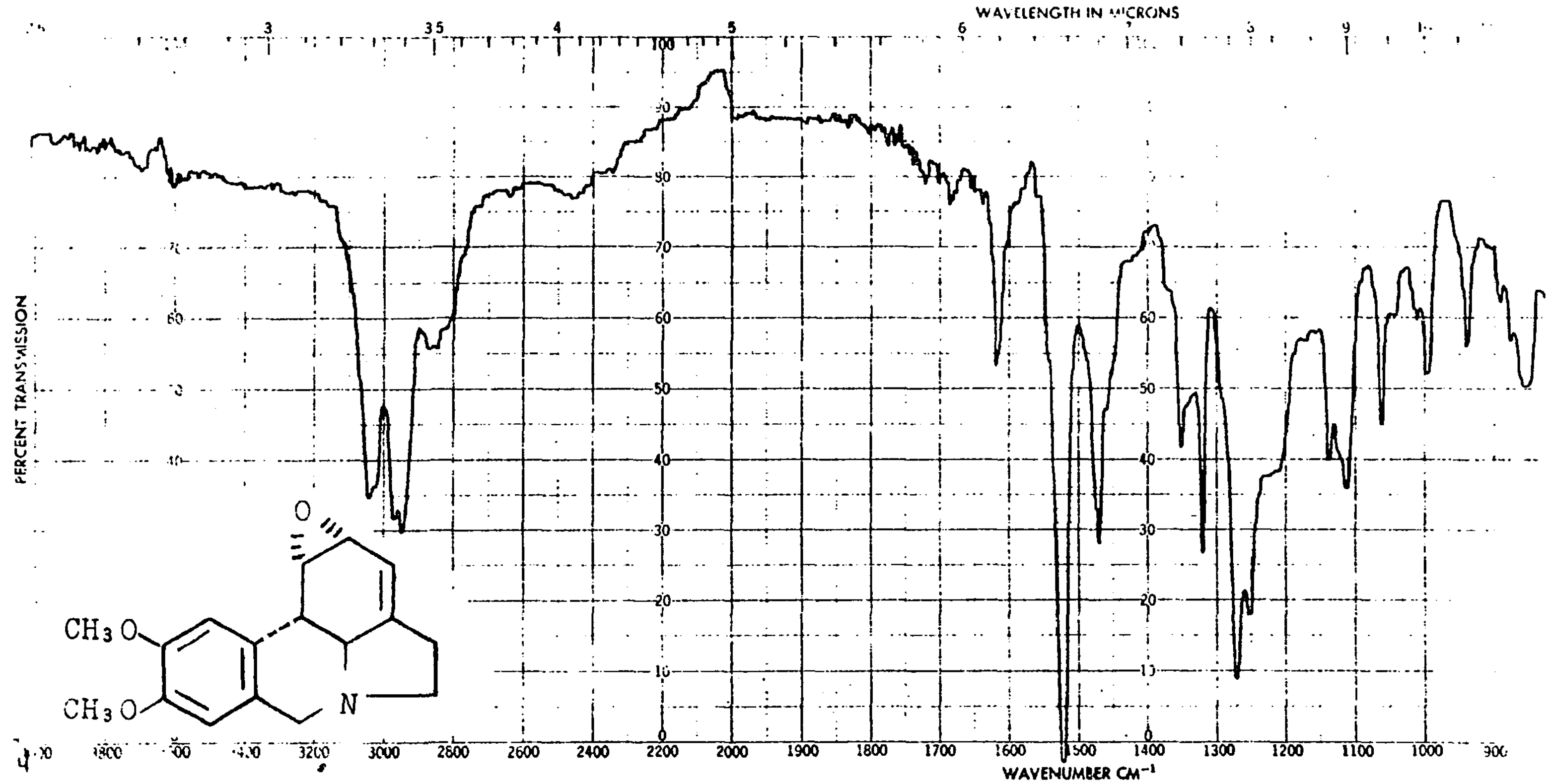

WAVELENGTH IN MICRONS
3
3.5
4
5
6
9
PERCENT TRANSMISSION
100
90
80
70
60
50
40
30
20
10
0
CH3O
CH3O
O
N
3000
2800
2600
2400
2200
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
WAVENUMBER CM⁻¹

Figure 37. NMR spectrum of anhydromethylpseudolycorine

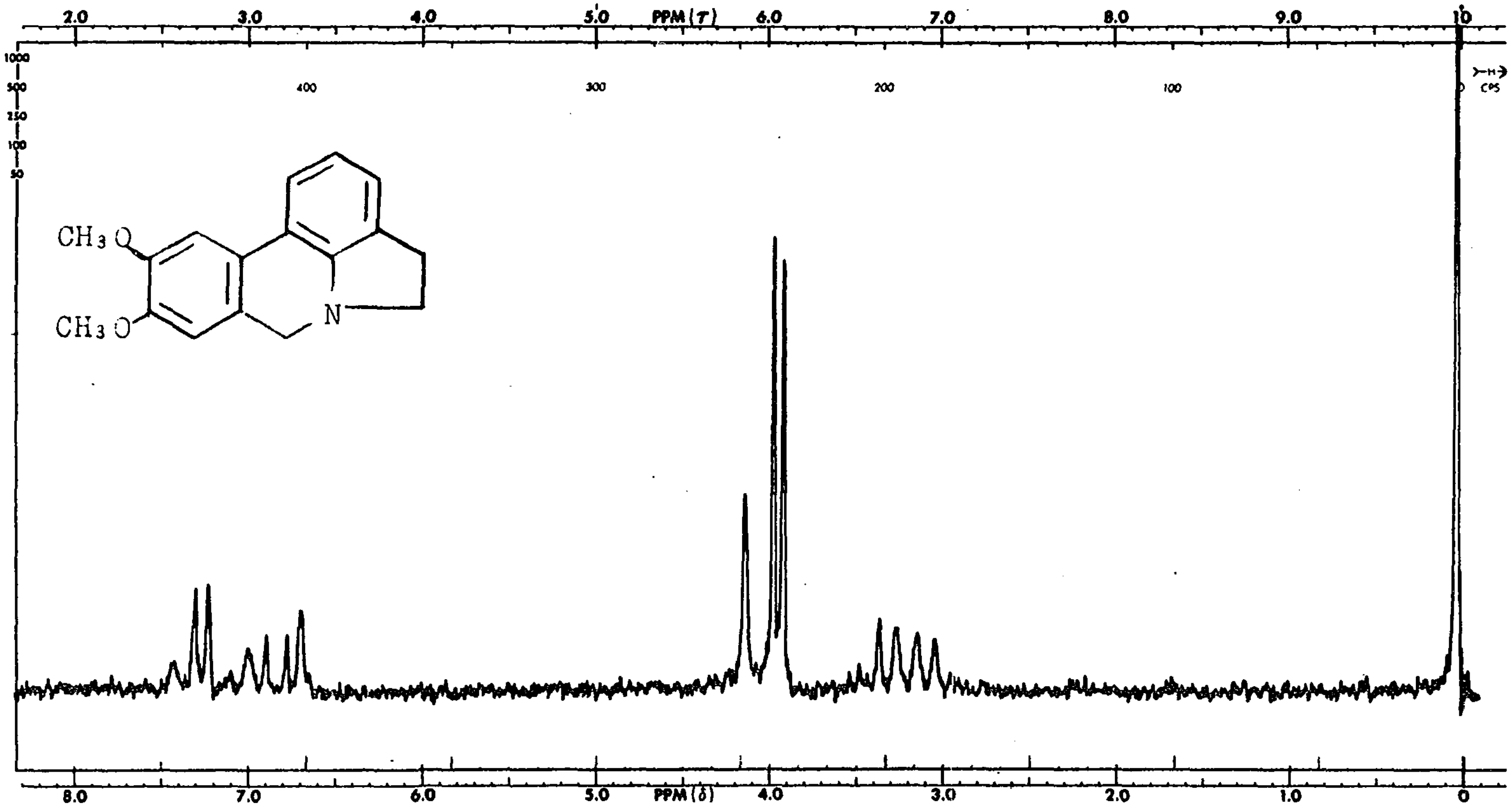

PPM (τ)
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10
1000
500
250
100
50
400
300
200
100
0 CPS
CH₃O
CH₃O
N
PPM (δ)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0

Figure 38. IR spectrum of anhydromethylpseudolycorine

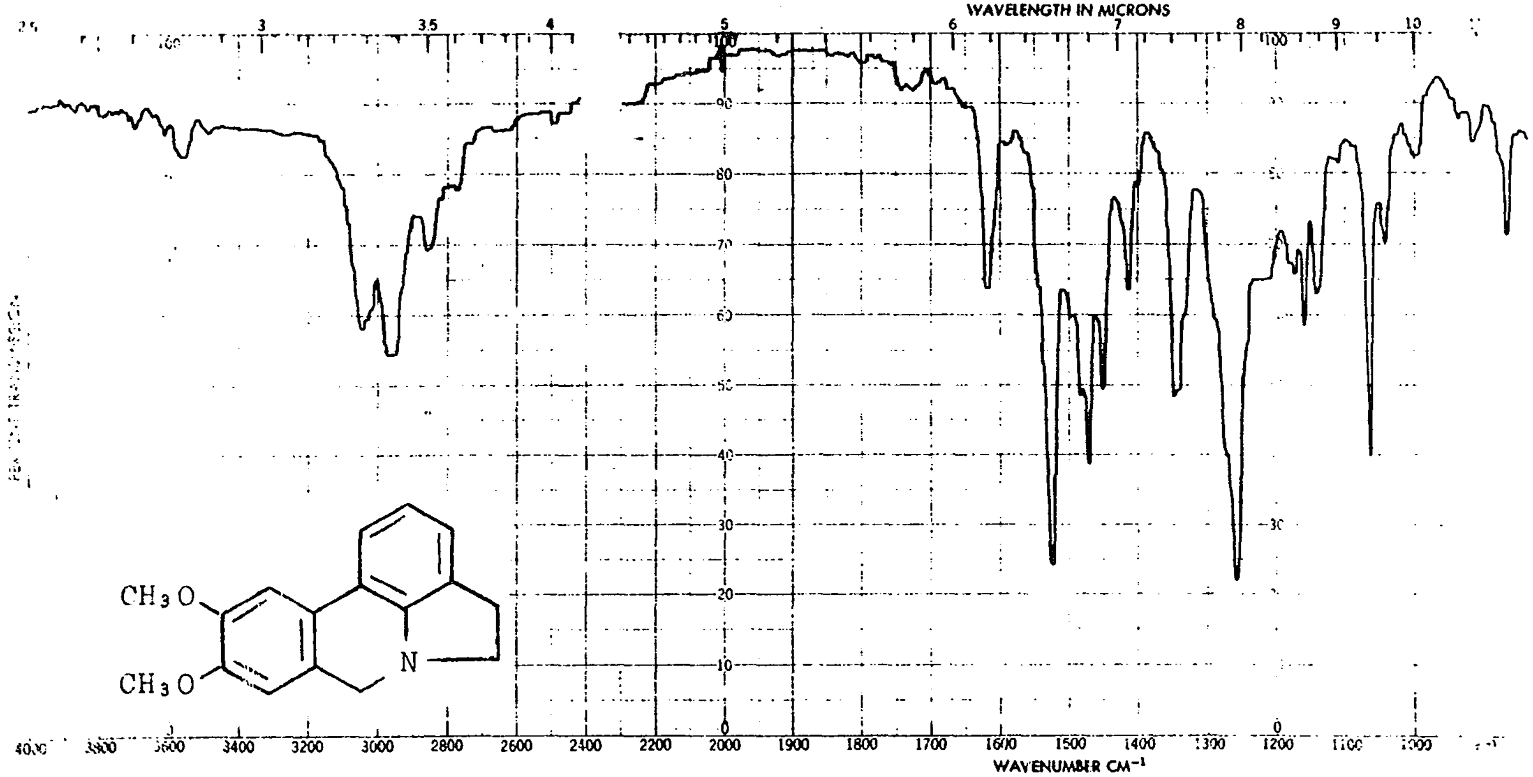
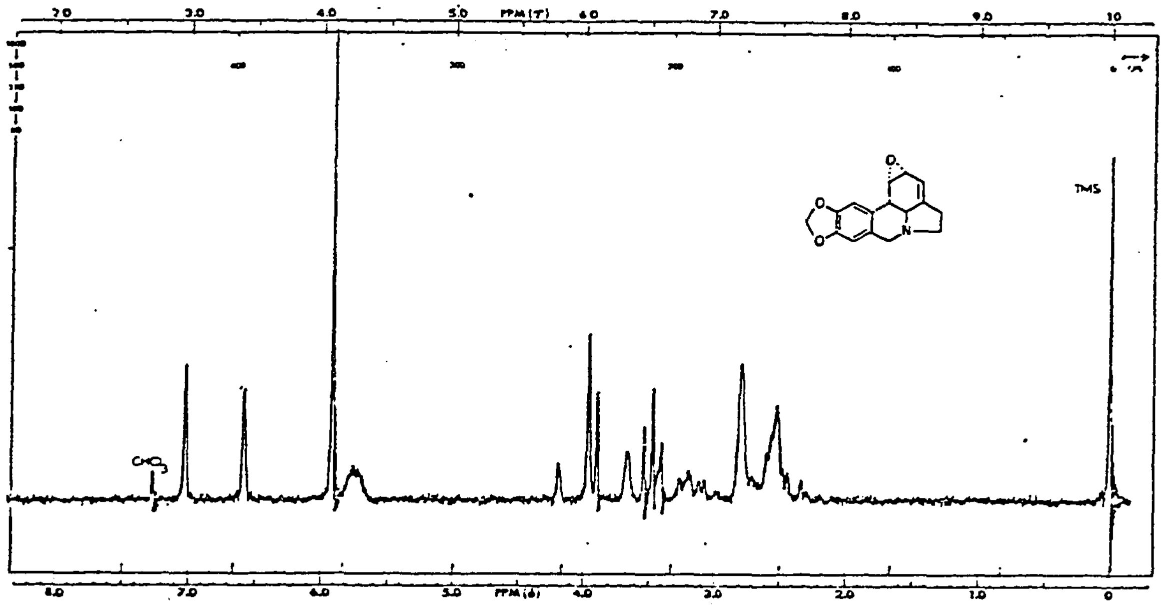

WAVELENGTH IN MICRONS
3
3.5
4
5
6
7
8
9
10
100
90
80
70
60
50
40
30
20
10
0
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
WAVENUMBER CM⁻¹
CH₃O
CH₃O
N

Figure 39. Spectra of lycorine α-epoxide


PPM (τ)
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10
CHCl3
TMS
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0

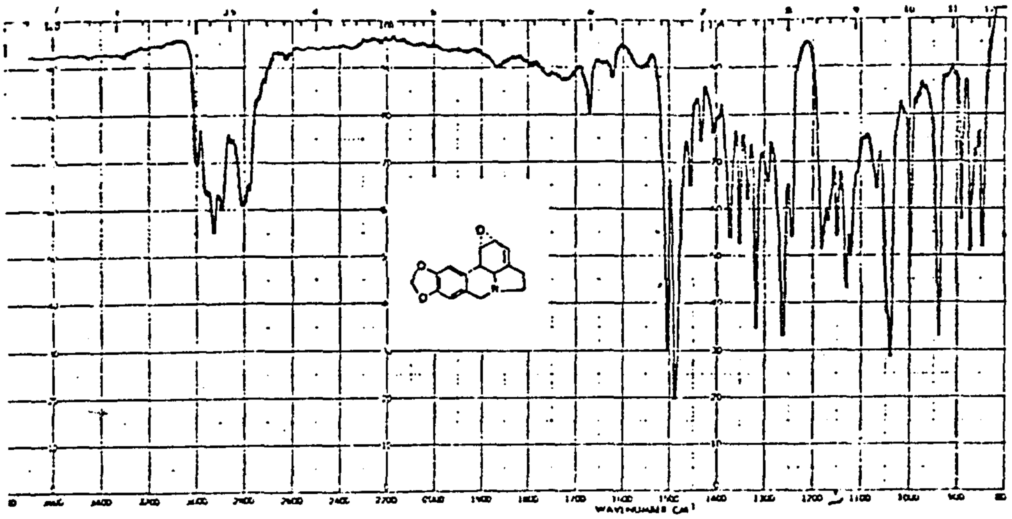
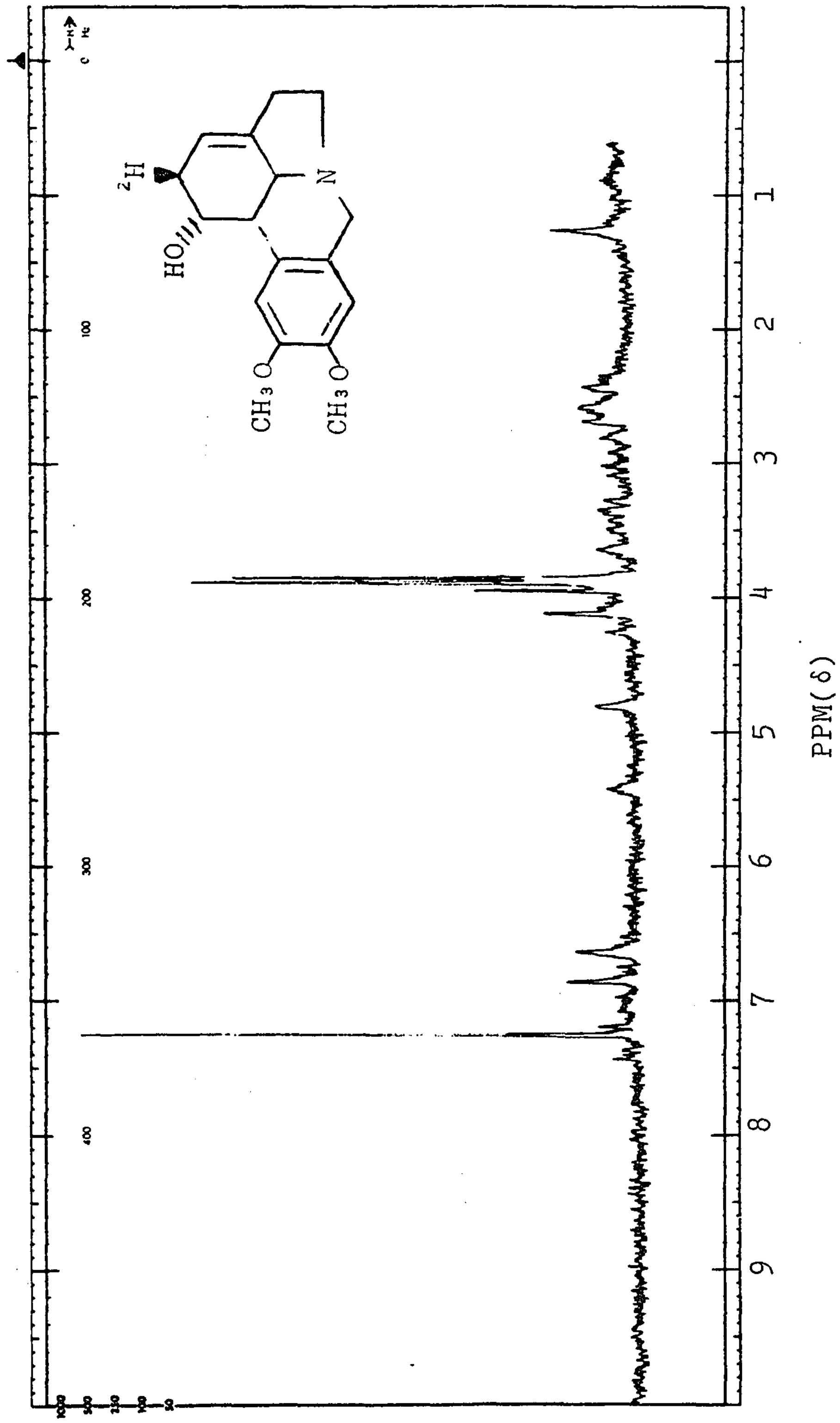

WAVENUMBER CM⁻¹

Figure 40. NMR spectrum of [2β-^{2}H]pluviine


^{2}H
HO
N
CH$_3$O
CH$_3$O
100
200
300
400
1
2
3
4
5
6
7
8
9
PPM(δ)

good agreement with lycorine α-epoxide, and the high resolution mass spectra showed the correct parent ion. Methylpseudolycorine α-epoxide was reduced with lithium aluminum hydride [^{2}H] or [^{3}H] to yield the labeled pluviine. The NMR spectra of [2β-^{2}H]pluviine was identical with authentic samples except in the region 2-3 δ where the C-2 deuterium would be expected to cause some differences (Figure 40). The infrared spectrum of the deuterated and authentic pluviine were again almost identical (Figure 41).

The stereochemistry of the hydrogen in the 2-position of the labeled pluviine was shown by acetylating the pluviine with acetic anhydride and pyridine to give [2β-^{2}H] O-acetyl pluviine (IR spectra, Figure 42). In the analogous acetylcaranine Wildman and Heimer (13) had shown a loss of m/e 60 (metastable) in both 2-deutero and nondeuterated acetylcaranine. This suggested that the label was trans to the acetate. Since the pyrolysis of acetates was known to proceed in a 1,2-cis manner, the stereochemistry was checked by pyrolyzing the O-acetylpluviine at 240° (Figure 43). Anhydromethylpseudolycorine was produced with 75% retention of the deuterium label. This required that 75% of the deuterium was in the β-position. The reaction sequence was repeated using lithium aluminum hydride [^{3}H] to yield [2β-^{3}H]pluviine which was mixed with [9-O-methyl-^{14}C]pluviine and recrystallized to constant activity. This material was

Figure 41. IR spectrum of [2β-^{2}H]pluviine

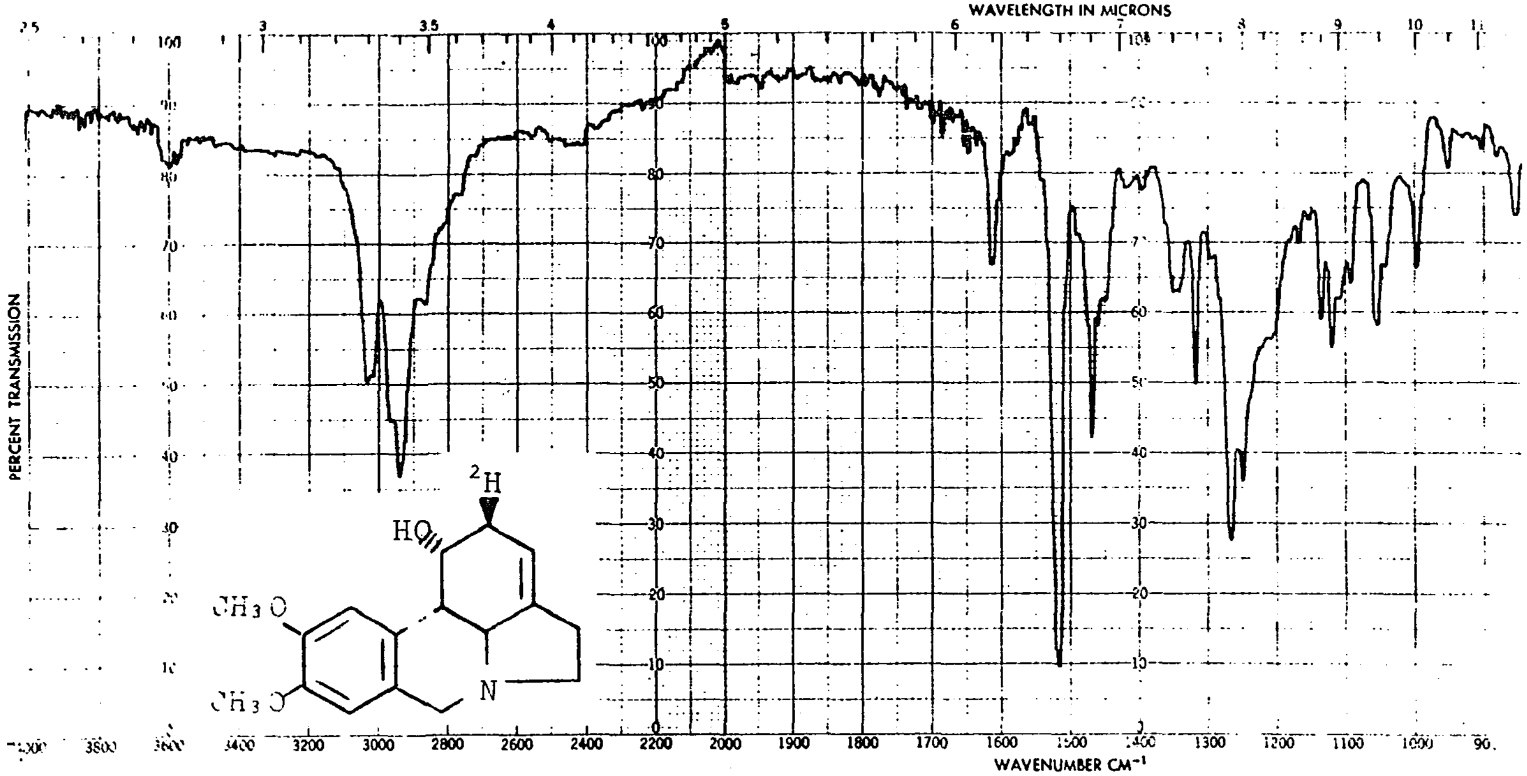
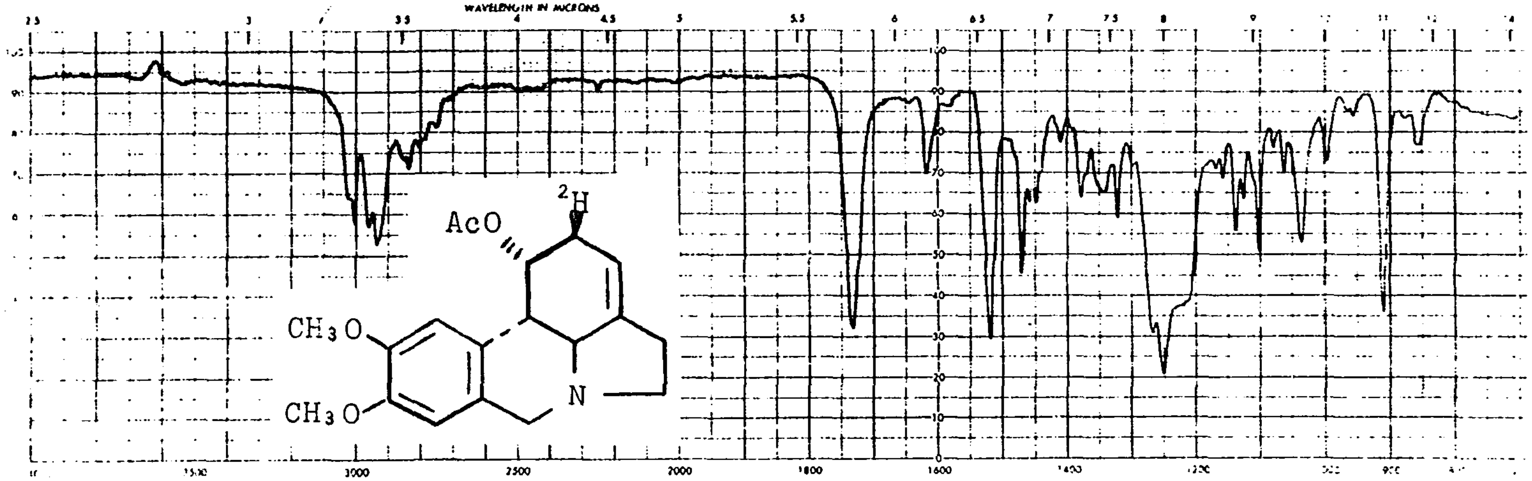

WAVELENGTH IN MICRONS
2.5
3
3.5
4
5
6
7
8
9
10
11
PERCENT TRANSMISSION
100
90
80
70
60
50
40
30
20
10
0
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
WAVENUMBER CM⁻¹
²H
HO
CH₃O
CH₃O
N

Figure 42. IR spectrum of [2β-^{2}H]acetylpluviine


WAVELENGTH IN MICRONS
AcO
2H
CH3O
CH3O
N
3000
2500
2000
1800
1600
1400

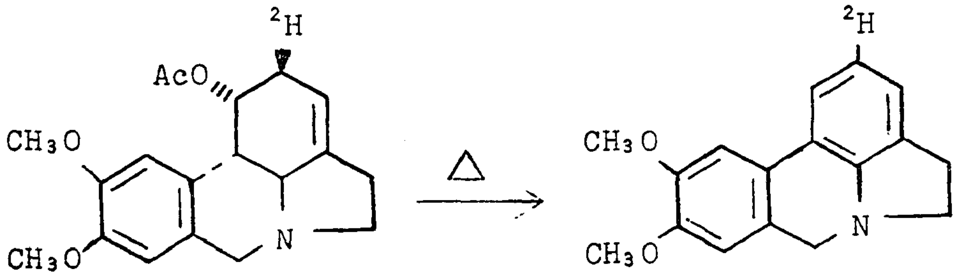


Figure 43

then fed to blooming "King Alfred" daffodils. After two weeks the daffodils were worked up in the usual manner. Thin layer chromatography allowed the separation of 29 mg of galanthine (6). The carbon-tritium ratio in the isolated galanthine showed a retention of 79% of the tritium. This clearly required that the hydroxylation of galanthine proceeded with inversion. This was in agreement with the original work by Wildman and Heimer (13) and the work of Bruce and Kirby (30, 31). However, it appears to be in conflict with the work of Fuganti and Mazza (32) on lycorine whose work definitely indicated that the stereochemical course of the biosynthetic hydroxylation was with retention of configuration. Resolution of this discrepancy is difficult. Since Fuganti and Mazza fed compounds whose stereochemistry was based on the work of Wildman and Heimer and of Bruce and Kirby, presumably, the stereochemistry was consistent in all these studies. This

Table I. Pluviine feeding to Narcissus "King Alfred"

Alkaloid	Quantity mg	Specific Activity (mci/mg)	Total Activity mci	% Incorporation	$^{3}H/^{14}C$ Ratio
Pluviine	9	$.77 \times 10^{-3}$ ^{3}H	6.9×10^{-3} ^{3}H		1.3
		$.578 \times 10^{-3}$ ^{14}C	5.2×10^{-3} ^{14}C		
Galanthine	29	$.73 \times 10^{-5}$ ^{14}C	2.1×10^{-5}	4.1 % C	1.0
		$.75 \times 10^{-5}$ ^{3}H	2.2×10^{-5}		

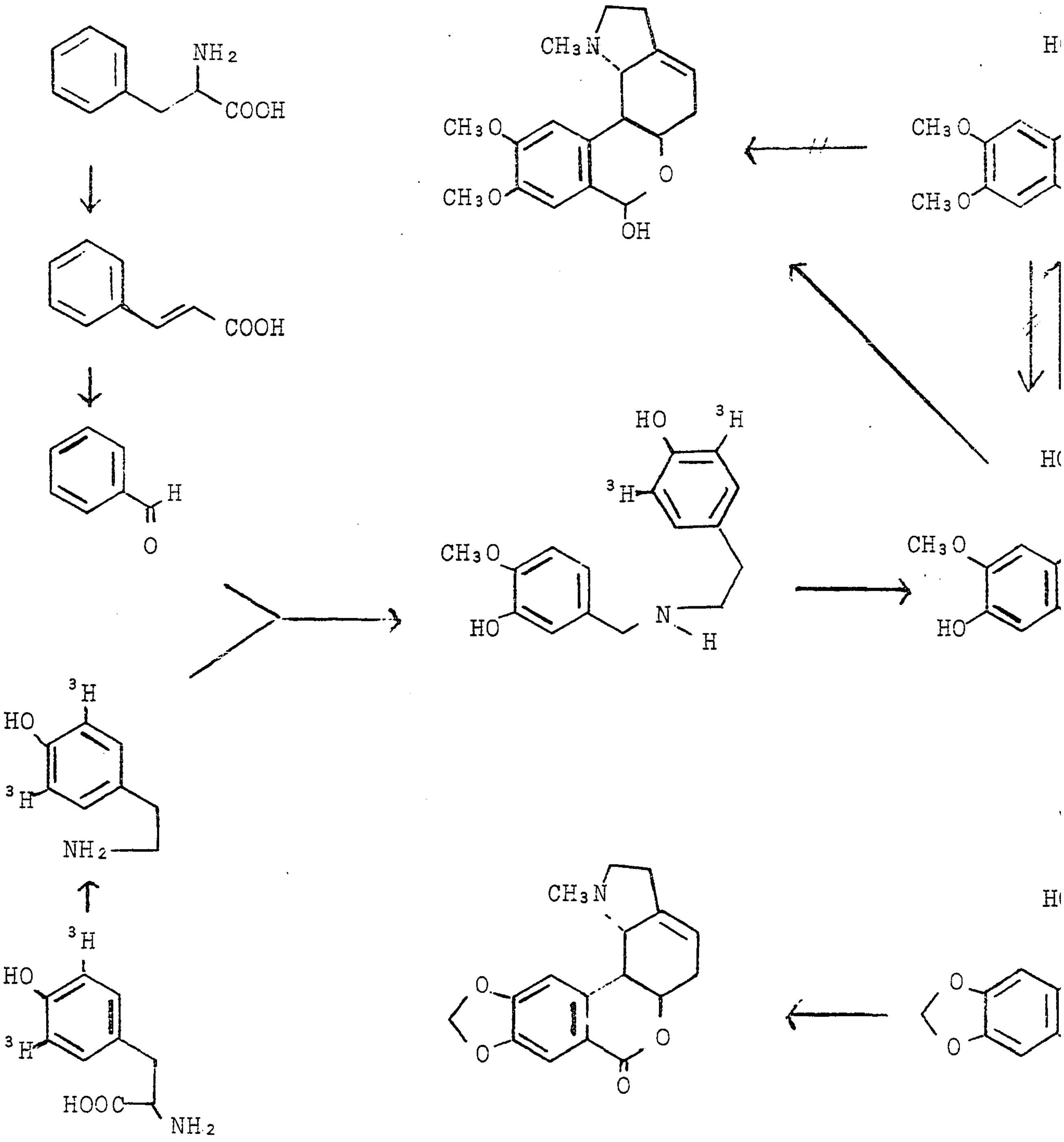
leaves as one possibility that different oxidative pathways for the same hydroxylation reaction occurred in different genera of the Amaryllidaceae. It should be noted that three groups feeding of differently labeled starting materials to different plants have found the C-2 hydroxylation to proceed with inversion and only the work of Fuganti and Mazza suggested that this hydroxylation proceeds with retention.

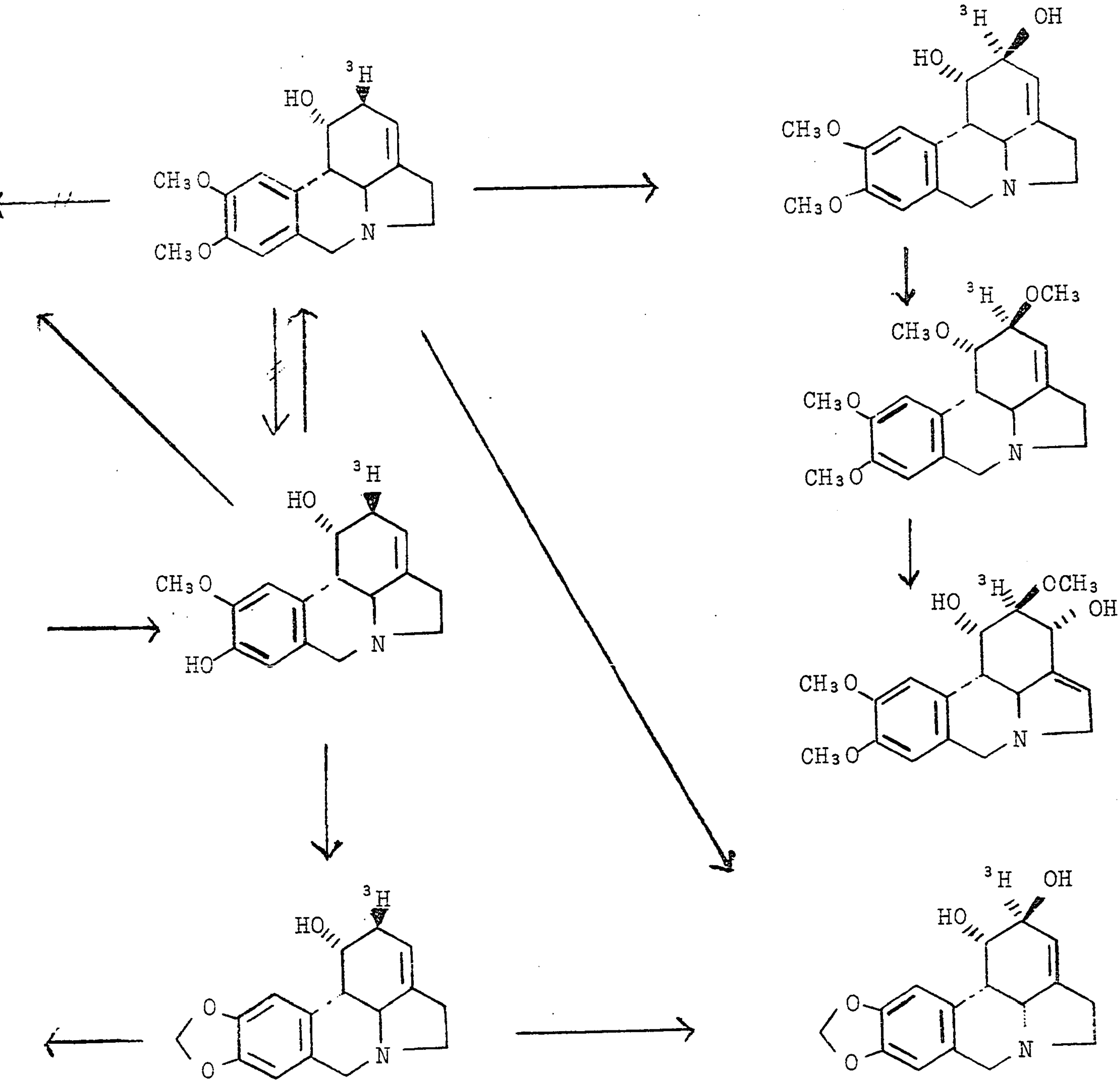
The problem of the biosynthesis of montanine and haemanthamine could be probed by feeding appropriately labeled O-methylnorbelladine to *Rodophiala bifida* which has been shown to contain vitattine, montanine, and haemanthamine (49). Since Feinstein (19) showed that vitattine was a precursor to both montanine and haemanthamine, if 11-hydroxyvitattine is the biosynthetic intermediate then both montanine and haemanthamine must retain the same hydrogen, i.e., pro R or pro S.

The results of the work presented in this dissertation when combined with the earlier work cited give a complete picture of the biosynthesis of the lycorine- and lycorenine-type alkaloids (Figure 44).

The only question still in doubt is the stereochemistry of the hydroxylations in narcissidine. The C-2 hydroxylation probably occurs with inversion since the work of Harken suggested that galanthine was the precursor of narcissidine.

Figure 44. Biosynthesis of lycorine- and lycorenine-type alkaloids


NH2
COOH
COOH
H
O
CH3N
CH3O
CH3O
O
OH
HO
³H
³H
CH3O
HO
N
H
³H
HO
³H
NH2
³H
HO
³H
HOOC
NH2
CH3N
O
O
O
O
CH3O
CH3O
CH3O
HO
O
O



³H
HO
CH₃O
CH₃O
N
³H
OH
HO
CH₃O
CH₃O
N
³H
OCH₃
CH₃O
CH₃O
CH₃O
N
³H
HO
CH₃O
HO
N
³H
OCH₃
HO
OH
CH₃O
CH₃O
N
³H
HO
O
O
N
³H
OH
HO
O
O
N

The stereochemistry of the arrangement of the double bond and the formation of the allylic alcohol at C-3 have not been determined.

Chemical Hydroxylation of C-7 in Lycorine

One of the useful applications of synthetic chemistry is the preparation of proposed biosynthetic intermediates which have not been isolated. As part of the continuing effort to elucidate the biosynthetic pathways in the Amaryllidaceae alkaloids, the preparation of 7-hydroxylycorine (43) was undertaken. The oxidation of lycorine (8) to the lactam (44) was achieved but reduction to 7-hydroxylycorine (43) by a variety of methods failed (Figure 45).

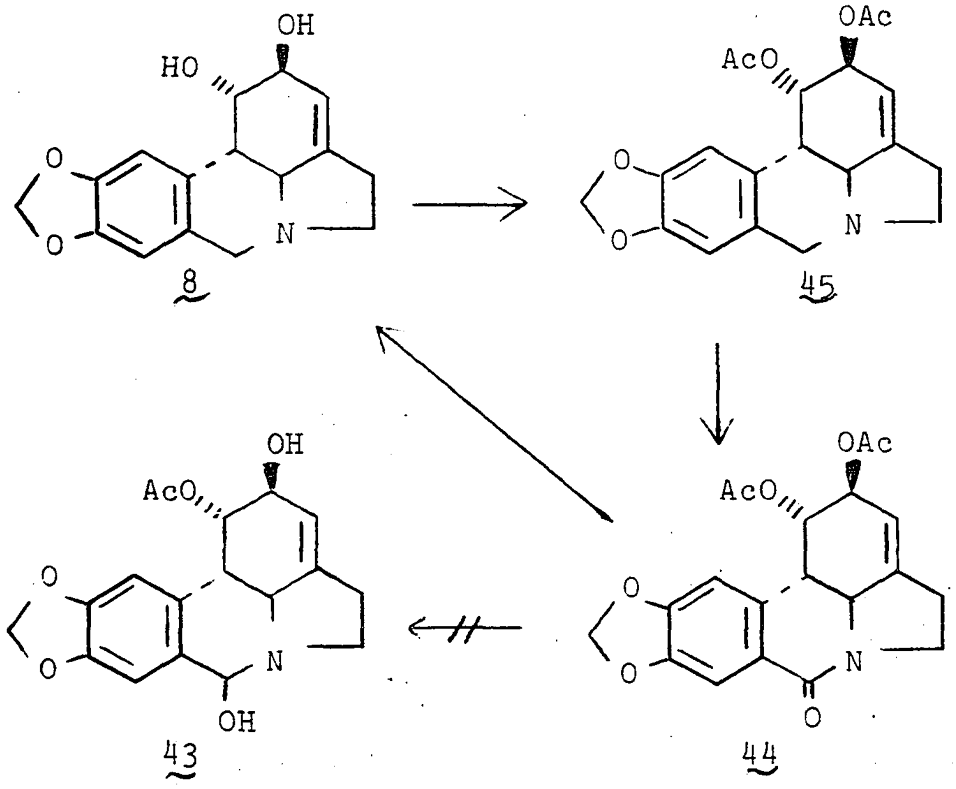


Figure 45. C-7 oxidation of lycorine via the lactam

Considering the work of Mizukami (16), it seemed that if the alcohol groups in lycorine were protected, the bromide intermediate might be isolated and converted to an aldehyde (Figure 46).

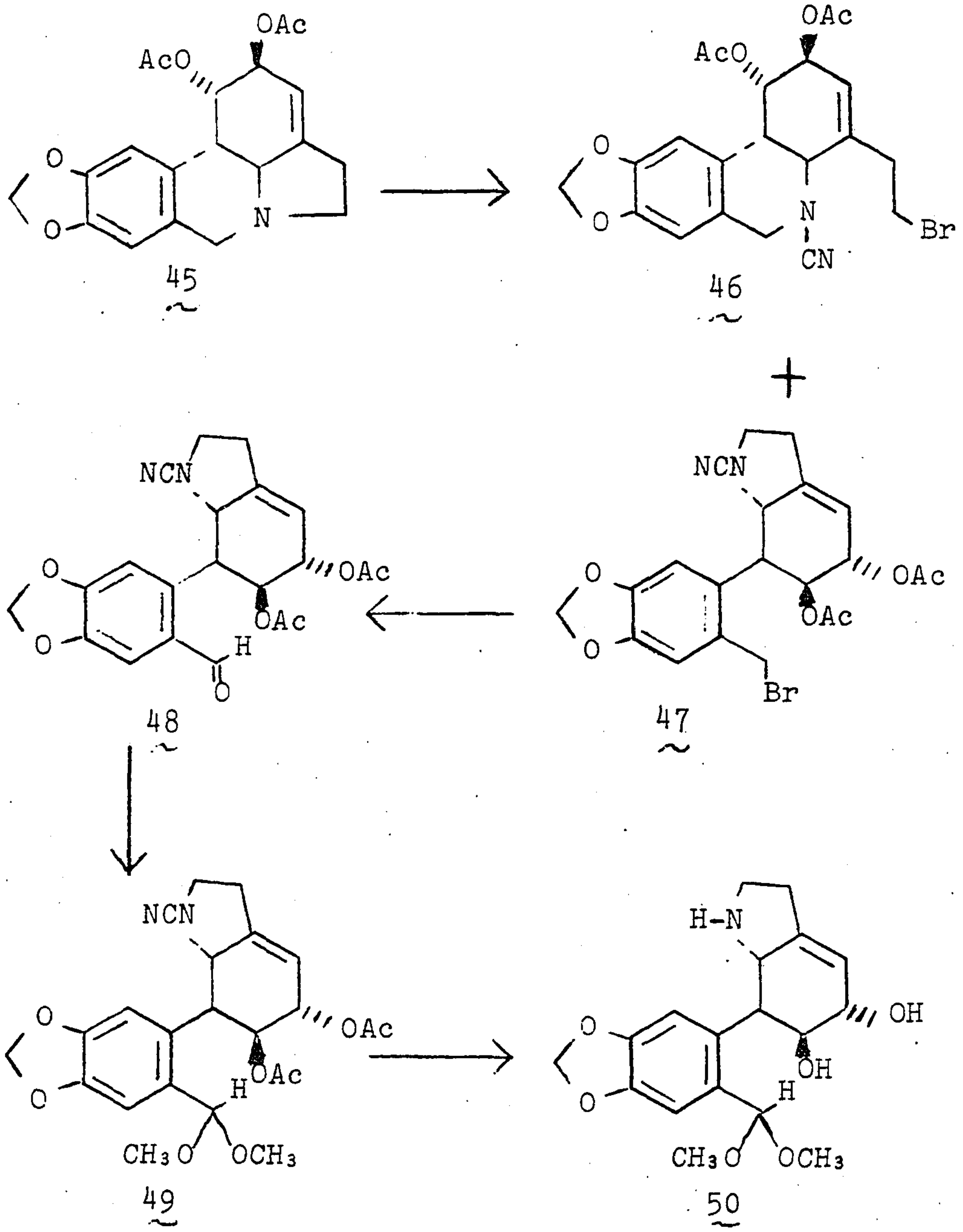

OAc
AcO
O
O
N
45
OAc
AcO
O
O
N
CN
Br
46
+
NCN
O
O
OAc
OAc
Br
47
NCN
O
O
OAc
OAc
H
O
48
NCN
O
O
OAc
OAc
H
CH3O
OCH3
49
H-N
O
O
OH
OH
H
CH3O
OCH3
50

Figure 46. Preparation and reduction of 7-hydroxylycorine

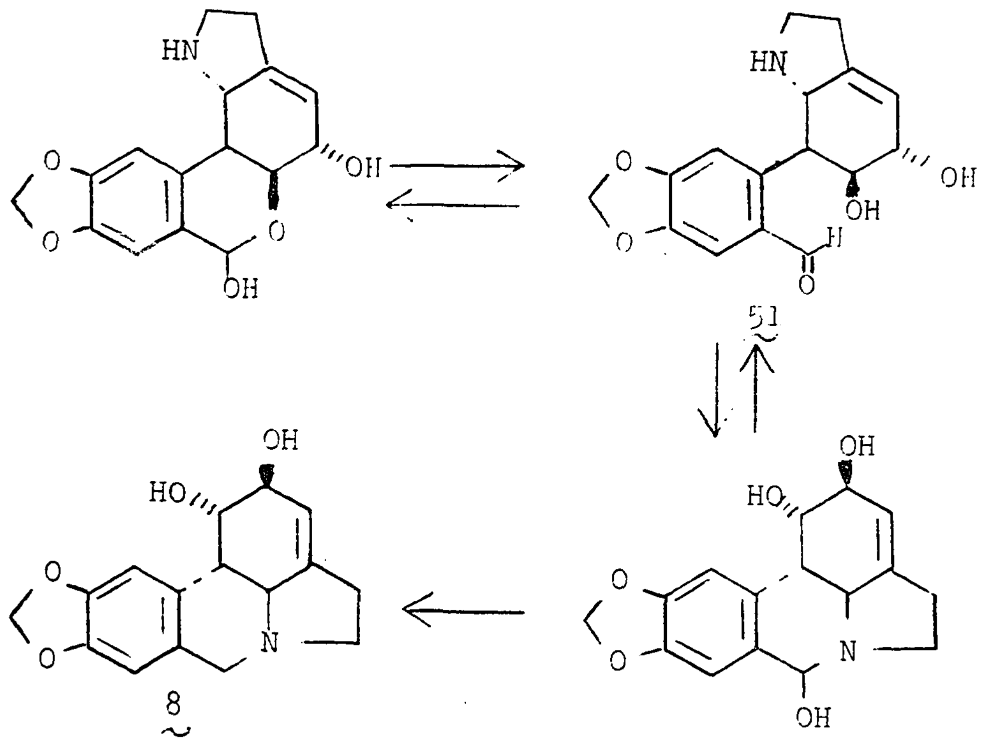


Figure 46. (Continued)

Starting with O,O-diacetyllycorine (**45**), the C-7-nitrogen carbon bond was cleaved using cyanogen bromide. The infrared spectrum showed the presence of a strong peak at 2100 cm^{-1} indicating the presence of the N-cyano group. The NMR spectrum indicated that the product was a mixture of isomers (**46** and **47**) and the mass spectrum was consistent with the desired product in molecular weight. Although Mizukami reported 25% of the desired cleavage using benzene as the solvent, the use of chloroform allowed the production of the desired isomer (**47**) in greater than 80% yield. The reaction

Figure 47. IR spectrum of aldehyde (48)

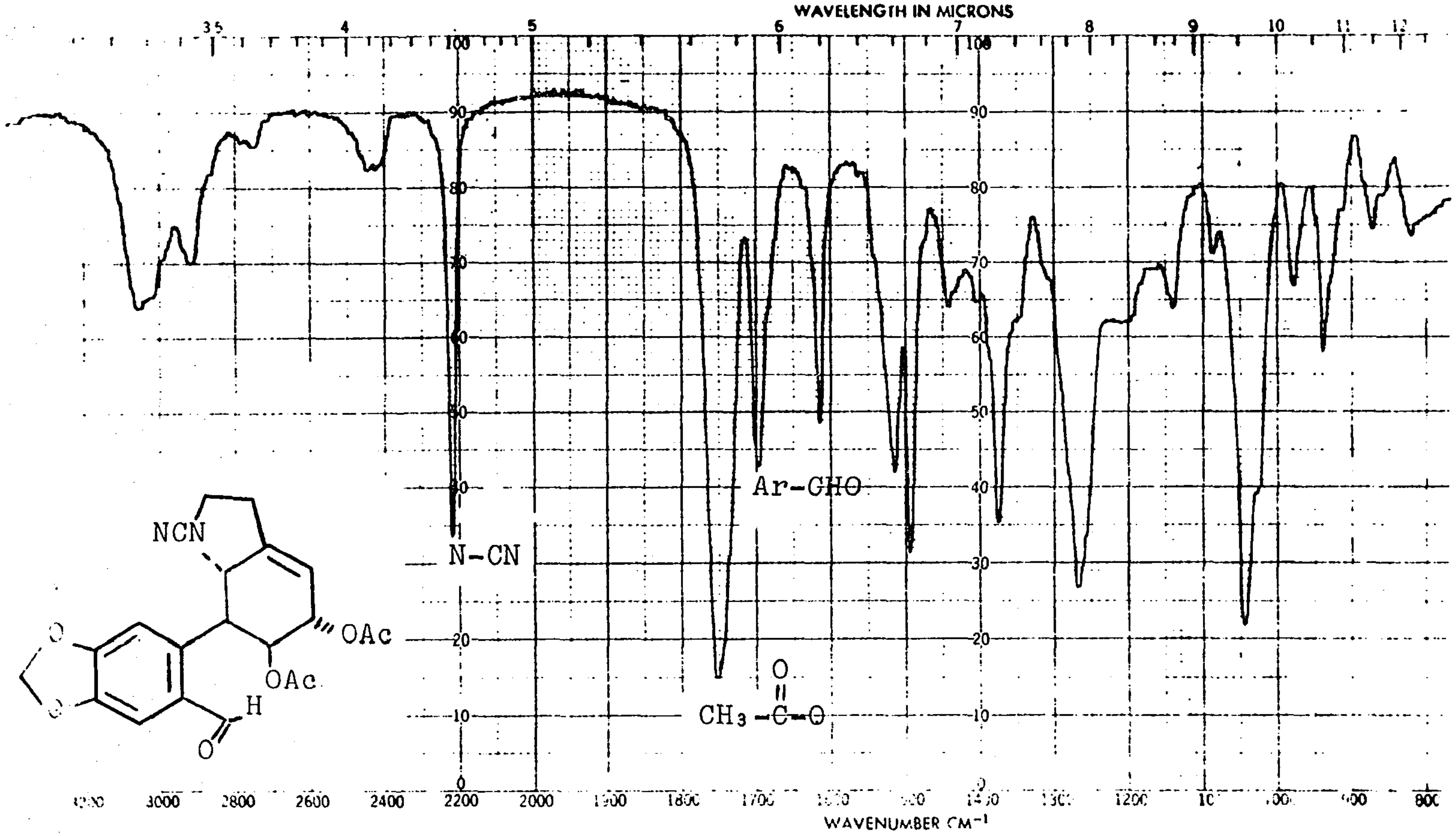
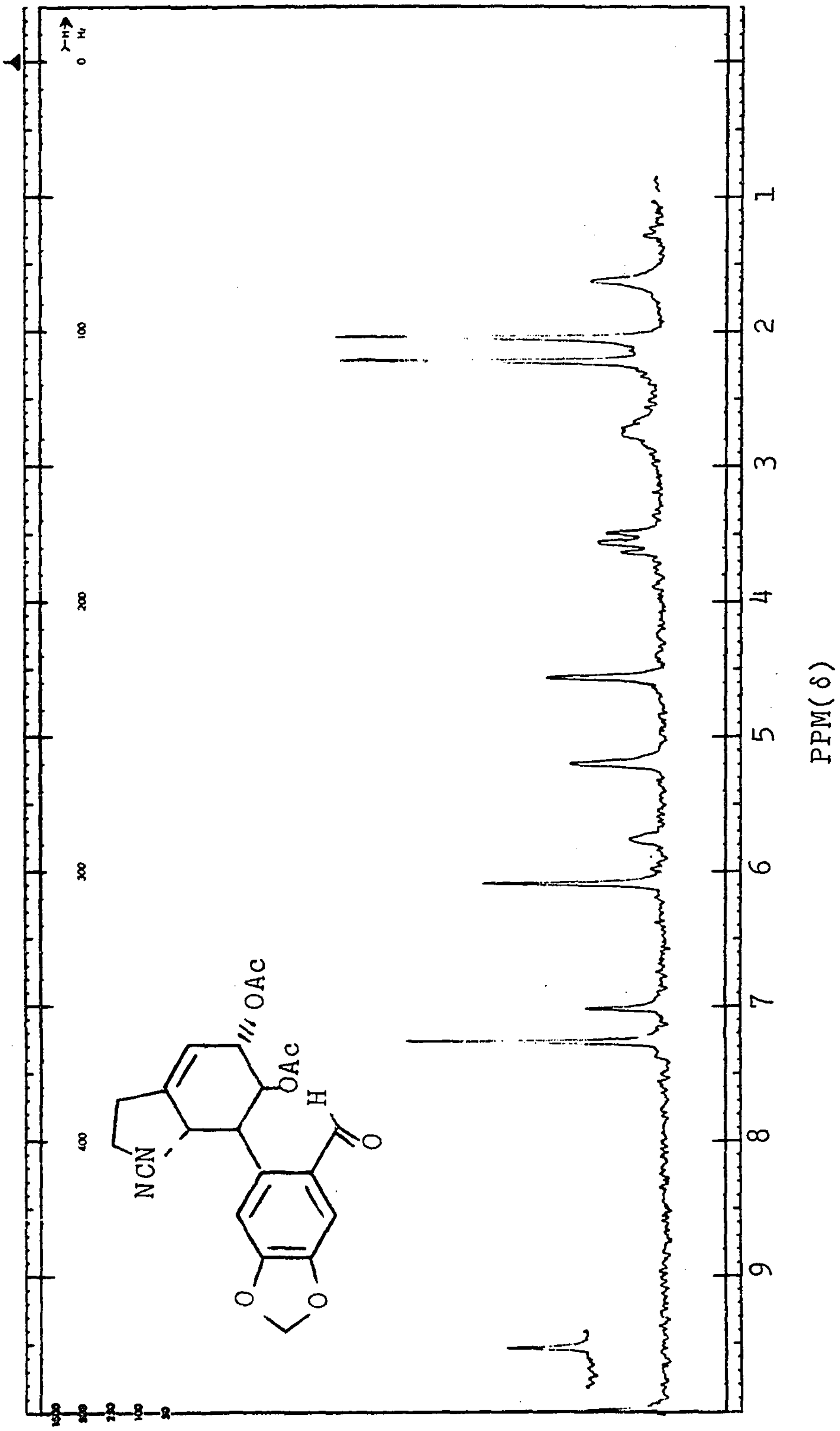

WAVELENGTH IN MICRONS
3.5
4
5
6
7
8
9
10
11
12
100
90
80
70
60
50
40
30
20
10
0
N–CN
Ar–CHO
CH3–C(=O)–O
NCN
OAc
OAc
H
O
3000
2800
2600
2400
2200
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
WAVENUMBER CM⁻¹

Figure 48. NMR spectrum of aldehyde ($\underset{\sim}{48}$)


0 Hz
100
200
300
400
1
2
3
4
5
6
7
8
9
PPM(δ)
NCN
OAc
OAc
H
O
O
O

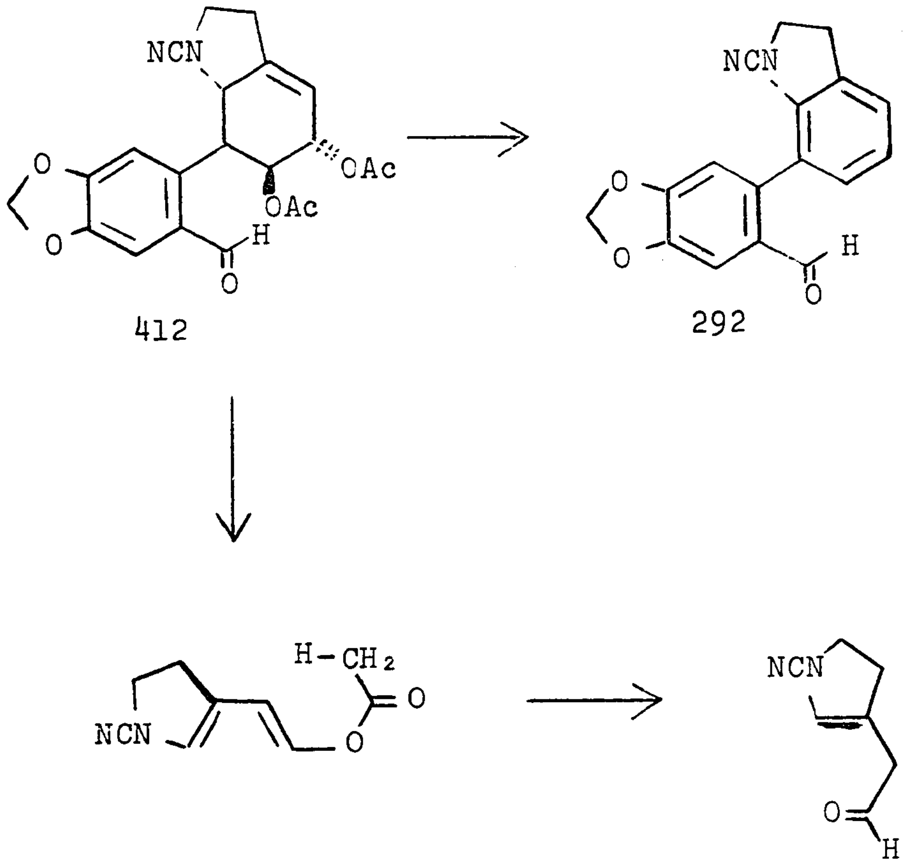

NCN
OAc
OAc
H
O
412
292
H-CH2
O

Figure 49. Mass spectra of aldehyde (48)

Figure 50. NMR spectrum of acetal (49)

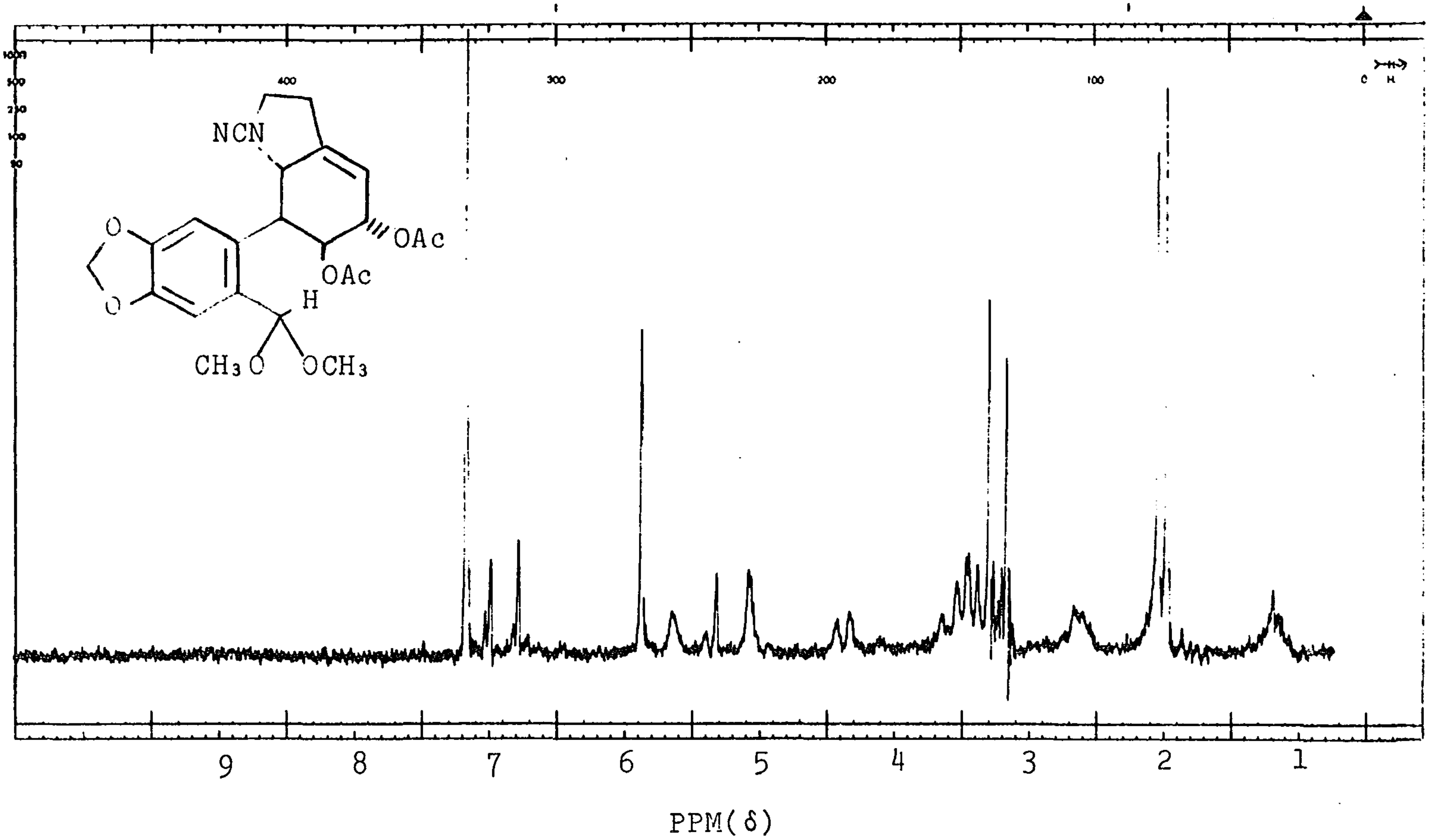

400
300
200
100
NCN
O
O
OAc
OAc
H
CH₃O
OCH₃
9
8
7
6
5
4
3
2
1
PPM(δ)

Figure 51. IR spectrum of acetal (49)

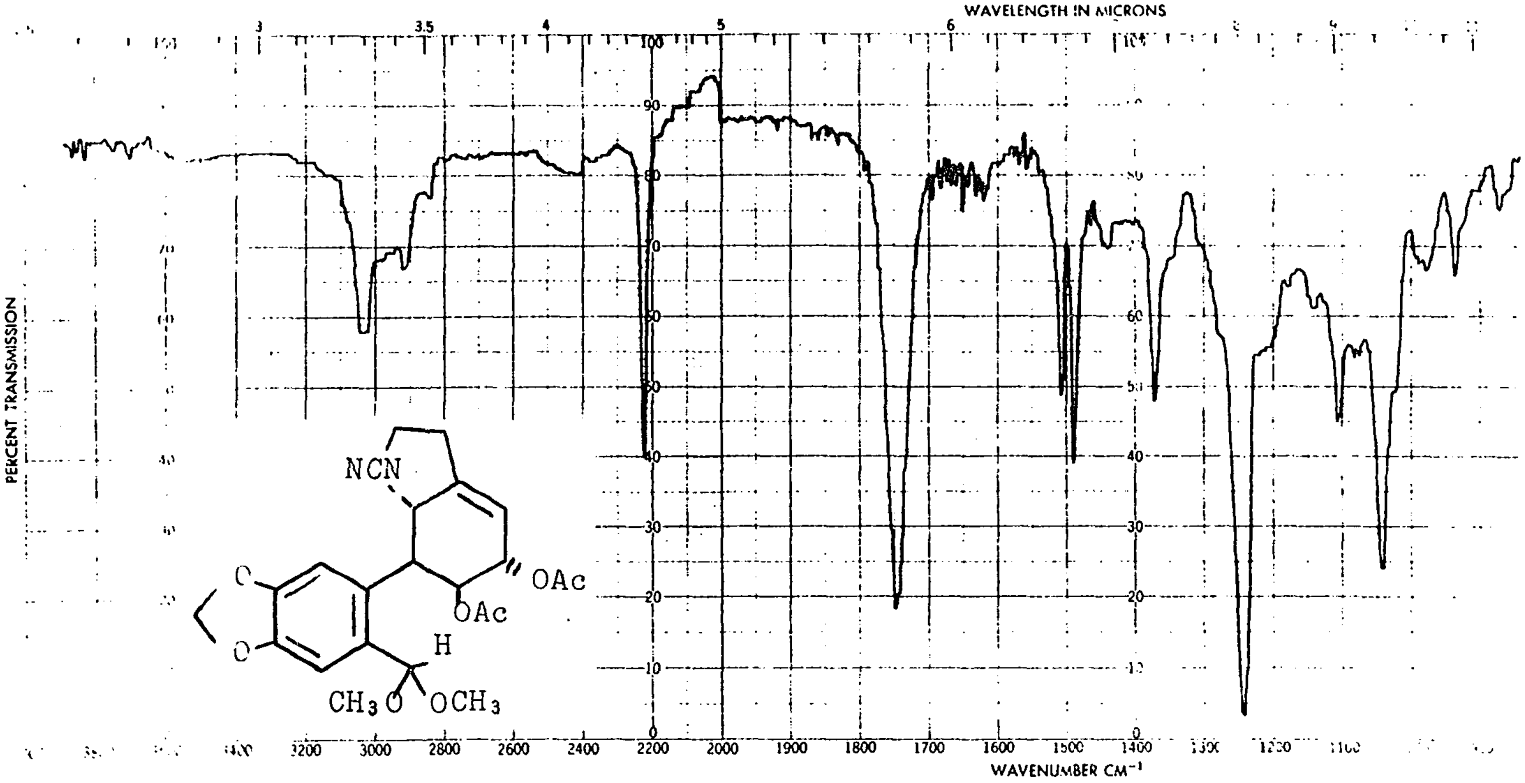
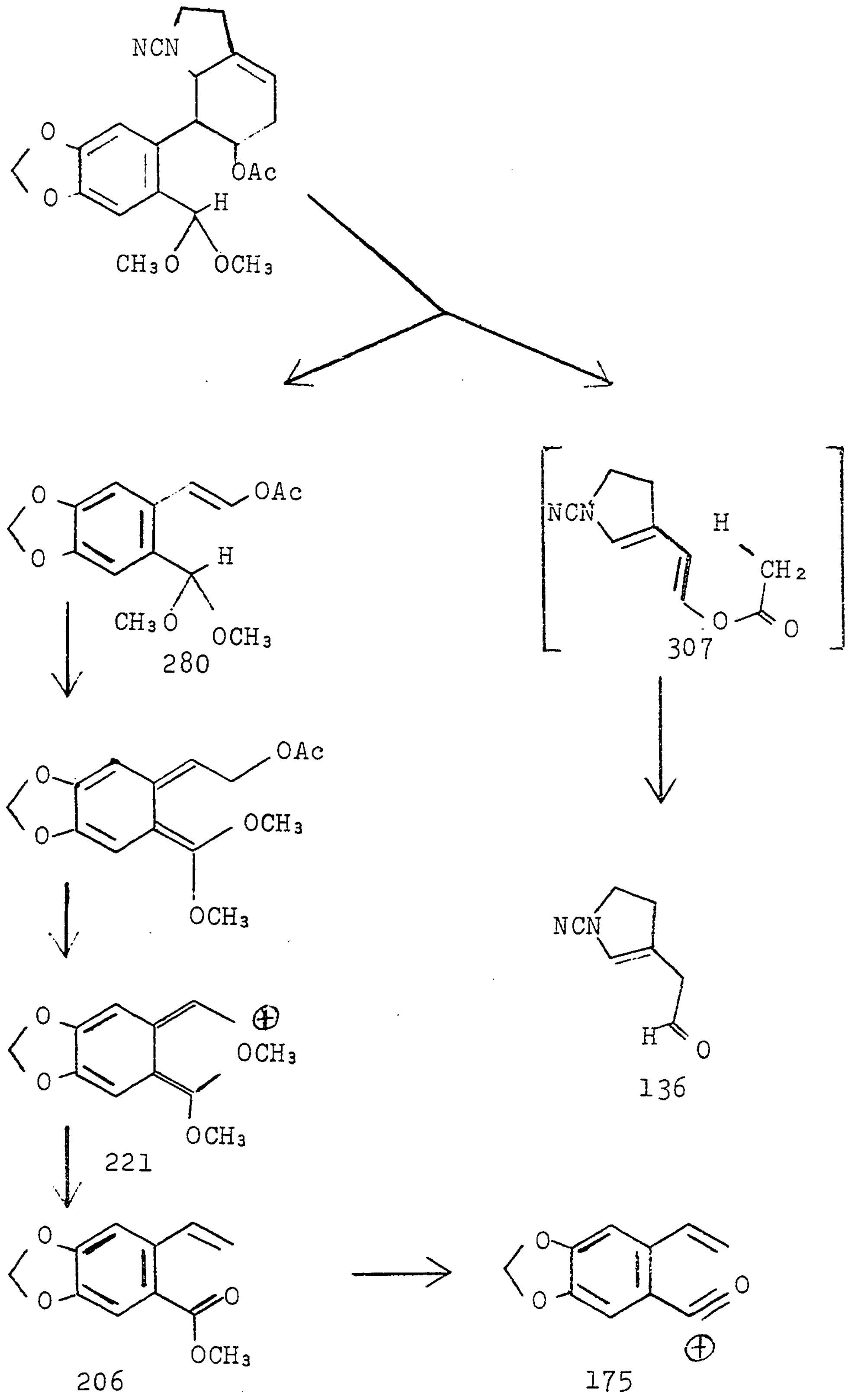
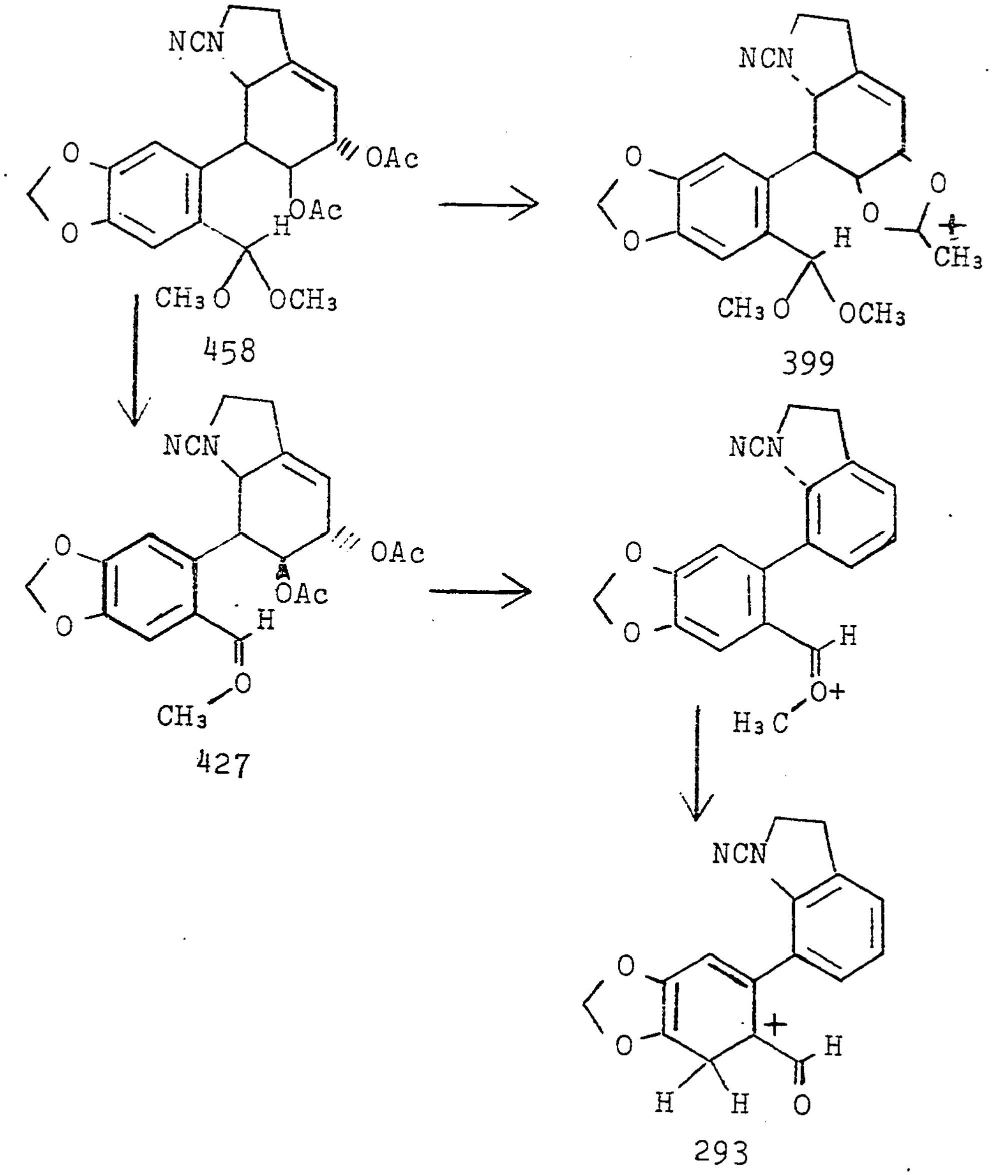

WAVELENGTH IN MICRONS
3
3.5
4
5
6
PERCENT TRANSMISSION
100
90
80
70
60
50
40
30
20
10
0
3200
3000
2800
2600
2400
2200
2000
1900
1800
1700
1600
1500
1400
WAVENUMBER CM⁻¹
NCN
OAc
OAc
H
O
O
CH₃O
OCH₃

Figure 52. Mass spectrum of acetal (49)

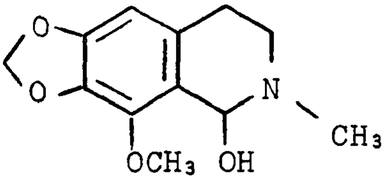

NCN
O
O
OAc
H
CH₃O
OCH₃
OAc
H
CH₃O
OCH₃
280
NCN
H
CH₂
O
O
307
OAc
OCH₃
OCH₃
⊕
OCH₃
OCH₃
221
NCN
H
O
136
O
O
OCH₃
206
O
⊕
175


NCN
OAc
OAc
H
CH₃O
OCH₃
458
NCN
O
O
H
CH₃
+
CH₃O
OCH₃
399
NCN
OAc
OAc
H
O
CH₃
427
NCN
H
O+
H₃C
NCN
+
H
H
H
O
293

product was not separated but was oxidized with dimethyl sulfoxide-sodium bicarbonate to the aldehyde (48). The aldehyde was separated via column chromatography from the undesired bromide isomer (46). The location of the aldehyde was confirmed by the observation of the aldehyde proton 9.95 δ as a singlet (Figure 47). The infrared carbonyl stretching frequency at 1698 cm^{-1} (Figure 48) and the ultraviolet absorption at 315 nm indicated a conjugated carbonyl group. The NMR spectrum also showed a shift in the downfield acetate methyl group relative to similar compounds without the aldehyde (Figure 47). The mass spectrum of the aldehyde (48) showed major peaks at m/e 412, 292, 178, and 136. These peaks could have been produced by the paths shown in Figure 47. The aldehyde (48) was converted to the acetal (49) with methyl orthoformate. The NMR (Figure 50) and infrared spectra (Figure 51) supported the assigned structure and the mass spectrum showed strong peaks at m/e 458, 427, 399, 307, 293, 280, 221, 206, 193, 175, 136. Possible routes to these ions are shown on Figure 52. This acetal (49) was reduced with lithium aluminum hydride to yield an acetal (50) without the O,O-diactyl groups and without the N-cyano group. The NMR and infrared spectra were consistent with the acetal structure (50). The compound decomposed in the heated probe of the mass spectrometer. This acetal (50) was hydrolyzed with dilute hydrochloric acid to yield the aldehyde (51).

The question of interest was, does this molecule (51) exist as the hemiacetal or the hemiaminal. In a similar case, the N-demethylpretazettine(52)-haemanthidine(20) tautomerism, the molecule was reported to exist almost exclusively in the haemanthidine-(hemiaminal) form, although the hemiacetal could be trapped as the acetal or the N-nitroso derivative (Figure 53).

NMR and CD studies seemed to offer an answer to this question. The NMR spectra of lycorenine showed a singlet at 6.04 δ for the benzylic proton. Pretazettine (21) which also has the hemiacetal linkage shows a singlet at 6.06 δ. In the hemiaminal linkage, as shown by cotarine (53),



(53)

the benzylic proton of interest absorbed at 5.39 δ. In 6-hydroxybuphanisine, however, two C-6 epimers exist with two absorptions for the benzylic protons (5.1 and 5.7 δ). Thus, it seemed that if the molecule existed as the hemiacetal, the benzylic proton should absorb at about 6 δ and if the molecule existed as a hemiaminal the absorption should be upfield between 5.7 and 5.3 δ. The NMR spectra of (51) showed a singlet at 6.02 δ which supported a hemiacetal structure.

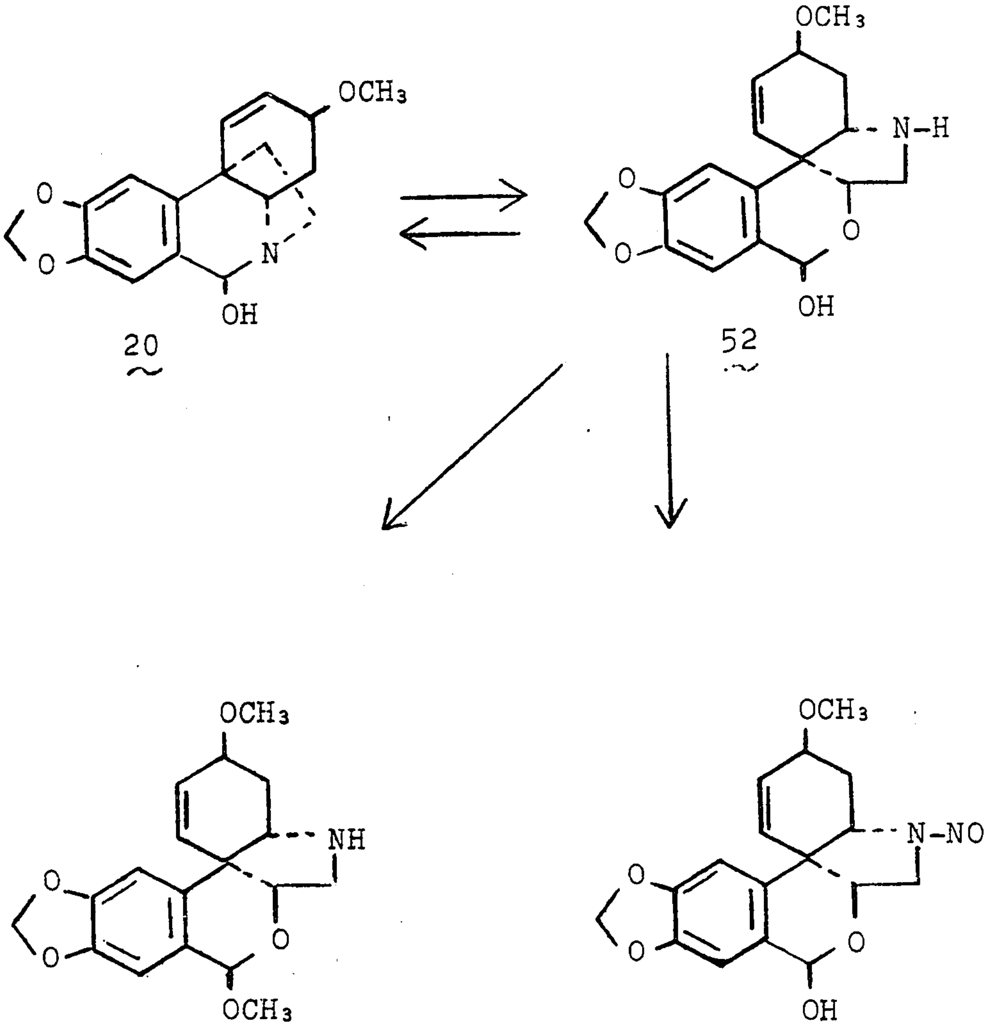
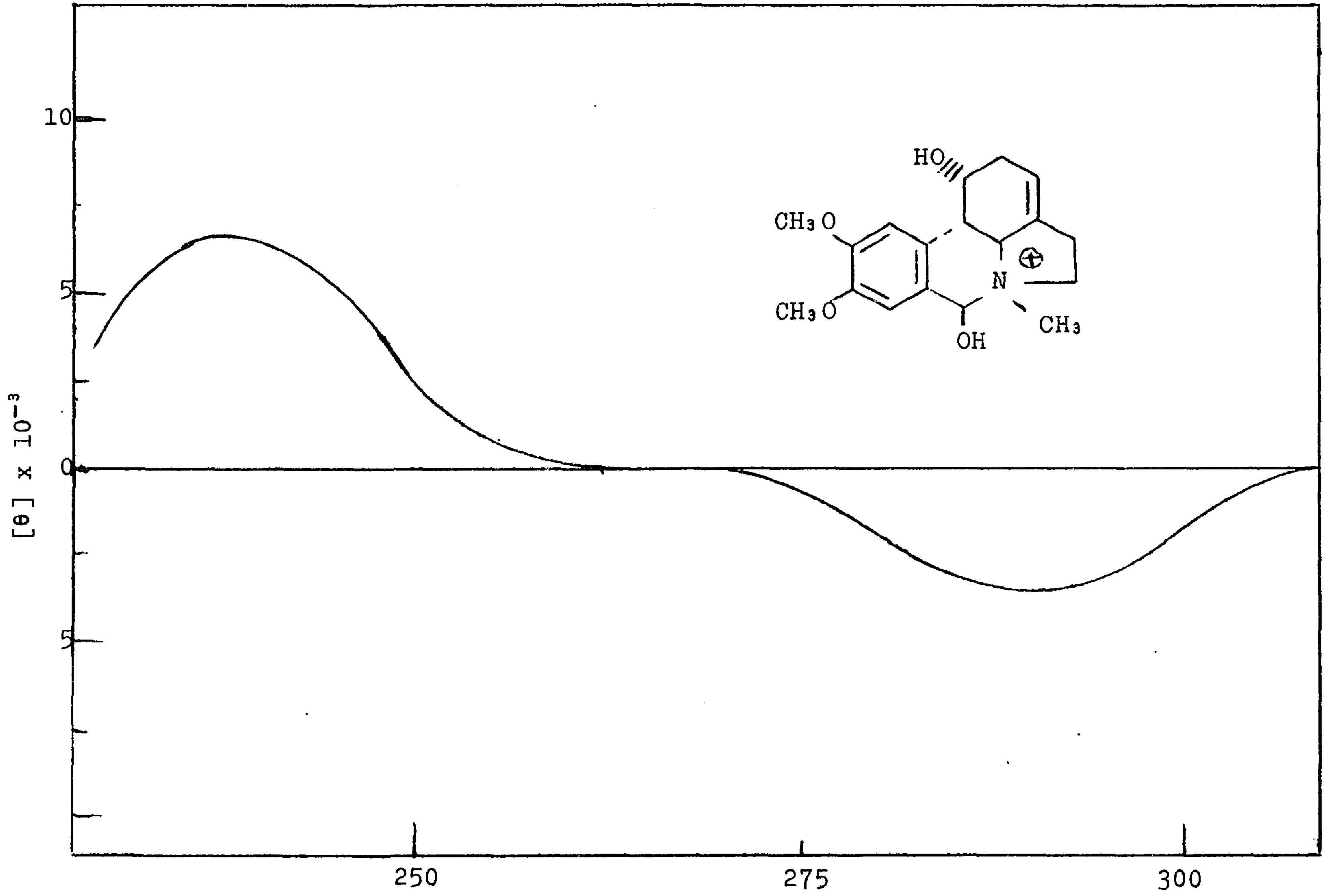

OCH₃
O
O
N
OH
20
OCH₃
N-H
O
O
O
OH
52
OCH₃
NH
O
O
O
OCH₃
OCH₃
N-NO
O
O
O
OH

Figure 53. N-demethylpretazettine-haemanthidine tautomerism

Figure 54. CD spectrum of lycorenine in methanol-water



[θ] x 10⁻³
10
5
0
5
250
275
300
HO
CH₃O
CH₃O
N
⊕
OH
CH₃

Figure 55. CD spectrum of lycorenine in ethanol

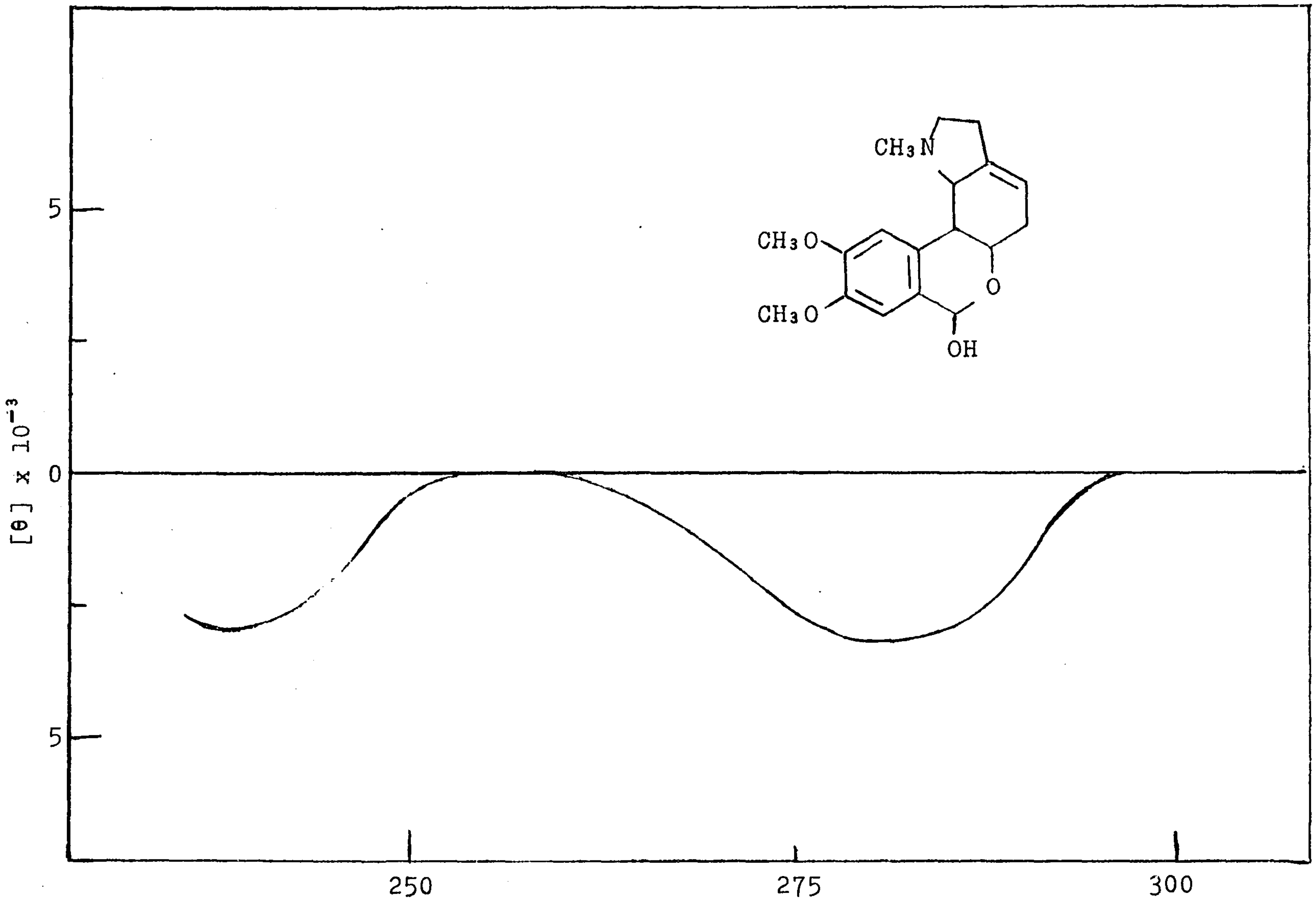
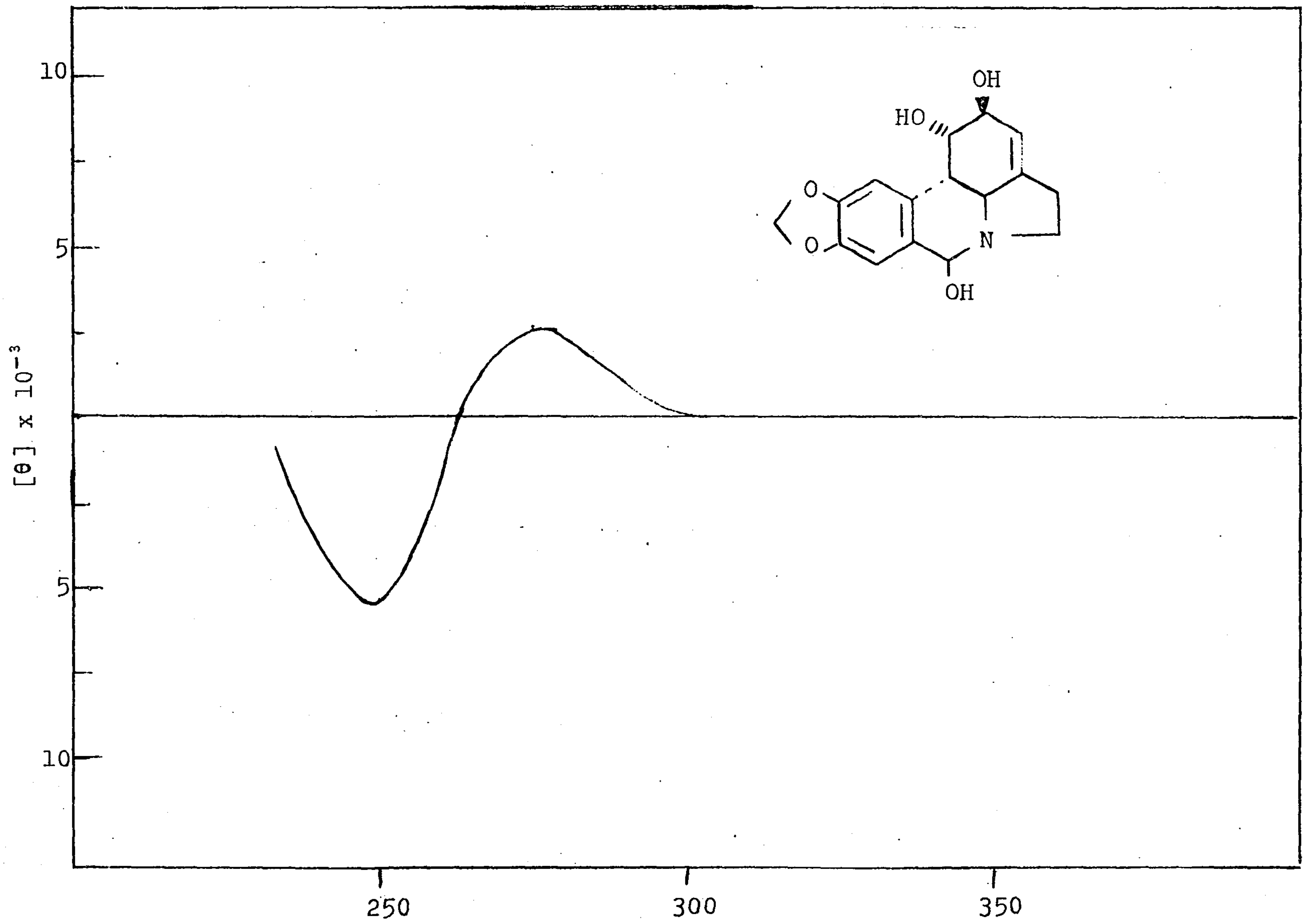

CH₃N
CH₃O
CH₃O
O
OH
[θ] x 10⁻³
5
0
5
250
275
300

Figure 56a. CD spectrum of 7-hydroxylycorine in water


10
5
5
10
[θ] x 10⁻³
250
300
350
OH
HO
O
O
N
OH

The CD spectra of lycorine and lycorenine (acetal form) show opposite signs for the ^1L_a Cotton effects and this offers a method of distinguishing between these ring systems. The CD spectra of lycorenine (Figures 54,55) are similar to those observed by Bailey (21) for pretazettine. In methanol-water (neutral or acidic) the CD curve resembles that of lycorine, but in ethanol the ^1L_a band inverts to give the sign predicted by the sector rule for a lycorenine-type structure. This interconversion from the hemiacetal to the hemiaminal structure in a polar solvent was also observed by Bailey for pretazettine (Figure 56b). The CD spectra of (51) showed a negative ^1L_a Cotton effect which supports a lycorenine (3) type structure, but the ratio of the absorption to rotation was small and made the magnitude of the Cotton effect uncertain (Figure 56a).

The reduction of (51) with lithium aluminum hydride yielded lycorine (8) and this suggests that the molecule exists as 7-hydroxylycorine to some extent.

Thus, the target molecule appears to exist as a mixture of the hemiacetal and the hemiaminal with the hemiacetal predominating.

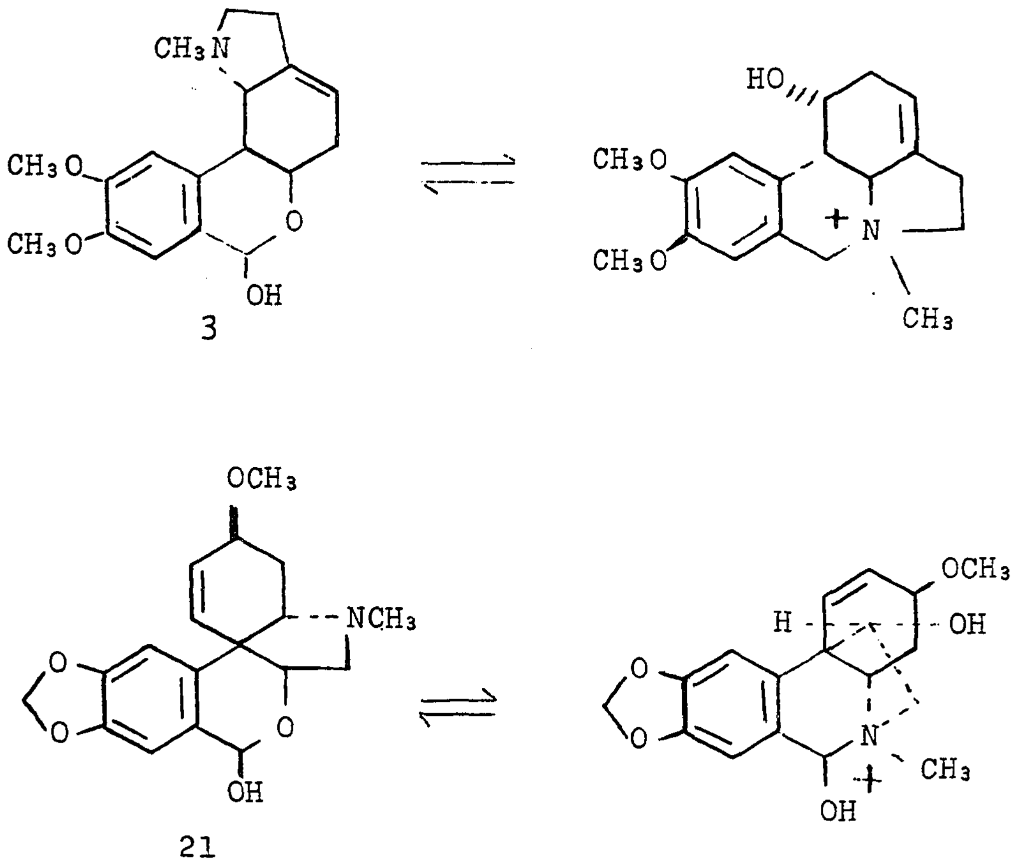
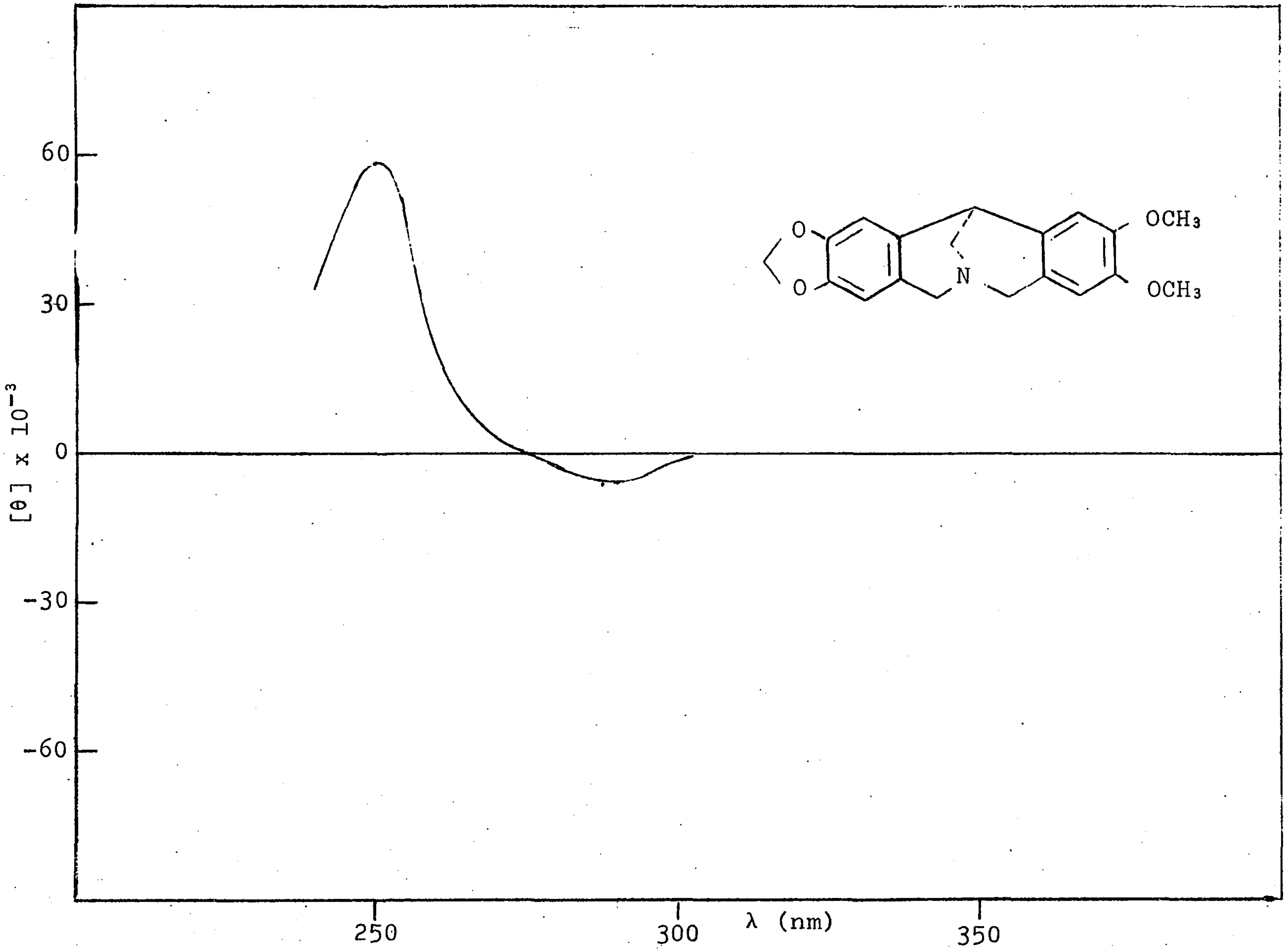

CH₃N
CH₃O
CH₃O
O
OH
3
HO
CH₃O
CH₃O
+ N
CH₃
OCH₃
NCH₃
O
O
O
OH
21
OCH₃
H
OH
O
O
N
+
CH₃
OH

Figure 56b. The equilibrium between N-methylhaemanthidine and pretazettine and lycorenine and 7-hydroxy-N-methylpluviine

Figure 57. CD spectra of azocine in methanol


60
30
0
-30
-60
[θ] x 10^{-3}
250
300
350
λ (nm)
O
O
N
OCH₃
OCH₃

X-ray Structure and CD Spectra of 2,3-Dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[c,f]azocine(17,31)

The X-ray structure of 2,3-dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[c,f]azocine-(17,31) was undertaken to clear up the question raised by Professors Mason (34) and Klyne (35) as to the compatibility of a strong CD effect (Figure 57) and a structure with as much symmetry as that proposed by Fales and Wildman (33) (Figure 58a). They suggested that an alternative structure as shown in Figure 58b might be more in keeping with the large observed rotation. The crystal structure (Figure 59) showed that the correct structure was as originally proposed. In the crystal the two benzene rings were not related by a mirror plane but were found to be twisted by seven degrees. This was a small distortion and would not imply that the molecule is in this conformation in solution. Indeed, the

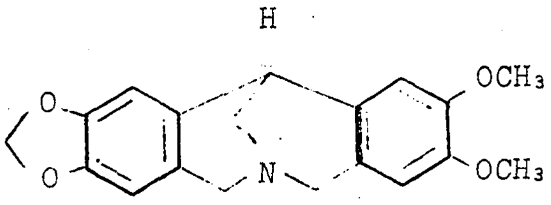


Figure 58a. Azocine

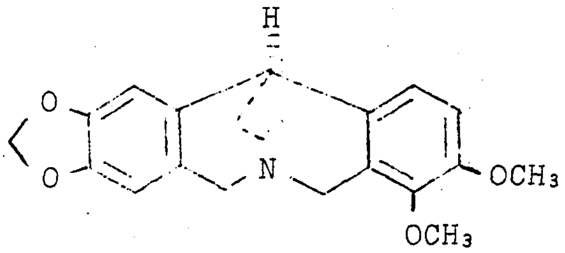
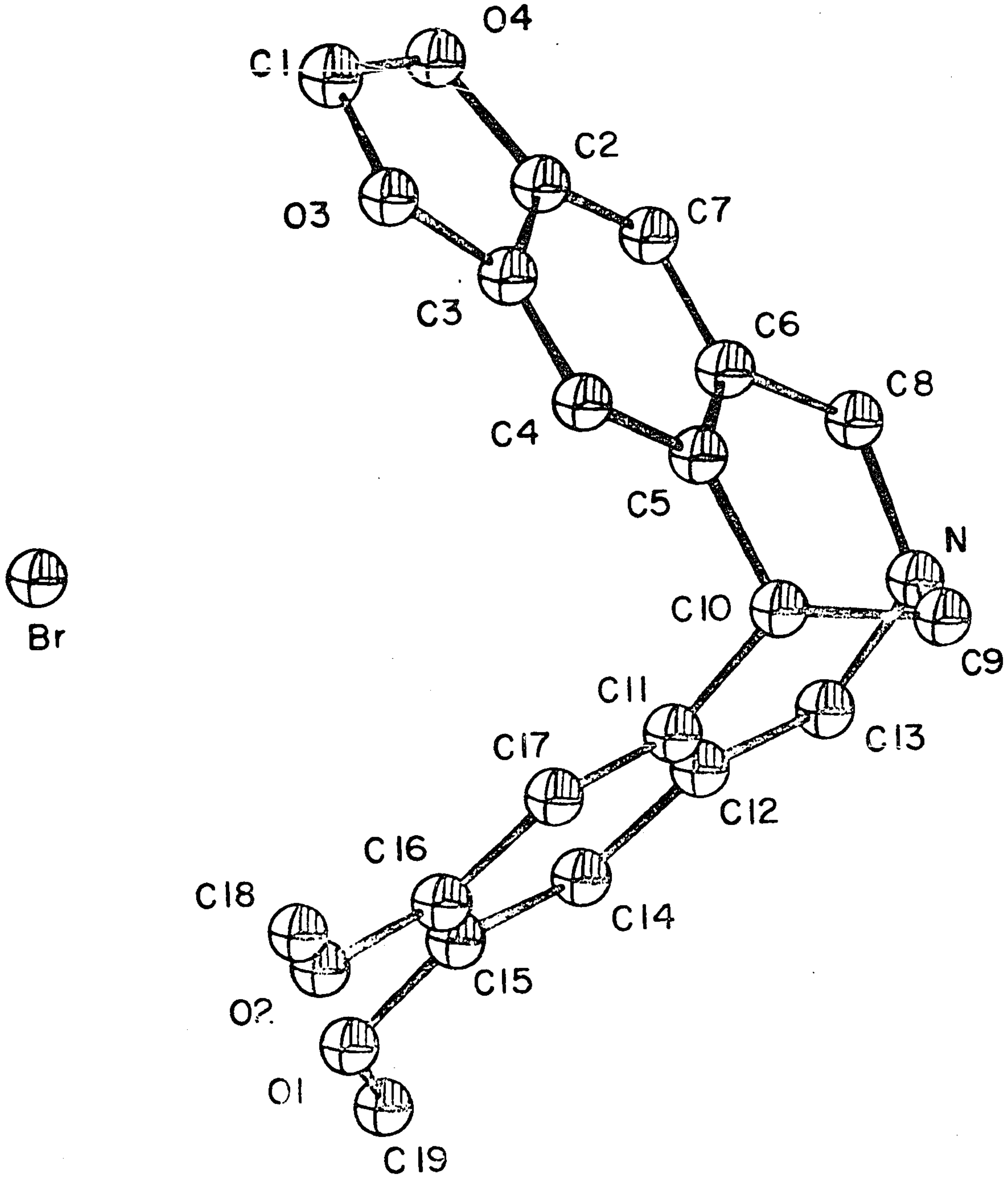


Figure 58b. Azocine alternative structure

Figure 59. 2,3-Dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[c,f]azocine


C1
O4
C2
O3
C7
C3
C6
C8
C4
C5
N
Br
C10
C9
C11
C13
C17
C12
C16
C18
C14
C15
O2
O1
C19

CD spectra is temperature dependent (Figure 57). The increase in the Cotton effect at low temperature would be consistent with a larger population of the more stable conformation at low temperature. The NMR spectra of the molecule was examined as a function of temperature but the freezing out of one conformation was not observed. These results are consistent with two twisted conformations in equilibrium with each other, each of which has a large Cotton effect.

Structure and Optical Activity in the Amaryllidaceae Alkaloids

These recent developments in sector rules, and the reports of the absolute configuration of several Amaryllidaceae alkaloids (50,51,52,53) prompted a reinvestigation of the sector rule for the oxygenated benzene chromophore.

It seemed that DeAngelis and Wildman (37) were correct when they chose the ^1L_a transition to correlate the Cotton effect with absolute configuration. However, the work of Schellman (42,43) suggested that the axis of interest was the local pseudosymmetry axis. This local pseudosymmetry axis corresponded to the X-axis in the system of DeAngelis and Wildman (Figure 60) for galanthamine. Since this was not known when the rule was developed, it was not used and this led to their inconsistencies. The work on the ^1L_b band seemed to offer no solution to the problems raised

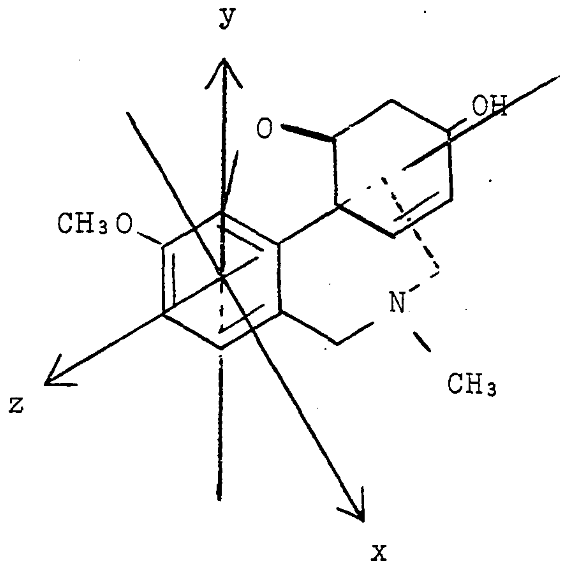


Figure 60. Galanthamine as viewed by DeAngelis and Wildman

by Verbit and Price (46) and by DeAngelis and Wildman concerning the consistency of this band. Indeed, it was suggested by Brewster and Prudence (48) that the direction of the transition moment was changing for this band and that the sector rule should be keyed to the direction of this moment. While this may be true, it suggested that the ^1L_b band was not a reliable source of information about absolute configuration. The solution to this problem appeared to lie in the ^1L_a transition. If it could be shown that the correct sector rule was based on the local pseudosymmetry axis and was octant in form, then the inconsistencies in the rule of

DeAngelis and Wildman would be removed and the new rule would be in agreement with the rule proposed by Verbit and Price.

A rule which satisfies these requirements is shown in Figure 61. The local pseudosymmetry axis was found by considering the benzene ring and the two or three oxygens attached to it. This axis was taken as the Z-axis. The X-axis was chosen to be in the plane of the ring, bisecting the ring, perpendicular to the Z-axis. The Y-axis was then defined to give a right handed Cartesian coordinate system. This coordinant system defined eight sectors bounded by the planes X=0, Y=0, and Z=0 which were used to predict the sign of the Cotton effect for the ^1L_a transition (Figure 61 and Table II). The necessity for an octant rather than a

Table II. Signs of the sectors for the oxygenated aromatic chromophore

Sector number	Sign of coordinants (X,Y,Z)	Sign of the Cotton effect
I	+++	-
II	-++	+
III	--+	-
IV	+-+	+
V	++-	+
VI	-+-	-
VII	---	+
VIII	+--	-

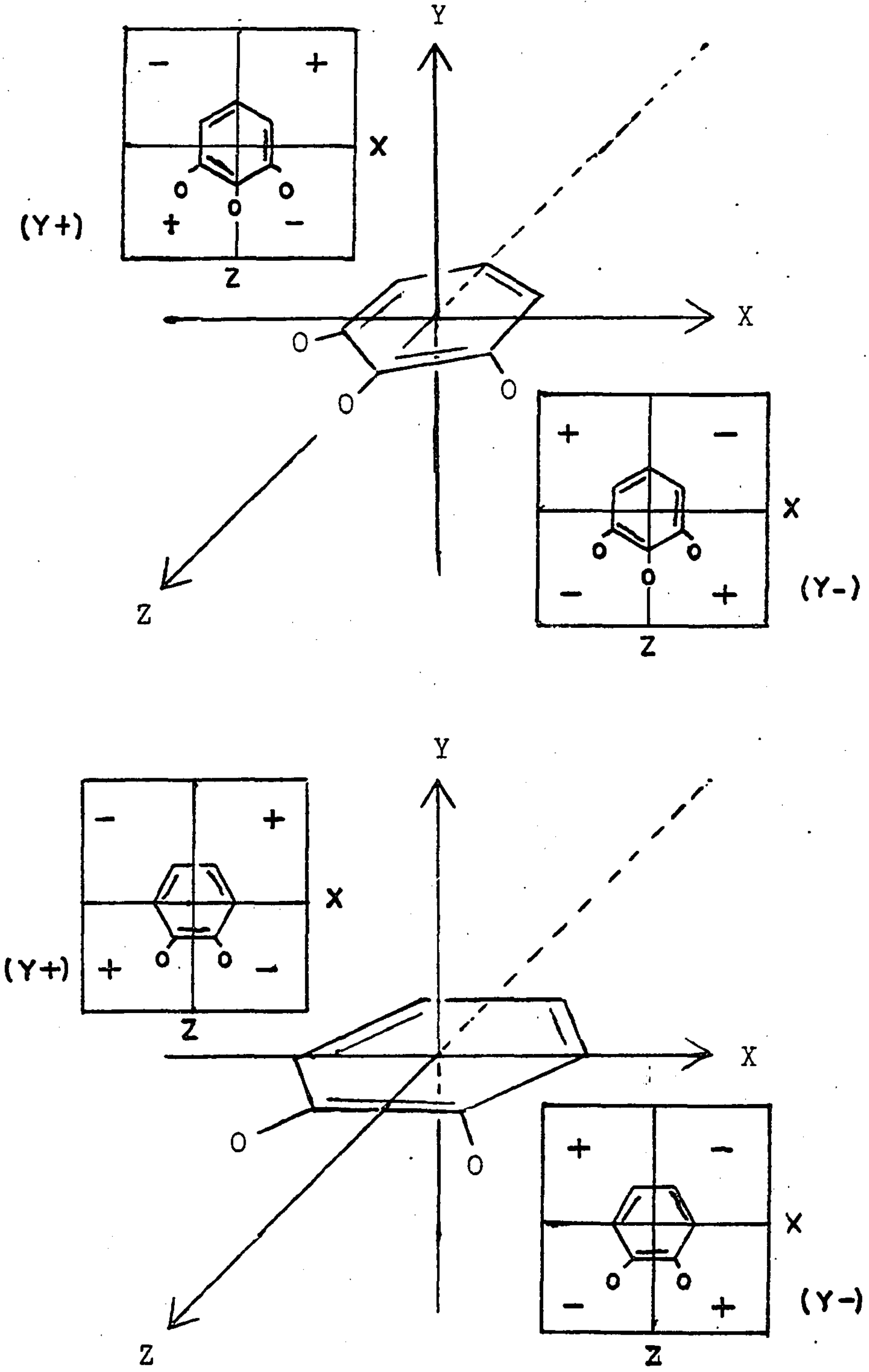

Y
−
+
X
O
O
O
(Y+)
+
−
Z
X
O
O
O
+
−
X
O
O
O
−
+
(Y-)
Z
Z
Y
−
+
X
(Y+)
+
O
O
−
Z
X
O
O
+
−
X
O
O
−
+
(Y-)
Z
Z

Figure 61. Sector rule for the oxygenated aromatic chromophore

quadrant rule was demonstrated by the morphine alkaloids and galanthamine where there were "front" sector (Z+) atoms. A quadrant rule would not have allowed the prediction of the ^1L_a Cotton effect for these molecules.

The application of the rule for galanthamine methiodide is illustrated below using the crystal coordinants which Professor Rogers (54) was kind enough to supply to generate the drawings. In practice a model of the system was made with Dreiding Stereomodels and the model was examined. It is very difficult to draw these projections accurately, therefore, only the computer generated drawings of galanthamine methiodide are illustrated. Figure 62 shows a galanthamine methiodide in perspective illustrating the relationship of the nodal planes to the benzene ring and the rest of the molecule. Figure 63 shows the molecule as viewed down the Z-axis. The view from the negative end of the X-axis (as viewed by DeAngelis and Wildman) is shown in Figure 64. Figures 62, 63 and 64 showed that sectors IV and V were the sectors occupied and thus a positive Cotton effect was predicted consistent with the observed spectra (θ_{238} = +2700).

If the rule was a quadrant rule, the sign of the Cotton effect would be very uncertain since the upper (I) and lower (IV) quadrants are both populated. However, if the rule was octant in form, the predicted sign of the Cotton

Figure 62. Galanthamine methiodide in perspective

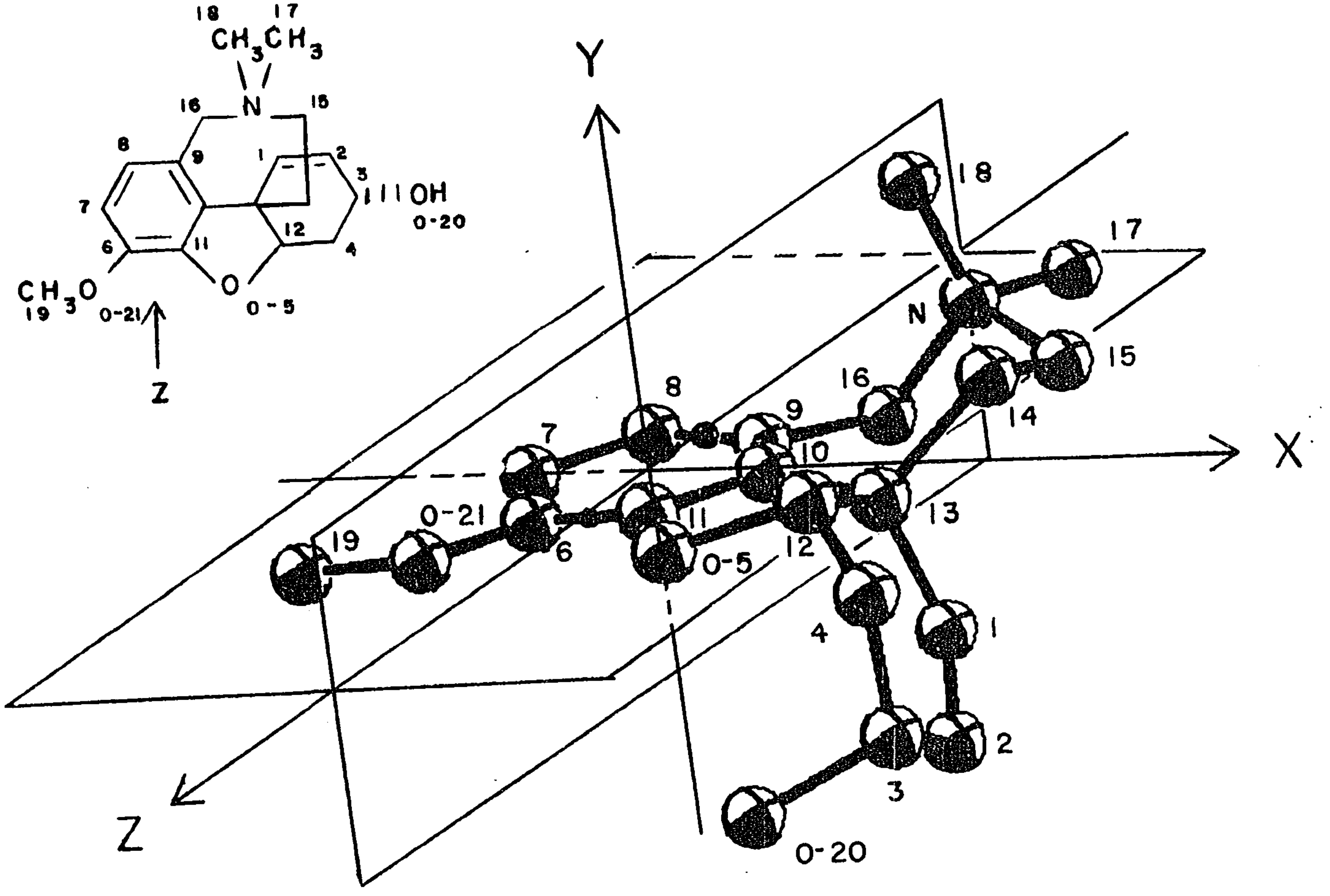
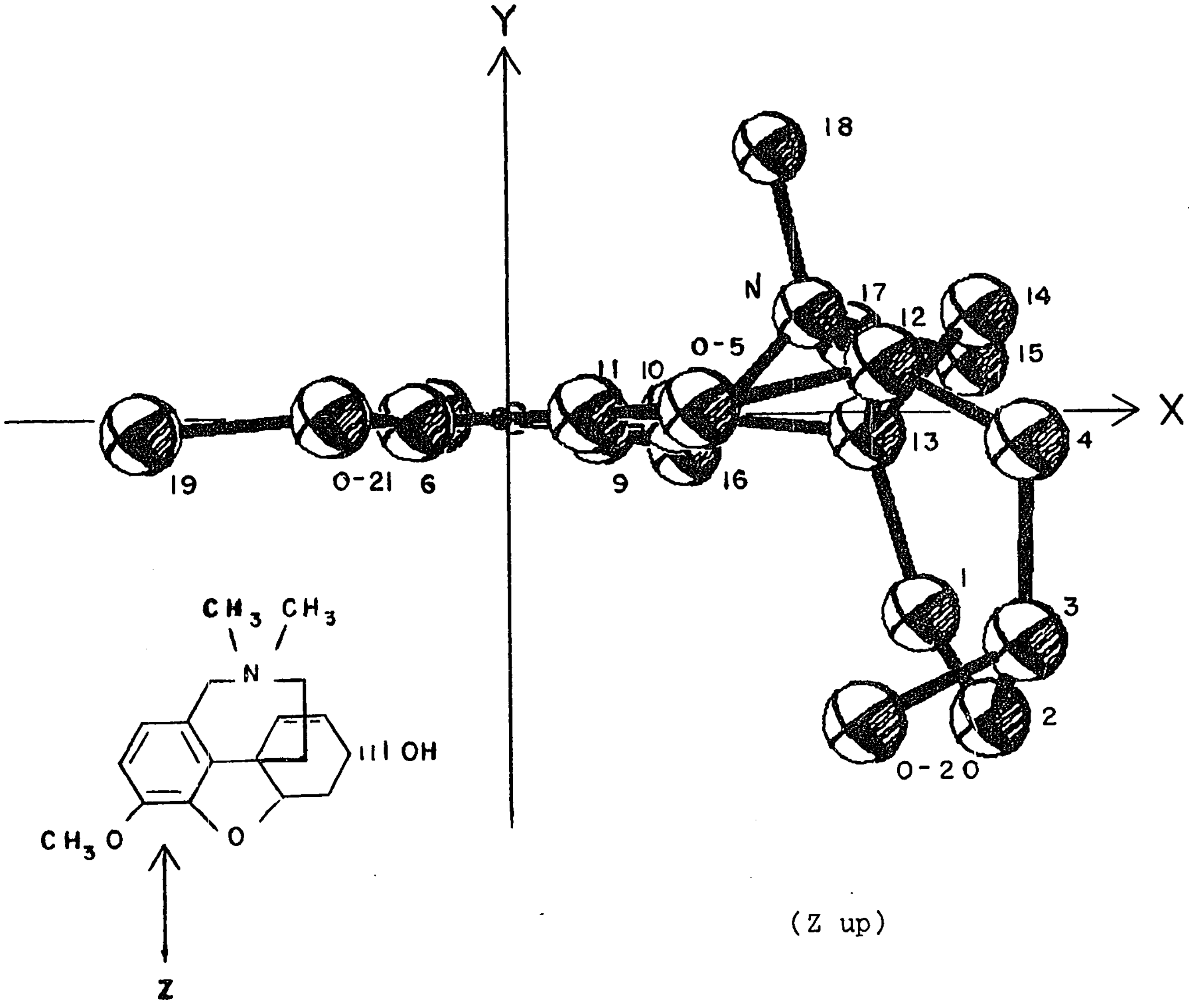
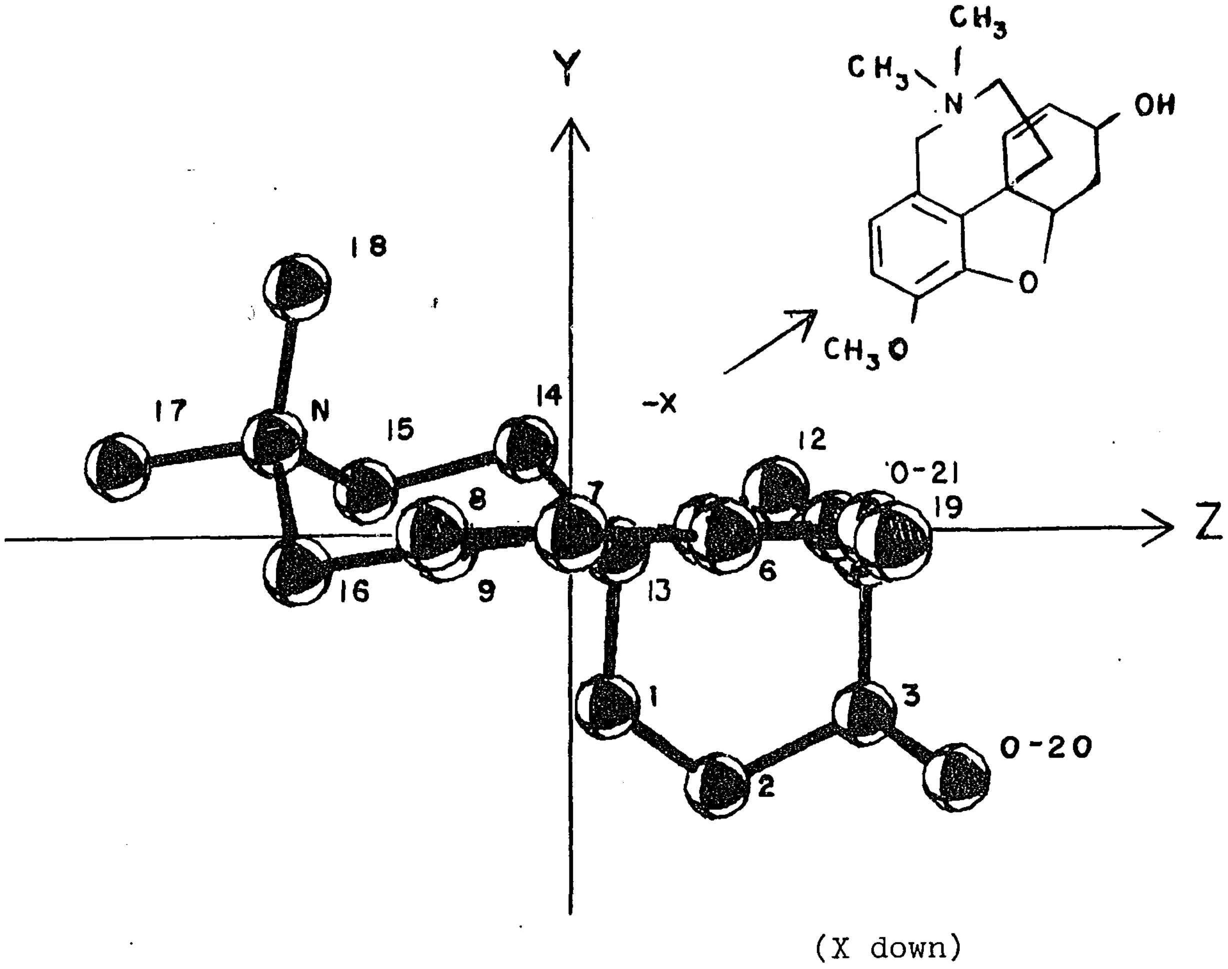

CH3CH3
N
OH
0-20
CH3O
0-21
O
0-5
Z
Y
X
Z
N
0-21
0-5
0-20

Figure 63. Galanthamine methiodide (from Z)


Y
18
N
17
12
14
O-5
11
10
15
X
4
13
19
O-21
6
9
16
1
3
2
O-20
CH₃
CH₃
N
OH
CH₃O
O
Z

(Z up)

Figure 64. Galanthamine methiodide (from -X)


Y
Z
-X
18
17
N
15
14
8
7
9
16
13
6
12
O-21
19
1
2
3
O-20
CH$_3$
CH$_3$
N
OH
O
CH$_3$O
(X down)

effect would be clear and correct, since the atoms were in the rear of quadrant I and the front of quadrant IV. These results were confirmed by the structure and spectra of codeine and several other morphine alkaloids (Table III). The rule was also able to correctly predict the sign of the Cotton effect for a variety of compounds as indicated in Table III.

An exception to the rule was found when extended to an unoxygenated benzene ring. $\Delta^{1,3,5(10)}$-Estratrien-17β-ol (54) as reported by Snatzke and Ho (44) has a positive ^1L_a Cotton effect and a ^1L_b Cotton effect with considerable fine structure whose overall sign is uncertain. From the known absolute configuration, however, a negative Cotton effect was predicted (Figure 65).

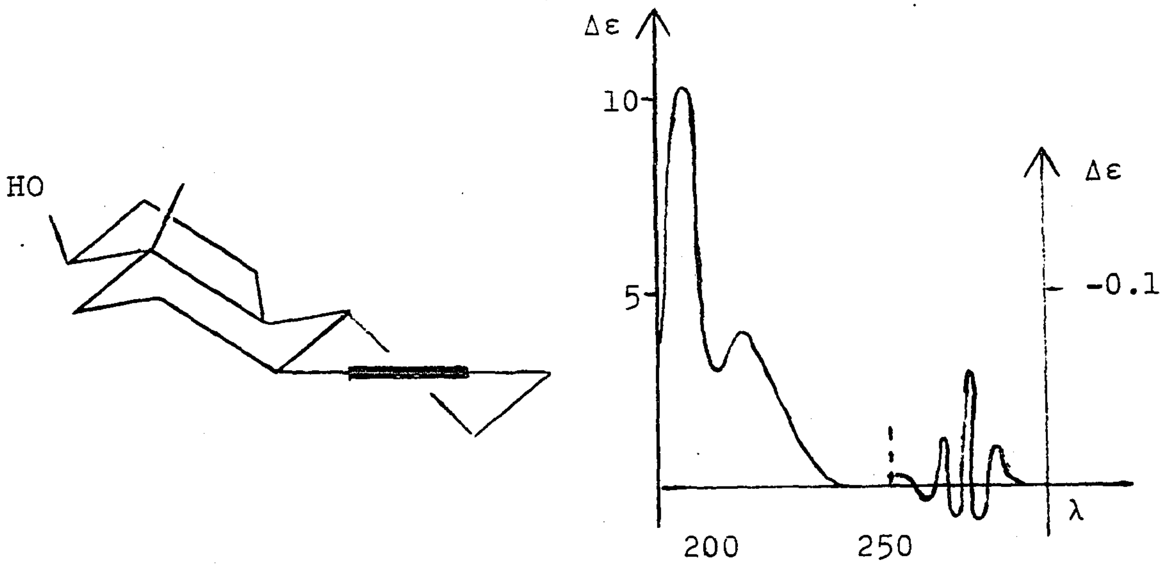


Figure 65. Drawing of $\Delta^{1,3,5(10)}$-estratrien-17β-ol

Table III. CD spectra

	^1L_b		^1L_a	
	Predicted (39)	Observed	Predicted	Observed
Lycorine	-	θ_{290} -6600	+	θ_{245} +4600[a]
Caranine	-	θ_{293} -7190	+	θ_{245} +5250[b]
Deoxydihydro-hippeastrine	+,-	θ_{297} - 3870	-	θ_{243} -2270[b]
Montanine	+	θ_{293} -3000	-	θ_{243} -57500[a]
Coccinine	+	θ_{295} -3300	-	θ_{240} -38000[a]
Galanthamine		θ_{285} +8200	+	θ_{238} +2700[a]
Galanthamine HCl		θ_{275} +7700	+	θ_{241} +4030[a]
Powelline	-	θ_{288} -1620	+	θ_{250} +4200[a]
Dihydropowelline	-	θ_{282} +380		θ_{245} +2840[a]
Powellane	-	θ_{280} +800	+	θ_{247} +1800[a]
Ambelline	-	θ_{279} -1350	+	θ_{245} +1300[a]
Dihydroambelline	-	θ_{285} +440	+	θ_{250} +585[a]
Buphanamine	-	θ_{288} +520	+	θ_{248} +1300[a]
Undulatine	-	θ_{280} -450	+	θ_{245} +500[a]
Dihydro-undulatine	-	θ_{280} +300	+	θ_{248} +1500[a]
Codeine		θ_{282} -8600	+	θ_{243} +41000[a]

[a] DeAngelis thesis (36).

[b] Kuriyama and coworkers (39).

To test the validity of the suggestion by Kuriyama et al. (39) that the nitrogen in deoxydihydrohippeastrine must be in a nodal plane since the CD curve is not greatly effected by conversion of the molecule to the hydrochloride salt, the spectra of several alkaloids whose nitrogen did not appear to be in a nodal plane were studied. The molecules studied and the results given in Table IV suggested that this conclusion was not valid. In none of the cases studied did the spectra change grossly, nor did there appear to be any general trend for the hydrochloride salts to have larger or smaller Cotton effects. These results and the spectra of crinane, dihydrocrinine and dihydroepicrinine supported the conclusion that nitrogen and oxygen, while they are important, do not play a dominant role in determining the magnitude and sign of the Cotton effect. Crinane may be considered the prototype of the crinine series. Crinane has a positive Cotton effect $\theta_{245} = +2010$. The nitrogen atom appears both from Dreiding Stereomodels and the X-ray structures of similar molecules to lie in the nodal plane of the benzene ring and, therefore, would not be expected to contribute to the Cotton effect. This leaves the Cotton effect of about 2000° to be caused by the hydrocarbon skeleton. The effect of an oxygen atom on the Cotton effect was examined by observing the spectra of dihydrocrinine and dihydroepicrinine. In both of these molecules the oxygen

Table IV. Affect of pH on the ^1L_a Cotton effect

Compound	^1L_a Cotton effect neutral/acidic
Lycorenine	
Hemiacetal	1.3
Hemiaminal	.83
Galanthamine	.8
Codeine	1.2
Tazettine	1.2

atom was in Sector VII (+ Cotton effect) and for both of these molecules the Cotton effect was larger than crinane indicating that oxygen was contributing to the Cotton effect in the normal manner.

These results were in accord with the report by Cook and Djerassi (56) in their study of ring heteroatoms and the octant rule for cyclohexanone. They also found that nitrogen and oxygen contributed to the Cotton effect as predicted by the normal octant rule for cyclohexanone.

In conclusion, the role of nitrogen and oxygen, while important, does not seem to be dominant and these heteroatoms appear to contribute to the Cotton effect in the normal manner.

The DeAngelis and Wildman rule for the oxygenated benzene chromophore as modified here appears to work well for dioxygenated and trioxygenated benzene rings and is in agreement with the rule proposed by Verbit and Price. However, caution should be exercised in applying the rule to new systems as was illustrated in the estratriene case. With these limitations the rule appears to be satisfactory and useful.

EXPERIMENTAL

Methods and Procedures

Instrumentation

The proton magnetic resonance spectra were obtained on a Varian A-60 or HA-100 in chloroform-d_1 unless another solvent was indicated. The infrared spectra were run on a Beckman Model IR-12 or IR18A in chloroform solution or as a potassium bromide pellet. The mass spectra were recorded on an MS-902 (high resolution) mass spectrometer. The CD spectra were observed in the indicated solvent on the Jasco Model 5 ORD spectrophotometer. Melting points were observed on a Köfler hot stage and are uncorrected. The measurements of radioactivity were made on a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3002) at ambient temperature. Toluene POPOP and internal standards as described by Virnig (24) were used.

Preparation of methylpseudolycorine

One gram of lycorine was added to a solution of 2 ml of boron tribromide in 25 ml of methylene chloride at -80°. After two hours, the solution was allowed to warm to ambient temperature and the reaction was stirred overnight. The solution was evaporated to dryness under reduced pressure and the residue was dissolved in methanol and treated twice with 10 millimoles of diazomethane to yield methylpseudolycorine.

The crude product was dissolved in chloroform and extracted with 10 percent sodium hydroxide solution. After removing the solvent, the methylpseudolycorine was recrystallized from ethanol, yield 300 mg (28%): mp 233-235°. The identity of the product was confirmed by comparing its spectra with those obtained from natural material.

Preparation of [9-methoxy-^{14}C]pluviine

Diazomethane (0.6 mmoles) was generated from N-methyl-^{14}C-N-nitroso-p-toluenesulfonamide (1.0 mmole) by adding an ethereal solution of the precursor into a solution of ethanolic potassium hydroxide at 60°. The labeled diazomethane was distilled into a cold trap at 0° containing 100 mg of norpluviine in methanol. The reaction was stirred overnight and evaporated to dryness. The residue was dissolved in chloroform and extracted with one percent sodium hydroxide and the resulting chloroform solution was dried and evaporated to dryness. The labeled pluviine was recrystallized from benzene-hexane, yield 18 mg (17%): mp 225-226°.

Methylpseudolycorine α-epoxide (40)

The synthesis of (40) was patterned after the approach of Wildman and Heimer (13) to lycorine α-epoxide. To a mixture of 200 mg of sodium chloride and 150 mg of methylpseudolycorine, 1 ml of phosphorus oxychloride was added. The slurry was heated to 35° with stirring; after five minutes two drops of 6 N hydrochloric acid was added, and the reaction was stirred for another thirty minutes. The reaction mixture was hydrolyzed in ice water and neutralized with sodium hydroxide. The aqueous solution was extracted with ether and evaporated to yield methylpseudolycorine α-epoxide (50 mg).

M.S. calculated 284.1287 observed 284.1282

NMR Figure 34 IR Figure 35

Anhydromethylpseudolycorine (41)

Galanthine (100 mg) was dissolved in 6 N hydrochloric acid and refluxed for four hours. The reaction mixture was neutralized and extracted with chloroform to yield anhydromethylpseudolycorine which was recrystallized from ethanol, yield 75 mg: mp 170-173°, recrystallized 176° dec above 230°.

M.S. calculated P-1 266.1181 observed 266.1178

NMR Figure 36 IR Figure 37

[2β-^{2}H]Pluviine (4)

The methylpseudolycorine α-epoxide (50 mg) was dissolved in 10 ml of dry ether and added to 25 mg of lithium aluminum hydride ^{2}H in dry ether. The reaction mixture was refluxed for one hour and the excess lithium aluminum hydride was hydrolyzed with wet ether. Saturated sodium potassium tartrate was then added and the [2β-^{2}H]pluviine was extracted with chloroform and purified by TLC (R_f = 0.6; 6/2/2: chloroform/acetone/methanol), 25 mg (50%); mp 222-225°.

M.S. calculated 288.1584 observed 288.1594

NMR Figure 39 IR Figure 40

[2β-^{2}H]Acetylpluviine

[2β-^{2}H]Pluviine was dissolved in 1 ml of 1:1 acetic anhydride-pyridine. After 24 hours the solution was evaporated to dryness under reduced pressure and the [2β-^{2}H]-acetylpluviine was sublimed at 160°, 0.001 Torr.

M.S. calculated 330.1667 observed 330.1668

IR Figure 42

Pyrolysis of [2β-^{2}H]acetylpluviine

The sample was sealed in a Pyrex tube at 0.001 Torr and heated in a 240° oven for 45 minutes. The volatile fraction was shown by MS to contain acetic acid and the nonvolatile fraction contained [2-^{2}H]anhydromethylpseudolycorine. Experiments on unlabeled material produced anhydromethylpseudolycorine which compared favorably with that produced by the dehydration of galanthine.

The feeding of the doubly-labeled pluviine

The labeled pluviine (27 mci/mg-^{3}H, 58 mci/mg-^{14}C, 9 mg) was dissolved in dilute hydrochloric acid and injected into the flower stalks of nine blooming *Narcissus* "King Alfred" plants and the plants were harvested after two weeks. The bulbs and the flower stalks (514 g) were processed in the manner described by Harken (12). The pH 8 and 10 chloroform extracts were combined and chromatographed on silica gel in 60/20/20 chloroform/acetone/methanol to yield galanthine (29 mg). The identity of the galanthine was confirmed by comparison with an authentic sample.

O,O-Diacetyllycorine (**45**)

A solution containing 5 ml of acetic anhydride and 5 ml of pyridine was added to 1 g of lycorine. The mixture was stirred for two days and the excess pyridine and acetic anhydride was removed *in vacuo*. The residue was recrystallized from ethanol. Yield 1.1 g, mp 220-221° (reported: 219-221°).

Addition of cyanogen bromide to O,O-diacetyllycorine

A mixture of 1 g of O,O-diacetyllycorine and 1 g of cyanogen bromide was dissolved in 50 ml of chloroform and after adding 500 mg of potassium carbonate, the solution was refluxed for 6 hours. The potassium bromide was removed by filtration and the mixture was evaporated to dryness. The product was a mixture of two isomers (46, 47) which were not separated but were used for the next reaction, yield: 1.2 g, mp 186-187°.

M.S. calculated 476.0583 observed 476.0602

The infrared spectrum showed a N-CN bond at 2210 cm^{-1}; acetate carbonyl (1750 cm^{-1}); methylenedioxy (1510, 1490 cm^{-1}); and C-O-C (1250 and 1050 cm^{-1}).

The NMR spectrum showed aromatic protons at 7.7, 7.8 δ; methylenedioxy protons at 6.0 δ; a vinyl proton at 5.8 δ; the protons adjacent to the acetate at 5.2 δ; the benzyl bromide protons as a quartet at 4.5 δ; two triplets at 3.5 and 2.8 δ; and the acetate protons at 2.1 δ.

DMSO oxidation of 47 to the aldehyde (48)

To 5 ml of DMSO and 500 mg of sodium bicarbonate, 500 mg of the cyanogen bromide adduct of O,O-diacetyllycorine was added and the reaction was heated at 120° for one hour. The DMSO was removed under reduced pressure and the residue was dissolved in a mixture of chloroform and water. The chloroform layer was removed, dried and chromatographed on a silica gel column. The undesired isomer from the starting material was eluted first in chloroform:benzene (3:1) and the desired aldehyde (48) was eluted in chloroform, yield 420 mg (80%), mp 186-187°.

M.S. calculated 412.1271 observed 412.1266

IR Figure 46a NMR Figure 46b

Conversion of the aldehyde (48) to the acetal (49)

To a solution of 200 mg of the aldehyde (48) in 1 ml of acidic methanol, 3 ml of methyl orthoformate was added and the solution was refluxed for 3 hours. The excess methyl orthoformate and methanol were removed under vacuum and the residue was recrystallized from acetone, yield 220 mg (95%); mp 81-83°.

M.S. calculated 458.1688 observed 458.1710

NMR Figure 48a IR Figure 48b

Reduction of the acetal (49)

A solution of the acetal (100 mg) in dry tetrahydrofuran was added to lithium aluminum hydride (200 mg) and refluxed for three hours. The excess lithium aluminum hydride was destroyed with saturated sodium potassium tartrate and the organic layer was separated. The aqueous layer was extracted twice with tetrahydrofuran and the organic layers were combined, dried and the solvent was removed under reduced pressure to yield the 50 mg of the acetal (50). This compound was not crystalline and the high resolution mass spectra was unobtainable. The infrared spectrum showed the loss of the acetate carbonyl and the N-cyano band. The NMR showed the aromatic protons at 7.3 and 7.1 δ; the methylenedioxy protons at 5.95 δ. The vinyl proton at 5.6 δ; the acetal proton at 5.5 δ; and the methoxy protons at 3.3 δ.

Hydrolysis of the acetal (50)

A sample (25 mg) of the acetal (50) was dissolved in tetrahydrofuran and water (1:1) and a drop of 6 *N* hydrochloric acid was added. The solution was warmed on a steam bath and allowed to stand overnight. On evaporation at room temperature the aldehyde (51) appeared as a crystalline solid. The NMR spectrum [acetonitrile/D_2O (1/1)] showed aromatic protons at 7.2 and 7.4 δ; the methylenedioxy protons at 6.2 δ; the hemiacetal protons at 6.0 δ; and the vinyl proton at 5.8 δ, yield 10 mg; mp 205-210 dec.

The aldehyde (51) (10 mg) was reduced with 50 mg of lithium aluminum hydride in tetrahydrofuran for one hour. The excess lithium aluminum hydride was destroyed with saturated sodium potassium tartrate and the product was extracted with ethyl acetate. The ethyl acetate was removed under reduced pressure and the product was identified as lycorine (8) by comparison of the mass spectra and the infrared spectra with known spectra.

SUMMARY

The stereochemistry of the C-2 hydroxylation of pluviine in the biosynthesis of galanthine was shown to proceed with inversion of configuration. This is in agreement with the previous work of Wildman and Heimer and of Bruce and Kirby and contradicts that of Fuganti and Mazza.

The ^1L_a Cotton effect was shown to correctly predict the absolute configuration of Amaryllidaceae and morphine-type alkaloids using an octant rule developed in this thesis. The structure of 2,3-dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[*c*,*f*]azocine(17,**31**) was shown to have been correctly assigned and its large Cotton effect was suggested to be due to a twisting of the molecule in which the two aromatic chromophores perturb each other.

Finally, a partial synthetic route to 7-hydroxylycorine was explored and evidence for both a hemiacetal and a hemiaminal structure was presented.

LITERATURE CITED

1. W. C. Wildman, 'Alkaloids of the Amaryllidaceae', in "The Alkaloids", R. H. F. Mamske, Ed., Academic Press, Inc., New York, N.Y., 1968, Vol. 11, pp. 307-405.

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

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

4. A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, J. Chem. Soc., 1595 (1965).

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

6. J. A. Miller, Biosynthetic Studies on Tazettine and Ambelline, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1966.

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

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

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

10. D. W. Jeffs, Proc. Chem. Soc., 80 (1962).

11. D. H. R. Barton and T. Cohen, 'Some Aspects of Phenol Oxidation', in "Festschrift: Arthur Stoll", Birkhauser, Basel, Switzerland, 1957, pp. 117-143.

12. R. D. Harken, The Biosynthesis of Lycorine, Narciclasine, and Dimethoxy Analogues of Lycorine, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1970.

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

14. H. G. Boit and H. Ehmke, Berichte, 89, 2093 (1956).

15. T. Kitigawa, S. Uyeo, and N. Yokoyama, J. Chem. Soc., 3741 (1959).

16. S. Mizukami, Tetrahedron, 11, 89 (1960).

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

18. F. Sandberg and K. Michel, Lloydia, 26, 79 (1963).

19. A. I. Feinstein, The Incorporation of ^{14}C-Labeled β-Phenylethylamine Derivatives and ^{3}H-Vitattine into Amaryllidaceae Alkaloids, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1967.

20. C. L. Brown, The Mass Spectra of Montanine- and Galanthamine-Type Alkaloids; The Structures of Pancracine and Habranthine, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1968.

21. D. T. Bailey, The Chemistry of Pretazettine and Related Compounds, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1968.

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

23. C. Fuganti and M. Mazza, Perkins Trans. I, 954 (1973).

24. M. J. Virnig, Benzylic Hydroxylations of the Amaryllidaceae Alkaloids, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1974.

25. H. R. Battersby, J. E. Kelsey, and J. Staunton, Chem. Commun., 183 (1971).

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

27. G. W. Kirby and J. Michael, J. Chem. Soc., Perkins Trans. I, 115 (1973).

28. C. Fuganti, D. Ghiringhelli, and P. Grasselli, Chem. Commun., 430 (1973).

29. N. E. Heimer, The Mechanism of the Biosynthetic Oxidation of Caranine and Derivatives, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1968.

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

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

32. C. Fuganti and M. Mazza, Chem. Commun., 936 (1972).

33. H. M. Fales and W. C. Wildman, J. Org. Chem., 26, 1617 (1961).

34. S. F. Mason, University of East Anglia, Norwich, England, personal communication, 1967.

35. W. Klyne, University of London, personal communication, 1967.

36. G. G. DeAngelis, Dichroism and Dispersion Studies of the Optically Active Aromatic Chromophore, Unpublished Ph.D. Thesis, Library, Iowa State University of Science and Technology, Ames, Iowa, 1966.

37. G. G. DeAngelis and W. C. Wildman, Tetrahedron, 23, 3449 (1967).

38. W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, J. Amer. Chem. Soc., 83, 4013 (1961).

39. K. Kuriyama, T. Iwata, M. Moriyama, K. Kotera, Y. Hameda, R. Mitsui and K. Takeda, J. Chem. Soc. (B), 46 (1967).

40. K. Kotera, Y. Hameda, K. Tori, K. Aono, and K. Kuiryama, Tetrahedron Letters, 2009 (1966).

41. M. Shiro, J. Sato, and H. Koyama, Chem. and Ind., 1229 (1966).

42. J. A. Schellman, J. Chem. Phys., 44, 55 (1966).

43. J. A. Schellman, Acc. Chem. Res., 1, 144 (1968).

44. G. Snatzke and P. C. Ho, Tetrahedron, 27, 3645 (1971).

45. G. Snatzke, M. Kajtar and F. Werner-Zamojska, Tetrahedron, 28, 281 (1972).

46. L. Verbit and H. C. Price, J. Amer. Chem. Soc., 94, 5143, (1972).

47. H. E. Smith and T. C. Willis, J. Amer. Chem. Soc., 93, 2282 (1971).

48. J. H. Brewster and R. T. Prudence, J. Amer. Chem. Soc., 94, 1217 (1973).

49. J. F. W. McOmie and M. L. Watts, Chem. and Ind., 1658 (1963).

50. J. C. Clardy, W. C. Wildman, and F. M. Hauser J. Amer. Chem. Soc., 92, 1781 (1970).

51. J. C. Clardy, F. M. Hauser, D. Dahm, R. A. Jacobson, and W. C. Wildman, J. Amer. Chem. Soc., 92, 6337 (1970).

52. J. C. Clardy, J. A. Chan, and W. C. Wildman, J. Org. Chem., 37, 49 (1972).

53. T. Sato and H. Koyama, J. Chem. Soc. (B), 1070 (1971).

54. D. Rogers, Imperial College, London, personal communication, 1973.

55. D. Rogers and J. D. Williams, Proc. Chem. Soc., 95, 3678 (1964).

56. M. Cook and C. Djerassi, J. Amer. Chem. Soc., 95, 3678 (1973).

ACKNOWLEDGEMENTS

The author wishes to thank Dr. W. C. Wildman for suggesting this problem and for his guidance and encouragement during this investigation.

The author also wishes to thank Dr. J. Applequist and Dr. J. Clardy for their help on parts of this problem.

The author thanks the members of the research group for their suggestions and help during this investigation.

Deepest gratitude is extended to my wife for her patience and support.

The author wishes to thank Sue Musselman for her meticulous typing of this thesis.

This research was partially supported by Grant HE 7503 from the National Institute of Health.

APPENDIX

Crystal Structure of 2,3-Dimethoxy-6,12-methano-9,10-methylenedioxy-5,6,12,13-tetrahydro[7H]dibenz[c,f]azocine

done with Dr. J. Clardy and Dr. S. Porter

The crystal structure of azocine was done on the hydrobromide salt. This structure confirmed the structure reported earlier (17) (R=.080). The absolute configuration was also as originally proposed. The R factor of the alternate enantiomorph was 0.110 which gave a confidence level of .995. The bond distances are given in Table V.

Table V. Azacene bond distances

Atom	Number	Bond distances
C1	O4	1.46(2)
C1	O3	1.44(2)
C2	O4	1.36(1)
C3	O3	1.32(1)
C3	C2	1.43(2)
C2	C7	1.34(2)
C7	C6	1.39(2)
C6	C5	1.37(1)
C5	C4	1.37(2)
C4	C3	1.37(2)
C5	C10	1.53(1)
C10	C9	1.51(1)
C9	N	1.53(1)

Table V. Continued

Atom	Number	Bond distances
N	C8	1.48(1)
C8	C6	1.50(2)
N	C13	1.47(1)
C13	C12	1.45(2)
C10	C11	1.45(1)
C11	C12	1.37(2)
C12	C14	1.40(1)
C14	C15	1.33(2)
C15	C16	1.33(1)
C16	C17	1.36(1)
C17	C11	1.38(1)
C16	O2	1.38(1)
O2	C18	1.44(2)
C15	O1	1.37(1)
O1	C19	1.47(2)

The space group is $P2_1$. Of the 1502 reflections measured, 1415 were judged observed after background and LP corrections.