THE LADYBUG ALKALOIDS INCLUDING SYNTHESIS AND BIOSYNTHESIS

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Introduction

The polka-dotted beetles (family Coccinellidae), because of their voracious appetite for aphids, mealy bugs and scale insects have long been known as friends of man (1). This was recognized as early as the Middle Ages and people dedicated them to the Virgin Mary. They were then known as Beetles of the Blessed Lady, which today has been shortened to lady beetles, ladybirds, or ladybugs. The family Coccinellidae are extremely diverse in their habits and the biology of the Coccinellidae has been the subject of several reviews (1-4). The majority of the species are beneficial because of their predaceous nature, but some are injurious being phytophagous on agricultural crops. The insect-eating coccinellids (the typical reddish or orange-colored beetles make

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Dedicated to Professor R.B. Woodward on the occasion of his 60th birthday.

up only part of the 4000 species (5), the smaller, dark species usually go unnoticed) have played an important role in the development of biological control methods of plant pests (1-4). For example, in the late 1800's the Australian cottony cushion scale insect (Icerya purchasi) was accidently introduced into California and within a few years threatened to destroy the citrus orchards. Entomologists then imported its natural predator the Australian ladybug Rodolia cardinolis which within two years conquered the scale insect (1).

Coccinellid aggregations could provide a large food source for animals, birds or insect predators but rarely are the beetles eaten. Gaudy colors and strikingly contrasting design patterns are exhibited by many species of coccinellids. Such visual display in insects is frequently associated with the presence of chemical defenses (6) and is then referred to as aposematic (warning) coloration. Indeed, ladybugs which are molested or disturbed fall into thanotosis and exude a yellowish, bitter fluid from the femoro-tibial articulations. This well-described mechanism, known as "reflex bleeding" (6) has been shown to give efficient protection against vertebrate and insect predators (6,7,8). It might well be that ladybugs, which have been extensively and successfully utilized in agricultural control of crop-destroying aphids and scale insect pests, are efficient biological control agents partly because the beetles themselves are chemically protected against predators. The present article surveys the Coccinellidae defensive substances; a novel structural group of alkaloids. A survey of 30 species of coccinellids has shown that the presence of alkaloids

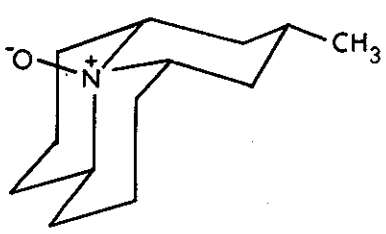
is associated with aposematic coloration and that the alkaloids constitute an effective defense against predators such as ants and quail (8). Annual summaries of new developments have appeared previously (9). In this review we describe first the isolation and structure elucidation of the defensive substances, briefly discuss their biosynthesis, and then conclude with a discussion of the laboratory syntheses.

Elucidation of Structures

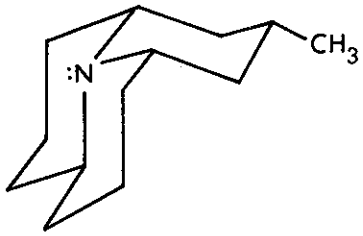
Coccinelline-precoccinelline

In 1971 Tursch and coworkers reported the first studies directed toward the identification of the defensive substances (10, 11). A methanol extract of the European ladybug Coccinella septempunctata was fractionated by chromatography over alumina. The active constituents were monitored by tasting the fractions in order to locate the exceedingly bitter taste of the beetles and by testing the fractions for efficiency as a repellent against the ant Myrmica rubra. Repeated fractionations led to the isolation of a white, crystalline alkaloid, $C_{13}H_{23}NO$, which was named coccinelline. Coccinelline is responsible for the bitter taste of C. septempunctata but not for its peculiar odor. Its efficiency as a defensive substance was demonstrated by its repellant activity towards ants: water containing 0.1 to 0.5% coccinelline was almost completely refused by thirsty M. rubra and a 2 mg extract of coccinelline on filter paper placed around their food discouraged hungry ants from feeding.

The mass spectrum of coccinelline (I) shows a prominent peak at M^+ -16, characteristic of a N-oxide, and reduction with either ferrous sulfate or hydrogen over platinum provides the free amine,



I



II

precoccinelline (II), $C_{13}H_{23}N$, which is also present in the haemolymph of C. septempunctata. The relationship between I and II as an N-oxide free-base pair was confirmed by oxidation of II to I with monoperphthalic acid. Precoccinelline readily forms a methiodide, whose ^{1}Hmr spectrum displays an N-methyl signal and a complex, three proton multiplet at δ 4.17 indicating the presence in precoccinelline itself of only three protons on carbons α to the tertiary nitrogen atom.

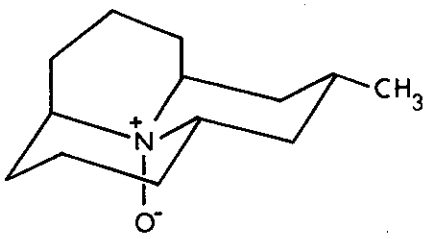
Since coccinelline is optically inactive the evidence presented indicates that it must be a tricyclic, racemic amine oxide or a tricyclic amine oxide possessing a plane of symmetry with the nitrogen atom, one α-carbon and the $-CHCH_3$ group in the symmetry plane. The existence of such an element of symmetry is consistent with the proton-decoupled ^{13}Cmr spectrum which contains only 8 signals: 5 singlets of relative intensity 2 and 3 singlets of relative intensity 1. The above data suggested, among others, structure I (without stereochemical implication) for coccinelline

and thus II for precoccinelline. The structural assignment as a 2-methylperhydro-9b-azaphenalene as well as the stereochemistry has been firmly established by an x-ray study of coccinelline hemihydrochloride (12).

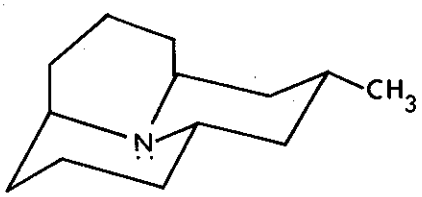
Since the preliminary report of Tursch and coworkers (10,11), coccinelline (I) and/or precoccinelline (II) have been found in several other species of the tribe Coccinellini (8,13,14). Although precoccinelline has been reported (15) to be present in Coleomegilla maculata no direct comparison was made and the spectral evidence reported for the defensive compound of this beetle is more consistent with that of the stereoisomer myrrhine (14).

Convergine - hippodamine

The abundant American ladybug Hippodamia convergens yielded two other alkaloids: convergine (III) and hippodamine (IV) (16,17). It has been demonstrated that convergine (III) has a repellent power 1.5 times that of coccinelline (I) towards the ant M. rubra (8). The initial structural assignment for convergine (16) has been corrected by a single-crystal x-ray diffraction analysis on convergine hydrochloride (17) which established III to be a stereoisomer of I.



III



IV

Hippodamine (IV) and convergine (III) are another free-base N-oxide pair. Hippodamine (IV) is readily oxidized to III with peracid and convergine (III) can be transformed to IV by reduction with lithium aluminum hydride (16) or by pyrolysis (13).

The mass spectra of III and IV are nearly identical with those of coccinelline (I) and precoccinelline (II), respectively, which precludes the use of this method for identification purposes. Comparison of the fingerprint region of the infrared spectra of III with I and IV with II allows discrimination and is the most reliable identification criterion. It is important to note that natural convergine (III), in contrast to coccinelline (I), when isolated by standard procedures is always obtained in its hydrated form as indicated by intense ir bands at 1680 and 3200 cm^{-1}. When convergine is transformed to its hydrochloride these bands disappear with concomitant appearance of characteristic salt bands at 1520 and 2600 cm^{-1}.

Although convergine (III) and hippodamine (IV) were first reported (16) to be optically inactive and thus racemic (the dissymmetry of the molecule could be inferred from the ^{13}Cmr spectrum, which shows 12 signals (2 signals superimposed) for the 13 carbon atoms), the x-ray results clearly indicated a chiral molecule and established the absolute configuration shown in III and IV (17). It was then shown that convergine hydrochloride does have an extremely small optical rotation which is strongly solvent dependent (17).

Convergine and/or hippodamine have been detected in other species of coccinellids, notably Hippodamia caseyi (13) and Anisosticta 19-punctata (8).

Myrrhine

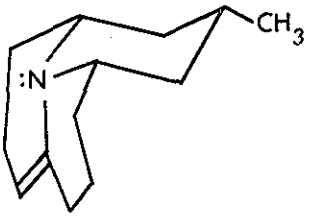
Because two of the three possible ring junction stereoisomers of 2-methylperhydro-9b-azaphenalene had been identified as ladybug alkaloids, an extensive search for the third stereoisomer (ring junction H's all cis) was undertaken by Tursch and coworkers (14). An alkaloid V, named myrrhine, was detected in Myrrha octodecimpunctata which was readily distinguishable by tlc (R_f 0.70, alumina, ethyl acetate) from precoccinelline (II) (R_f 0.29), and hippodamine (IV) (R_f 0.31).

Myrrhine (V), $C_{13}H_{23}N$, shows a mass spectral fragmentation pattern almost identical with that of II and IV. The ir spectrum, unlike that of II and IV, shows intense Bohlmann bands, possible only when the ring junction H's are all cis. This data led to the hypothesis that myrrhine was the stereoisomer V.

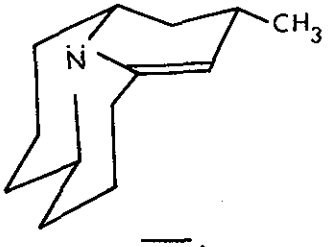
This structure, including the stereochemistry of the methyl group, was confirmed by chemical correlation of coccinelline and myrrhine using the Polonovski reaction (14) and by comparison with a synthetic sample (vide infra). Treatment of coccinelline at room temperature with acetic anhydride or ethyl chloroformate yielded an unstable enamine (VI) (ir 1650 cm^{-1}, ^{1}Hmr δ 4.4, 1 H, m), which gave a 9:1 mixture of myrrhine (V) and precoccinelline (II) on catalytic hydrogenation. Enamine VIa was expected to be formed preferentially on the basis of the known preference for trans elimination (18) in the Polonovski reaction. Had either enamine VIb or VIc resulting from cis elimination been formed, hydrogenation would not have led to the formation of myrrhine.

Myrrhine (V) forms an N-oxide (VII) which has not yet been isolated from natural souces. Compound VII does not undergo the

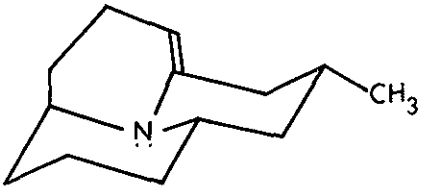
V



VI a

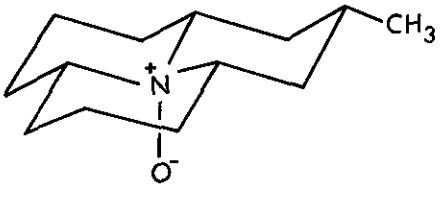


VI b



VI c

Polonovski reaction under the conditions described for coccinelline nor can it be reduced catalytically under mild conditions (14).



VII

Although myrrhine has not been reported present in any other species of coccinellid, it seems likely from the reported data (15) that myrrhine is the alkaloid present in Coleomegilla maculata.

Propyleine

Propyleine (VIb), $C_{13}H_{21}N$, is an amorphous unstable laevorotatory base which appears to be the only alkaloid present in the beetle Propylaea quattuordecimpunctata (19). The spectral properties of VIb (ir 1668 cm^{-1}, ^{1}Hmr δ 0.92, 3 H, d; 3.05-3.20, 2 H, br; 4.78, 1 H, m) suggest it is an enamine with one olefinic proton (β to nitrogen) and a secondary methyl group.

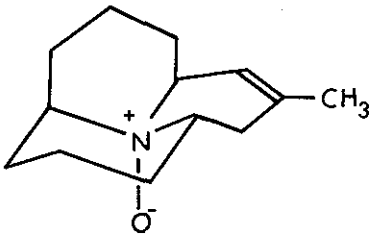
Catalytic hydrogenation of propyleine (VIb) yields precoccinelline (II). There are three possible enamine dehydroprecoccinellines (VIa, VIb and VIc). Propyleine shows only end absorption in the uv indicating that the lone pair on nitrogen does not overlap effectively with the π electron system of the enamine double bond. This condition is met in VIb and VIc, however only VIb would be expected to lead exclusively to precoccinelline on catalytic hydrogenation. On this basis propyleine has been assigned structure VIb.

Hippocasine oxide - Hippocasine

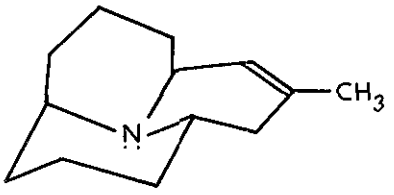
Hippocasine oxide (VIII) and hippocasine (IX) are two new alkaloids found along with convergine and hippodamine in the western Canadian ladybug Hippodamia caseyi Johnson (13).

Hippocasine oxide (VIII), $C_{13}H_{21}NO$, is an optically active substance which was characterized as the crystalline hydrochloride. Pyrolysis of hippocasine oxide (VIII) gave hippocasine (IX) whereas oxidation of hippocasine with hydrogen peroxide yielded hippocasine

oxide, establishing the N-oxide-free base relationship between the two. Hippocasine is isomeric with propyleine. The position of the



VIII



IX

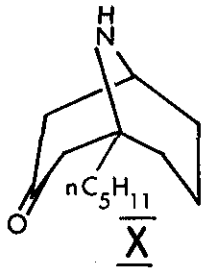
double bond was revealed by the presence of a vinyl methyl group in the ^{1}Hmr. The vinylic proton was vicinally coupled (J = 4.5 Hz) to a proton α to nitrogen. The size of this vicinal coupling constant suggested the stereochemistry shown in VIII and IX for hippocasine oxide and hippocasine respectively. This assignment was confirmed by x-ray crystallographic analysis of hippocasine oxide hydrochloride (13).

Adaline

A chemical defense associated with reflex bleeding in the European ladybug Adalia bipunctata L. was initially reported by Rothschild in 1961 (6). In 1973, Tursch and co-workers (20,21) reported the isolation and characterization of adaline (X), the defensive alkaloid present in A. bipunctata L., A. quadrimaculata Scopoli and A. pantherina L.

Adaline (X), $C_{13}H_{23}NO$, is an amorphous, laevorotatory base whose ir spectrum shows the presence of a carbonyl (1710 cm^{-1}) and an N-H (3300 cm^{-1}) group. The ^{1}Hmr spectrum of X shows a poorly

resolved triplet attributed to the terminal methyl of at least a two-carbon chain as well as one low field proton on a carbon α to the nitrogen atom.



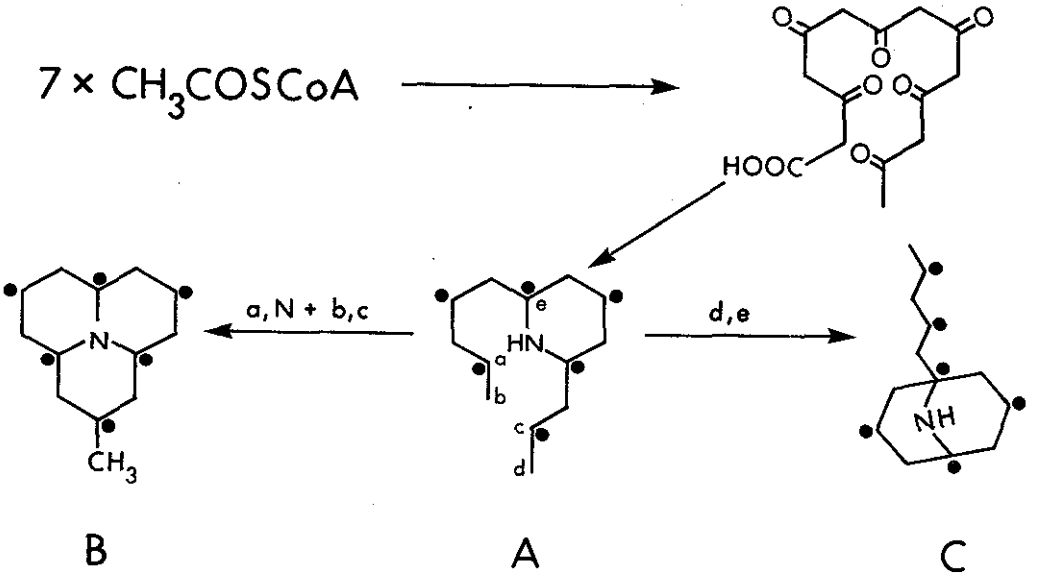
X

Adaline forms an N-acetyl derivative which gives a stereoisomeric mixture of alcohols on reduction with lithium aluminum hydride. Oxidation of the alcohol mixture gives N-ethyladaline. The data indicates that adaline is a bicyclic aminoketone with a carbon skeleton different from that found in other coccinellids. An x-ray analysis of adaline hydrochloride established structure X (20). A more highly refined x-ray analysis of adaline hydrochloride confirmed that the absolute configuration (as suggested by the positive Cotton effect observed in the o.r.d.) is that depicted in structure X (21).

Biosynthesis

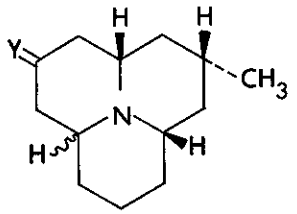
The carbon-nitrogen skeleton of alkaloids I-X may be generated by linear condensation of seven acetate units as illustrated in Scheme 1. Decarboxylation and combination with ammonia or its equivalent can lead to an intermediate A (dotted carbons represent carbonyl carbons of the acetate units). Further bond formation between the nitrogen and carbon "a" as well as C-C bond formation

between "b" and "c" leads to the skeleton (B) of the tricyclic alkaloids I-IX, whereas bond formation between carbons "d" and "e" leads to the bicyclic skeleton (C) of adaline (X). Support for the polyketide origin of the defensive substances has been provided (14)

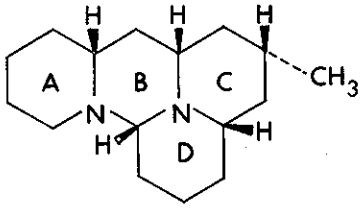


Scheme 1

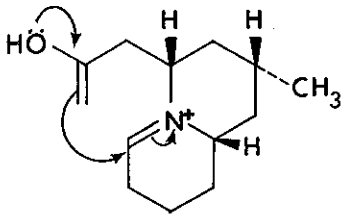
by feeding experiments with Coccinella septempunctata. When these ladybugs were fed with either C-1 or C-2 labelled (^{14}C) acetate, labelled coccinelline (I) was obtained. Kuhn-Roth oxidation furnished, in each case, labelled acetic acid which accounted for about one-seventh of the activity present in the coccinelline. The fact that the C-2 labelled acetate leads to labelled acetic acid rules out the possibility that the methyl group of the alkaloids comes from the one carbon pool and strongly supports a biosynthesis as broadly outlined in Scheme 1.



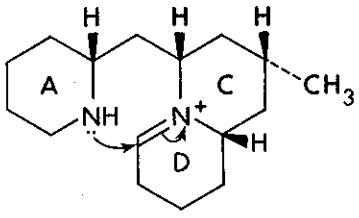
II, V



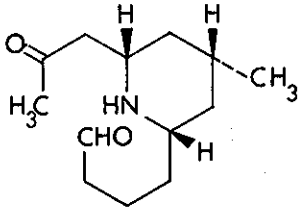
dihydrodeoxyepiallocernuine



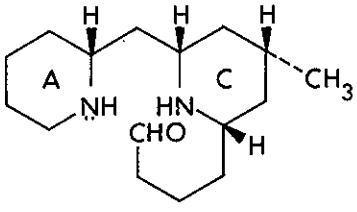
XII b



XI b



XII a



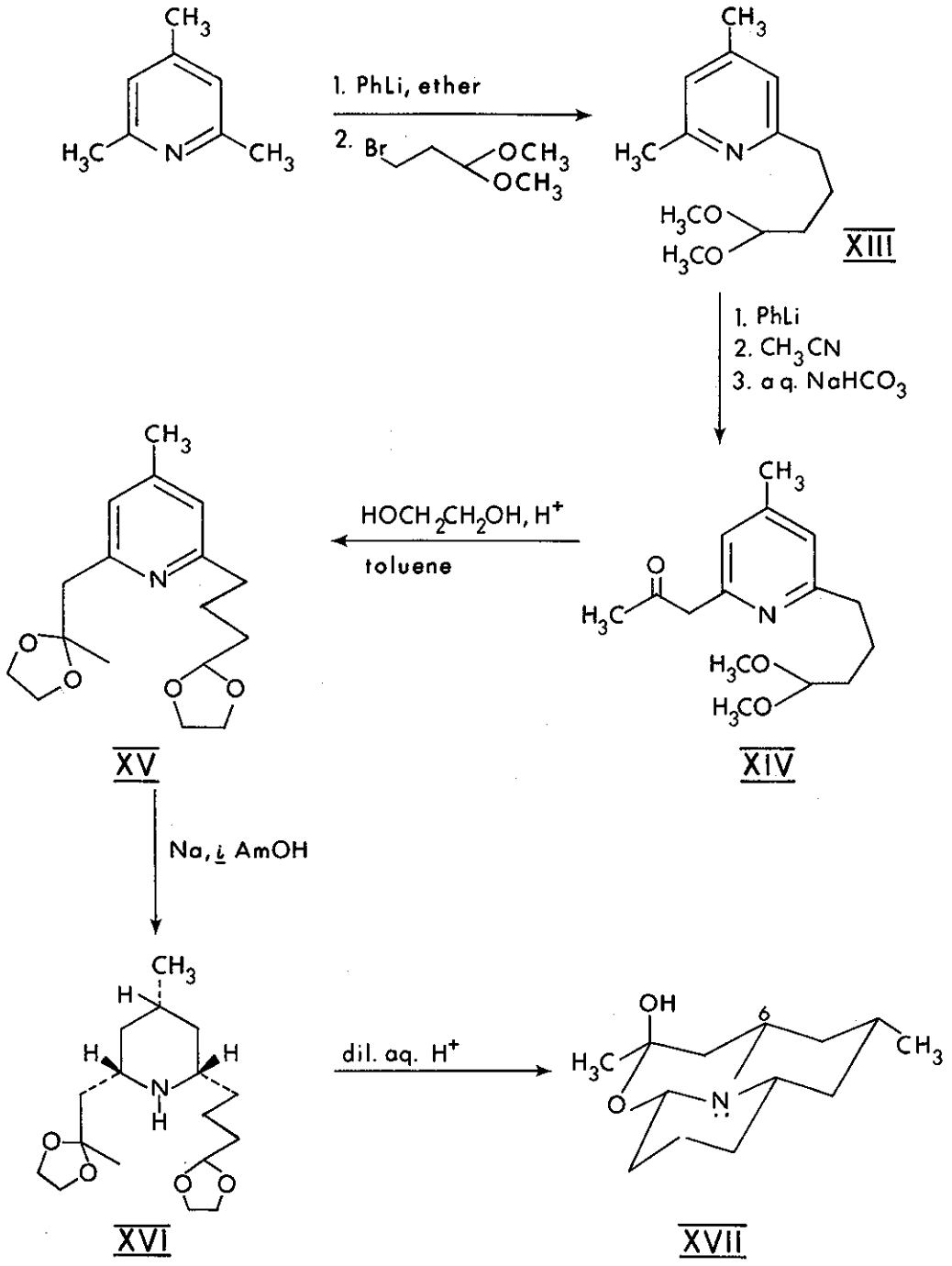
XI a

Scheme 2

Synthesis

Our interest in the synthesis of the tricyclic ladybug defensive substances was aroused when we noted the striking similarity (Scheme 2) between these substances (eg. II, V (Y = H, H)) and the BCD ring system of dihydrodeoxyepiallocernuine, a transformation product of the Lycopodium alkaloid cernuine which we had synthesized previously (22). A key step in the synthesis of dihydrodeoxyepiallocernuine was the cyclization of the diaminoaldehyde XIa, presumably involving nucleophilic addition of the ring A nitrogen to the intermediate immonium ion XIb as shown in Scheme 2. A similar sequence for the generation of the tricyclic system of myrrhine and/or precoccinelline is also illustrated in Scheme 2, where the nucleophilic nitrogen of XIb is replaced by an enol or enolate (XIIb) generated from the ketoaldehyde XIIa. Cyclization would then lead to the tricyclic system shown in Scheme 2 (II, V, (Y = O)).

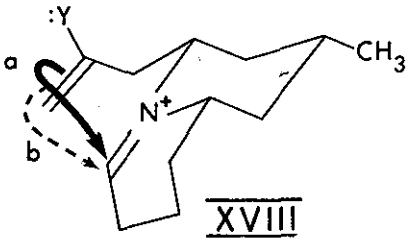
The synthesis of the protected form of ketoaldehyde XIIa, starting from 2,4,6-collidine, is shown in Scheme 3 (23). Collidyllithium was generated in ether and alkylated with β-bromopropionaldehyde dimethyl acetal to give acetal XIII. The introduction of an acetyl group into the 6-methyl group of XIII proved rather troublesome. However, treatment of XIII with phenyllithium in ether, followed by very slow addition of one equivalent of acetonitrile provided, after workup, the ketone XIV (1720 cm^{-1}) which was best isolated as the bis ethylene acetal XV. Proof that acetylation had indeed occurred at the 6-methyl group rather than the 4-methyl group of XIII is provided by the ^{1}Hmr spectrum of XV which clearly shows the presence of a pyridine γ-methyl group (δ 2.38).


1. PhLi, ether
2. Br
OCH3
OCH3
XIII
1. PhLi
2. CH3CN
3. a q. NaHCO3
HOCH2CH2OH, H+
toluene
XIV
XV
Na, i AmOH
dil. aq. H+
XVI
XVII

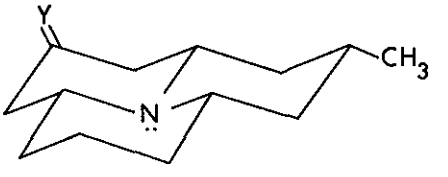
Scheme 3

Reduction of the diacetal XV with sodium in isoamyl alcohol provided a mixture of stereoisomers of which the desired all-cis piperidine XVI was the major component. The stereochemistry of XVI was determined mainly on the basis of its ^{13}Cmr spectrum, which indicated that all three substituents on the piperidine ring were equatorial (and thus cis). Diacetal XVI is the protected form of the desired ketoaldehyde XIIa. However, hydrolysis of XVI with dilute acid gave rise to a crystalline compound which did not show carbonyl absorption in the ir, but which did exhibit hydroxyl absorption and intense Bohlmann bands. On the basis of this and additional spectroscopic data, the hydrolysis product was shown to be XVII, the hemiketal of the carbinolamine form of XIIa. The presence of the intense Bohlmann bands confirmed that indeed the 2,6-substituents of piperidine XVI are cis, and the ^{13}Cmr indicated the equatorial nature of the methyl groups in XVII.

We now turned our attention to methods for generating the immonium ion XVIII in its enol (Y = OH) or enamine (Y = NR_2) form which should be capable of cyclization as shown in XVIII. Previous experience (22) did not allow us to predict, a priori, whether



XVIII



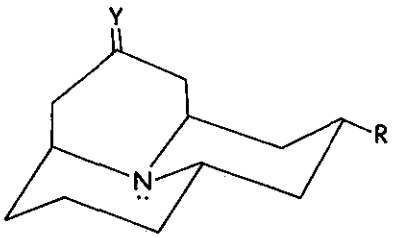
XIX Y = O

XX Y = -SCH$_2$CH$_2$S-

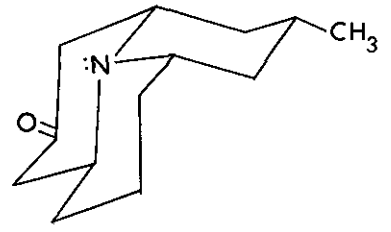
V Y = H,H

cyclization would occur by "topside" attack (arrow a in XVIII) to generate the coccinelline stereochemistry, or by "bottomside" attack (arrow b) to give the myrrhine stereochemistry. However, treatment of the hemiketal XVII with two equivalents of p-toluenesulfonic acid in hot toluene afforded in high yield the ketone XIX (ir 1728 cm^{-1}) possessing the myrrhine stereochemistry, as indicated by the presence of intense Bohlmann bands in the ir spectrum. Ketone XIX (which is unstable in the presence of air) was transformed into the crystalline, stable thioacetal XX. Raney nickel desulfurization of XX provided myrrhine (V) in ca. 40% overall yield from XVII. Thus the acid-catalyzed cyclization of XVII, presumably through the enol immonium ion XVIII, appears to proceed completely via "bottomside" attack (arrow b in XVIII), although this may be the result of a thermodynamically controlled process.

In an effort to obtain the coccinelline (I) stereochemistry other methods of cyclization were investigated. When the hemiketal XVII was heated with pyrrolidine and acetic acid in tetrahydrofuran, the product consisted of two ketones, one having the same R_f as ketone XIX, the other being more polar. The mixture of ketones was treated with ethanedithiol-BF_3 and the resulting mixture of thioacetals desulfurized over Raney nickel to give a mixture of myrrhine



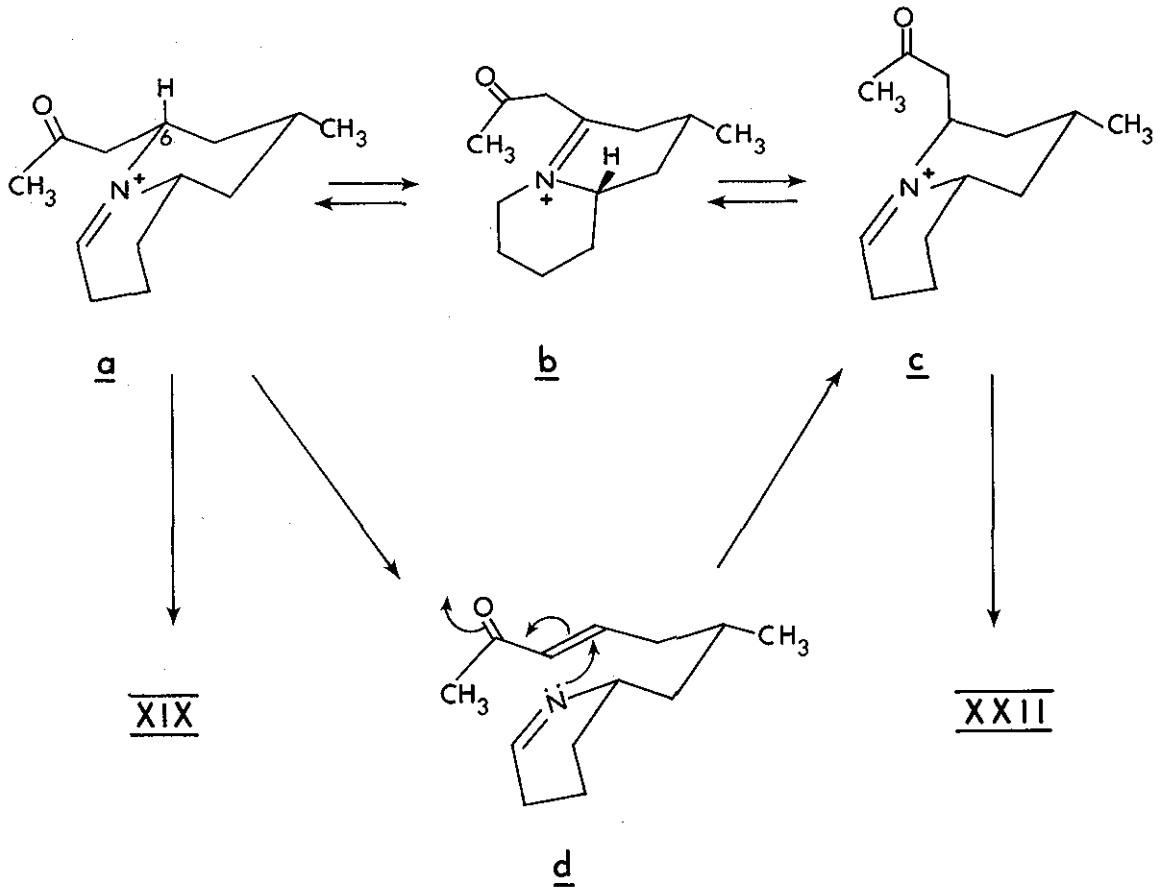
XXI Y=H,H R=CH_3
XXII Y=O R=CH_3
XXIV Y=O R=H



XXIII

and, much to our surprise, hippodamine (IV = XXI). Oxidation of hippodamine with peracid gave convergine (III). The overall yields of myrrhine and racemic hippodamine from XVII were 33% and 23%, respectively.

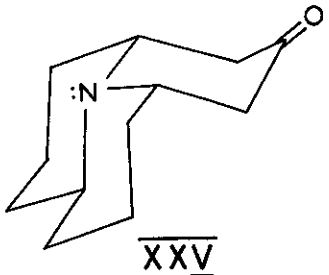
The formation of hippodamine (IV = XXI) requires isomerization at C-6 during the cyclization. In the presence of both acid and base this may involve a reversible shift of the immonium double bond via b as illustrated in Scheme 4. Although ion c is expected



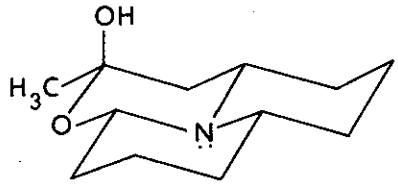
Scheme 4

to be a minor component in the equilibrium mixture, the axial side chain is ideally situated for ring closure to ketone XXII and the rate of cyclization of c may well be much greater than the rate of cyclization of a. Alternatively, ion a (Scheme 4) may undergo β-elimination to d and readdition to give ion c. We were unable to find conditions which enabled us to cyclize XVII to the ketonic precursor (XXIII) of precoccinelline (23).

At this stage, however, we realized that the ketone XXIV, which is also represented by XXV, requires only replacement of the carbonyl group by an equatorial methyl group to give precoccinelline. If the unexpected isomerization (Scheme 4) which allowed us to synthesize hippodamine occurred in a series lacking the 4-methyl group (i.e., in a series starting from 2,6-lutidine rather than 2,4,6-collidine) the product would be ketone XXV (= XXIV). Indeed, when 2,6-lutidine was subjected to the same series of reactions outlined



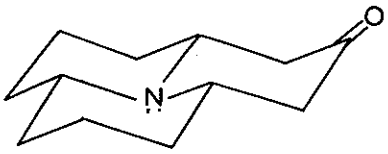
XXV



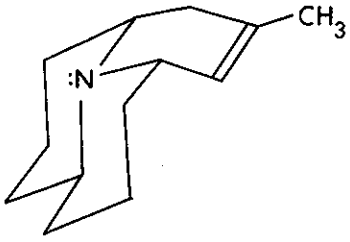
XXVI

in Scheme 3 for 2,4,6-collidine the crystalline hemiketal XXVI was obtained in good overall yield (24). When XXVI was subjected to the cyclization conditions (pyrrolidine-acetic acid-tetrahydrofuran) which led to the isomerization shown in Scheme 4, a 1:1 mixture of

ketone XXV (= XXIV) and XXVII (= XIX without the C-methyl group) was obtained in 86% yield. The ir spectrum of ketone XXV shows carbonyl absorption at 1710 cm^{-1} and lacks Bohlmann bands. Ketone XXVII absorbs at 1730 cm^{-1} and displays intense Bohlmann bands in the ir.



XXVII



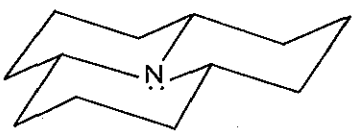
XXVIII

Treatment of ketone XXV with methyllithium followed by dehydration of the resulting carbinol with thionyl chloride in methylene chloride gave the air sensitive olefin XXVIII which on catalytic hydrogenation gave precoccinelline (II), identical with the natural material. Oxidation of synthetic II with m-chloroperbenzoic acid gave coccinelline (I). Treatment of ketone XXVII under similar conditions (methyllithium, dehydration, hydrogenation) provided another route to myrrhine (V) (24).

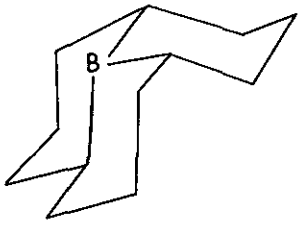
An elegant synthesis of the parent perhydro-9b-azaphenalene system (XXIX) from the readily available perhydroboraphenalene (XXX) has been reported (25). This paper also describes two methods for the transformation of XXIX to the precoccinelline related stereoisomer XXXI.

The bicyclic alkaloid adaline (X) has been synthesized (21) by an adaptation of the method first used to synthesize 9-aza-1-methyl-

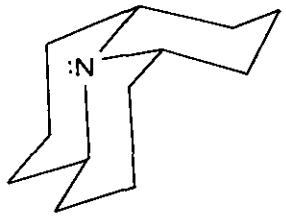
bicyclo[3.3.1]nonan-3-one (26). Thus oct-1-en-3-one underwent Diels-Alder addition with methyl vinyl ketone to give the adduct



XXIX

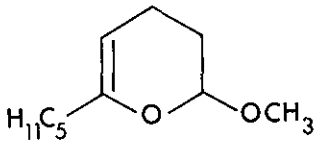


XXX

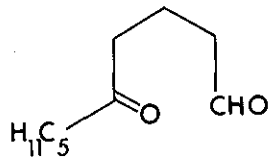


XXXI

XXXII. Acid hydrolysis provided the keto aldehyde XXXIII, which on condensation with ammonia and acetone dicarboxylic acid with concomitant decarboxylation afforded dl-adaline (X) (21).



XXXII



XXXIII

With the exception of the unsaturated tricyclic compounds propyleine (VIb) and hippocasine (IX) all of the known defensive substances are now available by means of total synthesis.

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