# RECENT PROGRESS IN THE CHEMISTRY OF PHENANTHROINDOLIZIDINE ALKALOIDS†

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This review gives a survey of developments since 1972 in the field of phenanthroindolizidine alkaloids under the following sub-divisions -
(1) Isolation, structure, stereochemistry
(2) Synthesis (3) Biogenesis (4) Biological activity.

The phenanthroindolizidine alkaloids comprise a small group of alkaloids isolated mainly from plants belonging to the Asclepiadaceae family. The best known sources are plants of the genus Tylophora and the plant Cynanchum vincetoxicum. Some of these alkaloids have also been isolated from Ficus septica which belongs to the Moraceae family. Their chemistry has been reviewed on two previous occasions[1,2], in 1967 and 1973, and the object of the present review is to document subsequent developments in this field. The literature is covered till the end of 1977 under the following four sub-divisions :

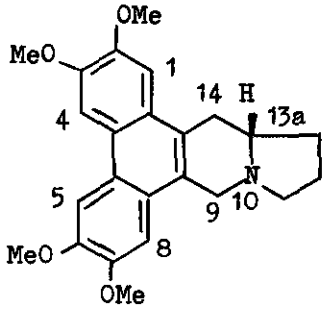
† Dedicated to Professor Tetsuo Nozoe on the occasion of his 77th birthday.

1. Isolation, structure, stereochemistry
2. Synthesis
3. Biogenesis
4. Biological activity

## 1. Isolation, structure, stereochemistry

### Tylophorine

The gross structure of tylophorine, the major alkaloid of Tylophora asthmatica Wight et Arn. (syn. T. indica) had been shown by degradation and synthesis to be 9, 11, 12, 13, 13a, 14-hexahydro-2,3,6,7-tetramethoxydibenzo[f,h]pyrrolo[1,2-b]isoquinoline (1). The stereochemistry with S-configuration at $C_{13a}$ was determined[3] by exhaustive ozonolysis of tylophorine which yielded (S)-pyrrolidine-2-acetic acid (2) identical with a sample synthesized from (S)-proline through the intermediates 3a to 3f. Because of the poor yield in the ozonolysis, the acid was converted to the methyl ester and coupled with N-trifluoroacetyl - (S)-prolyl chloride to give the dipeptide (4) which was examined by g.l.c. with the synthetic diester.

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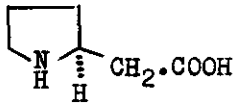


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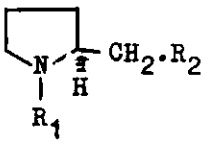

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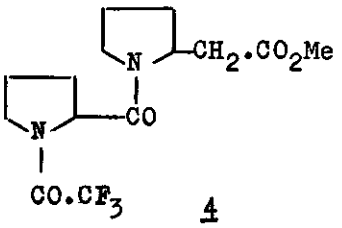


4

a : $R_1$ = H; $R_2$ = OH

b : $R_1$ = Ph.$CH_2$; $R_2$ = OH

c : $R_1$ = Ph.$CH_2$; $R_2$ = Cl

d : $R_1$ = Ph.$CH_2$; $R_2$ = CN

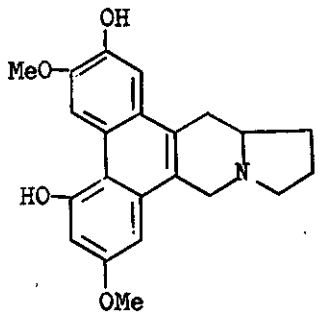
e : $R_1$ = Ph.$CH_2$; $R_2$ = $CO_2$Me

f : $R_1$ = H; $R_2$ = $CO_2$Me

The O.R.D. of tylophorine shows a negative Cotton effect in the region near 260 nm and this serves as a tool for determining the absolute configuration at $C_{13a}$ of the alkaloids lacking functionality at $C_{14}$.

Tylophorinidine, tylophorinine

Tylophorinidine, $C_{22}H_{23}NO_4$, was initially assigned[4] structure 5 which was incompatible with its spectral characteristics. Reinvestigation[5] of the structure of tylophorinidine led to the revision of its structure to 6a.

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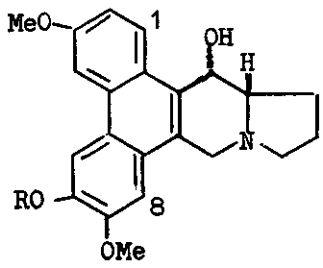


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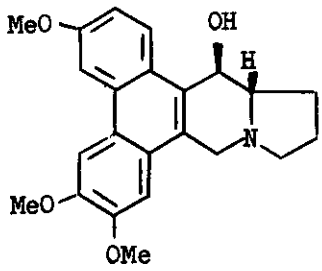
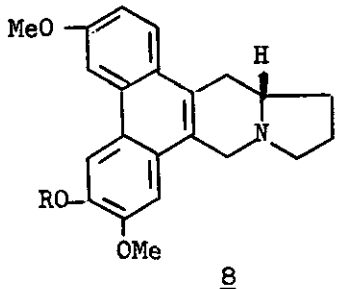



7

a : R = H
b : R = Me

The N.M.R. spectrum of O-methyltylophorinidine (6b) was found to differ from that of its diastereoisomer tylophorinine (7). In 6b, in $CDCl_3$, $C_1$-H is deshielded and the $C_8$-H is heavily shielded. This has been rationalised on the basis of a hydrogen-bonded dimer of 6b arising from two interactions between the $C_{14}$ hydroxyls and the nitrogen lone pair. An X-ray study[6] of the methiodide of diacetyltylophorinidine confirmed this assignment of the relative stereochemistry at $C_{13a}$ and $C_{14}$ and this also showed the absolute configuration as shown in 6a.

Attempts[7] to hydrogenolyse O-methyltylophorinidine (6b) gave only the racemic desoxy-base (8a) by racemization at $C_{13a}$. Tylophorinidine (6a) however yielded the optically active desoxy-base (8b) having a negative O.R.D. in the region 200-280 nm region in agreement with the X-ray study.




8

a : R = Me
b : R = H

Tylophorinine (7) had been shown to be a diastereoisomer of O-methyltylophorinidine. Examination of the O.R.D. of tylophorinine, its acetate and desoxy derivative showed that tylophorinine is racemic.[7] The $C_8$ hydrogen in tylophorinine is not shielded to the same degree as in O-methyltylophorinidine (6b) presumably because steric interference of the $C_{13a}$ hydrogen results in weaker hydrogen bonding in dimeric tylophorinine. The N.M.R. spectra of acetyltylophorinine and acetyl O-methyltylophorinidine are virtually superposable and show a low coupling of 2 Hz between $C_{14}$-H and $C_{13a}$-H. In O-methyltylophorinidine the $C_{14}$-hydroxyl and the $C_{13a}$-hydrogen are known to be axial. It appears that in tylophorinine also the $C_{14}$-hydroxyl is axial, the $C_{13a}$-hydrogen being equatorial to account for the low $C_{13a}$-$C_{14}$ coupling.[5] It has been suggested[8] that a satisfactory dimer of tylophorinine may be obtained when ring D is in a flattened boat conformation thus forcing the $C_{14}$-hydroxyl into a pseudo-equatorial disposition. An answer to the problem seems possible only from an X-ray study of tylophorinine.

Pergularia pallida

Mulchandani and Venkatachalam[9] have obtained from the plant Pergularia pallida (Asclepiadaceae) five phenanthroindolizidine alkaloids - tylophorine, tylophorinidine, two new bases designated as pergularinine and desoxypergularinine and an unidentified base.

Pergularinine, m.p. 233-235°(d), $[\alpha]_D^{25}$ -16° (c 0.25, $CHCl_3$), $\lambda_{max}^{MeOH}$ 260, 287, 313, 341, 357 nm (log ε 4.7, 4.4, 3.9, 3.2, 2.8), $C_{23}H_{25}NO_4$ ($M^+$ m/e 379) has a hydroxyl at $C_{14}$ as shown by its mass spectral fragmentation. Its I.R. spectrum is nearly superposable with O-methyltylophorinidine. It yields an acetate, m.p. 177-178° (d), $[\alpha]_D^{25}$ -17.4° (c 0.112, $CHCl_3$), $\nu_{max}^{KBr}$ 1725, 1615, 1539, 1510, 1250 $cm^{-1}$. The alkaloid was assigned structure 7 or its mirror image. Since this actually represents tylophorinine, pergularinine is only optically active (-)-tylophorinine and there does not seem to be need for a new name for the alkaloid.

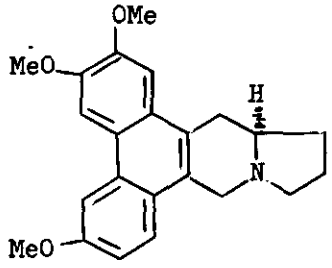
Deoxypergularinine, m.p. 208°(d), $[\alpha]_D^{25}$ -13.6° (c 0.25, $CHCl_3$), $\lambda_{max}^{MeOH}$ 258, 286, 311, 341, 360 nm (log ε 4.3, 3.9, 3.5, 3.1, 2.6), $C_{23}H_{25}NO_3$ ($M^+$ m/e 363) was found to be identical with the hydrogenolysis product of pergularinine and was hence assigned structure 8a. The stereochemistry at $C_{13a}$ follows from its negative Cotton effect at 270 nm of the same order of magnitude as tylophorine. Deoxypergularinine hence represents optically active (-)-desoxytylophorinine.

The fifth alkaloid from the plant, m.p. 210-212°(d), $[\alpha]_D^{25}$ -9.4° (c 0.04, $CHCl_3$), $\lambda_{max}^{MeOH}$ 258, 287, 302, 339, 355 nm (log ε 4.4, 4.2, 3.6, 3.1, 2.9), $\nu_{max}^{KBr}$ 3480, 1614, 1535, 1510,

1250 $cm^{-1}$, $C_{24}H_{27}NO_5$ ($M^+$ m/e 409) has a hydroxyl at $C_{14}$ as shown by its mass spectral cleavage and four methoxyl groups. Its U.V. spectrum is very close to that of tylophorine and the alkaloid is probably 14-hydroxytylophorine.

Antofine (9)

Antofine, isolated from Cynanchum vincetoxicum L. Pers, had been assigned the R-configuration at $C_{13a}$ since ozonolysis yielded D-proline.[10]

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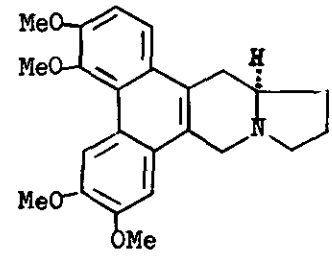



10

The O.R.D. of antofine is in agreement with this assignment.[3]

Partially racemic antofine has been found to be the major alkaloid in young trees of Ficus septica.[11] Other minor bases of unknown structure were also isolated.

Isotylocrebrine

(+)-Isotylocrebrine (10), obtained as a minor alkaloid from T. asthmatica[5], has been shown to have the R-configuration at $C_{13a}$ by O.R.D.[3]

Recent investigations of Tylophora spp.

The genus Tylophora comprises some 50 species and most of the known phenanthroindolizidine alkaloids have been obtained from T. asthmatica and T. crebriflora.

T. dalzelli has a low alkaloid content, demethyltylophorinine being the major alkaloid.[12]

Samples of leaf, stem and root of some Tylophora species from Sri Lanka have been screened for their alkaloid content.[8] The presence of tylophorinine and three unknown alkaloids is indicated in T. cordifolia and tylophorine, tylophorinine and four unknown alkaloids in T. flava. Eight different samples of T. asthmatica were screened and tylophorinine shown to be the major alkaloid in contrast to Indian T. asthmatica in which tylophorine is the major alkaloid.

T. hirsuta Wight is reported to have alkaloids but none has been identified.[13]

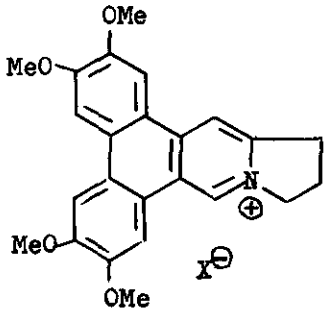
Seventeen species of Tylophora have been screened for their alkaloid, flavonoid, sterol and tannin content. Examination of the total percentage of tylophorine and tylophorinine in T. asthmatica as a function of growth phase revealed the highest content in the leaves during the flowering period.[14]

The isolation of two furoquinoline alkaloids, γ-fagarine and skimmianine, from the roots and aerial parts of T. asthmatica, may be of taxonomic importance.[15]

T. mollissima has a low alkaloid content, caffeine being the major alkaloid and tylophorine and tylophorinine being minor constituents.[16]

Quaternary alkaloids

Besides the tertiary bases reported earlier, water-soluble quaternary alkaloids have been isolated from T. asthmatica.[17] Conversion to the perchlorate gave a yellow salt, $C_{24}H_{24}NO_8Cl$, m.p.>280°, $\lambda_{max}^{EtOH}$ 287, 330 nm (log ε 4.56, 4.01), whose N.M.R. spectrum (in $CF_3CO_2H$) showed the presence of six aromatic protons as singlets, four methoxyl groups and three methylene groups. Catalytic reduction of the salt yielded dl-tylophorine thus showing the perchlorate to have structure 11 (X = $ClO_4$).

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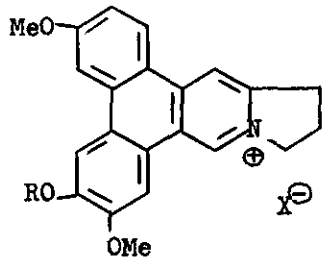



12

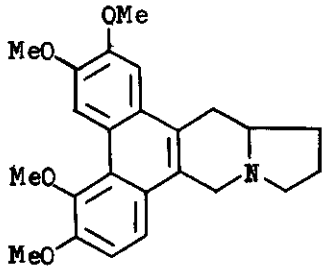
a : R = Me
b : R = H

Catalytic reduction of the crude perchlorate obtained from the mother liquors of 11 yielded dl-tylophorine contaminated with dl-desoxytylophorinine and dl-desoxytylophorinidine. This indicated the presence of the salts 12a and 12b in the crude salt.

Phenanthroindolizidine alkaloids are known to be unstable when exposed to light. Exposure of a solution of tylophorine in chloroform to light and air gives the crystalline chloride

11 (X = Cl) which can be converted to the perchlorate 11 (X = $ClO_4$) identical with the salt obtained from the plant. In view of this facile oxidation, it is likely that the salts 11, 12a and 12b are artifacts formed during the isolation.[17]

Oxidation of (-)-tylocrebrine (13) and (-)-septicine (14) with N-bromosuccinimide in chloroform yields the corresponding tetradehydroiminium salts 15 and 16.[18] The oxidation proceeds via the didehydroiminium salt such as 17, which is obtained with insufficient reagent or time. Reduction of the quaternary salts 15 and 16 with sodium borohydride gave the racemic bases 13 and 14 respectively.

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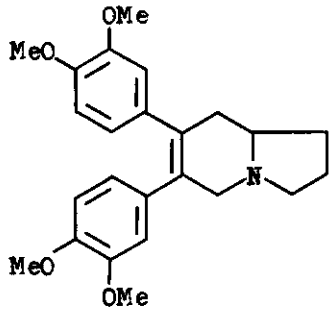


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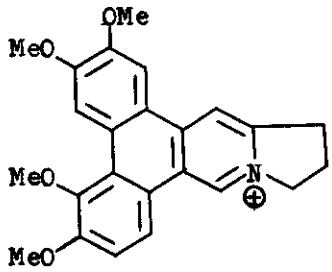


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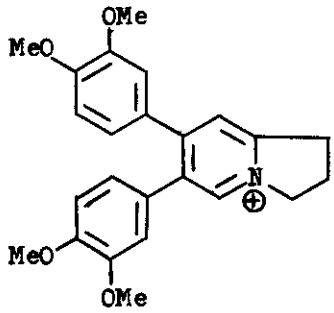


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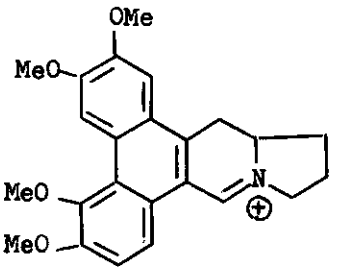
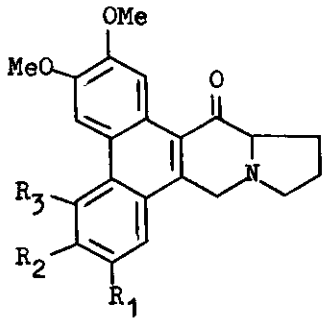



17

## 2. Synthesis

1. Chauncy and Gellert[19] have described the syntheses of racemic antofine, tylocrebrine, tylophorine and 2,3-dimethoxyphenanthroindolizidine. These involve the condensation of the appropriate chloromethylphenanthrenes with benzyl prolinate, hydrolysis, cyclization to the ketones 18a - 18d and reduction of the tosylhydrazones with sodium borohydride.

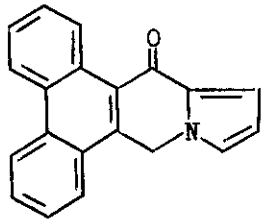



18

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| a | H | OMe | H |
| b | H | OMe | OMe |
| c | OMe | OMe | H |
| d | H | H | H |

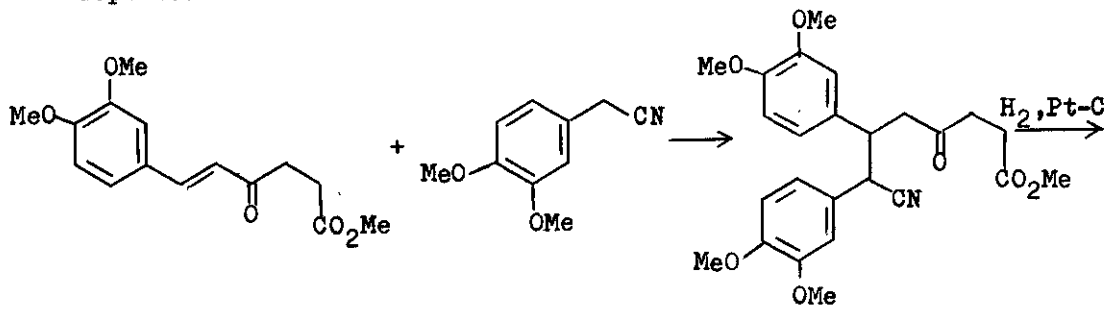
Shah and Trivedi[20] have synthesized the unsubstituted parent phenanthroindolizidine by this method, using sodium dihydrobis-(2-methoxyethoxy)aluminate for the reduction of the carbonyl group. The reduction however could not be carried out on the 6-methoxy analogue.

2. The pyrroloisoquinoline (19) was synthesized[21] by Friedel-Crafts cyclization of 1-(9-phenanthrylmethyl) pyrrole-2-carbonyl chloride.



19

3. Vanadium oxytrifluoride was found to convert a variety of 1,2-diarylethylene derivatives into phenanthrenes in high yield and provided the means for a new synthesis of dl-tylophorine as depicted below.[22]

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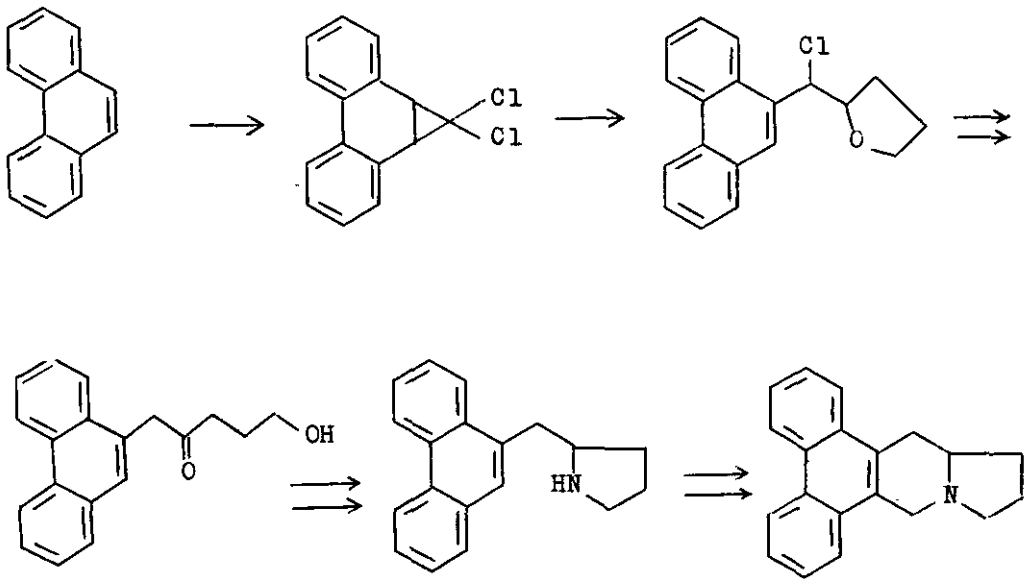
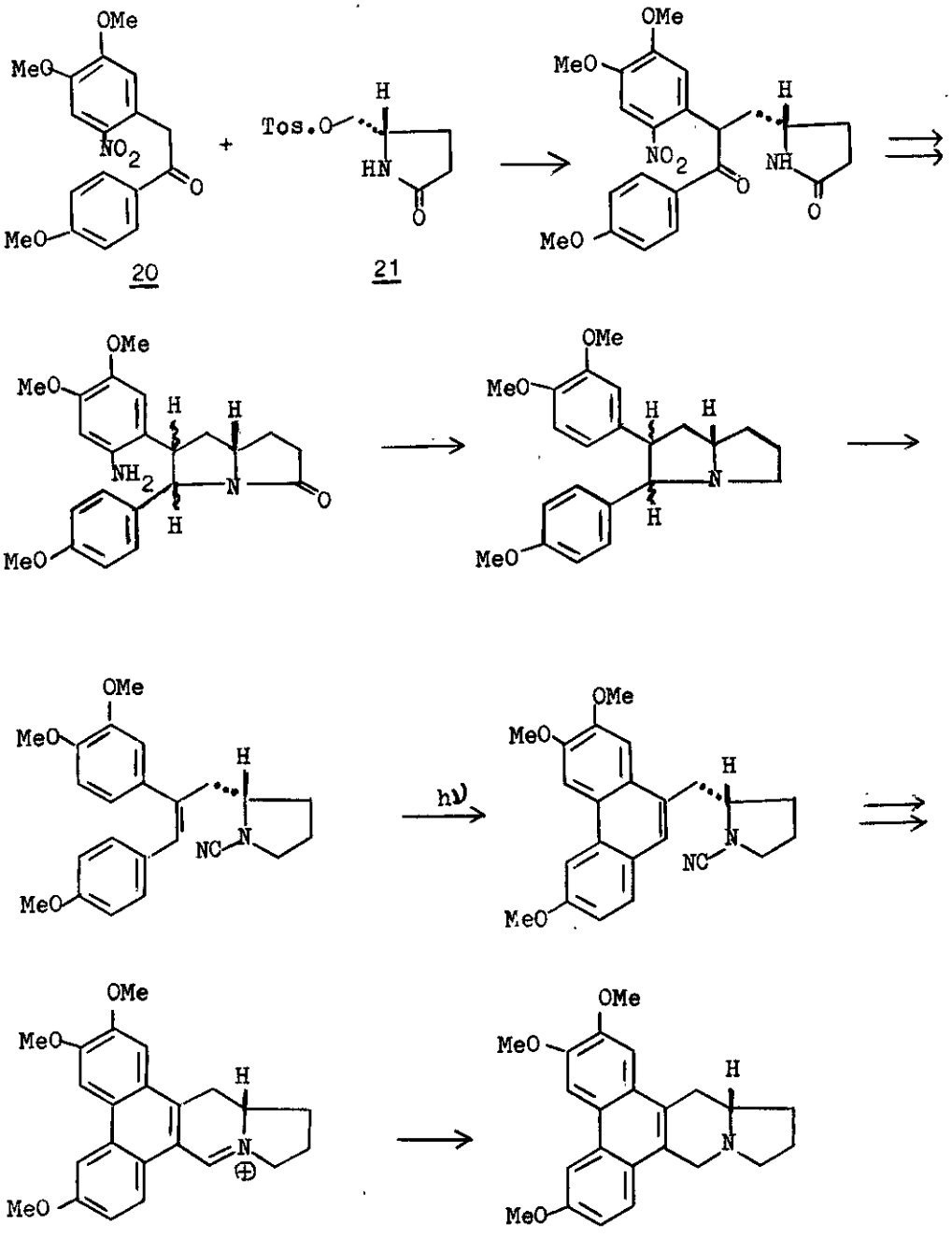
4. A novel synthesis of the phenanthroindolizidine ring system from phenanthrene has been described.[23] The sequence involves the addition of dichlorocarbene to the 9,10-double bond of phenanthrene and further elaboration of the side chain as outlined below.




5. Previous syntheses of the phenanthroindolizidine alkaloids had given racemates because they involved hydrogenation of a pyrrole derivative or because the intermediates had a carbonyl α to the asymmetric carbon atom at $C_{13a}$ Faber and Wiegrebe[24] have carried out the stereospecific synthesis of the optical antipode of (-)-antofine (9) from the desoxybenzoin (20) by the route outlined below. The chiral starting material, (S)-(2-pyrrolidon-5-yl) methyl p-toluenesulphonate (21) was only 50% optically pure and the final product had also 50% of the optical rotation expected for the pure optical antipode.


OMe
MeO
NO2
O
MeO
20
+
Tos.O
H
HN
O
21
NH2
N
NC
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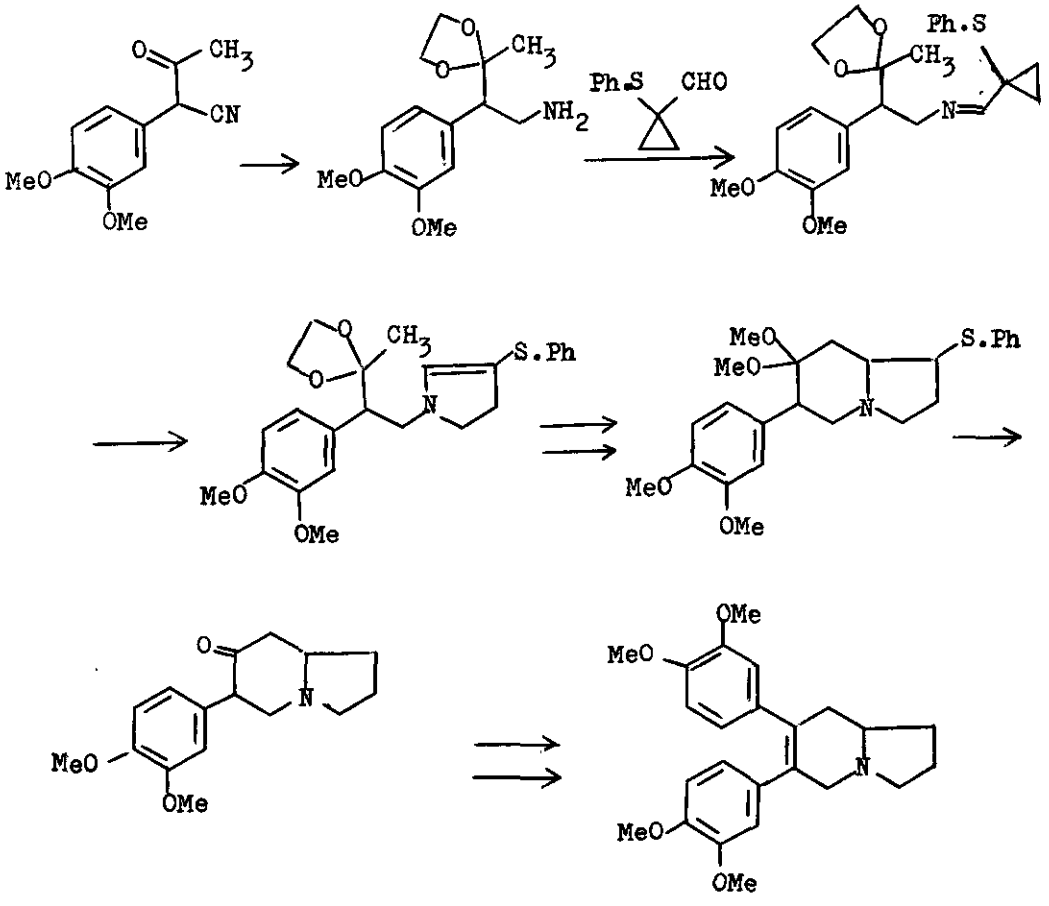
antipode of 9

The synthesis established that natural antofine (9) has the R configuration at $C_{13a}$.

6. A new synthesis of the seco-phenanthroindolizidine alkaloid septicine (14) has been published.[25] The synthesis, involving the acid-catalysed rearrangement of a cyclopropylimine to generate the 3-phenylthio-2-pyrroline synthon, is briefly depicted below.



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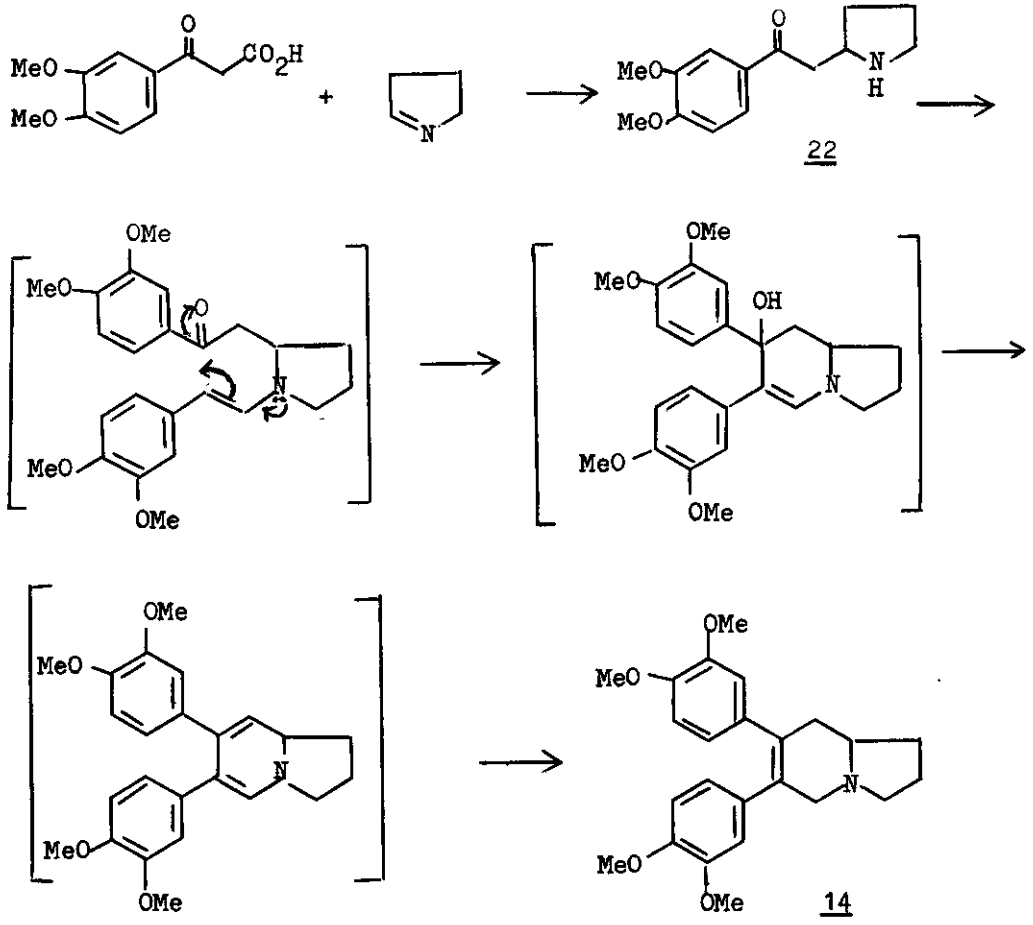
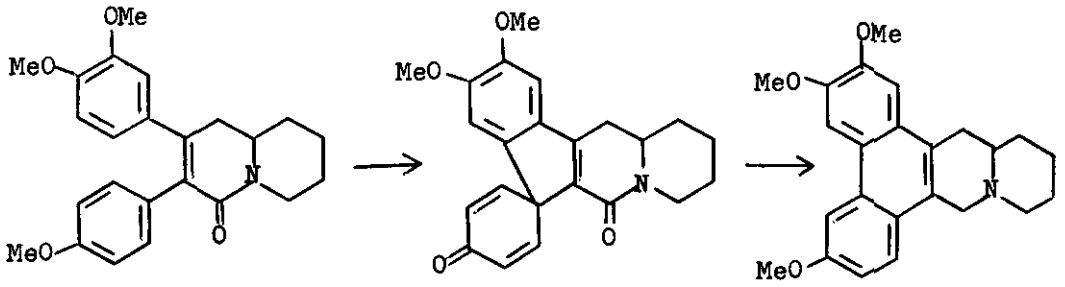
7. A new synthesis of septicine which is patterned on the likely biosynthetic pathway has been described.[26] Reaction of 3,4-dimethoxybenzoylacetic acid with $\Delta^1$-pyrroline gave the phenacylpyrrolidine (22). Condensation of 22 with 3,4-dimethoxyphenylacetaldehyde and subsequent reduction with sodium borohydride yielded dl-septicine (14).



Since $\Delta^1$-pyrroline may be prepared from either ornithine or putrescine, both of which are available with a variety of labels, the route lends itself to the synthesis of labelled compounds of biosynthetic interest.

8. The phenanthroquinolizidine alkaloid cryptopleurine (24) has been synthesized from the stilbene derivative (23) by anodic oxidation followed by a dienone-phenol rearrangement.[27]

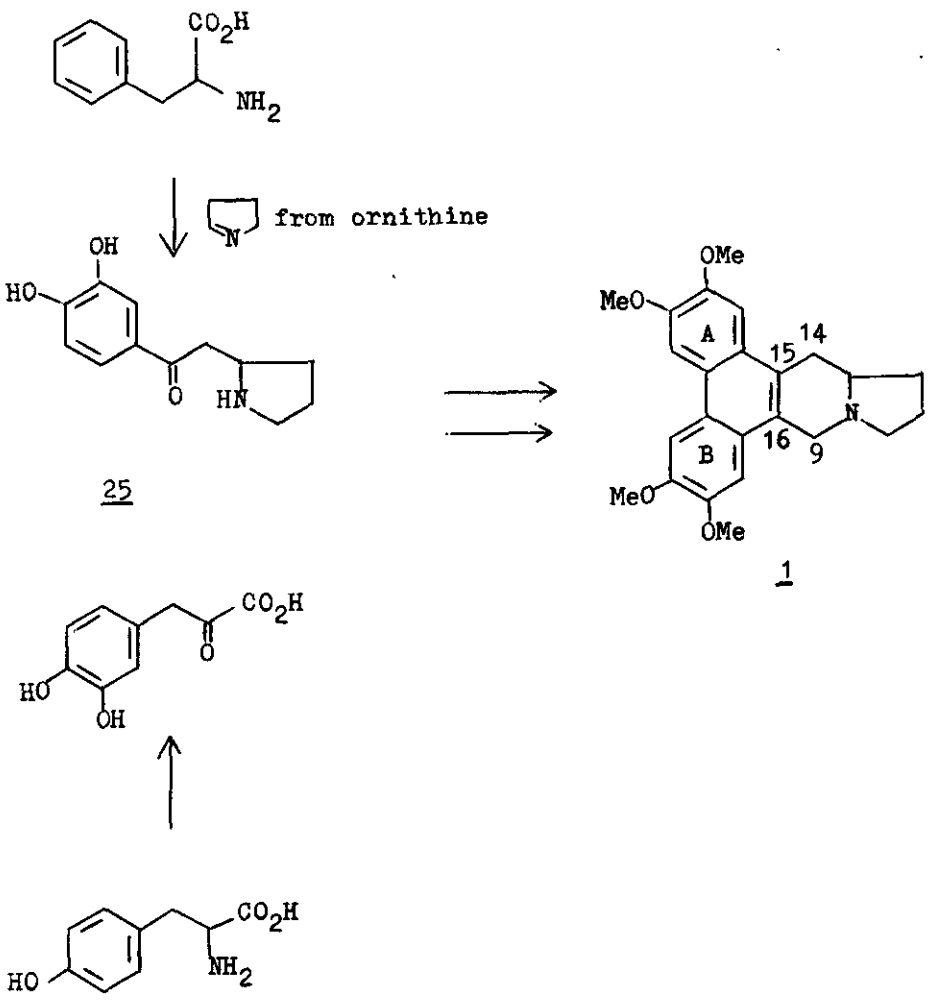



23 24

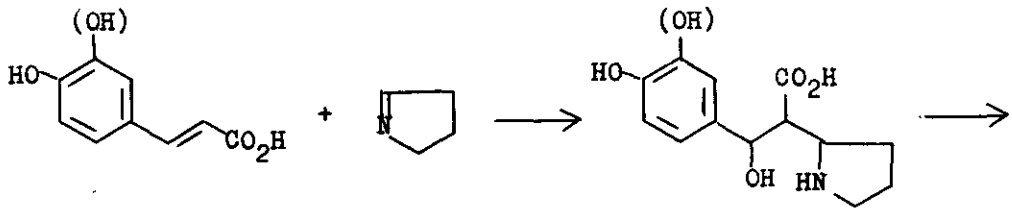
The method offers some advantages over the chemical phenol-coupling reactions and is capable of being extended to the synthesis of phenanthroindolizidine alkaloids.

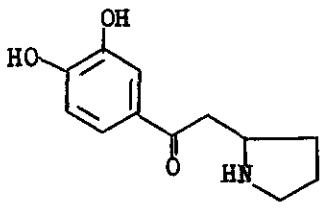
## 3. Biogenesis

Previous studies[28,29] had shown that the phenanthro-indolizidine alkaloids are derived from phenylalanine, tyrosine and ornithine according to the general scheme depicted below.



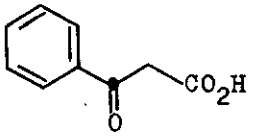
By using tyrosine-[2-$^{14}$C] and phenylalanine-[2-$^{14}$C] and degradative studies on the radioactive tylophorine, it was established that ring B and $C_{16}$ and $C_9$ arise from tyrosine whereas ring A, $C_{15}$ and $C_{14}$ are derived from phenylalanine. Transformation of phenylalanine to tyrosine did not take place during the administration of the former precursor. It was therefore suggested that phenylalanine could possibly be incorporated via cinnamic, p-coumaric and caffeic acids. Cinnamic acid -[2-$^{14}$C] has been found to be incorporated into tylophorine more efficiently than phenylalanine[30] and gave the alkaloid labelled at $C_{14}$. The genesis of the 2-phenacylpyrrolidine (25) has been visualised as follows.



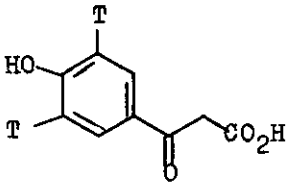




25

The intermediacy of benzoylacetic acids in the formation of 2-phenacylpyrrolidines has been demonstrated by the finding that compounds 26 (• = $^{14}$C) and 27 are incorporated into tylophorinine in T. asthmatica.[31]

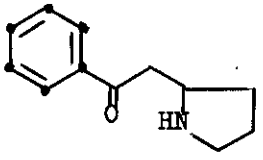


26

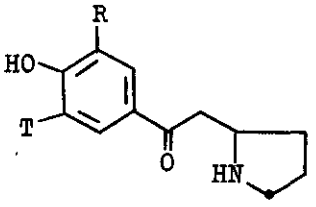


27

The phenacylpyrrolidines 28, 29a and 29b have been found to be intact precursors for tylophorinine.[31]



28

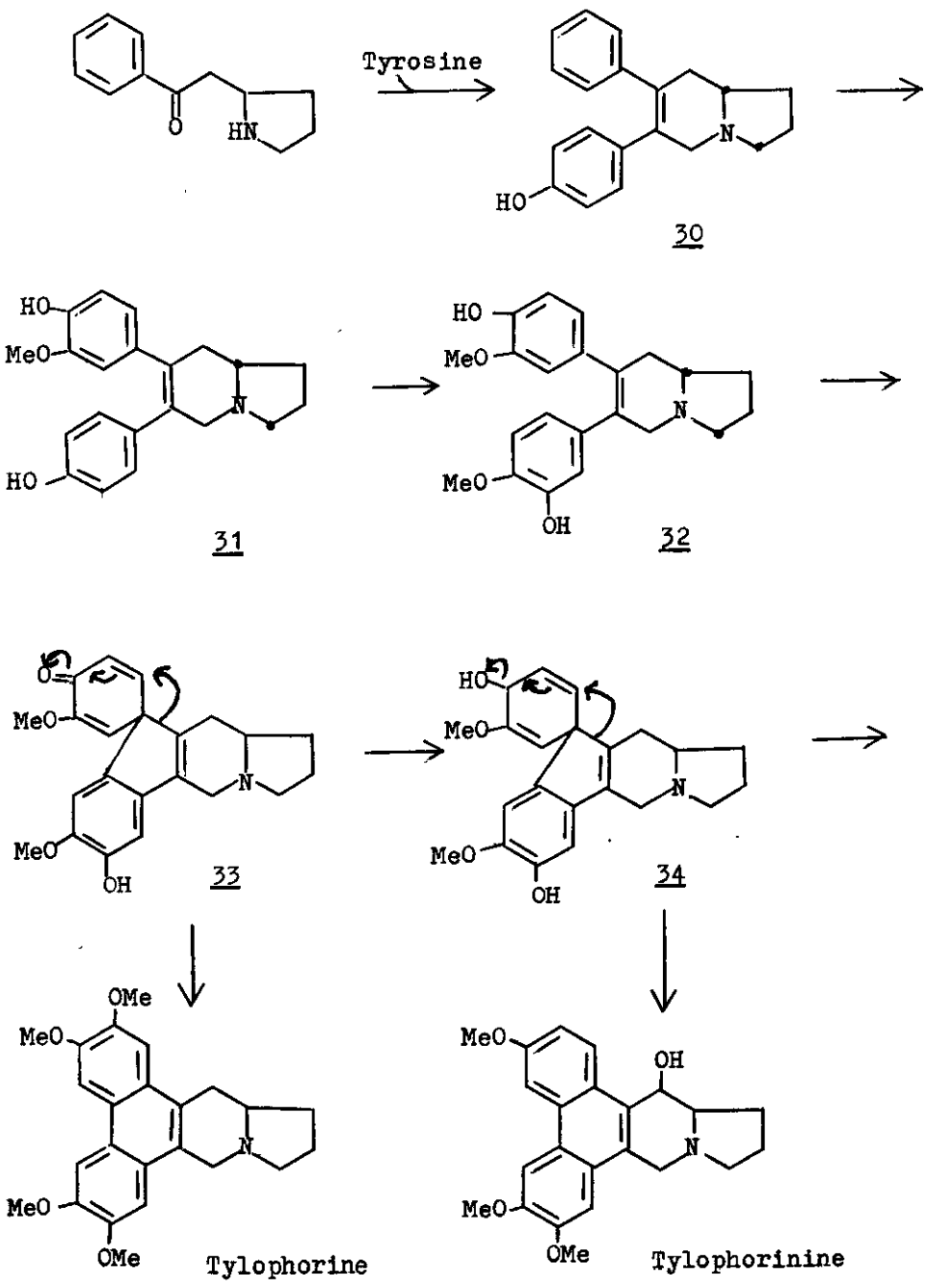


29

a : R = T

b : R = OMe

The incorporation of 29a into tylophorinine resulted in loss of half the tritium and implies entry of a hydroxyl group at one of the tritiated positions. This study and the subsequent one using labelled 6,7-diphenylhexahydroindolizines[32] lead to the following scheme for the biogenesis of tylophorine and tylophorinine.

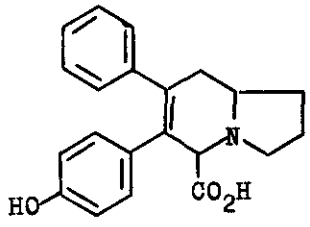

Tyrosine
30
31
32
33
34
Tylophorine
Tylophorinine

Condensation of the 2-phenacylpyrrolidine with tyrosine could lead to the hexahydroindolizine 30 which can undergo oxygenation to give first 31 and then 32. Oxidative phenol-coupling of 32 would lead to the dienone 33. Rearrangement of the latter gives tylophorine whereas reduction to 34 followed by rearrangement gives ultimately tylophorinine.

Convincing proof for the above scheme was obtained by the finding that the 6,7-diphenylhexahydroindolizines 30, 31 and 32 labelled with $^{14}C$ at the sites indicated and tritiated at the positions ortho to the free phenolic hydroxyls are incorporated into tylophorine, tylophorinine and tylophorinidine.

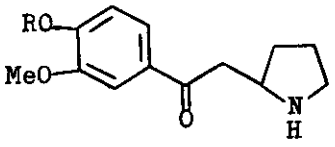
The dienone 33 provides an opportunity for either a styryl migration which leads to tylophorine or an aryl migration which would lead to isotylocrebrine (10) which is a minor alkaloid of T. asthmatica.

Amino-acids of the type 35 have been proposed as possible intermediates between 2-phenacylpyrrolidines and 6,7-diphenyl-hexahydroindolizines.[32]

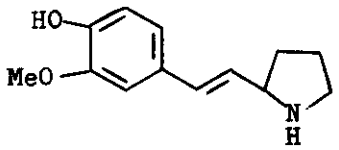



35

The recent isolation[33] of the 2-phenacylpyrrolidine alkaloids ruspolinone (36a) and norruspolinone (36b) and a styrylpyrrolidine norruspoline (37) from the plant Ruspolia hypercrateriformis M. R (Family Acanthaceae) fits neatly into the above biogenetic scheme.




36

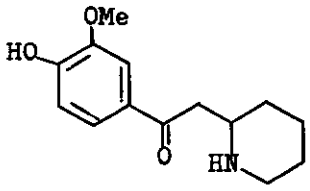



37

a : R = Me

b : R = H

The biogenesis of the phenanthroquinolizidine alkaloid cryptopleurine (24) which co-occurs with pleurospermine (38)[34] in Cryptocarya pleurosperma offers a close parallel to the above scheme.




38

Callus tissue[35] of T. indica was γ-irradiated and both control and irradiated groups lacked the ability to synthesize alkaloids.

## 4. Biological activity

The antitumour activity of the phenanthroindolizidine alkaloids has been well established but the high toxicity of these precludes their use in therapy. The mechanism of their effect on protein synthesis has been studied. Tylocrebrine irreversibly inhibits protein biosynthesis in He La cells and rabbit reticulocytes and the main effect was on chain elongation[36]. Mutants resistant to tylophorine and tylocrebrine have been isolated from the yeast Saccharomyces cerevisiae. The mode of action of these alkaloids has been examined and they appear to inhibit the translocation phase of protein synthesis[37]. In vitro amoebicidal activity has been observed for tylocrebrine and the alkaloid has been found to inhibit protein synthesis in E. histolytica[38].

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