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Synthetic and biosynthetic approaches to cherylline and related compounds

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Synthetic and biosynthetic approaches

to cherylline and related compounds

by

Richard Duane Shaffer

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INTRODUCTION

Improved techniques of natural product isolation and separation have caused reexamination of numerous plants of the Amaryllidaceae family. In this investigation, application of new methodology resulted in the discovery of several unique, structurally related phenolic alkaloids in numerous Crinum species of the Amaryllidaceae. Initial investigations suggested that these compounds were intermediate in complexity between previously identified alkaloids and their known precursors, simple aralkylamines and amino acids. Questions concerning the structures of these phenols and their importance in biosynthetic schemes stimulated further investigation. Experimental effort was directed into both areas of interest. This dissertation is concerned with the results and implications of those investigations. It further seeks to define the importance of this particular group of compounds in alkaloid biogenesis in the Amaryllidaceae and to examine the process of their formation in the plant.

HISTORICAL

Isolation

Phenols by classical methods

Application of the term 'classical methods' to scientific investigation in any field can be misleading, since the dynamic process of technological improvement makes it difficult to assign distinct temporal boundaries to methodology. Alkaloid isolation is no exception, as literature reports of phenolic compounds isolated from the Amaryllidaceae essentially span the duration of serious investigation of alkaloids from members of this plant family. An arbitrary distinction is convenient which designates as classical those separative methods available prior to the general introduction of preparative scale thin layer chromatography. The bulk of isolative investigation of the Amaryllidaceae was conducted prior to that time, and 1965 is taken as an approximate terminus.

Classical separative procedures consisted primarily of extractive partitioning, fractional crystallization, and column chromatography. Characterization of newly isolated compounds was likewise limited by the scarcity of investigative procedures initially available to investigators. Phenolic alkaloids, which distinguished themselves by their amphoteric solubility behavior, could be confirmed by classical color reactions. Zinc dust distillation and other standard degradative processes might produce information about skeletal

Table 1. Phenolic compounds isolated from the Amaryllidaceae

Phenolic Compound	Structure	Separation Procedures	Year Reported	Plant Source	Reference
Pseudolycorine	I	Unavailable	1932	Lycoris radiata	1
Crinin	II	Phenolic extraction Fractional crystallization (Acetone)	1954	Crinum moorei	2
Chlidanthine	III	Phenolic extraction Fractional crystallization (Chloroform)	1956	Chlidanthus fragrans	3
Norpluviine	IV	Phenolic extraction Fractional crystallization (Methanol)	1959	Lycoris radiata	4
Demethyl-homolycorine	V	Phenolic extraction Column chromatography (Alumina) Fractional crystallization (Ethyl acetate)	1959	Lycoris radiata	4
Goleptine	VI	Phenolic extraction Fractional crystallization (Acetone)	1963	Narcissus jonquilla	5
Golceptine	VII	Phenolic extraction Fractional crystallization (Acetone)	1963	Narcissus jonquilla	6
4-O,N-Dimethyl-norbelladine	VIIIc	Dilution Column chromatography (Alumina)	1963	Narcissus pseudonarcissus "King Alfred"	7

3

Table 1. Continued

Phenolic Compound	Structure	Separation Procedures	Year Reported	Plant Source	Reference
4-O-Methyl-norbelladine	VIIIb	Phenolic extraction Dilution	1964	Narcissus pseudonarcissus "Twink"	8
Amaryllisine	IX	Column chromatography (Florisil)	1964	Amaryllis belladonna	9
Narciclasine	X	Butanol extraction Column chromatography (Silica gel)	1968	Narcissus incomparabilis	10
Narciprimine	XI	Butanol extraction Column chromatography (Silica gel)	1968	Narcissus incomparabilis	10
Lycoricidinol	X	Ethyl acetate extraction Column chromatography (Silica gel)	1968	Lycoris radiata	11
Arolycoricidinol	XI	Ethyl acetate extraction Column chromatography (Silica gel)	1968	Lycoris radiata	11
Cherylline	II	Preparative thin layer chromatography	1970	Crinum powellii	12

4
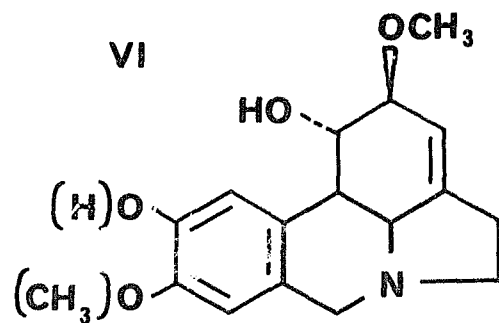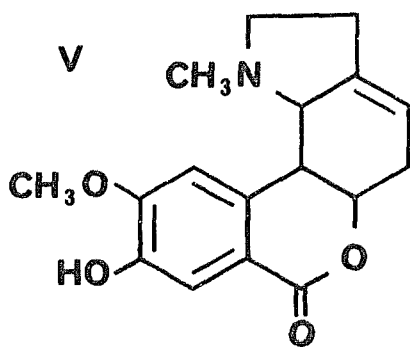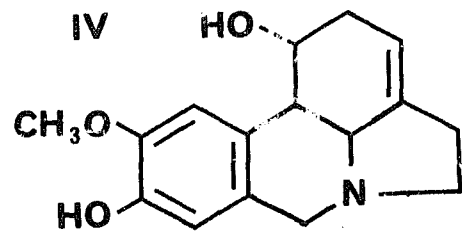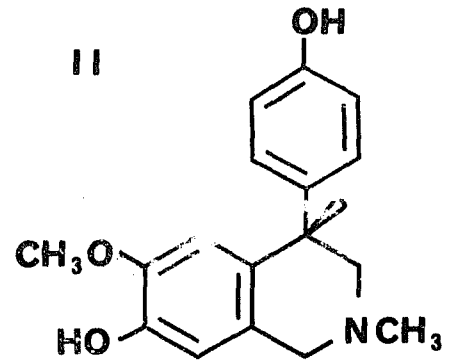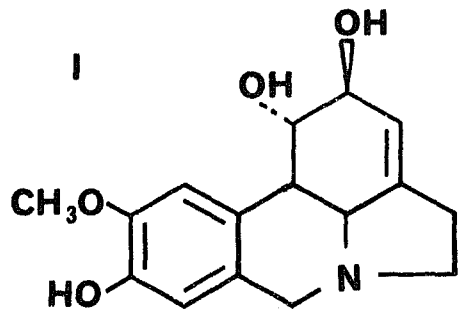
Figure 1. Early phenols of the Amaryllidaceae

VII

VIII

a) R_1, R_2 = H

IX

b) R_1 = CH_3, R_2 = H

c) R_1, R_2 = CH_3

X

XI
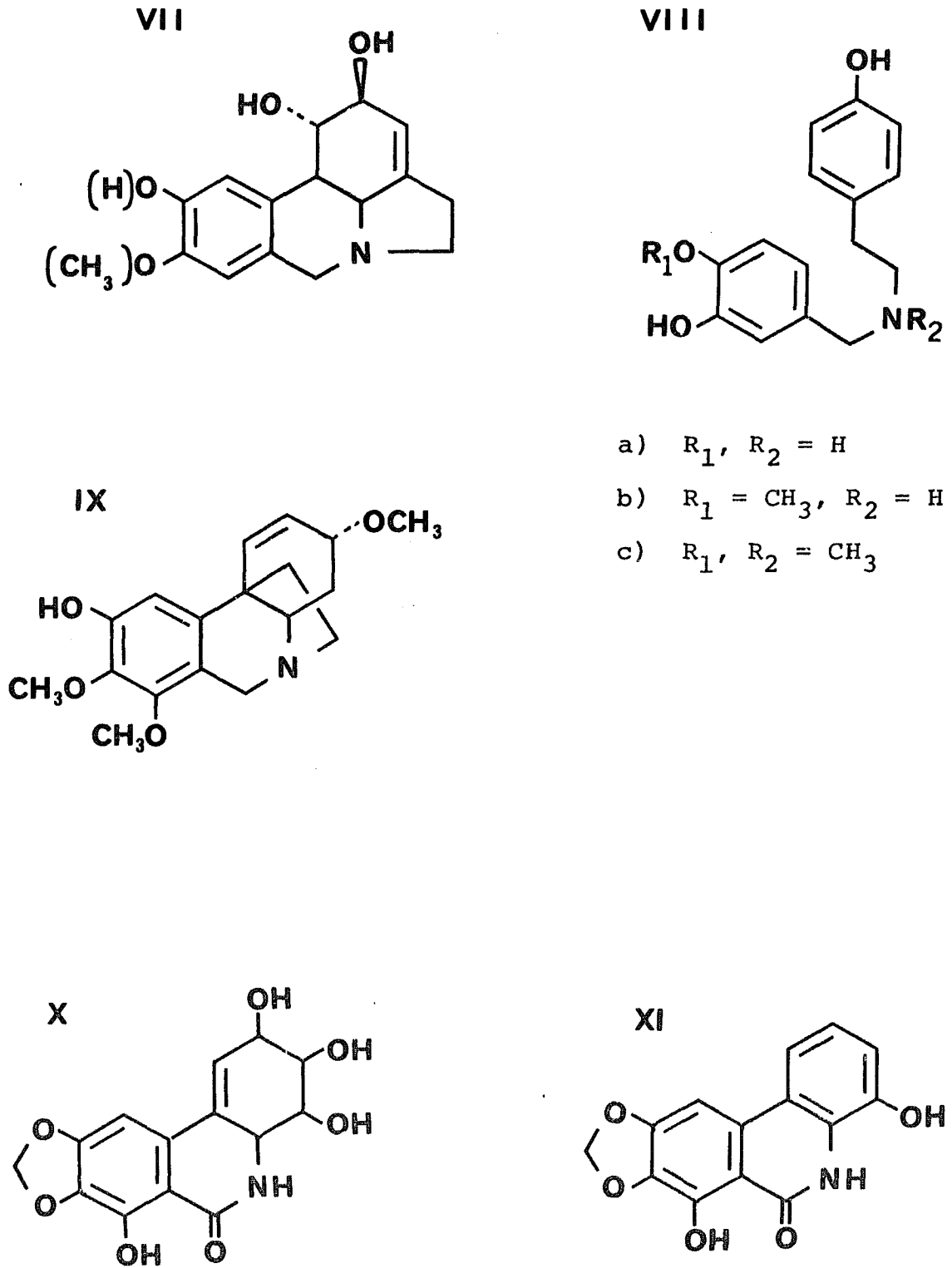
Figure 2.   Later phenols of the Amaryllidaceae

structure. Classical functional group analyses, such as N-methyl, methoxyl, and methylenedioxy, could also yield valuable substituent information, as could formation of standard functional group derivatives, such as amine salts and alcoholic acetates. However, the lack of tools for detailed structure determination of large and complex molecules limited the scope of most investigations predating 1953.

The initial isolation and characterization of crinin(II) from _Crinum moorei_ in 1954 by Boit(2) illustrates the classical plant workup procedure used for phenolic alkaloids. Figure 3 outlines the extraction sequence for separation of fractions. As with most other published phenolic isolations of that period, the phenolic fraction which was obtained required only fractional crystallization to complete the separation of a crystalline product. Boit's crinin, which constituted 9% of the alkaloidal material, was precipitated by trituration of the phenolic fraction with acetone. The purified compound was characterized as a levorotatory, tertiary base melting at 213-214°C which contained one methoxyl group and one N-methyl group, but no methylenedioxyl group. Elemental analysis indicated that the molecular formula was $C_{17}H_{19}NO_3$. Color reactions were negative with both ferric chloride and concentrated sulfuric acid. Refluxing the compound with excess methyl iodide produced a crystalline methiodide which melted at 258-259°C.

The information disclosed in this article provides a

<u>Crinum moorei</u> var. J. D. Hook
|
Pulverize with ethanol
Filter
```
         ┌─────────────────────────────┐
    Filtrate                    Plant residue
         |
    Evaporate
    Acidify (H₂SO₄)
    Filter
    Ether partition
    ┌──────────────┐
 Ether          Aqueous
    |              |
 Fraction      Chloroform partition
(Neutrals)     ┌────────────────────┐
          Chloroform          Aqueous
               |                 |
           Fraction          Neutralize (NH₄OH)
    (Chloroform soluble salts) Chloroform partition
                          ┌──────────────────────┐
                      Chloroform            Aqueous
                          |
                       Filter
                    ┌──────────────────┐
                Filtrate           Lycorine
                    |
            Dilute H₂SO₄ partition
        ┌───────────────────┐
   Chloroform           Aqueous
                           |
                        Filter
                   Neutralize (NH₄OH)
                   Chloroform partition
               ┌─────────────────────────┐
          Aqueous                    Filtrate
                                        |
                             Dilute NaOH partition
                        ┌────────────────────────────┐
                   Aqueous                       Chloroform
                        |                            |
        Neutralize (H₂SO₄, NH₄OH)              Fraction
            Chloroform partition          (Nonphenolic bases)
        ┌────────────────────┐
   Chloroform            Aqueous
        |
     Fraction
(Phenolic bases)
```

Figure 3. Plant workup for crinin isolation

reasonable physical characterization and functional description of the newly isolated compound. It would certainly be useful to another investigator for procedural duplication or sample comparison purposes. But there is little in the way of structural information which would suggest a molecular skeleton or spatial arrangement of functional groups. Methods to probe such details were simply not available to researchers for routine characterization at that stage of the development of chemical instrumentation.[1] The elucidation of structural detail was largely dependent upon straightforward chemical conversions to compounds of known structure.

The following decade produced an abundance of publications on isolations and numerous new instrumental methods to expand the capabilities for definition of structure. These innovations were quickly incorporated into isolation schemes. The initial isolation of amaryllisine(IX) from Amaryllis belladonna in 1964 by Fales and Highet(9) provides a sharp contrast to the example of Boit's crinin in two respects. Both the method of isolation and the characterization data differ markedly from earlier techniques.

----

[1]Professor Boit was associated with the Humbolt University(East Berlin) during the period of his isolation studies and had little access to the instrumental facilities available in non-communist countries. This limitation is also reflected in his analytical capabilities.

Although most individual phenols were initially discovered using isolation procedures with a phenolic extraction, as indicated in Table 1, amaryllisine stands out as an exception. The Amaryllis bases obtained by extractive methods were not processed by further extraction, but were instead subjected to column chromatography. The report contains an element of contradiction, since the text states "...chromatography of the alkaloid extract...over alumina produced a phenolic compound...", whereas the experimental section reports "...this fraction was chromatographed over 300 g. of Florisil. Elution with 4% ethanol in chloroform produced 470 mg of crude amaryllisine." Despite this uncertainty, the isolation by column chromatography is noteworthy. The phenolic alkaloid, representing 5.5% of the total alkaloid content, was eluted before all other alkaloids reported in the isolation. This evidence that amaryllisine is relatively nonpolar (in spite of the presence of the free phenol) is reflected in the assigned structure, which has no free aliphatic hydroxyl groups to supplement molecular polarity. This combination of structural features, together with the choice of a neutral, relatively inactive chromatographic support, presuming the experimental version to be correct, undoubtedly was largely responsible for the success of the isolation.

The changes effected by ten years of instrumental advances are illustrated dramatically by the evidence presented in the structure elucidation of amaryllisine. Combustion analysis

and neutralization equivalent of this phenol corresponded to the formula $C_{17}H_{21}NO_4$, and analysis showed three methoxyl groups and no N-methyl. Combustion analyses and neutralization equivalents of methyl ether, dihydro, and acetyl derivatives supported the formula. Analytical data were also reported on optical rotation, and ultraviolet and infrared spectra. Routine proton magnetic resonance spectra provided a direct probe to the type and number of functional groups present and their immediate chemical environment. More specific information on exact substituent location on the aromatic ring was drawn from detailed experiments with the compound, its dihydro derivative, and anionic salts derived from both. However, the workers found no possible structure to conform to all their information. The answer was finally provided by mass spectroscopic determination of molecular weight. Structure X was assigned even though standard elemental analyses gave unsatisfactory results for this molecular formula. The choice of ring system and stereochemistry are supported convincingly by mass spectroscopic comparisons of the compound and its dihydro and methyl ether derivatives with analogous compounds with the known molecular skeleton.

Phenols by dilution

Not all phenolic alkaloids in the Amaryllidaceae resulted from strictly isolatory investigations. In two notable instances concentrated searches were conducted for specific

phenolic compounds. The driving force behind this type of
search was biosynthetic in nature. Early investigations of
paths of biosynthesis in the Amaryllidaceae were directed at
providing evidence on the validity of particular biosynthetic
postulates. Simple tracer experiments quickly disproved most.
The phenolic oxidation concept of Barton and Cohen(13)
emerged as the primary prospect from feedings of radioiso-
topically labelled aralkylamines and amino acids such as
phenylalanine, tyrosine, and tyramine. The patterns and
specificities of labelled compound incorporation directed
attention at the family of more complex phenolic amines re-
lated to norbelladine(VIIIa). Successful specific incor-
porations of several compounds of this structure type into
various alkaloids confirmed their importance as biosynthetic
intermediates. Reported in published biosynthetic experi-
ments were efforts to isolate these phenols from the plants.

Work by Barton et al.(7) sought to determine possible
intermediacy of three different substituted norbelladine
derivatives by feeding solutions of each proposed precursor
mixed with radioactive tyrosine to King Alfred daffodils.
The experimentors intended to demonstrate diminished tyrosine
incorporation only in alkaloids for which the accompanying
norbelladine derivative served as specific precursor. This
scheme was unsuccessful, since incorporation was inhibited
even for processes for which the specific compounds had been
demonstrated not to be precursors. The explanation offered

was that "all the norbelladine phenols can compete with one another for the enzymes controlling the oxidative coupling step."

A second expectation was that recovery of the diluting norbelladine derivative would give radioactive material if the phenol were a true biosynthetic intermediate. Reported isolation attempts were limited to 4-O,N-dimethylnorbelladine (VIIIc), considered to be the most "tractable" member of the group. Recovery of 29% of the fed material was accomplished by chromatography of the extracted bases on activity grade V neutral alumina, instead of the usual more retentive activity grade III basic alumina. The compound was eluted with 5% ethanol in chloroform after less polar alkaloids had been recovered from the column. An incorporation level of 0.0016%, though low, did demonstrate the presence of 4-O,N-dimethyl-norbelladine in King Alfred daffodils.

In an independent effort, Battersby et al. (8) searched for the same family of compounds in "Twink" daffodils. The nature of "aromatic intermediates" was examined in a somewhat different manner. The known presence of norpluviine(IV) in the plant logically directed isolation attempts to the struc-tural analog, 4-O-methylnorbelladine(VIIIb). Separation was accomplished by performing a phenolic extraction with dilute aqueous sodium hydroxide in the presence of added 4-O-methylnorbelladine(156 mg) on a portion of the basic chloroform extract from daffodils which had been fed

[2-^{14}C]tyrosine. Adjustment of pH and extraction recovered

66% of the diluent. An incorporation level of 0.0008% docu-

mented the actual existence of this compound in "Twink"

daffodils.

Recent isolations

The fortuity of timing can be a primary factor in deter-

mining the impact of a particular technological improvement

on a specific field of scientific investigation. Had the

popularization of preparative thin layer chromatography

occurred early in the 1950's, in coincidence with the initia-

tion of the bulk of investigative isolation work in the

Amaryllidaceae, and in conjunction with the rapid expansion

in instrumentation which occurred thereafter, there is little

doubt that its immediate effects would have been enormous.

Isolative attention, which was necessarily restricted to major

and readily isolable compounds, could have been directed to

more of the minor constituents as well. A much more complete

and, presumably, more understandable picture of alkaloid abun-

dance and distribution would surely have been the result.
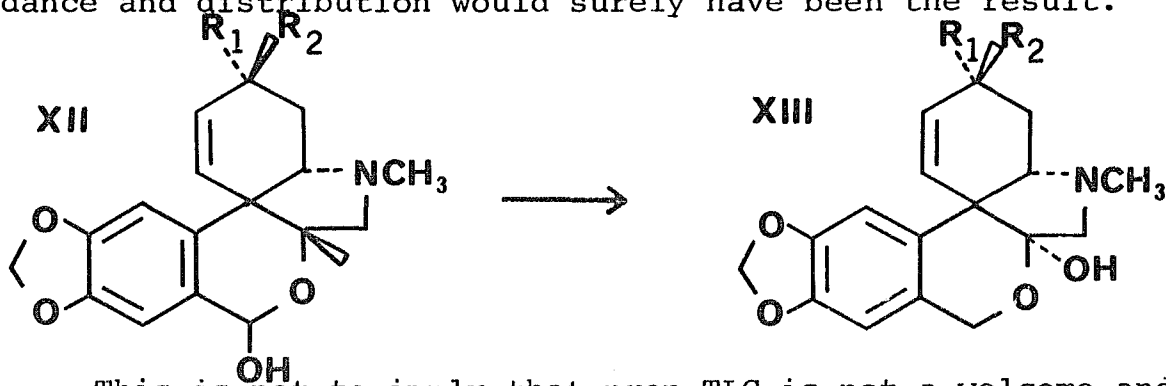
This is not to imply that prep TLC is not a welcome and

powerful addition to the analytical tools available to the

modern investigator, but rather that there is less impetus now
to undertake extensive isolation work when it seems a dupli-
cation of a previously completed investigation. The bounty
that such reexaminations can produce was documented by Wildman
and Bailey(14) with the discovery of pretazettine(XIIa)
and precriwelline(XIIb). After a plant workup which avoided
strongly basic conditions, application of successive prepara-
tive separations helped to isolate the new compounds. The
total absence of tazettine(XIIIa) and criwelline(XIIIb) was
taken as an indication that mild plant workup prevented their
formation, and that pretazettine and precriwelline were the
actual compounds present in the plant.

An extension by Wildman and Bailey of this combination
of mild workup procedure and preparative TLC led to publica-
tion in 1970 of the isolation of a phenolic alkaloid, cherylline,
from Crinum powellii(12). The extracted alkaloidal bases
were separated by silica gel preparative TLC with ethyl
acetate-methanol solvent, then recovered and chromatographed
again with chloroform-methanol-diethylamine as solvent to give
material which crystallized readily. Spectroscopic character-
ization by optical rotatory dispersion, ultraviolet, infrared,
proton magnetic resonance, and mass spectrometric means led
readily to the assignment of structure II. Confirmation was
provided after methylation of the phenols by comparisons with
synthetic material of known structure. An eventual comparison
of isolated samples of cherylline and Boit's crinin indicated

the two were identical, and that Boit's crinin finally had a structure.

Biosynthetic Significance of Phenols

Alkaloidal compounds which occur in the Amaryllidaceae demonstrate wide variations in structure and substituent functionality. The observation that each carbon skeleton can be produced by oxidative phenolic coupling of precursors of the norbelladine type provided the unifying concept to inter-relate such seemingly dissimilar ring systems. The chemical credibility of the idea is derived from the high reactivity and directing capability of the phenolic groups of the pro-posed precursors. This biogenetic hypothesis was presented in Festschrift Arthur Stoll as part of a more general consider-ation of phenol oxidations by D. H. R. Barton and T. Cohen(13). It was extensively tested, extended, and corrected in detail through observations from many biosynthetic experiments. For a thorough and competent summary of this development process, the review of W. C. Wildman(15) is recommended.

The concept involved is demonstrated sufficiently with information presented in Figure 4 from an evaluation by Barton of early tracer studies(16). Production of the three 'main' ring systems by oxidative phenolic coupling of methylated derivatives of norbelladine stems from the ortho and para directing character of the phenolic substituents, which form the skeletons of lycorine[para-ortho](XIV), crinine[para-para] (XV), and galanthamine[ortho-para](XVI). The skeletons of
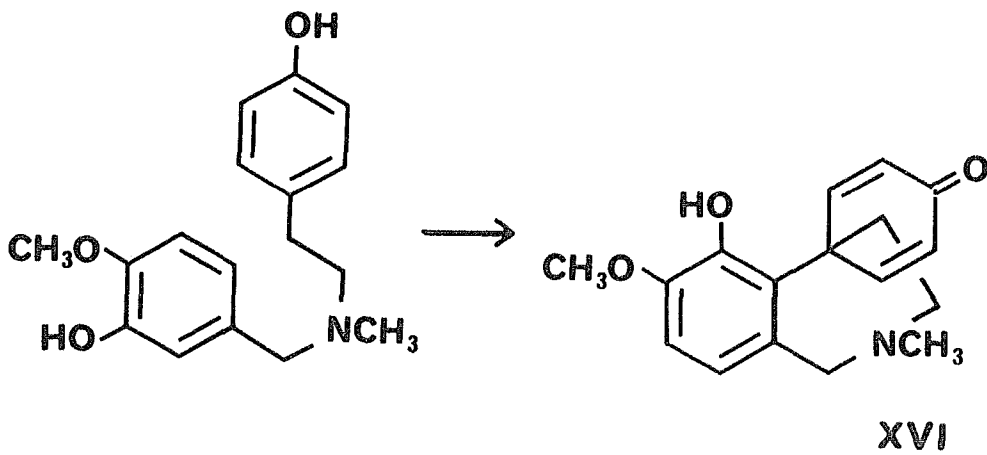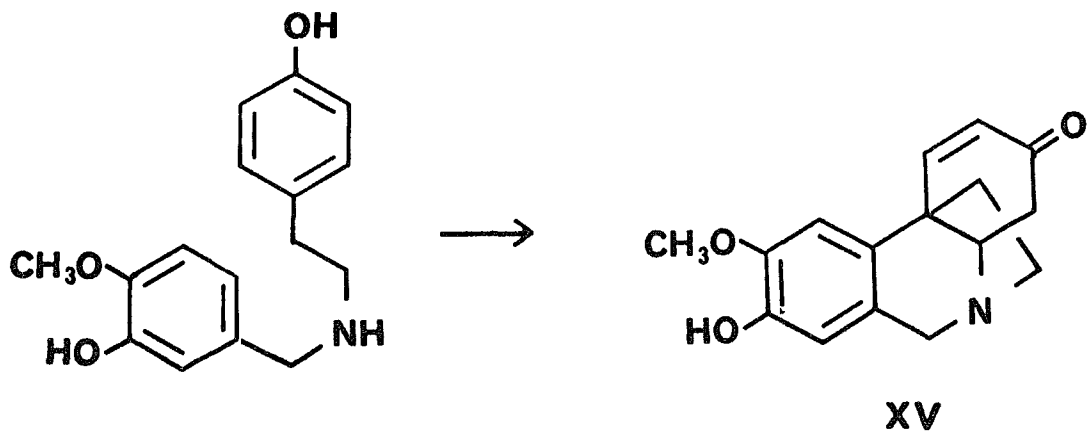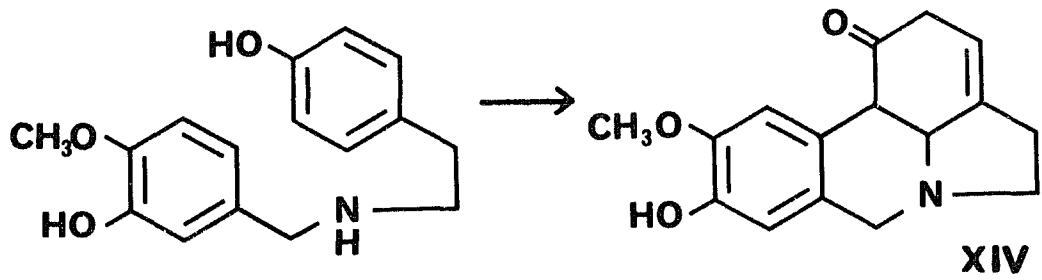
Figure 3. Phenolic oxidative coupling

lycorenine(XVII), tazettine(XIIIa), and montanine(XIX) do
not retain the intact norbelladine carbon framework. However,
subsequent feeding experiments have shown that norpluviine(IV)
is converted to several lycorenine-type alkaloids(17), and
that haemanthamine(XXa) and haemanthidine(XXb) are precursors
for tazettine(18). Formation of the montanine ring system by
skeletal rearrangement of crinine derivatives by chemical
methods has also been demonstrated(15). Every ring system
known at that time has thus been related to some member of the
norbelladine family.

XVII

XVIII

XIX

XX

XXI
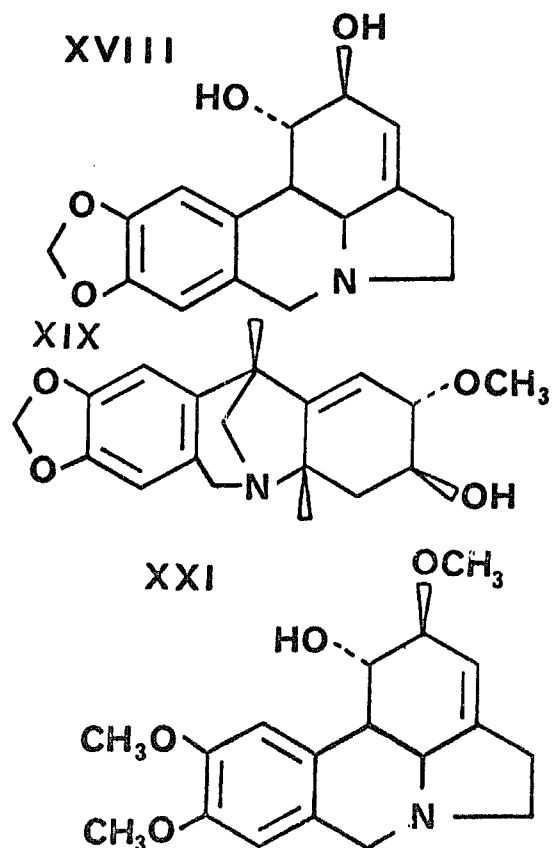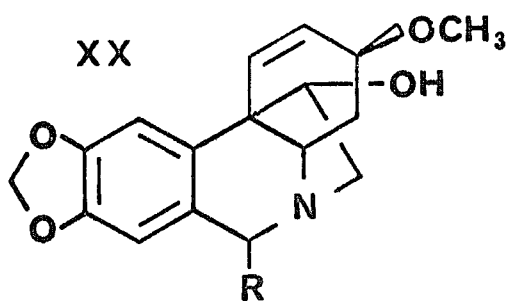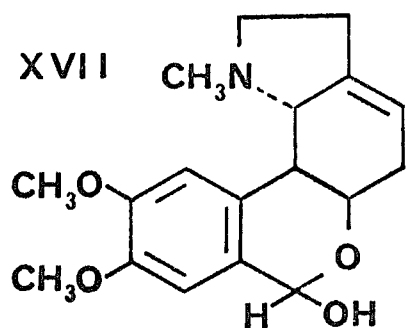
Of the phenolic alkaloids definitely shown to exist in
the plants of the Amaryllidaceae family, the previously men-
tioned methylated derivatives of norbelladine, VIIIb and VIIIc,
occupy a unique position. Interest sparked by successful
incorporation of tracers preceded the actual demonstration

of their presence. Thus, their function as general precursors of prime importance had been firmly established with isolation accomplished as a subsequent interest.

Norpluviine(IV) has also been a subject of considerable biosynthetic interest. As the simplest representative of the lycorine-type alkaloid and a relatively abundant compound in its own right, it was of primary concern from two considerations—both as a possible biosynthetic product from norbelladine and as a precursor for more highly substituted compounds. Norpluviine has since been related by tracers to 4-O-methylnorbelladine(VIIIb), norbelladine(VIIIa), tyrosine, and phenylalanine. Successful incorporation of norpluviine has been reported into several compounds of the lycorine system and of the lycorenine system. Hence, norpluviine, besides being an alkaloid of fairly high abundance in some plants, serves as a ring system precursor for these two important classes of alkaloids.

Evidence gleaned from early tracer studies with ortho methoxyphenols demonstrated the likelihood that even more complex phenolic alkaloids of the family could function as precursors, but of a more specific nature. Both the methylenedioxy group of haemanthamine(XXa) and the methoxyl of galanthamine(XVI) are introduced as an aromatic methoxyl prior to the norbelladine stage of alkaloid formation(19). The dimethoxyl functionality of galanthine(XXI) also retains a methoxyl label introduced in 4-O-methylnorbelladine(7). This

indirect evidence indicates that phenolic alkaloids with the substitution pattern of 4-O-methylnorbelladine can be converted both to dimethoxy compounds and, by oxidative cyclization of the _ortho_ methoxyphenol, to methylenedioxy compounds related to their specific structures. This concept is applicable to several of the known phenols. Although tracer experiments have not been performed, the idea of specific precursors within the ring system relates pseudolycorine(I), demethylhomolycorine(V), goleptine(VI), and golceptine(VII) to other known alkaloids as specific precursors for the last step of formation.

For two phenolic compounds, amaryllisine(IX) and chlidanthine(III), the location of the substituent phenol on the aromatic ring precludes action as a precursor in the ring system. In each case the substitution pattern opposes that of 4-O-methylnorbelladine. These compounds must be formed later in biosynthesis as mature alkaloids in their own right, or as the result of degradation of other alkaloids back to the phenolic stage.

The structural features of narciclasine(X) and narciprimine(XI) indicate a more extensive degree of degradation, since the two carbon bridging fragment of the molecular skeleton is no longer present. The first evidence on the origin of these two compounds was obtained when a small incorporation of norpluviine into narciclasine was observed(17). A much more extensive and definitive study with varied labels in

4-O-methylnorbelladine and oxocrinine(20) argues convincingly
that the crinine ring system provides the principal path to
narciclasine.

No tracer studies have been published to define the
importance of cherylline in the biosynthetic scheme of the
Amaryllidaceae. The only example of the 4-phenyltetrahydro-
isoquinoline ring system associated with this plant family is
a chemical degradation product of the montanine ring system,
but the dearth of positive biosynthetic information on that
group of compounds sheds no light on cherylline. The variety
of functions served by other phenols in the family indicates
several possibilities. The obvious structural similarity to
norbelladine suggests a simple biosynthetic derivation. The
recent appearance of a facile 'biosynthetically patterned'
synthesis of cherylline(21) through a benzylically hydroxy-
lated norbelladine derivative(XXII) bespeaks the reasonability
of such an approach. Speculations on further transformations
of the cherylline ring system must be restrained due to lack
of experimental information.
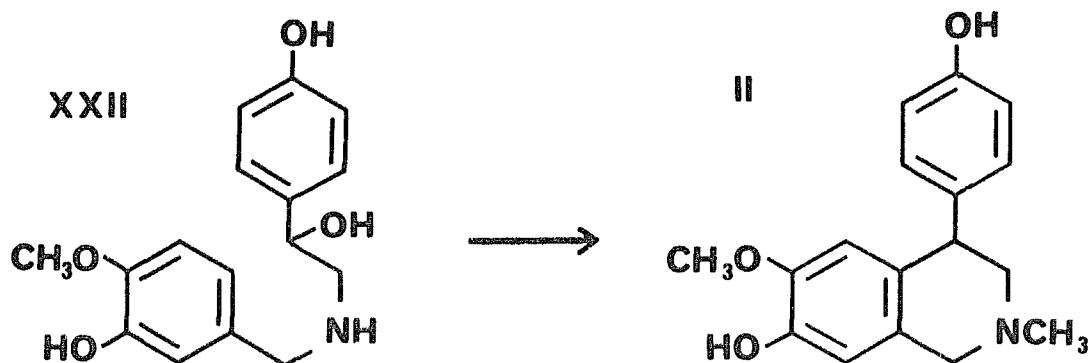
Synthetic Background of Cherylline-like Compounds

The isolation and subsequent structure elucidation of cherylline added an uncommon and noteworthy ring system to the variety found in the Amaryllidaceae family. Although this compound does contain both the C_6-C_1 and C_6-C_2 skeletal units characteristic of alkaloids of the family, the direct aryl-aryl linkage of the two fragments which dearomatizes ring C for other ring systems is absent. Insertion of a bridging carbon between the A and C rings produces a diphenylmethane nucleus and permits the retention of the second aromatic ring.
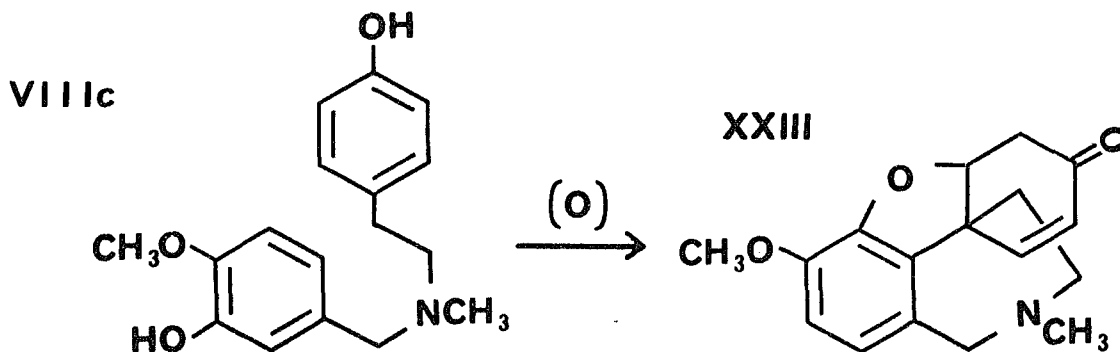
There are two examples of synthetic research which should be mentioned in considering approaches to cherylline. In an effort to provide experimental synthetic support for the phenol oxidation mechanism of biogenesis, Barton and Kirby performed oxidations of 4-O,N-dimethylnorbelladine(VIIIc) under various conditions(22). Small yields of narwedine(XXIII) were obtained with several oxidizing agents(manganese dioxide, lead dioxide, silver oxide, and potassium ferricyanide). Optical resolution and reduction of the product ketone gave galanthamine(XVI). Polymerization was indicated to be the primary competitive

reaction observed, but the oxidative process might well have produced some cherylline.

In an ensuing paper(23), Abramovitch and Takahashi rationalized the low yields; "...attack by the nitrogen with its lone-pair of electrons upon the α,β-unsaturated ketone formed in the first step of the oxidation gave indole systems which did not, or could not, undergo coupling of the aromatic nuclei." They sought to prove that initial phenol coupling was facile if nitrogen was blocked in the first stage. The methanesulfonamide derivative(XXIV) was prepared and treated with potassium ferricyanide in base to form the α,β-unsaturated ketone(XXV) in 81% yield, rather than the narwedine analog. Mild efforts to displace the sulfonamide failed. The ketone functionality which eliminates possible formation of a cherylline compound was characterized by infrared data(1655 cm^{-1}) and derivative formation(2,4-DNP). Attempts to duplicate the cyclization on the corresponding phenolic benzamide, 3-mesylbenzamide, and completely demethylated compound gave no pure products. These two illustrations serve to emphasize that the biosynthetically patterned synthetic methods do not lead necessarily and exclusively to the cherylline nucleus.
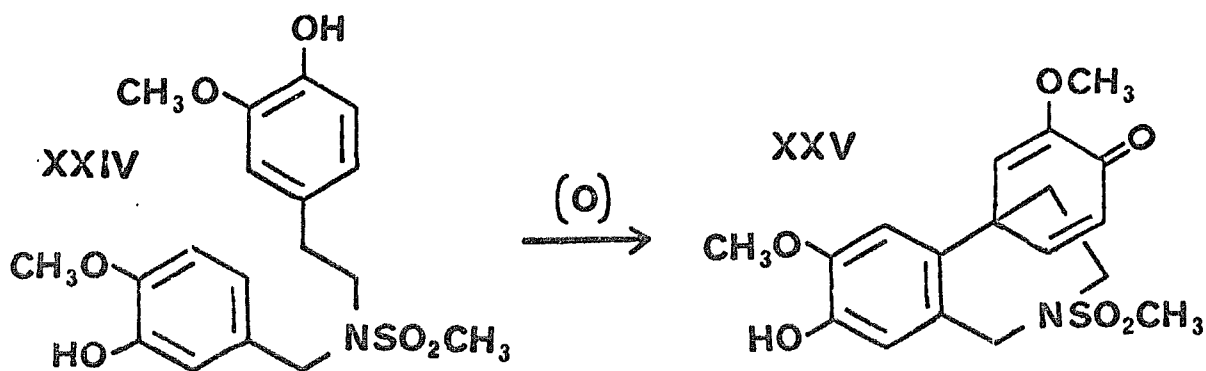
As the first example of a natural product with the 4-phenyltetrahydroisoquinoline nucleus, cherylline does possess a unique skeletal composition. However, the isoquinoline moiety has long been recognized as a fundamental structural unit for many natural products. In addition to simple isoquinolines, an assortment of substituted representatives have been discovered. Attachment of fused rings at various positions leads to extended ring systems from this foundation which also are known to occur naturally.

Synthetic interest led to development of numerous methods for production of these natural systems and to structural analogs which might possess interesting physiological properties. The summation of definitive work in this area is provided in Organic Reactions, Volume VI (24), which has chapters designated for specific consideration of generation of the isoquinoline (Pomeranz-Fritsch cyclization), the 3,4-dihydroisoquinoline (Bischler-Napieralski cyclization), and the 1,2,3,4-tetrahydroisoquinoline (Pictet-Spengler cyclization) entities. The successive chapters of this treatise, which appeared in 1951, examine, summarize, and tabulate attempts to utilize each particular mode of cyclization. Since that time, the volume of literature published in this broad area has increased markedly.

The C-4 phenyl substitution pattern has drawn only the modicum of curious interest of a structural variation not closely related to naturally occurring compounds. Prior to

the appearance of cherylline, the number of literature syntheses of 4-aryl isoquinolines was very limited. The early efforts which did appear concentrated on generation of the ring system and did not feature substituted aromatic rings. The entries itemized below demonstrate the dimension of possible ring closure variations available for this system.

R = H or Ph

R' = H, Me, or Ph
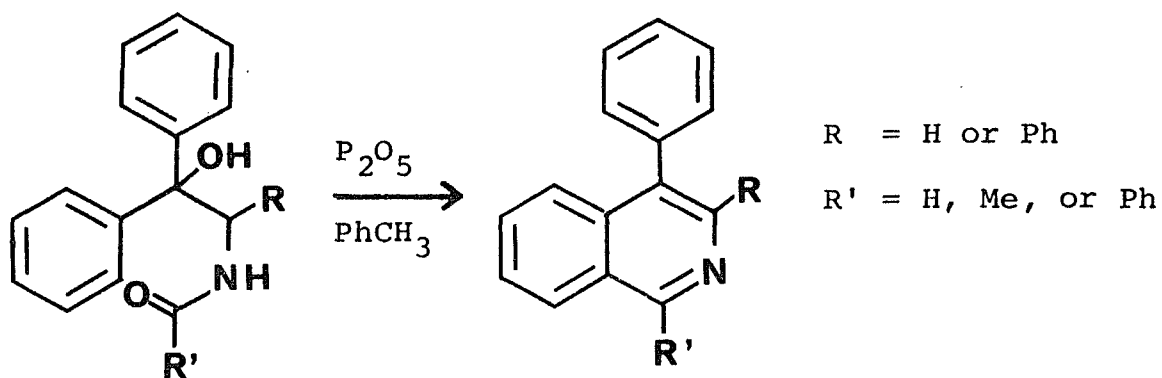
1938-W. Krabbe(25)-Preparation.

R	R'	R"	R'"	Yield
Ph	H	H	Me	28%
Ph	H	H	Ph	53%
Ph	H	H	$PhCH_2$	9%
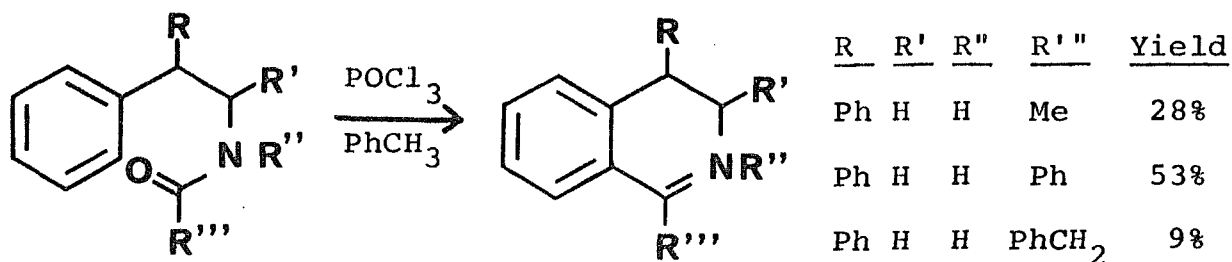
1943-B. B. Dey and V. S. Ramanathan(26)-Study of effects of side chain substitution on closure of the isoquinoline ring. Double bond reducible with zinc in 4 N. sulfuric acid.
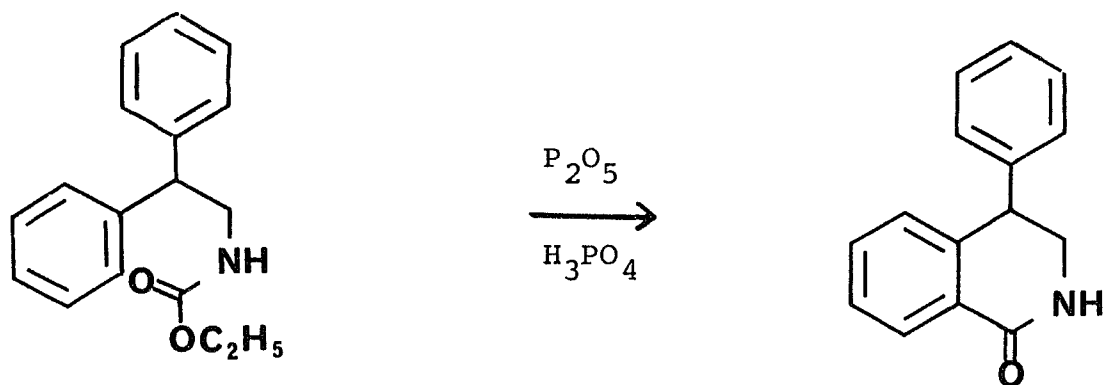
1956-D. F. M. Stephenson(27)-Preparation.

R = Me, Et, etc.
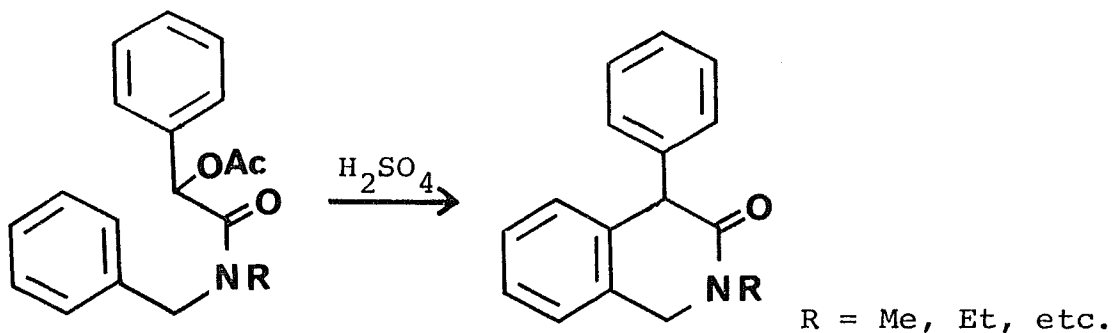
1957-M. E. Speeter(28)-Intermediates converted to 4-lithio salts and treated with tertiary aminoalkyl halides for pharmacological testing.
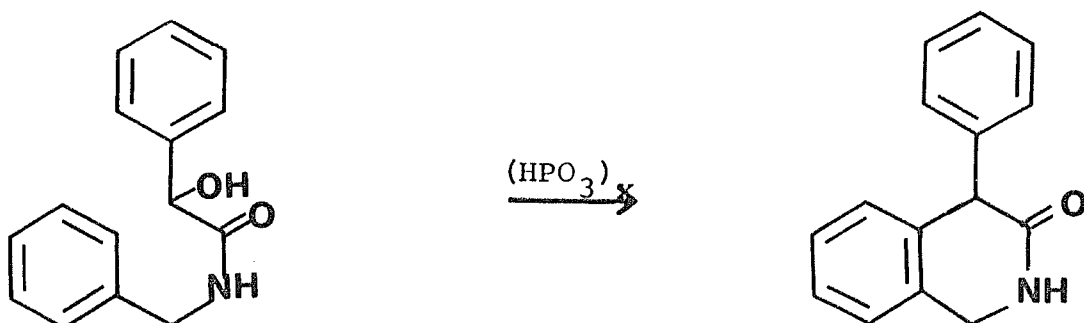
1966-J. Gardent and M. Hamon(29)-Preparation and properties.
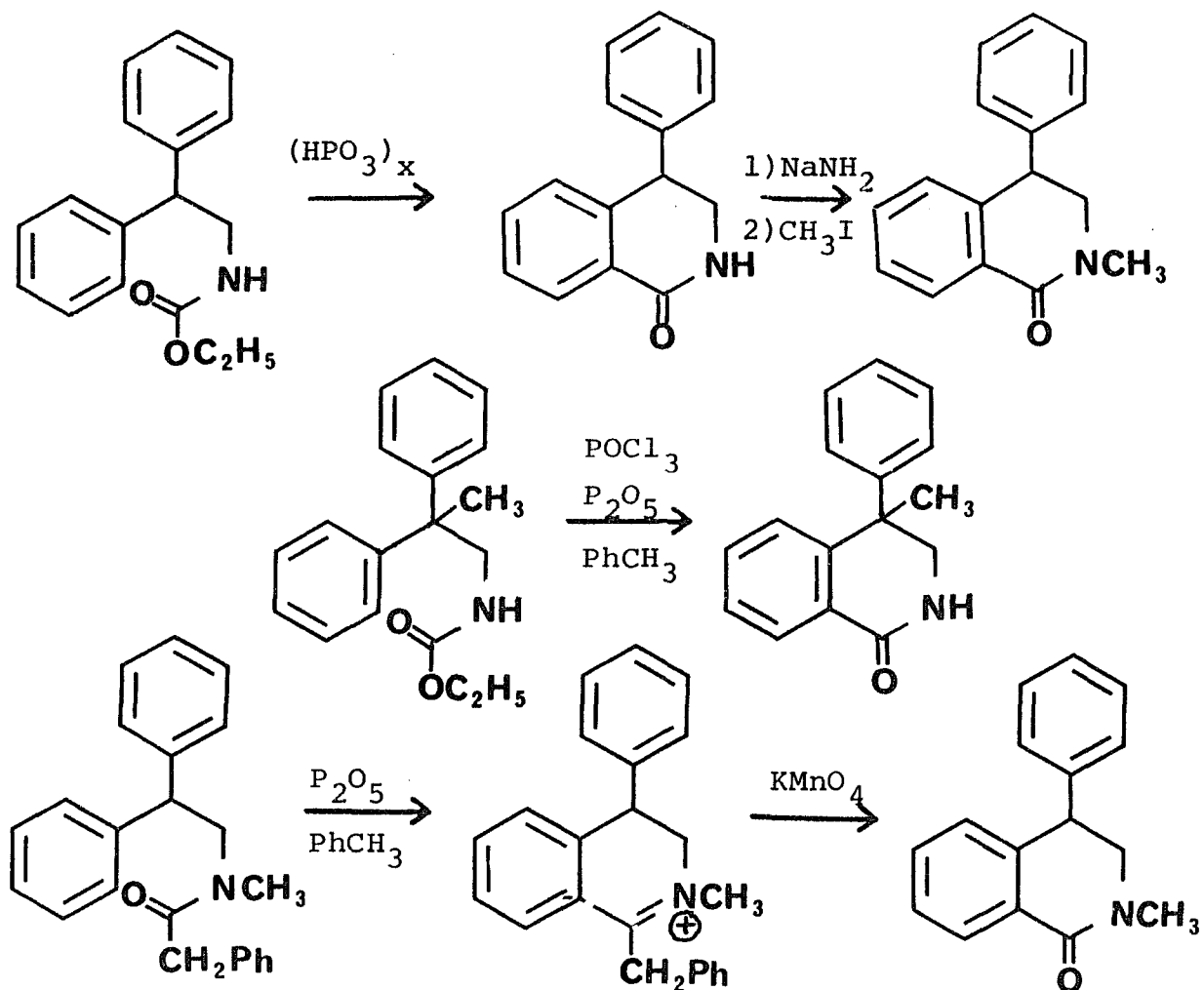
1960-G. Berti(30)-Preparation and structure proof.

(MeO)   - 4'

(MeO)$_2$ - 6,7; 2',4';7,4'

(MeO)$_4$ - 6,7,3',4'
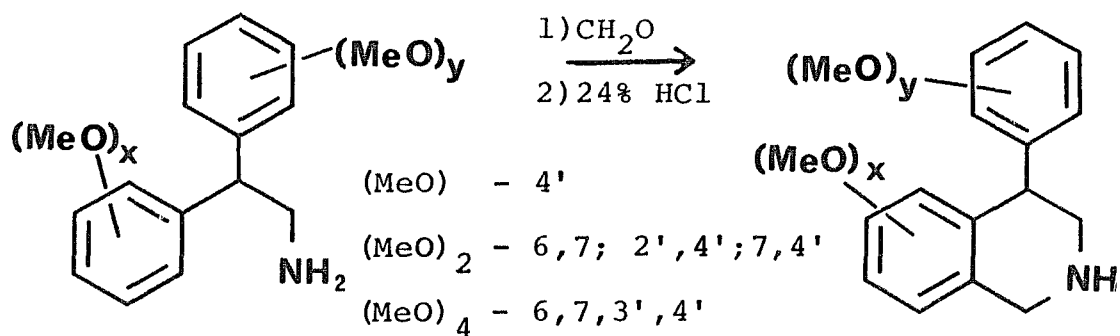
1958-V. N. Deshparde and K. S. Nargund(31)-Preparation, characterization, dehydrogenation, and derivative formation.

The original cherylline isolation paper included a synthesis of the dimethyl ether of cherylline(XXVII)(12). The compound was a vital element for comparisons in characterization of cherylline. The starting 4-isoquinolone(XXVI) was prepared by a sequence utilizing a Dieckmann condensation(32) and subsequent decarboxylation of the ketoester. The straightforward sequence initiated by Grignard addition to the ketone neatly provides the proper functional groups to complete the synthesis.

XXVI

1) $4\text{-}CH_3OC_6H_4MgBr$

2) HCl

1) HCl
2) $NaBH_4$
3) H_2, Pd-C
4) $CH_2O\text{-}HCOOH$
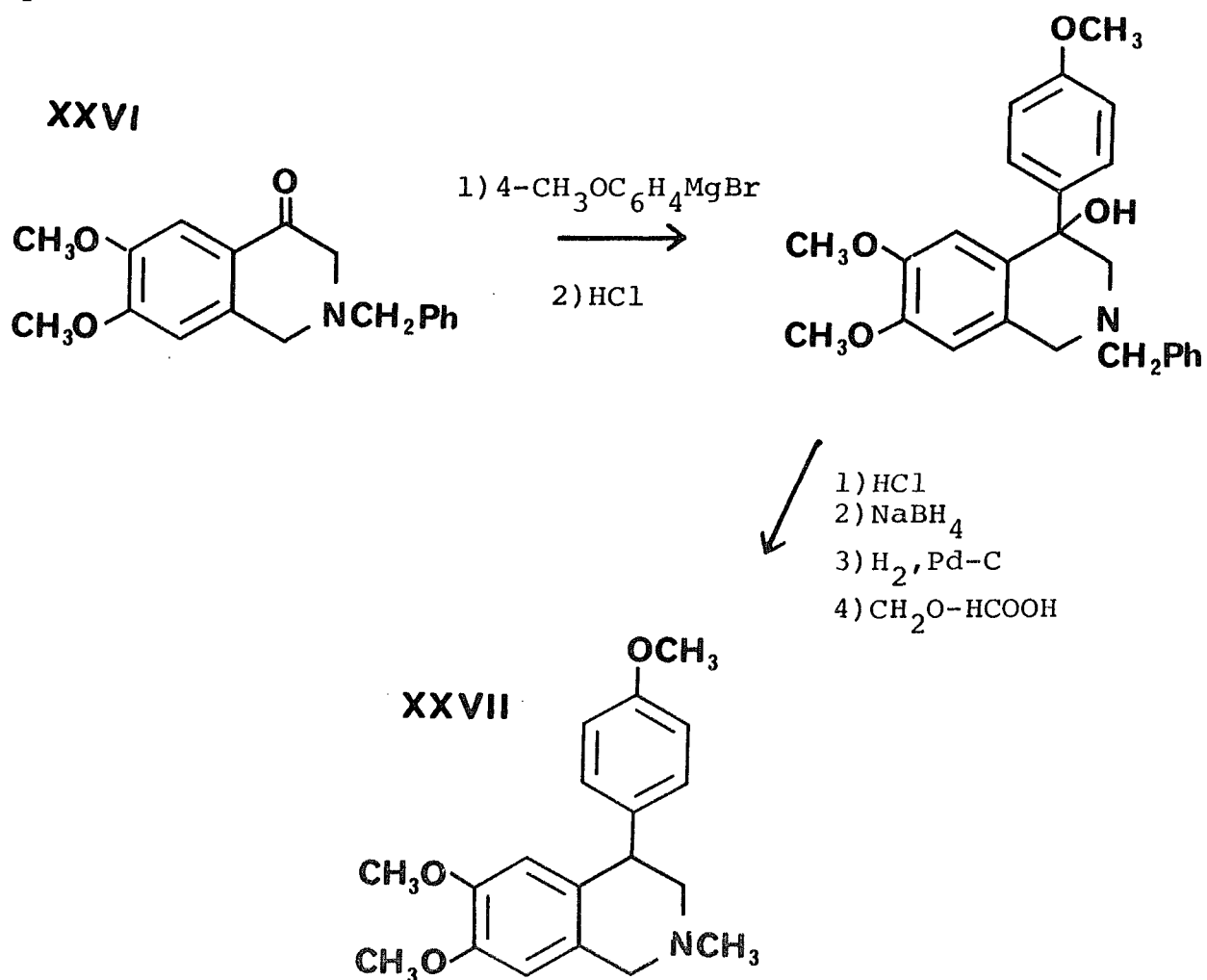
XXVII

The first actual synthesis of racemic cherylline was presented shortly thereafter by Brossi and Teitel(33). The formamide(XXVIII) was tediously constructed and subjected to Bischler-Napieralski cyclization with phosphorus oxychloride. Subsequent debenzylation, selective O-demethylation, quaternization with methyl iodide, and reduction produced the desired product(XLf).
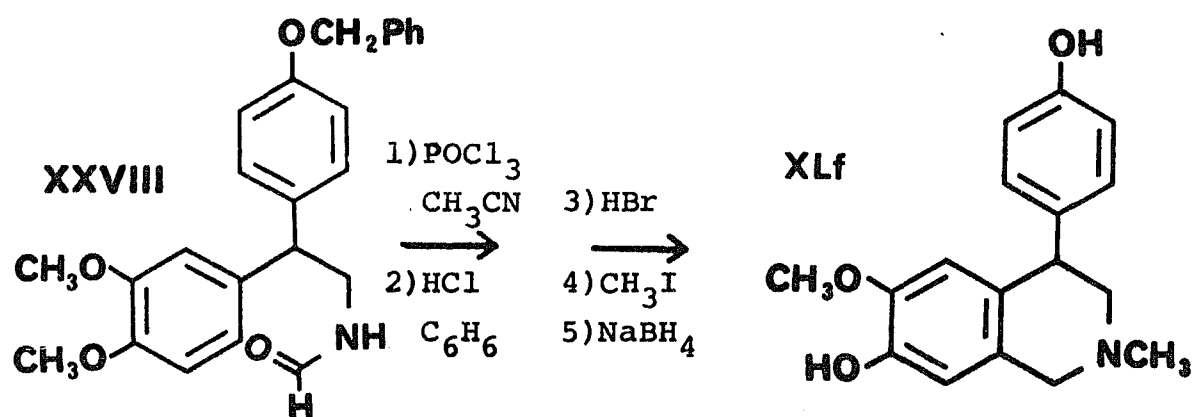
In independent work which appeared concurrently with publication of the cherylline isolation and structure determination, Bobbitt demonstrated a facile synthetic method for some phenolic 4-phenyltetrahydroisoquinolines(34). The acid catalyzed cyclization of a substituted benzylaminoacetal, as shown by the example(XXIX), was followed by coupling with a phenol. Without foreknowledge of the existence of cherylline, Bobbitt's publication mentioned condensations only with ortho-substituted phenols, and consequently did not actually include N-demethylcherylline, but the method is certainly applicable.

R = CH$_3$, OCH$_3$

XXIX

6 N HCl

$\longrightarrow$

2-RC$_6$H$_4$OH
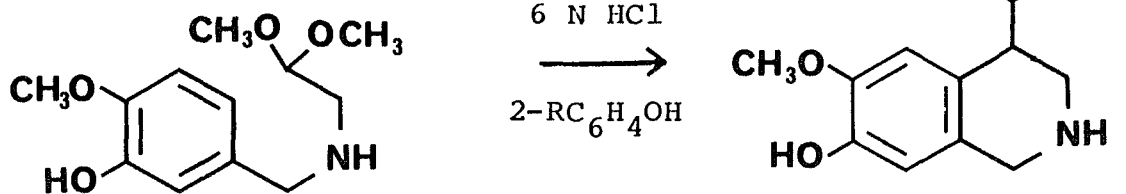
Schwartz, after the appearance of the cherylline paper, followed a somewhat similar tack in devising a 'biogenetically patterned' synthesis of cherylline(21). The O-methylhydroxy-norbelladine(XXII) was converted to the formamide, and reduced with lithium aluminum hydride. In the reduction workup, base assisted cyclization of the N-methyl product to cherylline occurred spontaneously. A footnote acknowledged information that the ring closure can also be performed with acid catalysts.

XXII

1) HCOOEt

$\longrightarrow$

2) LiAlH$_4$

3) NaOH

XLf
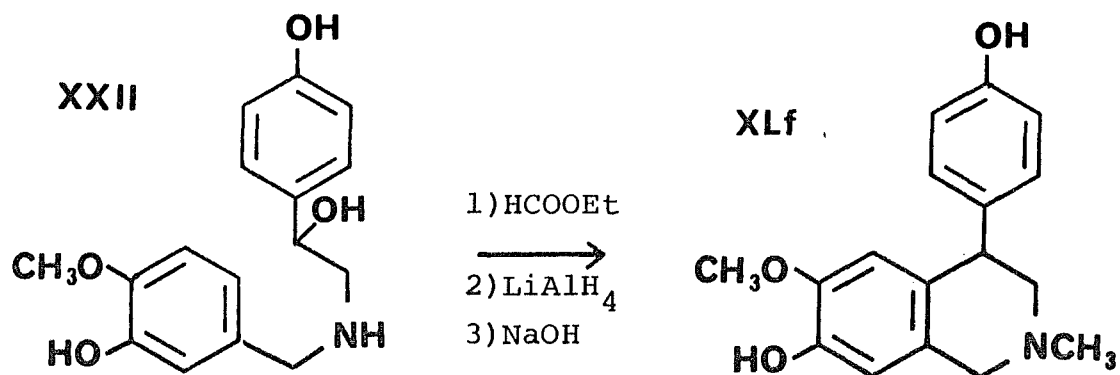
RESULTS AND DISCUSSION

Isolation Investigations

Isolation technology

Earlier discussion mentioned that recent work has brought
out inadequacies in accepted data on alkaloid occurrence and
abundance in the Amaryllidaceae. The demonstrations by
Bailey that strong bases or alumina columns can induce struc-
tural rearrangements(14), and that an alumina column can
completely retain a relatively abundant alkaloid(12) document
the presence of imperfections in previous research. These
discoveries were the initial products of a program to update
the technology of isolation techniques and subsequently to
seek less abundant and more difficultly isolable alkaloids.
Alterations have extended into both plant processing and
alkaloid separation areas and seem to have provided more
sensitive and versatile procedures which better profit from
the capabilities of modern instrumentation.

Changes in plant workup were designed to help retain the
chemical integrity of the components originally present.
Primary concerns were air oxidation, high temperature, and
extremes in pH. The revised plant workup presented in Figure
5 is similar to that of Boit shown in Figure 3, but control
of pH is emphasized and the use of strong acids and bases
minimized. Additional care is taken to eliminate prolonged
heating during solvent removal and to reduce delays in workup.

Column chromatography has long been the standard procedure

Plant Bulbs
|
Pulverize with ethanol
Stir and filter
Repeat extraction twice

Filtrate   Plant residue
|
Evaporate
Acidify (Tartaric acid-pH=4)
Filter
Benzene partition

Benzene   Aqueous
|
Fraction   Chloroform partition
(Neutrals)

Chloroform   Aqueous
|
Fraction   Neutralize (NH$_4$OH-pH=7)
(Chloroform soluble salts)   Chloroform partition

Chloroform   Aqueous
|
Filter

Fraction   Filtrate
(Chloroform insoluble bases) |
Dilute NaOH partition

Aqueous   Chloroform
|
Neutralize (HCl, NH$_4$OH)   Fraction
Chloroform partition   (Nonphenolic bases)

Chloroform   Aqueous
|
Fraction
(Phenolic Bases)

Figure 5. 'Mild' plant workup

for alkaloid separations. High retention for efficient separations has made alumina a favorite support for columns. The optimum conditions for a particular separation can then be provided by judicious choice of column size, eluting solvents, and polarity gradients. While high retentivity is a useful property in separation of the many-component mixtures which are encountered with alkaloid crudes, it also necessitates seeking other methods for many situations. The basicity characteristics of the support are directly responsible for some molecular rearrangements and for the strong retention of phenols. The development of preparative thin layer chromatography as an analytical method provides an alternative with greatly increased efficiency and versatility in separation. The utilization of what is, by comparison, a surface phenomenon increases the sharpness of separations and facilitates the recovery of components. Since elution of the support is not a problem, more polar elution solvents can also be used. The mass scale of TLC cannot compare with the column, since the quantity of material separated on a plate is limited by the absorptive capacity of the support and the necessity of applying a narrow band for effective separation. The unit separation can be expanded only by repetition. Abandonment of alumina column chromatography in favor of prep TLC represents an exchange of simplicity and capacity for increased efficiency and a broadened scope of application.

Exploratory isolations

Investigations of several Crinum species have been
undertaken utilizing these revisions in isolation technique.
The initial effort was generally exploratory. The source
under consideration was a garden variety Crinum identified
by the supplier only as having "pure white flowers with
strap-shaped petals". The 'mild' processing procedure out-
lined in Figure 5 was followed, but no separation of phenolic
and nonphenolic bases was included. A preliminary two-
dimensional spot separation detected the presence of at
least thirty compounds. No single dominant species was
evident, but at least twelve compounds appeared to have
abundances in the 2-10% range. This multiplicity suggested
that an effective separation would require large scale appli-
cation of prep TLC.

A 13.3 gram portion of the 'white' Crinum alkaloid
crude was separated into major bands by prep TLC with solvent
A(see experimental). Table 2 shows the distribution of
material in the recovered bands, which totaled to 79% re-
covery. Cursory examination of infrared spectra indicated
band IA5 was mostly cherylline, which was of considerable
interest. Further separation was therefore first initiated
on the high R_f fractions with solvent B. Separation of IA5
and IA6 produced three compounds which could be purified and
characterized. The major constituent proved to be cherylline
(IA53), but the other two, consolidated and designated as

Table 2. Isolation data

Plant:	Unidentified Crinum	
Fraction	Amount (g)	Percentage
IA	13.3	100.0
Primary separation		
IA1	0.3416	2.6
IA2	2.3479	17.7
IA3	4.4700	33.6
IA4	2.3180	17.5
IA5	0.8030	6.0
IA6	0.2002	1.5
Total	10.4907	78.9
Secondary separations		
IA63	0.067	0.50
IA52	0.093	0.70
IA53 (Cherylline)	0.534	4.0
Phenolic separation		
IB	9.517	100.0
IBP	1.517	16.6
IBP40 (IA52)	0.104	1.1
IBP51	0.531	5.8
IBP52 (IA63)	0.047	0.51
Plant:	Crinum erubescens	
Cherylline	0.147	8.5
Lyrechine	0.008	0.46
4-O-Methylnorbelladine	0.017	0.98
Plant:	Crinum powellii album	
Cherylline	0.191	6.2
Lyrechine	0.009	0.28
4-O-Methylnorbelladine	0.016	0.52

IA52 and IA63, were not readily identified. Both were
shown by ultraviolet to be phenolic, and both had spectral
similarities with cherylline, but each was distinctly differ-
ent in essential details.

With the clear evidence that there were a number of
related phenols present in the plant, a more concentrated
effort in that direction was undertaken. A phenolic separa-
tion was performed on another fraction of the same alkaloidal
crude(IB). This phenolic fraction(IBP) was subjected to the
same twofold separation as the previous fraction. The same
three purified compounds were again isolated, as shown in
Table 2. Various spectral data were secured to initiate
structure determination efforts.

Structural analysis

The structure of compound IA52(IBP40) proved to be the
easier of the two to compose. The similarities of infrared
(Figure 6) and ultraviolet spectra with those of cherylline
indicated the same general chromophores, but the proton NMR,
while confirming most functionalities, revealed the conspi-
cuous absence of N-methylation. Further enlightening
differences were provided by mass spectrometry. The mole-
cular ions of IA52 and its N-methyl derivative appeared
respectively at 273 and 287 mass units. Since cherylline,
at 285, has no nonbenzenoid double bonds, this provided a
broad hint that a ring-opened structural analog should be
considered. Comparisons with synthetic material led to the

Figure 6.   Infrared spectra

        (KRS-5 film)

a)   4-O-methylnorbelladine(VIIIb)

b)   Cherylline(II)

c)   Lyrechine(XXX)
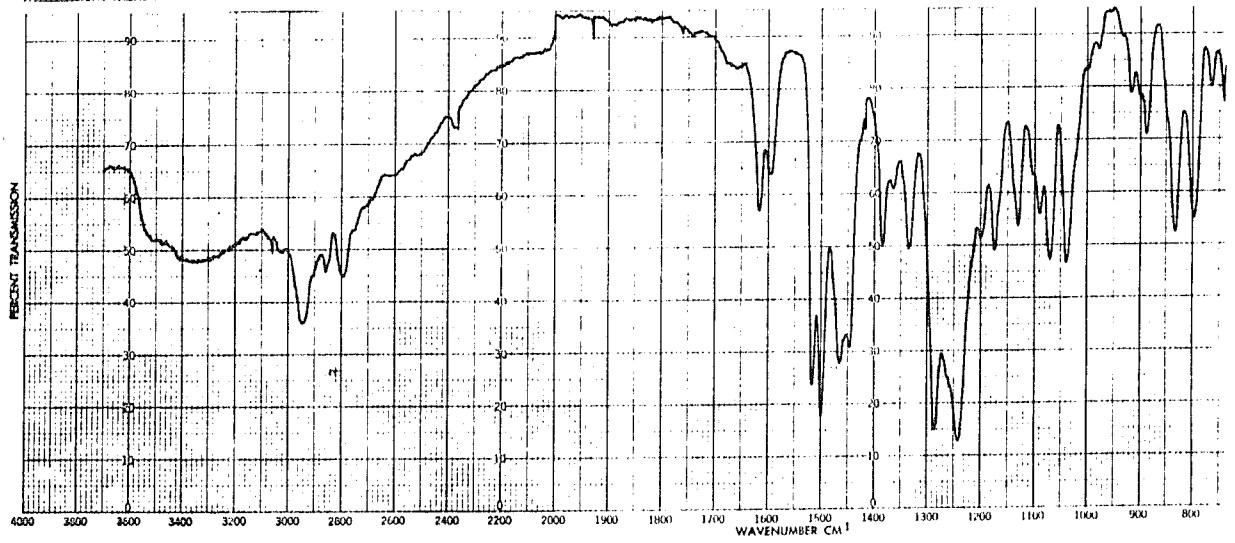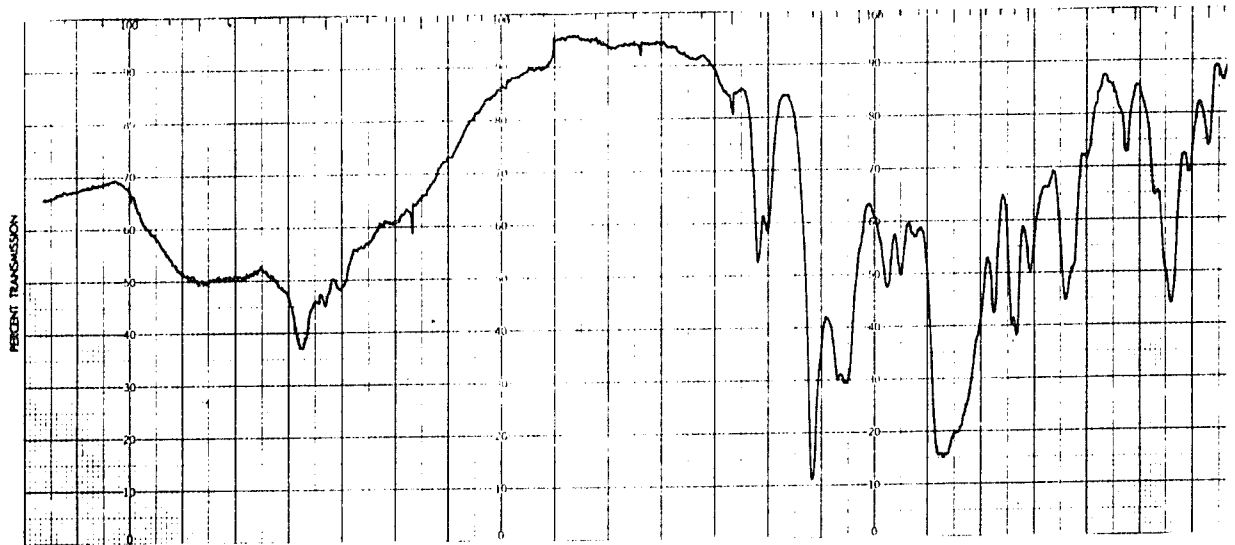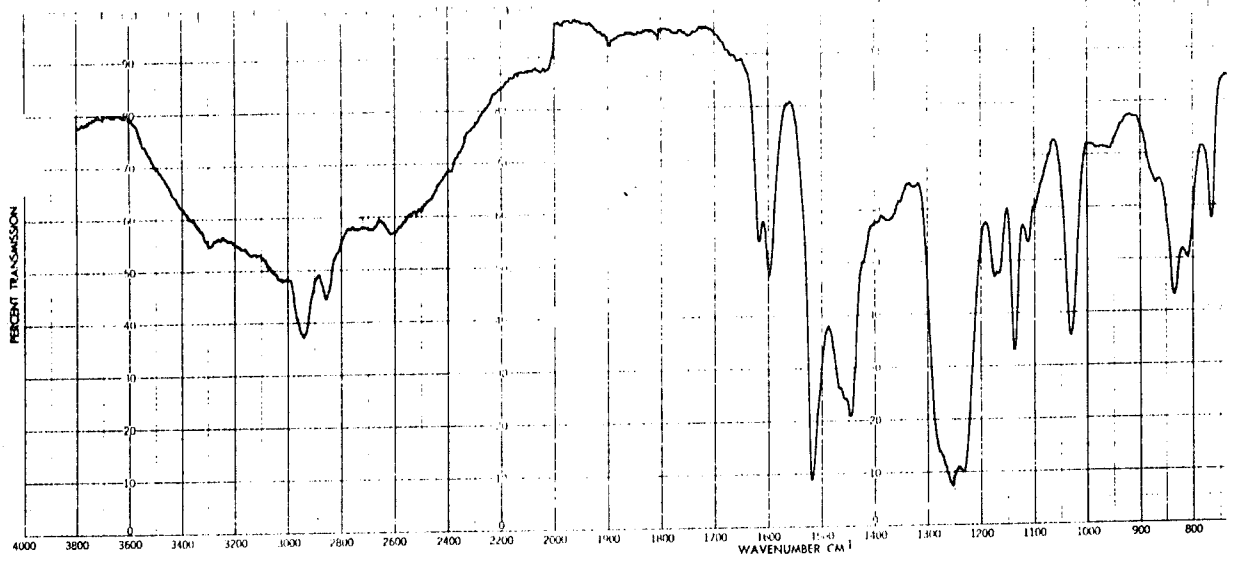
subsequent identification of the compound as 4-O-methylnor-belladine(VIIIb).

Compound IA63 proved to be even more intriguing. Treatment with diazomethane gave a nonphenolic dimethyl derivative which was not identical with the like product from cheryl-line. Various spectral studies were conducted on the natural compound. Since general similarities with cherylline over-shadow the differences, each type of spectral characterization is presented with like information for cherylline. Comparisons and contrasts of the two provide informative insights into structural relationships.

The optical rotatory dispersion and circular dichroism data for the two compounds are practically superimposable. The curves for IA63 parallel those of cherylline, but with a displacement of 10 nm. to shorter wavelength. The S configuration which was confirmed for cherylline by X-ray crystallography(12) can also be assigned to IA63 by analogy. The ultraviolet maxima exhibited by cherylline are those expected from the additive effects of two independent phenolic benzenoid chromophores in a single molecule. The reinforcing nature of these groups give extinction coefficients about double that of an isolated chromophore. Formation of the phenolate anions by addition of base causes the expected bathochromic shift. Table 3 shows that the differences in wavelengths of absorption for the new compound are small or negligible for all maxima. Although the extinction coefficients

Table 3. Comparison of ultraviolet spectra

Ethanol solution			
Cherylline		IA63	
Wavelength(nm.)	Extinction Coefficient	Wavelength(nm.)	Extinction Coefficient
225 (sh)	15,000	234 (sh)	20,500
281	3,960	279	4,620
285	4,050	283 (sh)	4,360
294 (wk sh)	2,480		
Base added			
		244	20,200
299	----	296	7,790

are somewhat larger than those of cherylline, they are still well within the range expected for a molecule with the same chromophores. The overall similarity of the ultraviolet data is obvious.

Comparative analysis of the infrared spectra in Figure 6 reveals a few more differences. Absorption assignments in the functional group regions are generally similar; both molecules have strongly hydrogen-bonded hydroxyl groups, a variety of carbon-hydrogen stretch, and benzenoid rings with methoxyl substitution. Strong intramolecular hydrogen bonding of the hydroxyl absorption is retained for each compound even in dilute carbon tetrachloride solution(3545 cm^{-1} at 10^{-3} M). The 800-1500 cm^{-1} fingerprints are interesting, but not too revealing. The fine structure appearances differ

in this area, but mostly by small variations in position and relative intensities of band combinations. The principal spectral contrast between the two is found in the pattern of the major bands around 1500 cm^{-1}. The para-oriented methylenedioxy and dimethoxy oxygenation patterns normally associated with Crinum species exhibit recognizable and predictable absorption combinations in this region. The particular four band set from 1440-1530 cm^{-1} in the spectrum of IA63 resembles neither type. By contrast, the pattern of cherylline, dominated by a strong band at 1520 cm^{-1}, is comparable to that of the dimethoxy compounds whose substitution pattern it emulates. So infrared comparison establishes further close comparisons with cherylline, but intimates that ring A may have different positions of oxygenation.

Proton NMR comparison provides much more detailed and specific information of individual functional groups and the chemical environs of protons in the molecular structure. The obvious proton by proton correlation in the pyridine-d_5 spectra of the two compounds in Figure 7 is emphasized by the stepwise comparison shown in Table 4. The only difference of note outside the aromatic region is the singlet in the cherylline spectrum where an AB quartet appears for the C-1 methylene in IA63. This is readily reconciled, since both occurrences are well-precedented in this alkaloid family. The aromatic region of cherylline is readily interpreted, while the greater complexity in IA63 makes confident assignment

Figure 7. Nuclear magnetic resonance spectra

$(d_5$-pyridine)

a)   Cherylline(II)

b)   Lyrechine(XXX)
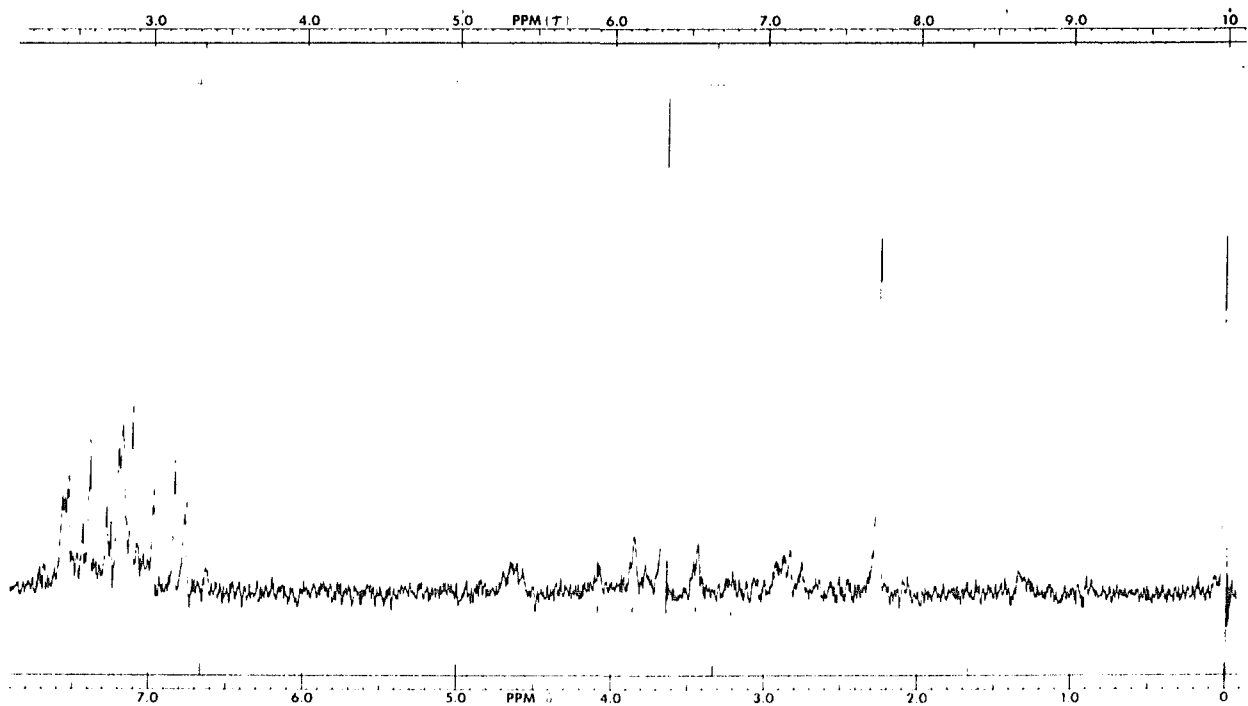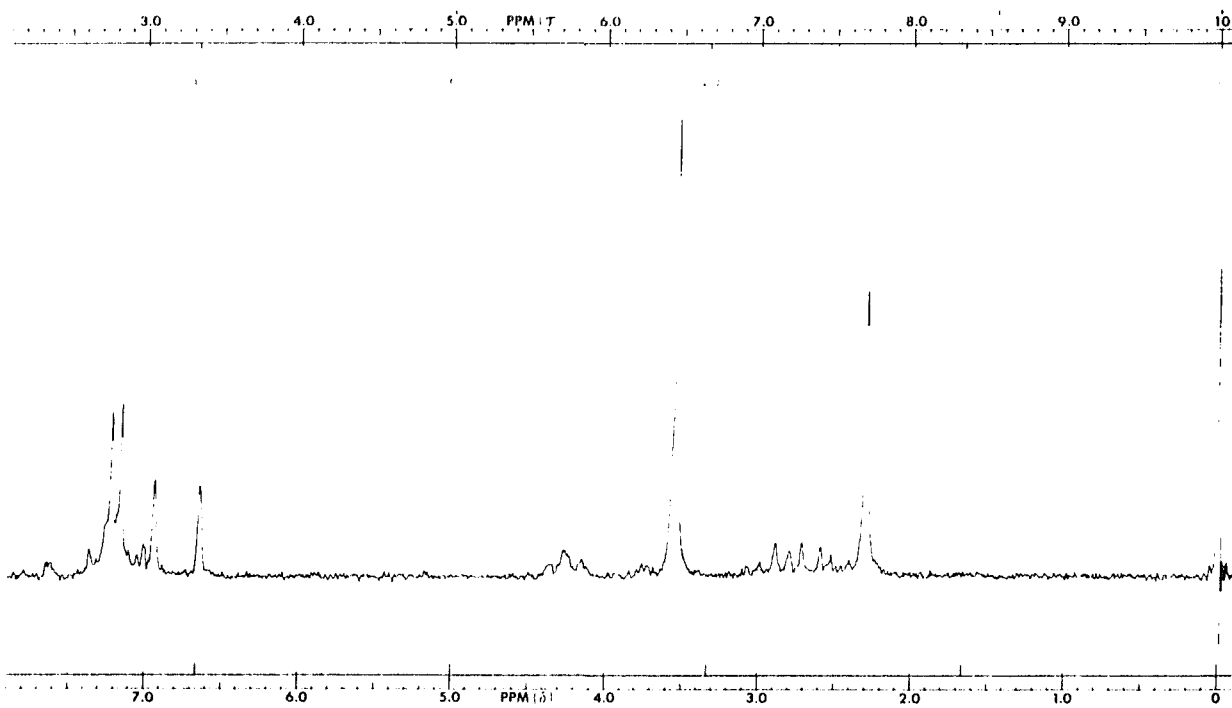
Figure 8. Nuclear magnetic resonance spectra

$(d_6\text{-DMSO})$

a) Lyrechine (XXX)

b) Cherylline (II)
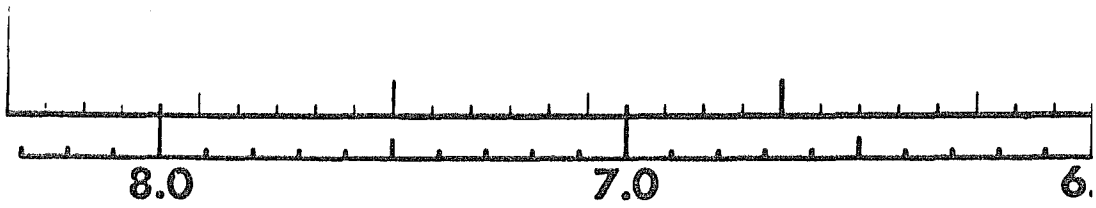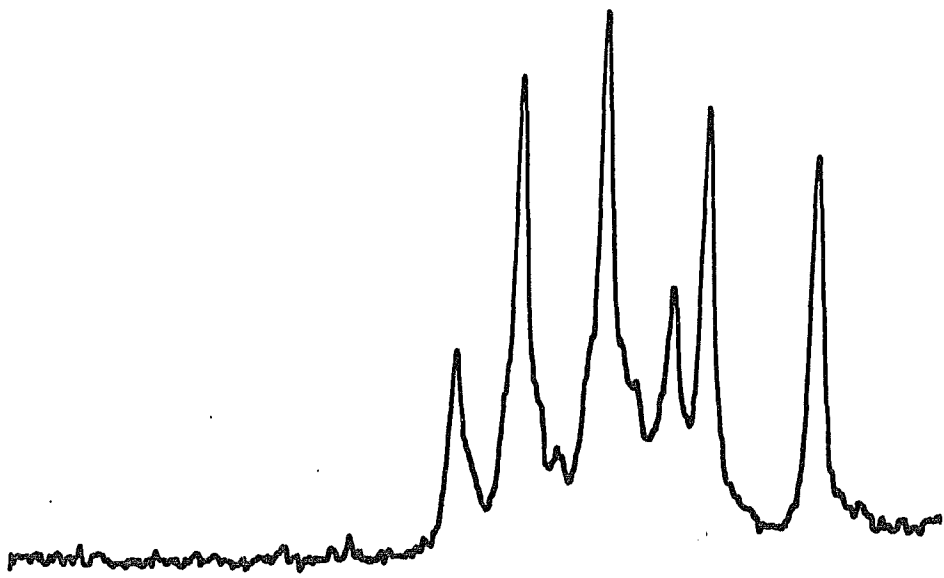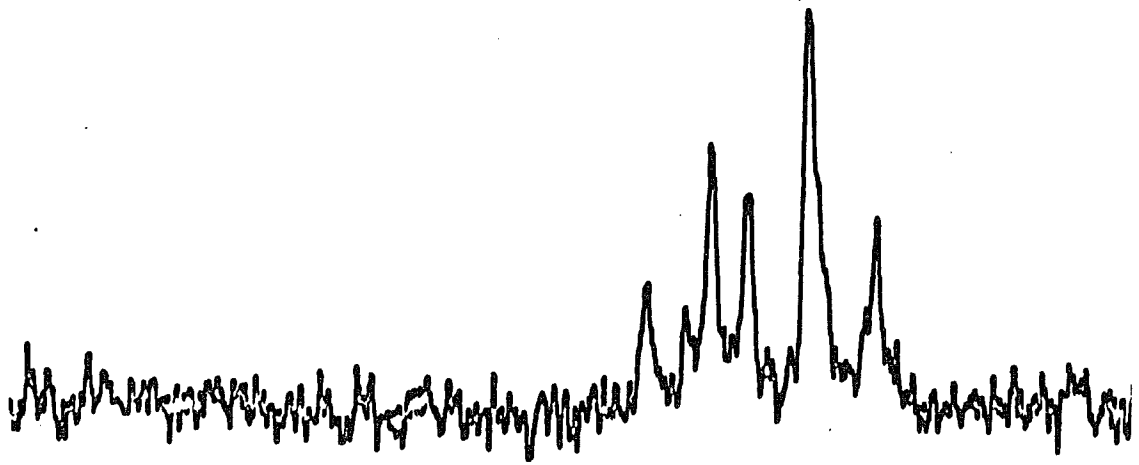
Table 4. Nuclear magnetic resonance assignments

	Cherylline				IA63	
Chemical Shift	Multiplicity	Relative Integral	Assignment	Relative Integral	Multiplicity	Chemical Shift
			Pyridine-d_5			
2.28	singlet	3	N-methyl	3	singlet	2.26
2.4-3.1	multiplet	2	C-3 methylene	2	multiplet	2.7-3.0
3.52	singlet	5	aromatic methoxyl	3	singlet	3.65
			C-1 methylene	2	quartet (J=14 hz)	3.65
4.27	broad triplet	1	C-4 methine	1	broad unresolved	4.65
6.64	singlet	1	C-5 aromatic			
6.93	singlet	1	C-8 aromatic			
7.0-7.4	quartet (AA'BB') (J=9 hz)	4	ring C aromatic	6	quartet(AA'BB') (J=9 hz)	7.0-7.6
			ring A ortho pair		quartet (AB) (J=9 hz)	6.6-7.0
			DMSO-d_6--Aromatic Region Only			
6.27	singlet	1	C-5 aromatic			
6.51	singlet	1	C-8 aromatic			
6.6-7.1	quartet (AA'BB') (J=8.5 hz)	4	ring C aromatic	6	quartet(AA'BB') (J=8 hz)	6.5-7.0
			ring A ortho pair		quartet (AB) (J=8 hz)	6.5-6.9

difficult. The additional information in Figure 8 on the
aromatic region provided by spectra in DMSO-d$_6$ proves useful.
The four proton AA'BB' pattern representing para disubstitu-
tion of IA63 is much more apparent, as is part of the pattern
demonstrating the ortho coupling of the two remaining aro-
matic protons. The assignment of spectral singlets in cheryl-
line to C-5 and C-8 is based on changes in chemical shift
upon addition of base. The downfield proton moved further
upfield than its counterpart when the phenolate anion was
formed, indicating that C-8 is ortho to the phenol, rather
than meta. The same treatment applied to IA63 caused the AB
pattern to expand. Hence the upfield proton is ortho or
para to the phenol.

In Table 5 are presented the masses and intensities of
the major ions from the mass spectra of cherylline and IA63
at several energies. The molecular ions of both compounds
are found at 285 m.u. The intensities of most major fragments
are comparable, although the 191 m.u. ion is much more pre-
valent for lyrechine than for cherylline. In the 16 ev.
spectra, cherylline appears to fragment almost exclusively
through the ion at 242 m.u., while IA63 has considerable ion
intensity at 191 m.u. The metastable ions from the 16 ev.
spectrum of lyrechine identify several major fragmentation
patterns. Reasonable structures for these decomposition pro-
ducts are given in Figure 9. In terms of these structures,
the low energy fragmentation of cherylline is almost exclusively

by way of the retro-Diels-Alder process which produces the 242 m.u. ion, while IA63 has a comparable alternate fragmentation directly to 191 m.u. The steric effects associated with an <u>ortho</u> substituent would reasonably account for this difference. The direct fragmentation of the molecular ion to the 166 m.u. ion in both spectra is of some interest. Although this is not a dominant decomposition path for either compound, it could represent a reversal of the biological process which results in the original ring closure to form these compounds as suggested by the ring-opened structure in Figure 9.

Consolidation of the spectral evidence gives enough information to propose a reasonable structure for the compound. The mass spectrum furnishes the same molecular formula and primary fragmentation patterns as for cherylline. The closeness of the individual proton correlations in the NMR are particularly strong evidence for a 4-phenyltetrahydroisoquinoline structure with the functional groups of cherylline. The <u>para</u>-hydroxyphenyl moiety also appears to be a shared feature, but the ring A substitution pattern differs. While the <u>para</u>-oriented protons are obvious in the cherylline spectrum, the more complex spectrum of IA63 appears to contain an <u>ortho</u>-coupled pair. The remaining hydroxyl and methoxyl substituents must be adjacent, due to the infrared evidence of strong intramolecular hydrogen-bonding. The remaining spectral data generally support such a structure, but provide no additional

insights to discriminate among the four possible hydroxy-
methoxy arrangements at C-5,C-6 or C-7,C-8.

OH

HO

CH₃O

N CH₃

XXX
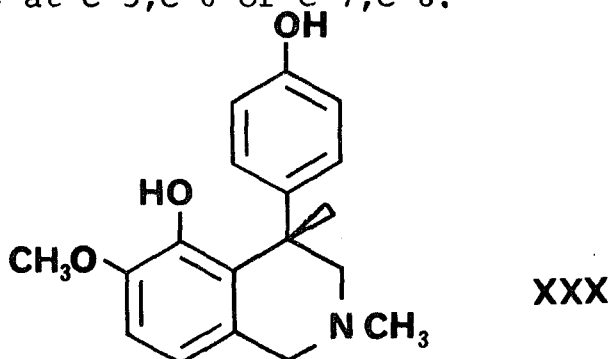
At this point it is necessary to inject the elements of
reason to single out the most likely structure for the com-
pound, though not necessarily to ascertain its correctness.
Consideration of the biosynthetic background of the source of
the compound argues for a structure derivable from 4-O-methyl-
norbelladine(VIIIb). The 5-hydroxy-6-methoxy alternative is
the only structure which fills this specification. This
structure is in harmony with the spectral observations, but
further proof is desirable. Chemical degradation is precluded
by lack of material. Synthesis of the compound seems to offer
the best possibility. In view of the proposed structural re-
lationship of the compound to cherylline, the name lyrechine
(XXX) is suggested. This structure proposal implies a close
chemical and biosynthetic relation between cherylline and
lyrechine. Chemically they become the favored products of an
intramolecular electrophilic attack on ring A; cyclization
occurs either _ortho_ or _para_ to the phenol. The predominance
of the _para_ substitution product in this plant follows normal
chemical tendency. Thus, a similar biosynthetic relationship

Table 5. Major mass spectral ions

Cherylline				IA63		
Intensity			Mass Units (m.u.)	Intensity		
70ev.	20ev.	16ev.		70ev.	20ev.	16ev.[a]
52	22	27	286	21	23	20
100	100	100	285	100	100	100
21	9	7	284	30	12	11
176	138	95	242	92	51	32
128	29	5	241	52	14	4
100	29	1	225	62	16	4
86	24	1	211	70	18	5
52	13	1	210	82	21	6
7	9	2	191	40	25	21
21	13	3	166	17	7	5
17	4	1	165	18	2	1

[a]Metastable ions at 16 ev.

$$96.7 \quad (285 \to 166)$$

$$128.0 \quad (285 \to 191)$$

$$184.0 \quad (242 \to 211)$$

$$205.0 \quad (285 \to 242)$$
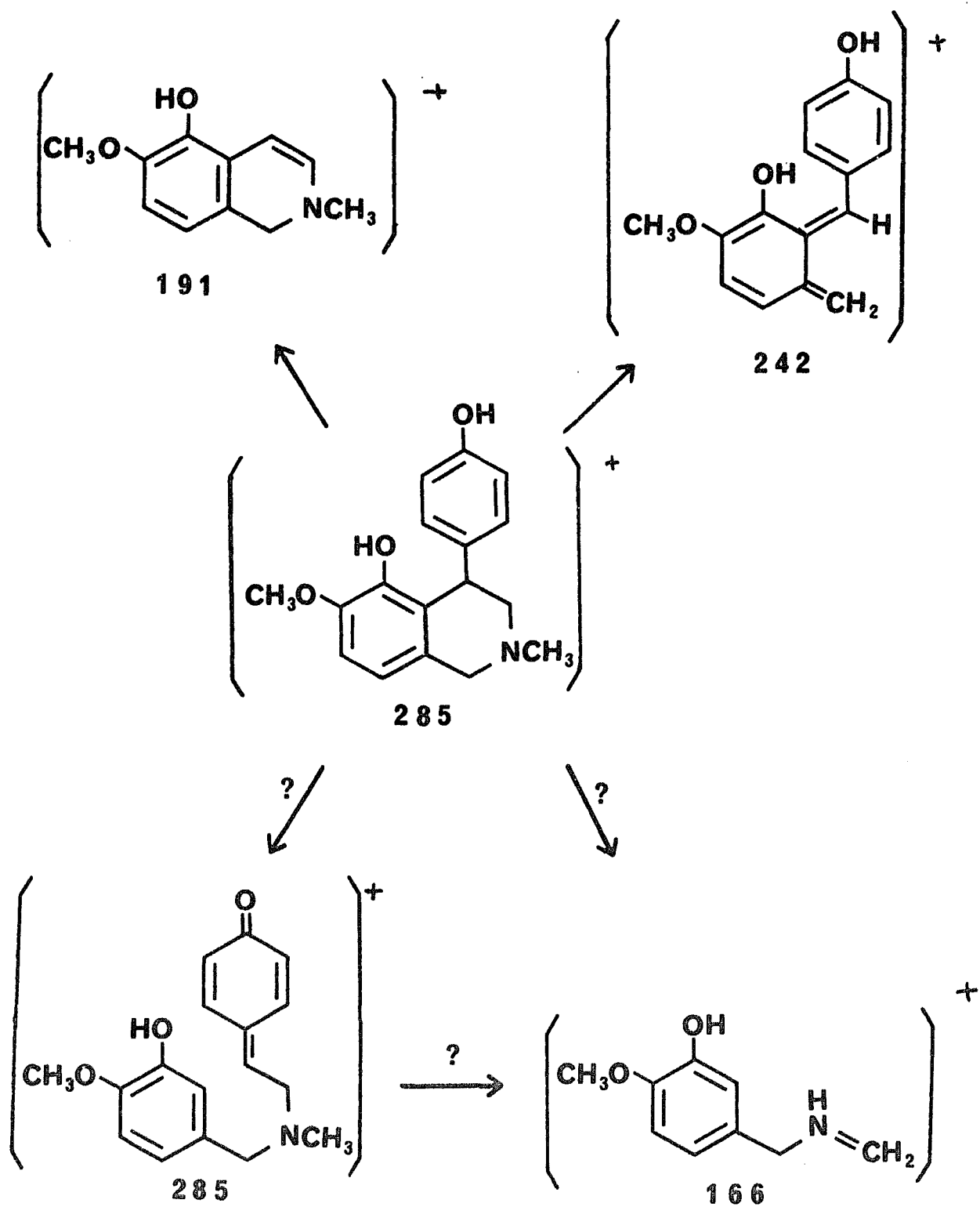
Figure 9. Mass spectral ions from lyrechine

based on a 4-O-methylnorbelladine would also be expected.

Further isolations

Two further isolation investigations of Crinum were under-
taken specifically to determine the extent of occurrence of
phenolic alkaloids in this species. Phenolic isolations were
carried out on single bulbs(app. 1 kg) of Crinum erubescens
and Crinum powellii album. Although separation of the phenolic
fraction produced other polar, but nonphenolic, alkaloids,
the only identifiable phenolic compounds in each plant were
the previous three. Quantities isolated are included in
Table 2. This family of compounds appears to be generally
distributed throughout Crinum. No attempts were made to in-
vestigate other species of the Amaryllidaceae.

Biosynthetic speculation

The presence of these three compounds together provokes
some speculation on the position and importance of the cheryl-
line system in biosynthesis of Amaryllidaceae alkaloids.
Several possibilities were mentioned in the background dis-
cussion of the functions of various other phenols. As was
stated, the newness of cherylline has thus far precluded
experimental studies on its relation to other compounds.
Speculation is initiated with the realization that the cheryl-
line ring system was not considered by Barton and Cohen under
the general topic of oxidative coupling of phenols(13). This
was due to lack of prior knowledge, rather than to inadequacy
of the concept. Simple inclusion of the benzylic carbon of
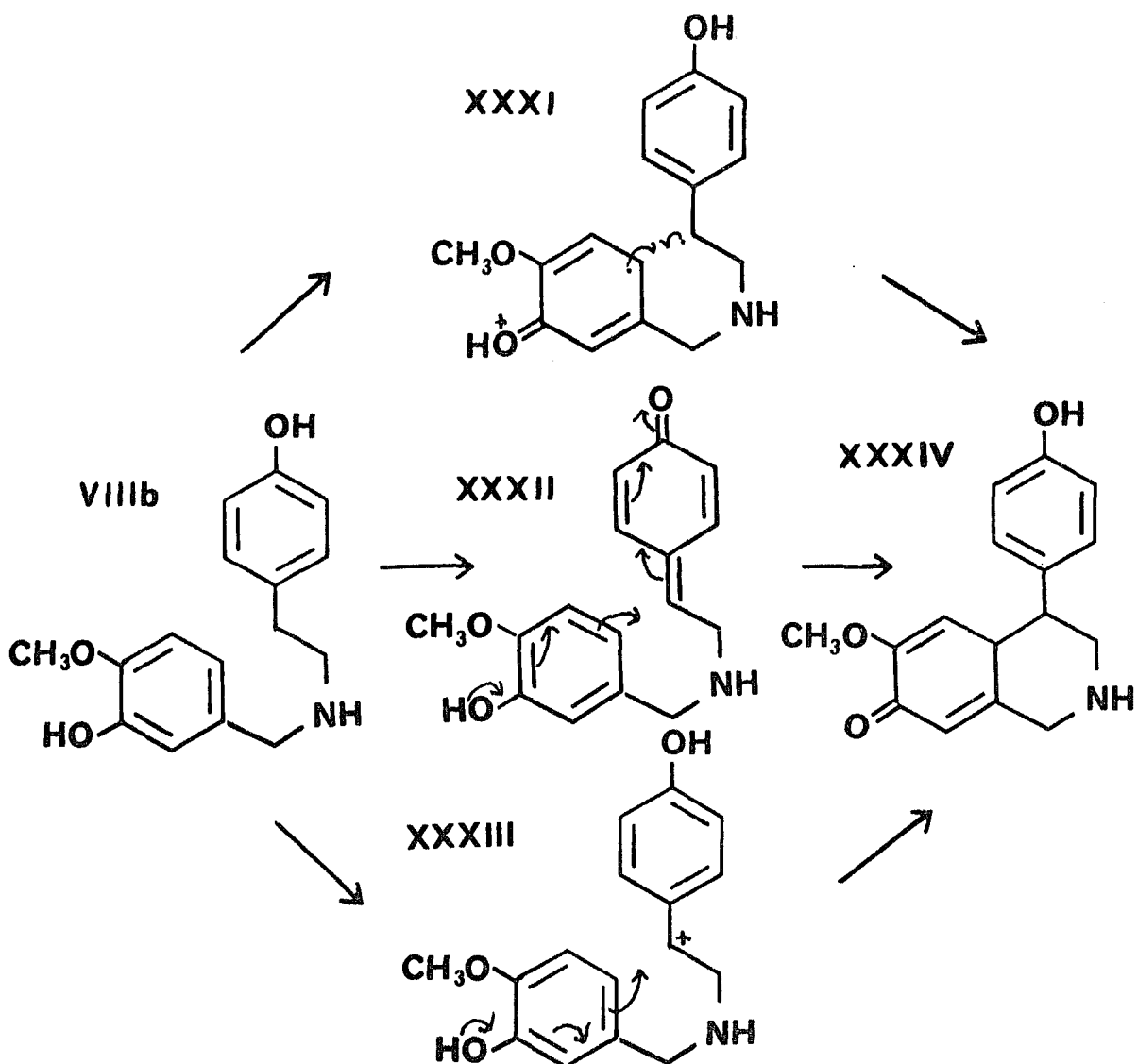
ring C in the oxidative process provides a proper basis. The

same degree of oxidation—two electrons per molecule—is

necessary to produce the desired transformation, but in this

case at least part of the oxidation must involve that benzylic

position. The resonance-stabilized process can proceed

through XXXI as two independent one electron oxidations with

radical coupling, or through XXXII as a two electron oxidation

to a quinone-methide structure with electrophilic attack. As

a plant process, the change might involve benzylic hydroxylation

(XXII) with dehydration to XXXII, or formation of the carbonium ion(XXXIII) and electrophilic attack. Although the formal representations of XXXII and XXXIII seem very different, chemical distinctness of the two species is questionable. The dipolar nature of the quinone-methide and unknown nature of bonding of ions in the plant could make them only shadings of each other or of another similar species. Although the illustration uses cherylline, the same processes produce lyrechine if cyclization is <u>ortho</u> to the phenol, instead of <u>para</u>.

There is no reason to suspect anything other than a straightforward biosynthetic path from norbelladine to cherylline and lyrechine. The particular interest in the order of processes of cyclization, O-methylation, and N-methylation can be satisfied only with tracer experiments with the potential precursors. The presence of 4-O-methylnorbelladine in isolable amounts suggests initial O-methylation and also indicates a likely starting place for tracer experiments.

Further biosynthetic interest in this pair of compounds stems from thought in other directions. Cherylline derivatives attract the most speculation in this species because of the domination of 6,7-oxygenation in <u>Crinum</u>. The most likely form for biosynthetic conversion is the N-demethylcherylline (xLe), since the norbelladine series has demonstrated repeatedly that N-demethylation is not an efficient process in the Amaryllidaceae. A possible biosynthetic change is a 1,2-aryl migration of ring A from C-4 to C-1' to produce XXXV, which leads

to the crinine ring system. The similar 1,4-migration to
produce XXXVI, and subsequently the lycorine ring system,
would appear to be less likely. There is little obvious
driving force for such rearrangements, since either process
generates an aryl carbanion on a ring which is already electron
rich, and each requires a subsequent hydride transfer.
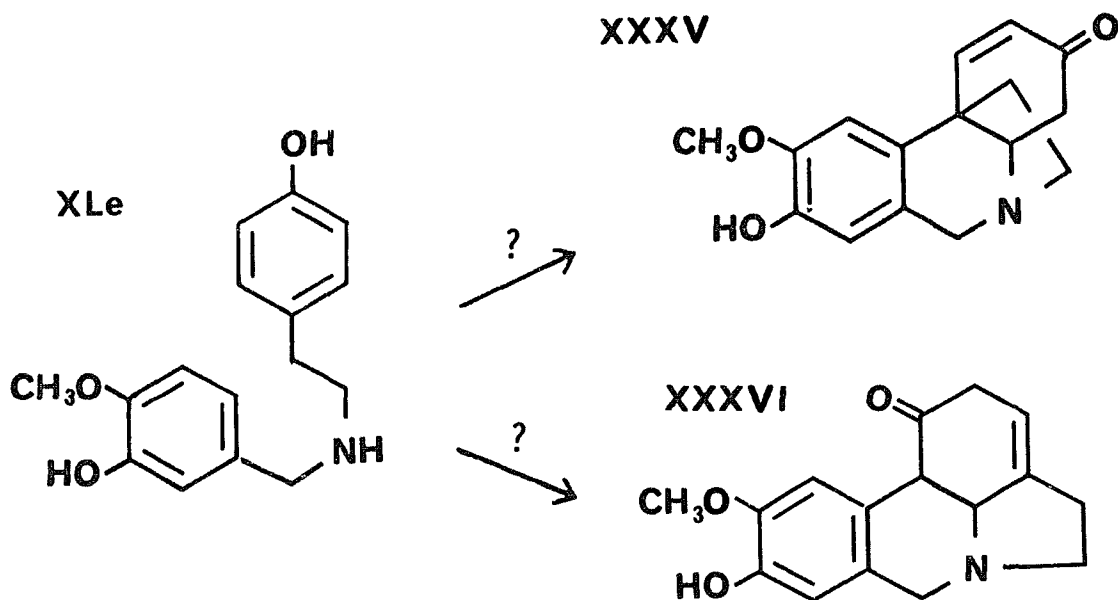
It is also notable that although an additional ring has
been formed by an oxidative process, both compounds still
possess intact the two independent phenolic aromatic rings
which are the necessary ingredients for the oxidative process
seen in the norbelladine series. A second phenolic oxidation,
accompanied by 1,2-aryl migration from C-4 to C-1', converts
XLe to an 11-hydroxy compound(XXXVII) with the crinine ring
system. Since the stage of biosynthesis at which this posi-
tion is hydroxylated has not been established, the process

has not been proved implausible.

XXXVII
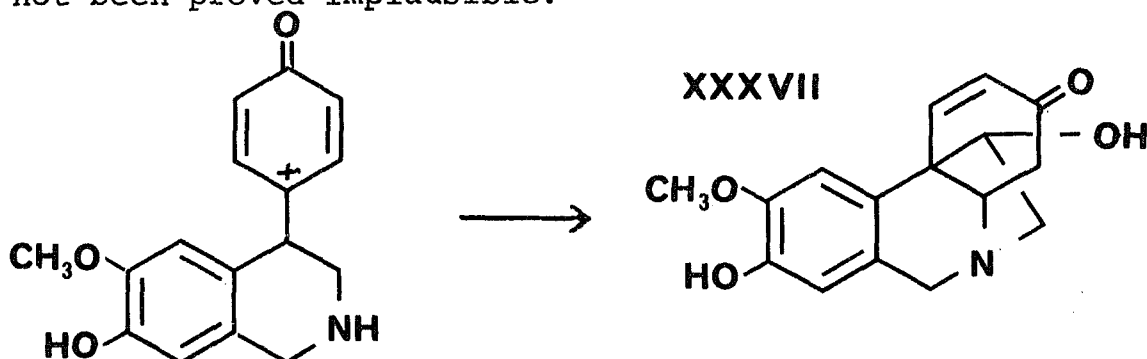
The lyrechine 5,6-oxygenation pattern is virtually unknown

in <u>Crinum</u>. It would seem, therefore, that the compound has

little potential to serve as a biosynthetic intermediate of

any significance in this species. It does, however, remain an

object of peculiarity and interest from the mere standpoint

of its formation in a species in which other oxygenation

patterns predominate. In other species with greater abundances

of compounds with similar substitution, lyrechine is a possible

biosynthetic intermediate.

Isolation summary

Three different <u>Crinum</u> species of the Amaryllidaceae

produced the same structurally-related set of three phenolic

alkaloids. The structural relationship of the two cyclized

N-methyl compounds and the open-chain, nonmethylated analog

was demonstrated. No evidence of the presence of the corre-

sponding N-methyl, open-chain compound was found, but no con-

certed effort was made to prove the presence or absence of

detectable amounts of that specific compound.

The possibility that cherylline and lyrechine might

serve as significant biosynthetic intermediates was considered.

Although the lyrechine 5,6-oxygenation pattern is relatively rare in this family, the 6,7-oxygenation of cherylline is standard. The necessity of N-demethylation makes cherylline an unlikely general precursor, but the possibility that the secondary amine analog might effectively serve as an intermediate for the very abundant crinine ring system was demonstrated.

Synthetic Investigations

Statement of aims

Specific syntheses of cherylline were mentioned earlier as background historical material. These syntheses have been published since the synthetic investigations reported in this dissertation were completed. Since the overall purposes of the present synthetic investigations differ considerably from those cited for cherylline, the usefulness of such syntheses to this investigation is small.

The obvious initial reason to undertake total synthesis of cherylline and lyrechine is to provide unequivocal proof of the correctness of the assigned structures, particularly that of lyrechine. The structure of cherylline had been reasonably well established by several methods before publication of the initial isolation, even though the compound had not actually been synthesized. The structure of lyrechine was a different matter, since several similar structures could reasonably fit the available information. No synthetic analogs of lyrechine were available, so differentiation between the

possible structures could prove to be more difficult. The main concern of any such proof of structure synthesis is that the reactions proceed in a well-understood manner to produce products which distinguish between the different possibilities.

A subsequent and considerably more confining purpose for undertaking such a synthesis stems from the desire to use the synthetic routes to produce radioactively tagged compounds for tracer experiments to study biosynthetic relations in the plant. Among general considerations, most radioactive syntheses utilize:

1. Label inclusion by well-defined (specific) reactions.

2. A convenient source of radioactive label.

3. Convenient (safe) workup of radioactive reactions.

4. Label introduction late in the synthesis.

Additional desired characteristics arise from particular limitations and requirements of the project to be undertaken. In light of the suspected structural relation of the desired products of this investigation with 4-O-methylnorbelladine (VIIIb), it was considered desirable to label separate parts of the molecule with different isotopes (^{14}C and ^{3}H). It was also intended that tracer experiments with both N-methyl and N-H analogs be conducted. The additional restrictions of the projected syntheses were that they:

1. Obtain both the N-H and N-methyl compounds of the respective series.

2. Allow labelling with ^{14}C and ^{3}H in separated parts

of the molecules.

Syntheses completed

The literature information previously covered offers numerous possible approaches to the desired ring system, but none are proven routes to specific phenolic compounds. Consideration of the restrictions just mentioned above does not clearly indicate a possible route which offers the best opportunity of successful synthesis. The greater danger is not that of exhausting all possible approaches without success, but rather that of becoming bogged down in an alternative with little chance of success while more likely candidates await. Several unsuccessful routes and numerous variations were investigated during the course of the synthetic work, but only those which gave some success will be considered here.

a) R_1 = OH
    R_2 = H

b) R_1 = H
    R_2 = OH
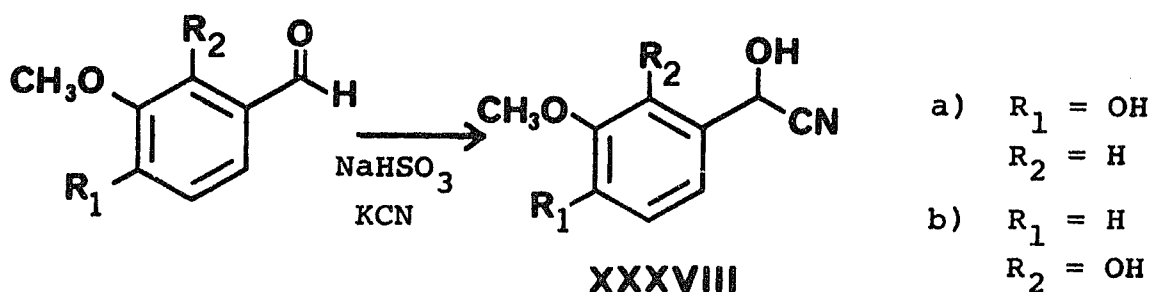
The condensation which initiated the first successful synthetic sequence is derived from a literature procedure(35) for condensation of methoxymandelonitriles with phenols and phenolic ethers in a reaction medium of 73% sulfuric acid. Later workers attempted to apply the method to phenolic mandelonitriles(36), but observed decomposition of the cyanohydrin

Table 6. Diphenylmethane structures
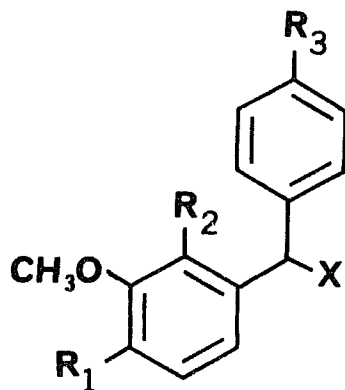
XXXIX

	R_1	R_2	R_3	X
a)	OH	H	OH	CN
b)	OH	H	OH	CH_2NH_2
c)	OH	H	OH	$CH_2NHCO_2CH_2Ph$
d)	OCO_2Et	H	OCO_2Et	$CH_2NHCO_2CH_2Ph$
e)	OH	H	OH	$CH_2NHCOPh$
f)	OCH_2Ph	H	OCH_2Ph	$CH_2NHCOPh$
g)	OCH_2Ph	H	OCH_2Ph	CH_2NHCH_2Ph
h)	OCH_2Ph	H	OCH_2Ph	CH_2NHCO_2Et
i)	OCH_2Ph	H	OCH_2Ph	CH_2NHCH_3
j)	OCH_2Ph	H	OCH_2Ph	CN
k)	OCH_2Ph	H	OCH_2Ph	CH_2NH_2
m)	H	OH	OH	CN
n)	H	OH	OH	CH_2NH_2
o)	H	OH	OH	$CH_2NHCO_2CH_2Ph$
p)	H	OCO_2Et	OCO_2Et	$CH_2NHCO_2CH_2Ph$
q)	OH	H	OH	CH_2NHCO_2Et

Table 7. Cyclized structures
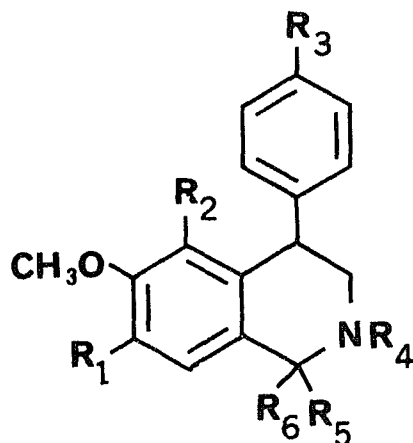
	R_1	R_2	R_3	R_4	R_5	R_6
a)	OCO_2Et	H	OCO_2Et	H	O	O
b)	OH	H	OH	H	O	O
c)	OCH_2Ph	H	OCH_2Ph	H	O	O
d)	OCH_2Ph	H	OCH_2Ph	H	H	H
e)	OH	H	OH	H	H	H
f)	OH	H	OH	Me	H	H
g)	OCH_2Ph	H	OCH_2Ph	CH_2Ph	H	H
h)	OCH_2Ph	H	OCH_2Ph	Me	H	H
i)	H	OCO_2Et	OCO_2Et	H	O	O
j)	H	OH	OH	H	O	O
k)	H	OCH_2Ph	OCH_2Ph	H	O	O
m)	H	OCH_2Ph	OCH_2Ph	H	H	H
n)	H	OH	OH	H	H	H
o)	H	OH	OH	Me	H	H

functionality, rather than aromatic condensation. This particular reaction was valued as an initial synthetic step, since a number of vital structural features can be incorporated with minimal effort. Using the appropriate initial aromatic aldehyde, a successful reaction introduces the desired substitution of ring A, the para-hydroxyphenyl moiety of ring C, and the properly positioned heteroatom for development of hydroaromatic ring B(XXXIXa) in a single step.

Attempted execution of the literature procedure with vanillin(XXXVIIIa) did indeed result in decomposition of the cyanohydrin, but because of the high potential usefulness of the sequence, a number of procedural modifications were attempted. A successful variation was found in which a catalytic amount of concentrated sulfuric acid was added to a stirred melt of the reactants at elevated temperature. Excellent yields of compounds with the substitution patterns of both cherylline(XXXIXa) and lyrechine(XXXIXm) were obtained in this manner. Exclusion of water from the reaction mixture appears to be a necessary condition for the successful reaction. Under neat conditions, decomposition of the mandelonitrile by water or hydroxide displacement of cyanide is evidently preempted by protonation and formation of the intermediate benzyl cation, which then condenses rapidly with phenol.

Completion of ring B seemingly involves just two steps; reduction of the phenolic nitrile(XXXIXa) to a phenolic amine (XXXIXb), and attachment of this amine to ring A by addition

of a one carbon fragment. Organic Reactions(24) offers several methods for this general isoquinoline closure. The simplicity of this approach becomes complicated when the functional group interactions of an amphoteric molecule are considered. The reactions and synthetic routes classically used for production of the isoquinoline nucleus are not directly applicable to molecules with phenolic substituents. Functional group manipulations are necessary.

Interference from the phenols complicated attempts to reduce the nitrile. Lithium aluminum hydride reduction failed, since phenoxide salts were precipitated before reduction could proceed. Neither sodium borohydride nor diborane reduces this functional group efficiently. Low pressure catalytic hydrogenation caused no significant amount of reduction. A literature method for reduction of nitriles with Raney nickel alloy in 5% ethanolic sodium hydroxide(37) did accomplish the reduction in high yield, but the amphoteric product was not separable from aluminum oxide coprecipitated by neutralization of the product mixture. This complication was adequately circumvented by derivatization of the amine before isolation was attempted.

The ethyl carbamate(XXXIXq) was the first in situ derivative prepared, and, for the sake of convenience, the corresponding carboethoxy esters of the phenols were added as protecting groups. The carbamate did not cyclize readily. Reaction conditions rigorous enough to decompose the carbamate

function resulted in formation of an isocyanate. This inter-
mediate could be induced to cyclize to the desired product(XLa)
only in poor yield. The benzyl carbamate(XXXIXc) was subse-
quently produced in an analogous manner. After addition of
carboethoxy esters(XXXIXd), cyclization was again attempted.
Compound XLa was produced smoothly and in high yield, although
much milder reaction conditions were employed. Utilization
of the benzyl leaving group for this type of cyclization
is not precedented in the literature, but it seems to allow a
very facile and specific transformation under relatively
mild conditions. With the ring system complete, removal
of the amide carbonyl was routinely accomplished by saponifi-
cation(XLb), benzyl ether formation(XLx), lithium aluminum
hydride reduction (XLd), and catalytic hydrogenolysis of the
protecting groups. The secondary amine product(XLe) was
readily converted to racemic cherylline(XLf) with formaldehyde
and sodium borohydride. Comparison of thin layer R_f values in
several solvents, as well as infrared, NMR and mass spectral
data, indicated that the racemic material had properties iden-
tical with those of natural cherylline.

This reaction sequence presents the synthesis of racemic
cherylline. A directly analogous scheme was found to effect
a successful synthesis of lyrechine(XLo). Except for the
necessity of initiating the mandelonitrile-phenol condensation
at 100°C. instead of 80°C., the same reaction conditions pro-
duced comparable yields in each step of the two sequences.

by TLC, NMR, infrared, and mass spectra demonstrated structural identity with natural lyrechine.

These unambiguous synthetic routes fully confirm the assigned structures of the naturally occurring alkaloids. In addition, they provide an excellent method of incorporation of a tritium label into each structure system. Reduction of the respective benzylated amides(XLc and XLk) with tritiated lithium aluminum hydride generates the tetrahydroisoquinoline which requires only removal of the protecting groups for completion. This route, however, does not allow convenient incorporation of radioactive carbon into the molecular skeleton, since numerous chemical manipulations must be performed after the skeletal construction is completed.

Since the previous synthetic route serves well to incorporate tritium into that portion of the molecule derivable from the C_6-C_1 fragment of 4-O-methylnorbelladine(VIIIb), the desired complementary synthesis allows introduction of ^{14}C into a position derivable from the C_6-C_2 portion of VIIIb. A more applicable synthetic route was found initiated from the nitrile(XXXIXa). Derivatization of the nitrile reduction product(XXXIXb) with benzoyl chloride gives the benzamide (XXXIXe), which, after benzylation of the phenols(XXXIXf), can be reduced with lithium aluminum hydride to the corresponding benzylamine(XXXIXg). Pictet-Spengler cyclization with formaldehyde to XLg is accomplished with methanol as solvent. Removal of the three protecting groups by catalytic

hydrogenolysis to give XLe is aided by addition of a small amount of perchloric acid.

Each reaction involved in this sequence produces a single product in good yield so that intermediate purifications are facilitated. With fewer manipulations of the protecting groups, the total number of steps involved is lessened. So despite the fact that the C_6-C_2 portion of the cherylline structure is introduced in the initial steps of the synthesis, this route provides a better method for introduction of a ^{14}C label. Relatively inexpensive potassium cyanide-^{14}C can be used in the initial cyanohydrin formation to introduce the radioisotope.

Several other synthetic variations on this same theme were also found to give cherylline. A route directly to the N-methyl compound(XLf) was found by treatment of the nitrile reduction solution with carboethoxy chloride to give the ethyl carbamate XXXIXq. In direct analogy to the previous route, the sequence of benzyl ether formation(XXXIXh), lithium aluminum hydride reduction to the N-methylamine(XXXIXi), Pictet-Spengler cyclization with formaldehyde(XLh), and catalytic hydrogenolysis of the protecting groups leads directly and cleanly to cherylline.

The shortest variation in this series is accomplished by protecting the phenolic groups of the original nitrile (XXXIXj), reducing the nitrile with lithium aluminum hydride (XXXIXk), cyclizing with formaldehyde to XLd, and regenerating the free phenol(XLe). Unfortunately, the nitrile reduction is

not a high yield reaction, and the product is not readily purified. The reaction sequence has been completed and the phenolic amine obtained, but in unimpressive yield.

As was indicated earlier, closure of the appropriate benzyl carbamate provides both a synthetic access to the lyrechine structure and also a convenient method of introduction of a tritium label into the compound for tracer experiments. Each of the other sequences which proved successful for the synthesis of cherylline were applied to attempts to synthesize lyrechine by other methods. All cyclizations failed completely. An indication of the source of these synthetic difficulties is provided in the proton NMR spectra of several benzyl ethers produced in intermediate steps. The methylene protons of the benzyl ether functions which appear in all cherylline analogs as a pair of two proton singlets near 5.0 ppm., differ in the lyrechine analogs. One methylene, logically assignable to the ring C _para_ position, is a normal singlet. The other methylene, however, is represented by a sharp AB pattern from 4.2 to 4.9 ppm. with a typical gem coupling constant. Evidently the bulky benzyl protecting group introduced _ortho_ to the ring-joining carbon severely restricts rotation of this methylene group. This rigid conformation could also prevent normal cyclization if the reactive amino group is directed away from ring A. That such difficulty is not encountered in the carbamate cyclization may be attributed to the different steric requirements of the less bulky carboethoxy protecting group

in use. A similar cyclization with more bulky carbobenzoxy

protecting groups was unsuccessful in the lyrechine system,

but was not attempted in the cherylline system, so no strong

implications can be based on that failure.

Labelled syntheses

Identical tritium labelling experiments were carried out

on the protected amides (XLc and XLk) of each substitution

pattern with ^{3}H-lithium aluminum hydride as described in the

experimental section. The procedure was not altered from that

used in unlabelled synthesis, but particular care was taken

to exclude moisture from the reaction in view of the small

scale of the reaction and of the water-sensitive nature of the

precursor material. The reaction proceeded smoothly to incor-

porate nearly 80% of the total tritium activity into the tetra-

hydroisoquinoline product in each case. This was considered

to be a highly successful result.

Carbon-14 labelling of cherylline was initiated with

vanillin, the starting aldehyde. Alteration of the literature

procedure for conversion to the cyanohydrin(38) was necessary,

since the required fourfold excess of potassium cyanide was

not a justifiable expense. A reverse addition was devised

which produced nearly 85% product based on cyanide as limiting

reagent, as compared with 80% for the usual reaction with

vanillin as limiting reagent. The sequence described earlier

was performed normally through the ring closure step(XXXIXe

to XLg). A glass breakage accident at that stage caused loss

of most of the material so that the overall yield in the labelling sequence could not be accurately determined, but trial experiments on the same scale had given overall yields of 20-30%. Enough labelled material was recovered to provide a confirmatory ^{14}C label for tracer experiments, but not at the level originally anticipated.

Attempts to incorporate a ^{14}C label into the lyrechine nucleus by the route used for tritium labelling were defeated. There were two principal contributory causes. Incorporation of the tracer label into the molecular structure in the initial stages of a multi-step synthesis inevitably decreases the probability of success. The more serious problem arose in reducing the scale of the reactions to that needed for labelling. Product purification problems were encountered. The nitrile reduction step (XXXIXm to XXXIXn) did not proceed properly, presumably due to the presence of unreacted phenol in the material to be reduced. In view of the questionable importance of the lyrechine compounds in biosynthetic processes in Crinum species, the single tritium label was judged sufficient to initiate feeding experiments with the tracer labelled compounds after two unsuccessful attempts at this synthesis.

Synthetic summary

Assigned structures of both cherylline and lyrechine were fully confirmed by unequivocal synthesis. Whereas only a single method was found for synthesis of lyrechine, a number proved successful for production of cherylline. The principal

consequence of this difficulty was that while radioisotopic labelled synthesis produced double labelled compounds for the cherylline substitution, the corresponding lyrechine compounds had only tritium labels. The labelled compounds produced were:

1. $1-^3H-3-^{14}C-N$-demethylcherylline (XLe).

2. $1-^3H-3-^{14}C$-cherylline (XLf).

3. $1-^3H-N$-demethyllyrechine (XLn).

4. $1-^3H$-lyrechine (Xlo).

Biosynthetic Investigations

Tracer experiments were carried out on available Crinum species which were known to contain cherylline and lyrechine. Crinum erubescens was used for the initial feeding and Crinum powellii album for all others. An appropriate quantity of the labelled compound was administered by injection into the bulbs as an aqueous solution of the hydrochloride salt. Feeding experiments were conducted in the fall on actively growing plants. A ten day growing interval was maintained between injection and harvesting of the plants.

Feeding results

A total of five feedings are included in this description. In addition to the four compounds for which syntheses have been described, a preliminary feeding of $1-^3H-4-O$-methylnorbelladine (VIIIb) was conducted. This experiment was a cooperative effort with Dennis L. Oltmans in conjunction with related tracer experiments he was conducting (39). The labelled synthesis of VIIIb, which is described in the reference, and

Table 8. Precursors for feeding experiments

Feeding	Compound	Quantity (mg)	Specific Activity $\frac{dpm}{mg}$	Total Activity (dpm)	Isotope	$^{14}C/^{3}H$ Ratio
O	VIIIb	4.12	1.59×10^{8}	6.55×10^{8}	^{3}H	
A	XLe	7.21	6.75×10^{6}	4.87×10^{7}	^{14}C	0.699
			9.65×10^{6}	6.96×10^{7}	^{3}H	
B	XLf	2.38	3.38×10^{6}	8.04×10^{6}	^{14}C	0.0134
			2.52×10^{8}	6.00×10^{8}	^{3}H	
C	XLn	3.08	1.82×10^{8}	6.00×10^{8}	^{3}H	
D	XLo	3.76	1.82×10^{8}	6.84×10^{8}	^{3}H	

Table 9. Radioactivity incorporations

Compound	Quantity (mg)	Specific Activity (dpm)	Total Activity $\left(\dfrac{dpm}{mmole}\right)$	Percent Incorporation	Isotope	$^{14}C/^{3}H$ Ratio
			Feeding O			
Cherylline	94	6.87×10^{4}	6.46×10^{6}	0.986	^{3}H	
4-O-Methyl-norbelladine	20	4.06×10^{4}	8.20×10^{5}	0.126	^{3}H	
Lycorine	675	4.34×10^{4}	2.93×10^{7}	4.47	^{3}H	
Lyrechine	33	3.27×10^{3}	1.08×10^{5}	0.0165	^{3}H	
			Feeding A			
Cherylline	191	2.33×10^{1}	4.45×10^{3}	0.00914	^{14}C	0.0187
		1.25×10^{3}	2.39×10^{5}	0.343	^{3}H	
Lyrechine	15	3.74×10^{1}	5.61×10^{2}	0.00115	^{14}C	0.00706
		5.30×10^{3}	7.95×10^{4}	0.114	^{3}H	
4-O-Methyl-norbelladine	15	1.20×10^{2}	1.80×10^{3}	0.000370	^{14}C	0.0917
		1.76×10^{3}	1.96×10^{4}	0.0282	^{3}H	
Lycorine	1000	<1	$<10^{3}$	$<10^{-3}$	^{14}C	
		<1	$<10^{3}$	$<10^{-3}$	^{3}H	
Crinine	620	<1	$<6 \times 10^{2}$	$<10^{-3}$	^{14}C	
		<1	$<6 \times 10^{2}$	$<10^{-3}$	^{3}H	

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Table 9. Continued

Compound	Quantity (mg)	Specific Activity (dpm)	Total Activity $\left(\frac{dpm}{mmole}\right)$	Percent Incorporation	Isotope	$^{14}C/^{3}H$ Ratio
Feeding B						
Cherylline	131	1.03×10^4	1.35×10^6	16.8	^{14}C	0.0163
		6.31×10^5	8.27×10^7	13.8	^{3}H	
Lyrechine	15	2.78×10^2	4.17×10^3	0.0517	^{14}C	0.00313
		8.87×10^4	1.33×10^6	0.218	^{3}H	
4-O-Methyl-norbelladine	14	3.90×10^1	5.50×10^2	0.0068	^{14}C	0.0562
		6.96×10^2	9.74×10^3	0.00162	^{3}H	
Lycorine	1000	<1	$<10^3$	$<10^{-3}$	^{14}H	
		<10	$<10^4$	$<10^{-3}$	^{3}H	
Feeding C						
Cherylline	102	1.10×10^4	1.12×10^6	0.187	^{3}H	
Lyrechine	15	8.34×10^4	1.27×10^6	0.208	^{3}H	
4-O-Methyl-norbelladine	13	9.92×10^4	1.29×10^6	0.215	^{3}H	
Lycorine	1000	0	0	0	^{3}H	
Feeding D						
Cherylline	191	5.12×10^4	9.80×10^6	1.43	^{3}H	
Lyrechine	15	6.24×10^6	9.36×10^7	13.7	^{3}H	
4-O-Methyl-norbelladine	15	3.18×10^3	4.77×10^4	0.00698	^{3}H	
Lycorine	1000	0	0	0	^{3}H	

ing experiment were conducted by Mr. Oltmans. Compound puri-
fication and radioassay were completed as part of the present
investigation. The results of that experiment have not been
published elsewhere and are included in this presentation to
provide documentation of the work. In addition, the results
provide information pertinent to the present work.

The specific activities of precursor compounds are given
in Table 8, with the incorporations determined for compounds
isolated from each feeding summarized in Table 9. Per cent
incorporations for ^{14}C and ^3H are entered separately for double
label feedings. For double label feedings, an intact incor-
poration of a precursor would be indicated by identical incor-
poration percentages for each label. Analysis of the results
of individual feedings follows.

Feeding O   Tritiated 4-O-methylnorbelladine(VIIIb)
was converted to both cherylline and lyrechine, but not as
efficiently as to lycorine(XIV). The total incorporation
into each phenol corresponds roughly to its relative abundance.

Feeding A   The ratios of incorporated isotopes indicate
that intact incorporation of N-demethylcherylline is not a
significant biosynthetic pathway to either cherylline or lyre-
chine. However, significant incorporation of degraded portions
of the molecule, particularly the C_6-C_1 portion, into all
three phenols is evident. The activity found in 4-O-methyl-
norbelladine could be the source of that in the other com-
pounds(as in Feeding O), but, if so, the majority of activity

was processed during the growing period. No evidence of significant conversion to crinine or lycorine was found. The activity level found for 4-O-methylnorbelladine was too low to be detectably transmitted to lycorine at reported levels for that transformation.

Feeding B   Even though the quantitative efficiency of alkaloid recovery for plant workup is not known, it appears that a large part of the labelled material was not utilized by the plant. Activity of 4-O-methylnorbelladine again suggests some degradative incorporation, but the isotope ratio is close enough to that of the precursor that incorporation of the intact molecular skeleton cannot be eliminated. Incorporation into lyrechine is surprising. Since this activity is greater than that found in 4-O-methylnorbelladine, another method of formation of lyrechine must be available. No incorporation into lycorine is evident.

Feeding C   Absence of the complementary ^{14}C label decreases the information derivable from this feeding. The C_6-C_1 fragment with its label is extensively incorporated into all three compounds. The high specific activity of 4-O-methylnorbelladine(relative both to Feeding A and to the other compounds in Feeding C) indicates that the suggested degradative incorporation is more prevalent in this feeding. This could logically be due to the lesser abundance of the corresponding methylated alkaloid in the plant(i.e.--slower conversion to lyrechine), or to the fact that the plant has

difficulty processing the intact compound, but can use some degraded fragments. No incorporation into lycorine is found.

Feeding D   The surprising result is not the high percentage of activity recovered in the precursor, but rather the high incorporation into cherylline. The lack of alkaloids with this oxygenation pattern in the species indicated a strong possibility that lyrechine was a terminal alkaloid. However, this high incorporation is evidence for a very facile conversion to the other methylated phenolic compound. This process cannot proceed through 4-O-methylnorbelladine; the obvious alternative is an O,N-dimethyl compound. No incorporation into lycorine is found.

Biosynthetic summary

Correlation and coordination of the results of the individual feedings does not yield a simple, clear-cut view of the biosynthetic processes involved, but a series of valid observations can be made.

1) Tritiated 4-O-methylnorbelladine was converted efficiently into both cherylline and lyrechine. In view of 3), only one or the other need be produced directly from 4-O-methylnorbelladine, but there is no experimental evidence of such a circumstance.

2) The N-H compounds are not converted intact into the N-methyl compounds, nor are they incorporated exclusively to the methylated compound of the same substitution. This fact, together with 1), suggests that the biosynthesis of cherylline

and lyrechine proceeds primarily by N-methylation of 4-O-methyl-norbelladine before cyclization. The necessary experiment to confirm this route is a feeding of the appropriately labelled 4-O,N-dimethylnorbelladine(VIIIc).

3) The reciprocal incorporations found for cherylline and lyrechine establish that a definite equilibrium relationship exists between the two compounds, and that it does not involve VIIIb as an intermediate. It is possible that the corresponding N-methyl compound (VIIIc) might be involved in the equilibrium, but this is unlikely in view of the oxidative and reductive processes required for such a condition to exist.

4) All four cyclized precursors exhibit a degree of degradative incorporation to 4-O-methylnorbelladine, but the N-H compounds are used with considerably greater efficiency. The double labelled experiments indicate that the molecular skeleton is not converted to 4-O-methylnorbelladine intact. This type of degradative incorporation has precedent in the Amaryllidaceae(19), but not for so complex a molecule.

5) No evidence of incorporation of any of the cyclized compounds into lycorine was found. Hence, none of these compounds serve as a general precursor of the lycorine ring system.

6) No incorporation of N-demethylcherylline into crinine was found. Hence, the compound did not serve as a general precursor to the crinine ring system.

SUMMARY

A unique structural and biosynthetic relationship has been found to exist among three phenolic alkaloids which occur in isolable amounts in several <u>Crinum</u> species of the Amaryllidaceae.

The discovery that such a group of compounds was present and could be isolated stemmed from recent improvements in plant isolation technology which stimulated renewed interest in investigation of alkaloid content of such plants. The initial detection of compounds which proved to be phenolic directed efforts specifically toward examination of the phenolic alkaloid content of several species. Of the three compounds consistently found, one, 4-O-methylnorbelladine(VIIIb), was a known general precursor to most alkaloids in the plant family. It had previously been isolated only by dilution. The second, cherylline(II), was a recently identified phenolic constituent whose initial isolation was reported twenty years ago. The third, lyrechine(XXX), had not been reported previously. A tentative structure was proposed based primarily on comparison of chemical, physical, and spectral properties of the compound with those of cherylline.

Efforts to accomplish syntheses of cherylline and lyrechine were initiated with two general purposes in mind. The initial aim was confirmation of the proposed structures, with the structure of lyrechine as the main concern. A second prime interest was utilization of the completed syntheses to produce

radioisotopically labelled cherylline and lyrechine, and the
N-demethyl analogs of each, for biosynthetic tracer experiments.
Accomplishment of the first purpose was fully realized. Unam-
biguous syntheses of both cherylline and lyrechine were com-
pleted which confirmed the proposed structures and the close
structural relationship between the two compounds. Only partial
success was attained in the effort to perform tagged syntheses.
The initial synthetic sequence served well to introduce tritium
into the compounds of each substitution pattern. The compounds
with the cherylline substitution pattern were also labelled
with carbon-14 by a second synthetic sequence which was not
successful for the lyrechine series. Thus, double-labelled
cherylline(XLf) and N-demethylcherylline(XLe) were synthesized,
while lyrechine(XLo) and N-demethyllyrechine(XLn) were synthe-
sized with tritium labels only.

Tracer studies of biosynthesis were undertaken with each
of the four synthesized compounds. An additional experiment
with ^{3}H-4-O-methylnorbelladine(VIIIb) provided additional
information about the biosynthetic processes involved. The
label of VIIIb was found to be efficiently incorporated into
both cherylline and lyrechine. The N-demethyl compounds ap-
peared not to undergo direct methylation, although fragments
were incorporated into all three phenols. This constitutes
indirect evidence that methylation precedes cyclization in
the formation of cherylline and lyrechine from 4-O-methylnor-
belladine. The respective methylated compounds were found to

be interconvertible in the plant. High incorporation of the label of each into the other demands a facile equilibrium between the two involving carbon-carbon bond cleavage, reorientation of the aromatic ring, and recyclization. No evidence was found that these phenols act as precursors for alkaloids from other ring systems.

EXPERIMENTAL

Instrumentation

Infrared spectra were run on a Beckman Model IR-12 spectro-
photometer in chloroform solution, or as a potassium bromide
pellet, or as a film on sodium chloride or a KRS-5 internal
reflectance plate (Wilks Scientific). The proton nuclear mag-
netic resonance spectra were run in the indicated solvents,
on either a Varian A-60 or Hitachi Perkin-Elmer R-20B spectro-
meter operating at 60 Mhz. Mass spectra were obtained through
the Department of Chemistry Instrument Services from either
an Atlas CH-4 (low resolution) or AEI MS-902 (high resolution)
mass spectrometer. Ultraviolet spectra were run on a Cary
14 spectrophotometer in ethanol solvent. Radioactivities were
measured with a Packard Tri-Carb Liquid Scintillation Spec-
trometer (Model 3002) operating at ambient temperature.

Thin Layer Chromatography

Alkaloids were separated by preparative scale thin layer
chromatography (TLC) on 20 cm X 20 cm glass plates with a 0.5
mm layer of silica gel containing an ultraviolet indicator
(Merck PF_{254}). Three general solvent systems were used for
separations; solvent A (75 parts ethyl acetate/25 parts meth-
anol), solvent B (90 parts chloroform/5 parts diethylamine/
5 parts methanol), and solvent C (85 parts ether/5 parts diethyl-
amine/10 parts methanol). These ratios were sometimes altered
slightly to optimize separation characteristics. Separated
bands were visualized at 254 nm, sectioned, and scraped from

the plates. Alkaloid recovery was accomplished by suspension of the material in 1 N. hydrochloric acid, neutralization with ammonium hydroxide, and extraction with chloroform.

Sample Plant Workup

Crinum erubescens

Bulbs totalling 1 kg in weight were macerated with ethanol in a Waring blender and filtered. The residue was extracted two additional times. The filtrates(total volume 4.7 l.) were evaporated to one-fourth their original volume under reduced pressure, acidified(pH 4) with tartaric acid, and filtered. Extraction of the filtrate with benzene(2 X 200 ml) gave a 'neutrals' fraction; extraction with chloroform(3 X 70 ml) gave a 'chloroform-soluble salts' fraction. The aqueous solution was made weakly basic(pH 8) with ammonium hydroxide and extracted with chloroform(5 X 70 ml) to give a 'bases' fraction. This chloroform solution was evaporated, redissolved in chloroform, and filtered to remove 'chloroform insoluble bases'. The chloroform solution was extracted with 0.5 N. sodium hydroxide(3 X 20 ml). Evaporation of the chloroform solution gave a 'nonphenolic bases' fraction. The combined sodium hydroxide extracts were acidified with hydrochloric acid and neutralized with ammonium hydroxide. Extraction of the aqueous solution with chloroform(4 X 25 ml) gave a 'phenolic bases' fraction. The residues obtained were:

1) 'Neutrals' 4.2 g.

2) 'Chloroform-soluble salts' 2.94 g.

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3)   'Chloroform-insoluble bases'   0.88 g.

4)   'Nonphenolic bases'   1.72 g.

5)   'Phenolic bases'   0.33 g.

A spot from the phenolic fraction was subjected to two dimensional TLC separation by development with solvent A followed by orthogonal development with solvent B. Presence of 10-15 constituents was detected, although only a few were present in significant quantities. Preparative scale separation of this fraction(317 mg) was undertaken with the same two solvents. Solvent A gave a streaky separation from which three bands were sectioned, collected, suspended in 1 N. hydrochloric acid, neutralized to pH 8 with ammonium hydroxide, and extracted with chloroform(4 X 25 ml). Infrared spectra directed interest at the two more mobile fractions. Each was developed with solvent B and bands recovered as before. Three significant fractions were recovered which contained cherylline (147 mg), lyrechine(8 mg), and 4-O-methylnorbelladine(17 mg).

Synthetic Procedures

Each of the synthetic sequences leading to cherylline and lyrechine proceed through a series of compounds which had not been prepared previously. Each of these compounds has been characterized to adequately demonstrate the presumed structure. High resolution mass spectroscopy was performed where feasible to confirm molecular formulas. Synthetic procedural information is presented together with pertinent spectral data. No attempt has been made to log complete

information, but rather to verify the chemical transformations at each step.

4-Hydroxy-3-methoxymandelonitrile (XXXVIIIa)

The compound was prepared from vanillin by the method of Shaw(37). The reported yield was 80%.

IR: 1520 cm^{-1}(s), 2250 cm^{-1}(vw), 3350 cm^{-1}(w), 3550 cm^{-1}(m).

NMR(CDCl$_3$): 3.8(s) 3H, 5.5(s) 1H, 6.8-7.1(c) 3H.

(4-Hydroxy-3-methoxyphenyl)(4-hydroxyphenyl)acetonitrile (XXXIXa)

To a stirred melt of 4.3 g(45 mmoles) phenol was added 7.7 g(43 mmoles) XXXVIIIa. The solution was heated to 80°C and five drops concentrated sulfuric acid added. Vigorous bubbling ensued; the temperature rose rapidly to 120°C. The melt was cooled, dissolved in 15 ml ethanol with stirring, 30 ml water added, and stirring continued until crystallization was complete. The product was collected by filtration and recrystallized from ethanol/water(1:2) to give 10.4 g(95%) product.

IR: 1520 cm^{-1}(s), 2250 cm^{-1}(w), 3550 cm^{-1}(m).

NMR(d$_5$-pyridine): 3.7(s) 3H, 5.5(s) 1H, 7.1-7.6(AA'BB') 4H J=9 hz.

MS: $C_{15}H_{13}NO_3$ 255.0895, measured 255.0869, difference 0.0026.

2-(4-Hydroxy-3-methoxyphenyl)-2-(4-hydroxyphenyl)ethylamine (XXXIXb)

One gram Raney nickel alloy was added in a single portion to a magnetically stirred solution of 1.0 g XXXIXa in 40 ml 5% sodium hydroxide. Vigorous gas evolution ensued and

continued for most of the additional hour of stirring. The solution was filtered with care taken to keep the flammable catalyst from igniting. The catalyst was rinsed with water and destroyed by addition of small amounts of dilute hydrochloric acid. The aqueous solution of phenolic amine was derivatized before isolation for further synthetic manipulations. The reduction product could be isolated in poor yield (16%) by adjusting pH to 8-9 with hydrochloric acid and ammonium hydroxide and extracting with chloroform. A heavy, flocculant precipitate of aluminum hydroxide severely limited recovery by extraction, but enough material was isolated to obtain melting point(223-226°C) and other analytical data.

IR: 1520 cm^{-1}(s), 3400 cm^{-1}(m), 3550 cm^{-1}(s).

NMR(pyridine): 3.4(d) 2H J=8 hz, 3.7(s) 3H, 4.1(t) 1H J=8 hz.

MS: $C_{15}H_{17}NO_3$ 259.1208, measured 259.1224, difference 0.0016.

<u>Benzyl 2-(4-hydroxy-3-methoxyphenyl)-2-(4-hydroxyphenyl)ethyl carbamate</u>(XXXIXc).

The filtered reduction solution of 5.0 g(16.9 mmoles) XXXIXb was diluted to 200 ml and chilled in an ice bath with magnetic stirring. A solution of 6.6 g(30.8 mmoles) carbobenzoxy chloride in 50 ml ether was added from a dropping funnel with the temperature maintained below 10°C. After overnight stirring, the solution was cautiously acidified with hydrochloric acid and extracted with chloroform(3 X 30 ml). Solvent was removed under reduced pressure, 100 ml 10% potassium hydroxide in 50% aqueous ethanol added, and the solution

refluxed fifteen minutes. The cooled solution was extracted with hexane (3 X 100 ml), acidified with hydrochloric acid and extracted with chloroform (3 X 40 ml). Reduced pressure evaporation of solvent gave 4.62 g (61% based on starting nitrile) urethane product as a gum.

IR: 1520 cm^{-1} (s), 1700 cm^{-1} (s), 3360 cm^{-1} (m), 3540 cm^{-1} (m).

NMR (d_5-pyridine): 3.7 (s) 3H, 4.3 (m) 2H, 5.3 (b) 1H, 5.3 (s) 2H.

MS: $C_{23}H_{23}NO_5$ 393.1576, measured 393.1547, difference 0.0029.

Benzyl 2-(4-carboethoxy-3-methoxyphenyl)-2-(4-carboethoxy-phenyl)ethyl carbamate (XXXIXd)

To a magnetically stirred solution of 4.6 g (11.8 mmoles) XXXIXc in 20 ml pyridine in an ice bath was added slowly 10 ml ethyl chlorocarbonate (11.4 g, 105 mmoles), so low temperature was maintained. The solution was allowed to stand overnight at room temperature, poured into 150 ml water in a separatory funnel, made strongly acidic with hydrochloric acid, and extracted with chloroform (3 X 40 ml). Extraction of the combined organic solutions with 1 N. hydrochloric acid (2 X 50 ml) and evaporation of the organic extracts under reduced pressure gave 6.029 g (96%) product as a gum. Attempts to induce crystallization were unsuccessful.

IR: 1765 cm^{-1} (s), 1720 cm^{-1} (m), 1515 cm^{-1} (m), 3400 cm^{-1} (w).

NMR (CDCl$_3$): 1.3 (t) 6H J=7 hz, 3.8 (s) 3H, 4.2 (q) 4H J=7 hz, 5.1 (s) 2H.

7-Carboethoxy-6-methoxy-4-(4'-carboethoxyphenyl)-3,4-dihydro-
isocarbostyril (XLa)

Cyclization of XXXIXd was accomplished by two hour reflux
of a solution of 8.0 g(14.9 mmoles) compound in 30 ml chloro-
form containing 10 ml phosphorus oxychloride and 5 g phosphorus
pentoxide. The supernatant liquid was decanted and evaporated
under reduced pressure. Both portions were cautiously decom-
posed with water, combined, and diluted to 150 ml. Since a
chloroform extract of this solution (3 X 40 ml) was turbid,
anhydrous magnesium sulfate was added with stirring, the solu-
tion filtered and evaporated under reduced pressure to give
6.9 g(88%) crude product. Recrystallization from methanol
gave analytical material with melting range 165-67°C.

IR: 1770 cm^{-1}(s), 1680 cm^{-1}(m), 1515 cm^{-1}(m), 3380 cm^{-1}(w).

NMR(CDCl$_3$): 1.3(t) 6H J=8 hz, 3.7(s) 5H, 4.2(q) 4H J=8, 6.5(s)
1H, 7.9(s) 1H.

MS: C$_{22}$H$_{23}$NO$_8$ 429.1424, measured 429.1403, difference 0.0021.

7-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-3,4-dihydroisocarbo-
styril (XLb)

A solution of 6.9 g(16.1 mmoles) XLa in 100 ml of 10%
potassium hydroxide in 50% aqueous ethanol was refluxed for
15 minutes. Acidification of the solution with hydrochloric
acid, extraction with ethyl acetate (3 X 30 ml), and evapora-
tion gave 5.7 g(83%) product. Crystallization from methanol
gave analytical material which melted with sublimation at
256°C.

IR: 1655 cm^{-1}(s), 1520 cm^{-1}(s), 3360 cm^{-1}(m), 3540 cm^{-1}(m).

NMR(d$_5$-pyridine): 3.7(s) 3H, 6.6(s) 1H, 8.0(s) 1H.

MS: C$_{16}$H$_{15}$NO$_4$ 285.1001, measured 285.0982, difference 0.0019.

7-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-3,4-dihydroiso-
carbostyril(XLc)

A solution of 5.7 g(20.0 mmoles) XLb in 100 ml anhydrous methanol containing 5.75 ml(6.38 g, 50.5 mmoles) benzyl chloride and 5.52 g(40 mmoles) potassium carbonate was refluxed overnight. The supernatant solution was decanted and evaporated under reduced pressure. Each portion was suspended in water, extracted with chloroform(3 X 40 ml), and the combined extracts evaporated under reduced pressure. Crystallization of the product from acetone gave 7.905 g(85%) product melting at 193-95°C.

IR: 1665 cm^{-1}(s), 1515 cm^{-1}(s), 3430 cm^{-1}(m).

NMR(CDCl$_3$): 3.6(s) 3H, 5.0(s) 2H, 5.2(s) 2H, 6.4(s) 1H, 6.8-7.2(AA'BB') 4H J=9 hz, 7.4(s) 10H, 7.7(s) 1H.

MS: C$_{30}$H$_{27}$NO$_4$ 465.1940, measured 465.1912, difference 0.0028.

7-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-1,2,3,4-tetra-
hydroisoquinoline(XLd)

To a magnetically stirred solution of 1.0 g(24 mmoles) lithium aluminum hydride in 30 ml dry tetrahydrofuran was added from a dropping funnel a solution of 2.75 g(6.1 mmoles) XLc at such a rate as to maintain controlled reflux. Reflux was continued four additional hours. Excess hydride was cautiously decomposed by dropwise addition of 1.0 ml water, 1.0 ml 15% sodium

hydroxide, and 3.0 ml water to the stirred mixture. The granular gray-white precipitate of aluminum oxide which formed was filtered and washed with 100 ml chloroform, and the combined filtrates evaporated under reduced pressure. The oily product was dissolved in 30 ml hot chloroform and anhydrous hydrogen chloride in ethyl ether added until the solution became turbid. Refrigeration caused precipitation of 2.463 g(83%) amine hydrochloride melting at 130-33°C.

IR: 1515 cm^{-1}(s), 3320 cm^{-1}(w).

NMR(CDCl$_3$): 3.5(s) 5H, 4.95(s) 2H, 5.05(s) 2H, 6.4(s) 1H, 6.6(s) 1H, 6.7-7.1(AA'BB') 4H, 7.3(s) 10H.

MS: $C_{30}H_{29}NO_3$ 451.2147, measured 451.2148, difference 0.0001.

7-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-1,2,3,4-tetrahydro-isoquinoline(Xle)

A solution of 1.05 g(2.16 mmoles) XLd hydrochloride in 40 ml 95% ethanol was hydrogenated with 100 mg 10% palladium-charcoal catalyst. The mixture was stirred at room temperature and atmospheric pressure under hydrogen atmosphere until uptake ceased. The solution was filtered and evaporated under reduced pressure. The residue was taken up in 10 ml ethanol and treated with ethereal hydrogen chloride solution until turbidity appeared. Refrigeration produced 0.641 g(97%) crystalline amine hydrochloride with melting point 228-30°C.

IR: 1520 cm^{-1}(s), 3300 cm^{-1}(w), 3555 cm^{-1}(m).

NMR(d$_5$-pyridine): 2.6-3.3(m) 2H, 3.5(s) 3H, 3.8(s) 2H, 3.9(t) 1H J=8 hz, 6.2(s) 1H, 6.4(s) 1H, 6.7-7.1(AA'BB') 4H J=9.

MS: $C_{16}H_{17}NO_3$ 271.1208, measured 271.1163, difference 0.0045.

<u>7-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl-2-methyl-1,2,3,4-tetra-</u>

<u>hydroisoquinoline</u>(XLf)

A solution of 1.50 g(4.90 mmoles) XLe in 10 ml isopropanol was treated with 1.0 ml 37% formalin(12.3 mmoles). The solution was stirred 30 minutes, and 0.200 g(5.3 mmoles) sodium borohydride added in small portions. After 30 minutes additional stirring the mixture was poured into 50 ml 3 N. hydrochloric acid, adjusted to pH 8 with ammonium hydroxide, and extracted with chloroform(3 X 40 ml). The extracts were evaporated under reduced pressure. The residue was dissolved in 30 ml hot ethanol and crystallized by adding ethereal hydrogen chloride to turbidity. Refrigeration induced crystallization of 1.350 g(86%) amine hydrochloride melting at 184-87°C. This material was spectrally identical in all respects with natural cherylline.

IR: 1520 cm^{-1}(s), 2800 cm^{-1}(m), 3540 cm^{-1}(m).

NMR(d_5-pyridine): 2.3(s) 3H, 2.4-3.1(m) 2H, 3.5(s) 5H, 4.3(t) 1H, 6.6(s) 1H, 6.6(s) 1H, 6.9(s) 1H, 7.0-7.4(AA'BB') 4H J=9.

MS: $C_{17}H_{19}NO_3$ 285.1365, measured 285.1364, difference 0.001.

<u>N-[2-(4-hydroxy-3-methoxyphenyl)-2-(4-hydroxyphenyl)ethyl]</u>

<u>benzamide</u>(XXXIXe)

The filtered solution(40 ml) from production of 1.7 g (6.67 mmoles) XXXIXb was diluted to 80 ml and cooled with an ice bath. To the stirred solution was slowly added a solution of 0.95 ml(1.15 g, 8.0 mmoles) benzoyl chloride in 20 ml ether.

The cooling bath was removed and stirring continued overnight at room temperature. The solution was made strongly acidic with hydrochloric acid, extracted with chloroform(3 X 40 ml), and the extracts evaporated under reduced pressure. A suspension of the solid in 100 ml 10% potassium hydroxide in 50% aqueous ethanol was refluxed for one hour. The cooled solution was acidified, extracted with chloroform, and the extracts evaporated. The desired product was obviously contaminated with crystalline benzoic acid. Since this impurity did not interfere with the next reaction, the product was utilized as isolated. Analytical material obtained by recrystallization of the benzamide from acetone melted at 195-97°C.

IR: 1520 cm^{-1}(s), 1655 cm^{-1}(m), 3300 cm^{-1}(w), 3550 cm^{-1}(w).

NMR(d_5-pyridine): 3.6(s) 3H, 4.1(b) 1H, 7.9(m) 2H.

MS: $C_{22}H_{21}NO_4$ 363.1470, measured 363.1498, difference 0.0028.
N-[2-(4-Benzyloxy-3-methoxyphenyl)-2-(4-benzyloxyphenyl)ethyl] benzamide(XXXIXf).

Impure XXXIXe was readily converted to the bis(benzyl) ether and purified. Dissolution in 25 ml anhydrous methanol and overnight reflux with 0.70 ml(6.2 mmoles) benzyl chloride and 0.90 g(6.9 mmoles) potassium carbonate accomplished the conversion. The supernatant was decanted and evaporated under reduced pressure. A suspension of both residues in 0.1 N. sodium hydroxide(50 ml) was extracted with chloroform(3 X 40 ml), and the extracts evaporated. Crystallization from methanol provided 2.32 g(64% based on nitrile XXXIXa) protected

benzamide which melted at 169-72°C.

IR: 1515 cm^{-1}(s), 1650 cm^{-1}(m), 3350^{-1}(w).

NMR(CDCl$_3$): 3.5(s) 3H, 5.0(s) 2H, 5.1(s) 2H, 8.0(m) 2H.

N-Benzyl-2-(4-benzyloxy-3-methoxyphenyl)-2-(4-benzyloxyphenyl)
ethylamine(XXXIXg)

A solution of 1.01 g(1.9 mmoles) XXXIXf in 20 ml dry
tetrahydrofuran was slowly added to a magnetically stirred
suspension of 0.300 g(8.0 mmoles) lithium aluminum hydride
in 20 ml dry tetrahydrofuran. An additional 24 hours reflux
period effected complete reduction of the amide. Subsequent
decomposition with 0.30 ml water, 0.30 ml 15% sodium hydroxide,
and 0.90 ml water, filtration, chloroform rinse(100 ml) of the
residue, and evaporation of the filtrates under reduced pres-
sure gave crude product. Crystallization was accomplished
by addition of ethereal hydrogen chloride to a hot solution
of the product in 20 ml chloroform to give 0.913 g(85%) amine
hydrochloride product melting at 191-94°C.

IR: 1510 cm^{-1}(s), 2800 cm^{-1}(m).

NMR(CDCl$_3$): 3.2(d) 2H J=8, 3.8(s) 5H, 4.2(t) 1H, 5.1(s) 2H,
5.2(s) 2H.

7-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-2-benzyl-1,2,3,4-
tetrahydroisoquiniline(XLg)

To 0.325 g(0.576 mmoles) hydrochloride of XXXIXg was added
3.0 ml 37% formalin and enough methanol to give a clear solu-
tion. Steam bath heat was used to reflux the mixture for 30
minutes. The solution was poured into 50 ml water, adjusted

to pH 8 with ammonium hydroxide, and extracted with chloroform (3 X 30 ml). The extracts were evaporated under reduced pressure, the residue dissolved in five ml hot chloroform, and ethereal hydrogen chloride added to turbidity. Refrigeration resulted in precipitation of 0.249 g(75%) amine hydrochloride.

IR: 1510 cm^{-1}(s), 2800 cm^{-1}(m).

NMR(CDCl$_3$): 2.4-3.1(m) 2H, 3.6(s) 7H, 4.1(t) 1H J=6, 5.0(s) 2H, 5.05(s) 2H, 6.4(s) 1H, 6.5(s) 1H, 6.7-7.1(AA'BB') 4H J=9, 7.2(s) 5H, 7.3(s) 10H.

7-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-1,2,3,4-tetrahydro-isoquinoline(XLe)

Hydrogenolysis was performed on 0.249 g(0.432 mmoles) XLg hydrochloride in 20 ml ethanol, 5.0 ml ethyl acetate, and 0.5 ml 71% perchloric acid with 10% palladium-charcoal catalyst (100 mg). The magnetically stirred mixture absorbed 28.2 ml (97%) theoretical hydrogen at room temperature and atmospheric pressure. The catalyst was filtered from the solution and the filtrate evaporated under reduced pressure. Recrystallization from ethanol with ethereal hydrogen chloride gave 0.119 g(90%) amine hydrochloride with identical spectral characteristics to and undepressed mixed melting point with XLe produced by the previous reaction sequence.

Ethyl 2-(4-benzyloxy-3-methoxyphenyl)-2-(4-benzyloxyphenyl)-
ethyl carbamate(XXXIXh).

The filtered reduction solution of 8.09 g(31.7 mmoles)
XXXIXb was diluted to 320 ml and chilled in an ice bath with
magnetic stirring. A solution of 10.4 g(48 mmoles) carboethoxy
chloride in 70 ml ether was added from a dropping funnel with
the temperature maintained below 10°C. After overnight stir-
ring, the solution was cautiously acidified with hydrochloric
acid and extracted with chloroform(3 X 30 ml). Solvent was
removed under reduced pressure, 100 ml 10% potassium hydroxide
in 50% aqueous ethanol added, and the solution refluxed fif-
teen minutes. The cooled solution was acidified with hydro-
chloric acid and extracted with chloroform(3 X 40 ml). Reduced
pressure evaporation of solvent gave 8.62 g(82%) urethane prod-
uct(XXXIXq) as a gum. This product in 200 ml anhydrous meth-
anol was refluxed overnight with 7.0 ml(60 mmoles) benzyl
chloride and 9.0 g(65 mmoles) potassium carbonate. The super-
natant was decanted and evaporated under reduced pressure.
A suspension of both portions in 75 ml water was extracted
with chloroform(3 X 30 ml), the combined extracts evaporated,
and the residue crystallized from methanol. The 11.55 g(87%)
protected carbamate product melted at 90-93°C.

IR: 1720 cm^{-1}(s), 1520 cm^{-1}(s), 3380 cm^{-1}(w).

NMR(CDCl$_3$): 1.1(t) 3H J=7 hz, 3.7(m) 2H, 3.7(s) 3H, 4.0(q) 2H
J=7 hz, 4.8(t) 1H J=6, 4.9(s) 2H, 5.0(s) 2H.

MS: C$_{18}$H$_{21}$NO$_5$ 331.1420, measured 331.1485, difference 0.0065.

C$_{32}$H$_{33}$NO$_5$ 511.2359, measured 511.2355, difference 0.0004.

N-Methyl-2-(4-benzyloxy-3-methoxyphenyl)-2-(4-benzyloxyphenyl)-ethylamine(XXXIXi)

A solution of 4.60 g(9.0 mmoles) XXXIXh in 25 ml dry tetra-hydrofuran was dripped into a stirred solution of 0.700 g(18.4 mmoles) lithium aluminum hydride in 25 ml tetrahydrofuran at such a rate as to maintain gentle reflux. Reflux was continued for four hours. Excess hydride was destroyed with 0.70 ml water, 0.70 ml 15% sodium hydroxide, and 2.10 ml water, the mixture filtered, and the residue washed with 50 ml chloroform. The combined filtrates were evaporated under reduced pressure, the residue dissolved in 50 ml hot chloroform, and ethereal hydrogen chloride added to turbidity. Refrigeration caused crystallization of 3.39 g(77%) amine hydrochloride melting at 200-05°C.

IR: 1515 cm^{-1}(s), 2800 cm^{-1}(m), 3400 cm^{-1}(w).

NMR(CDCl$_3$): 2.4(s) 3H, 3.1(d) 2H J=7, 3.8(s) 3H, 4.1(t) 1H J=7, 4.9(s) 2H, 5.0(s) 2H.

7-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(XLh)

To 0.450 g(0.90 mmole) XXXIXi hydrochloride was added 0.25 ml(3.1 mmoles) 37% formalin and enough methanol to form a clear solution. This solution was refluxed for 30 minutes, poured into 50 ml water, and extracted with chloroform(3 X 30 ml). The combined extracts were evaporated under reduced pressure and the residue crystallized from methanol to give 0.372 g(89%) free amine with melting range 140-2°C.

IR: 1515 cm^{-1}(s), 2800 cm^{-1}(m).

NMR(CDCl$_3$): 2.3(s) 3H, 3.5(s) 2H, 3.6(s) 3H, 5.0(s) 2H, 5.1(s) 2H 6.4(s) 1H, 6.6(s) 1H, 6.8-7.2(AA'BB') 4H, 7.4(s) 10H.

MS: C$_{31}$H$_{31}$NO$_3$ 465.2304, measured 465.2312, difference 0.0008.

7-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (XLf)

The 0.372 g(0.800 mmole) XLh was subjected to hydrogenolysis at room temperature and atmospheric pressure in 20 ml ethanol containing 10% palladium charcoal(50 mg) as catalyst. Uptake ceased after one hour with absorption of 17.6 ml(98%). Filtration of the catalyst, evaporation of solvent under reduced pressure, and crystallization of the residue from ethanol with ethereal hydrogen chloride gave 0.251 g(98%) material identical in all respects with that previously synthesized.

(4-Benzyloxy-3-methoxyphenyl)(4-benzyloxyphenyl)acetonitrile (XXXIXj)

A solution of 2.43 g(9.53 mmoles) XXXIXa in 50 ml anhydrous methanol containing 3.5 ml (30 mmoles) benzyl chloride and 2.5 g(18 mmoles) potassium carbonate was refluxed overnight. The supernatant was decanted and evaporated under reduced pressure. The portions were combined, suspended in water, and extracted with chloroform(3 X 30 ml). The combined extracts were evaporated and the residue crystallized from methanol to give 3.358 g(81%) benzylated nitrile which melted at 123-24°C.

IR: 1515 cm^{-1}(s), 2240 cm^{-1}(w).

NMR(CDCl$_3$): 3.5(s) 3H, 4.95(s) 2H, 5.05(s) 2H.

MS: C$_{29}$H$_{25}$NO$_3$ 435.1834, measured 435.1868, difference 0.0034.

2-(4-Benzyloxy-3-methoxyphenyl)-2-(4-benzyloxyphenyl)ethyl-
amine(XXXIXk)

A solution of 2.0 g(4.56 mmoles) XXXIXj in 15 ml dry tetrahydrofuran was dropped into a stirred suspension of 0.34 g (9.0 mmoles) lithium aluminum hydride in 20 ml dry tetrahydrofuran. The reaction mixture was refluxed for four hours, excess hydride destroyed(0.34 ml water, 0.34 ml 15% sodium hydroxide, and 1.0 ml water), the solution filtered, and the residue washed with 50 ml chloroform. The combined filtrates were evaporated under reduced pressure. Attempted crystallization was unsuccessful. Since the spectra obtained indicated the nitrile was completely reduced and product formed with the expected spectral properties, cyclization was attempted on the crude product mixture.

IR: 1520 cm^{-1}(s), 3390 cm^{-1}(w).

NMR(CDCl$_3$): 3.8(s) 3H, 5.0(s) 2H, 5.1(s) 2H.

7-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-1,2,3,4-tetrahydro-
isoquinoline(XLe)

A chloroform solution of the previous product(XXXIXk) was converted to the hydrochloride with ethereal hydrogen chloride and evaporated under reduced pressure. Formalin (2.0 ml, 24.7 mmoles) was added and a clear solution produced by addition of methanol. The solution was refluxed for 30

minutes, poured into 50 ml water, and extracted with chloroform(3 X 30 ml). The extracts were evaporated and crystallization unsuccessfully attempted. Again the spectral data indicated a substantial portion of the material to be the desired product(XLd), so removal of protecting groups was attempted. Hydrogenolysis was performed at room temperature and atmospheric pressure in 50 ml ethanol containing 10% palladium-charcoal(100 mg). The solution was then filtered, evaporated under reduced pressure, and the residue taken up in 50 ml 1 N. sodium hydroxide. The solution was extracted with chloroform(2 X 30 ml), acidified with hydrochloric acid, extracted with chloroform(2 X 30 ml), adjusted to pH 8 with ammonium hydroxide, and again extracted with chloroform(4 X 30 ml). The final extracts of phenolic amine were evaporated under reduced pressure and crystallized from ethanol with ethereal hydrogen chloride to give 280 mg of the desired tetrahydroisoquinoline. This constitutes a 20% yield for the three step sequence of nitrile reduction, cyclization, and debenzylation.

2-Hydroxy-3-methoxymandelonitrile(XXXVIIIb)

This compound was prepared from ortho-vanillin by the same method used to prepare XXXVIIIa. Recrystallization from ether-hexane gave 66% product melting at 85-7°C.

IR: 1495 cm^{-1}(s), 3540 cm^{-1}(m).

NMR(d_6-acetone): 3.8(s) 3H, 5.9(s) 1H, 6.8-7.4(m) 3H.

(2-Hydroxy-3-methoxyphenyl)(4-hydroxyphenyl)acetonitrile(XXXIXa)

To a melt of 3.06 g(32.6 mmoles) phenol was added 5.29 g (29.6 mmoles) XXXVIIIb. The magnetically stirred melt was heated with an oil bath to 100°C, and two drops concentrated sulfuric acid added. The solution turned reddish-brown, bubbling ensued, and the temperature rose to about 125°C. Stirring was continued for five minutes, the mixture cooled, and 50 ml chloroform added. Continued stirring caused precipitation of product. Recrystallization from ethanol gave 7.48 g (90%) white needles melting at 177-80°C.

IR: 2260 cm^{-1}(w), 3420 cm^{-1}(m).

NMR(d$_6$-acetone): 3.7(s) 3H, 6.0(s) 1H, 7.1-7.7(AA'BB') 4H.

MS: C$_{15}$H$_{13}$NO$_3$ 255.0895, measured 255.0878, difference 0.0017.

2-(2-Hydroxy-3-methoxyphenyl)-2-(4-hydroxyphenyl)ethylamine (XXXIXn)

Raney nickel alloy(2.3 g) was added in a single portion to a stirred solution of 2.3 g(9.01 mmoles) XXXIXm in 50 ml 5% sodium hydroxide. The mixture was stirred for one hour, the catalyst was filtered, and the solution used for derivatization. No isolation of the unsubstituted phenolic amine was attempted.

Benzyl 2-(2-hydroxy-3-methoxyphenyl)-2-(4-hydroxyphenyl)ethyl carbamate(XXXIXo)

The filtered solution from the previous reduction was diluted to 100 ml and cooled with an ice bath. The solution was treated with carbobenzoxy chloride in an analogous manner

to that used to form XXXIXc. The crude product obtained

(2.49 g, 66%) was used in the next reaction without further

purification, but an analytical sample crystallized from a

large volume of ether melted at 168-69°C.

IR: 1695 cm^{-1}(s), 3380 cm^{-1}(vw).

NMR(d$_5$-pyridine): 3.7(s) 3H, 4.3(m) 2H, 5.3(b) 1H, 5.3(s) 2H.

MS: C$_{23}$H$_{23}$NO$_5$ 393.1576, measured 393.1547, difference 0.0029.

Benzyl 2-(2-carboethoxy-3-methoxyphenyl)-2-(4-carboethoxy-

phenyl)ethyl carbamate(XXXIXp)

Carboethoxy ester protecting groups were added to the

molecule by treating XXXIXo as for synthesis of XXXIXd. The

2.94 g(6.34 mmoles) reactant gave 3.20 g(94%) product as a gum.

IR: 1770 cm^{-1}(s), 1730 cm^{-1}(m), 3400 cm^{-1}(w).

NMR(CDCl$_3$): 1.2(t) 3H J=7 hz, 1.3(t) 3H J=7 hz, 3.8(s) 3H,

4.2(q) 2H J=7 hz, 4.3(q) 2H J=7 hz, 5.1 (s) 2H.

5-Carboethoxy-6-methoxy-4-(4'-carboethoxyphenyl)-3,4-dihydro-

isocarbostyril(XLi)

A chloroform solution of 8.0 g(15 mmoles) XXXIXp was

treated with phosphorus oxychloride-phosphorus pentoxide as

for production of XLa. The 6.4 g(82%) crude product crystal-

lized from aqueous ethanol melted at 143-46°C.

IR: 1765 cm^{-1}(s), 1670 cm^{-1}(m), 3430 cm^{-1}(w).

NMR(CDCl$_3$): 1.2(t) 3H J=7 hz, 1.4(t) 3H J=7 hz, 3.9(s) 3H,

3.9-4.5(m) 5H, 7.0(d) 1H J=8 hz, 7.1(s) 4H, 8.1(d) 1H J=8 hz.

MS: C$_{22}$H$_{23}$NO$_8$ 429.1423, measured 429.1403, difference 0.0020.

5-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-3,4-dihydroisocarbo-
styril (XLj)

A solution of 6.4 g (14.9 mmoles) XLi was refluxed in 100
ml of 10% potassium hydroxide in aqueous ethanol for 15
minutes. Acidification of the solution with hydrochloric acid,
extraction with ethyl acetate (3 X 30 ml), and evaporation gave
4.2 g (82%) product. Methanol crystallized material sublimed
at 257°C, melted at 287-91°C.

IR: 1660 cm^{-1} (s), 3400 cm^{-1} (m).

NMR (d_5-pyridine): 3.7 (s) 3H, 8.2 (d) 1H J=9 hz.

MS: $C_{16}H_{15}NO_4$ 285.1001, measured 285.0991, difference 0.0009.

5-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-3,4-dihydroiso-
carbostyril (XLk)

Treatment of 0.800 g (2.8 mmoles) XLj with benzyl chloride
as for formation of XLc gave the amide (1.042 g, 80%), which
when crystallized from methanol melted at 171-73°C.

IR: 1670 cm^{-1} (s), 1515 cm^{-1} (s), 3200-3400 cm^{-1} (w).

NMR (CDCl$_3$): 3.9 (s) 3H, 4.3 (d) 1H J=11 hz, 4.9 (d) 1H J=11 hz,
4.9 (s) 2H, 7.9 (d) 1H J=9 hz.

MS: $C_{30}H_{27}NO_4$ 465.1940, measured 465.1920, difference 0.0020.

5-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-1,2,3,4-tetra-
hydroisoquinoline (XLm)

A solution of 1.042 g (2.24 mmoles) XLk was treated with
lithium aluminum hydride in the same manner as for the forma-
tion of XLd. Crystallization of the product from hot chloro-
form by addition of ethereal hydrogen chloride gave 0.948 g

(87%) amine hydrochloride with melting point 184-86°C.

IR: 1515 cm^{-1}(s), 1500 cm^{-1}(s).

NMR(CDCl$_3$): 2.7(d) 2H J=5 hz, 3.3(d) 1H J=14 hz, 3.8(s) 3H, 3.9(d) 1H J=14 hz, 4.0(d) 1H J=11 hz, 4.3(t) 1H J=5 hz, 4.9(d) 1H J=11 hz, 5.0(s) 2H.

MS: C$_{30}$H$_{29}$NO$_3$ 451.2147, measured 451.2202, difference 0.0055.

5-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-1,2,3,4-tetrahydro-isoquinoline(XLn)

The protecting groups of XLm hydrochloride(0.948 g, 1.93 mmoles) were removed by hydrogenolysis in 20 ml ethanol containing 100 mg 10% palladium-charcoal. A total of 87.0 ml hydrogen(102%) were absorbed. Filtration, evaporation of solvent, and crystallization from ethanol with ethereal hydrogen chloride gave 0.562 g(95%) amine hydrochloride melting with decomposition at 251-55°C.

IR: 1515 cm^{-1}(s), 1500 cm^{-1}(s), 3550 cm^{-1}(m).

NMR(d$_6$-acetone): 3.7(s) 2H, 3.8(s) 3H, 4.4(b) 1H.

MS: C$_{16}$H$_{17}$NO$_3$ 271.1208, measured 271.1219, difference 0.0011.

5-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(XLn)

N-methylation of XLm(0.562 g, 1.83 mmoles) was accomplished in direct analogy to formation of XLf from XLe. The 0.499 g(85%) amine hydrochloride product melted with decomposition at 201-05°C. Spectral characteristics were identical to those of material isolated from plants.

IR: 1515 cm^{-1}(s), 1500 cm^{-1}(s), 2800 cm^{-1}(m), 3545 cm^{-1}(m).

NMR(d_5-pyridine): 2.3(s) 3H, 2.7-3.0(m) 2H, 3.3(d) 1H J=14 hz, 3.7(s) 3H, 4.0(d) 1H J=14 hz, 4.6(b) 1H, 6.7-7.4(c) 6H.

MS: $C_{17}H_{19}NO_3$ 285.1365, measured 285.1333, 0.0032.

Tritium Labelled Syntheses

Specific tritium labelling was introduced at C-1 in each substitution pattern by reduction of the corresponding benzyl-protected dihydroisocarbostyril with ^{3}H-lithium aluminum hydride. The extreme water sensitivity of the reducing agent necessitated stringent precautions to exclude sources of moisture. The small scale of the reaction eliminated the need for gradual combination of reactants, so only minimal solvent necessary for reflux was used. Reaction was initiated with unlabelled lithium aluminum hydride to consume the secondary amide active hydrogen and any traces of moisture. Freshly opened ampoules of radioactive material were emptied into the reaction mixture and rinsed twice with small amounts of freshly distilled solvent. After reflux had been maintained for the usual reduction period with amide in calculated excess, additional unlabelled reducing agent was introduced and reflux resumed to ensure complete reduction. Normal workup was accomplished in a closed hood with efficient ventilation. The remaining reactions to complete synthesis of each labelled compound were conducted as previously described.

1-^{3}H-7-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-1,2,3,4-tetrahydroisoquinoline(XLd)

Unlabelled lithium aluminum hydride(6 mg, 0.63 meq) was combined with 212 mg(0.456 mmole) XLc in a dry 25 ml pear shaped flask equipped with a reflux condenser and drying tube. Tetrahydrofuran(3 ml) was distilled from lithium aluminum hydride directly into the reaction flask. Two ampoules (5.0 mg, 0.53 meq, spec. act. 75 mc/mmole) tritiated lithium aluminum hydride were opened, the dry contents transferred, and remaining traces rinsed into the reaction vessel with a small amount of dry solvent. Reflux was maintained under a slight positive pressure of dry nitrogen for one hour. An additional 10 mg unlabelled lithium aluminum hydride was added and reflux resumed for another hour. Excess hydride was destroyed by dropwise addition of water until gas evolution ceased. The reaction mixture and flask rinsings were suspended in 50 ml 1 N. hydrochloric acid and extracted with chloroform(4 X 25 ml). The extracts were evaporated under reduced pressure and crystallized from hot chloroform by addition of ethereal hydrogen chloride to turbidity. Complete reduction of the amide was indicated by the absence of any carbonyl infrared absorption at 1665 cm^{-1}. The reaction produced 177 mg(80%) amine hydrochloride with approximate specific activity of 4.1 X 10^{-2} mc/mg, indicating incorporation of roughly 72% of the total tritium activity.

1-^{3}H-5-Benzyloxy-6-methoxy-4-(4'-benzyloxyphenyl)-1,2,3,4-
tetrahydroisoquinoline (XLm)

Tritium was introduced into the lyrechine sequence in the
same manner by subjecting 206 mg (0.444 mmoles) XLk to reduc-
tion with 11 millicuries tritiated lithium aluminum hydride
as in the previous procedure. Reduction proceeded smoothly
to give 180 mg (81%) amine hydrochloride with approximate spe-
cific activity of 5.1 X 10^{-2} mc/mg, or incorporation of about
84% tritium activity. The infrared carbonyl absorption of
the starting material at 1670 cm^{-1} was completely removed.

Carbon-14 Labelled Synthesis

The 3-^{14}C synthesis of cherylline was based on the
sequence already presented (XXXVIIIa → XXXIXa → XXXIXb → XXXIXe
→ XXXIXf → XXXIXg → XLg → XLe) utilizing Pictet-Spengler cycli-
zation of an N-benzyl amine. Since the label was introduced
with ^{14}C-potassium cyanide in the first step of the synthesis,
some alterations of procedure were necessary. Purification
of intermediate compounds was limited to extractive methods
to avoid material losses.

3-^{14}C-7-Hydroxy-6-methoxy-4-(4'-hydroxyphenyl)-1,2,3,4-tetra-
hydroisoquinoline (XLe)

To a solution of 89 mg (1.35 mmoles, 5.0 millicuries)
^{14}C-potassium cyanide in 0.5 ml water in a centrifuge tube
was added 664 mg (2.70 mmoles) bisulfite adduct of vanillin.
The tube was stoppered and the suspension shaken five minutes
in an ice water bath. The mixture was then rinsed with three

successive ten ml portions of ether into a separatory funnel
and the ether washed with 4 N. sodium bisulfite(2 X 25 ml).
The ether layer was dried with 5 g anhydrous magnesium sulfate
and suction filtered through a sintered-glass filter into a
50 ml pear-shaped flask and evaporated. The reaction glass-
ware was rinsed with ether and the rinsings filtered into the
flask, 280 mg phenol(3.0 mmoles) added and the solution evapo-
rated(XXXVIIIa). The flask was heated with steam for five
minutes, three drops concentrated sulfuric acid added, and
steam heating continued 45 minutes with constant rotation of
the flask(XXXIXa). The nitrile product was transferred with
20 ml 10% sodium hydroxide to a 100 ml beaker. Magnetic stir-
ring was initiated and Raney nickel alloy(500 mg) added. After
one hour stirring, the solution was filtered and the catalyst
washed with water(XXXIXb). The solution was acidified with
concentrated hydrochloric acid and extracted with chloroform
(2 X 25 ml), then made basic with 50% sodium hydroxide. The
solution was chilled and 0.25 ml(2.1 mmoles) benzoyl chloride
in ten ml ether dropped in with stirring. The reaction was
stirred three hours, then heated with steam for 30 minutes
and transferred to a separatory funnel. The solution was
extracted with chloroform(25 ml), acidified, again extracted
with chloroform(4 X 25 ml), and the final extracts evaporated
(XXXIXe). The remaining sequence of benzylation(XXXIXf), amide
reduction(XXXIXg), cyclization(XLg) and debenzylation(XLe)
was conducted as described in previous synthetic procedures

with the scale of each reaction proportionately reduced.
The sequence was completed through final removal of the pro-
tecting groups at which point mechanical failure caused break-
age of the flask into a hot water bath. Attempts to recover
the product achieved limited success. The recovered material
was combined with tritium labelled material and crystallized
to constant activity for feeding.

Radioassay Method

The two channels of the scintillation counter were ad-
justed to detection windows which compromised between complete
discrimination of the two isotopes and efficient detection
of the desired isotope. Instrumental counting efficiencies
were determined daily on separate ^{3}H-toluene and ^{14}C-toluene
samples. Counting data were then corrected for the amount of
undesired isotope in each channel. Typical values were 39%
efficiency for carbon with a noise level of 30% of the tritium
counts and 11% efficiency for tritium with a noise level of
2.0% of the carbon counts.

All radioactivities were measured in 15 ml Bray's solu-
tion(40). A weighed amount of each sample was dissolved in
5.00 ml methanol and triplicate aliquots(1.0 ml) counted.
Each aliquot was counted ten times and the average value used.
Compounds were recrystallized and counted until constant activ-
ity was obtained.

Feeding Experiment Isolations

Radioactive alkaloid plant processing was carried out by the isolation methods previously described. Separation of the phenolic and nonphenolic bases of <u>Crinum</u> <u>powellii</u> <u>album</u> by prep TLC with solvents A and B gave a number of alkaloids. The compounds listed below are identified by the bands from which they were obtained in successive separations (bottom band = 1), rather than by experimental R_f values, which were variable.

ID	Phenolic fraction	mg	ID	Nonphenolic fraction	mg
21	Coranicine	19	23	Crinine	621
31	4-O-methylnorbelladine	15	32	6-Hydroxypowelline	17
42	Cherylline	191	33	Flexinine	65
51	Criwelline	20	34	Powelline	63
61	Lyrechine	15	35	Buphanisine	222
			41	Crinamidine	180
			42	6-Hydroxybuphanidrine	53
			42	Buphanidrine	113
			52	Undulatine	248
			63	Caranine	62
			73	Acetylcaranine	27

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