ISOLATION, STRUCTURE AND SYNTHETIC STUDIES ON SOME β-CARBOLINE AND IBOGA ALKALOIDAL SYSTEMS

THESIS SUBMITTED

FOR

THE FULFILMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

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MOHAMMAD BASHIR

DEDICATED TO MY PARENTS

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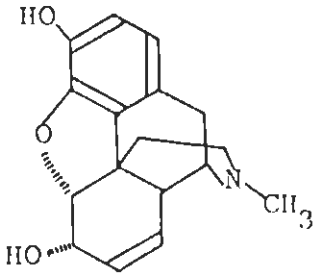
contd.

1.0 THE INDOLE ALKALOIDS

1.1 INTRODUCTION

Alkaloids, which are basic nitrogen containing compounds of plants origin, often manifest significant pharmacological activity. Examples of well known alkaloids are morphine (1),[1] nicotine (2),[2] quinine (3),[3] reserpine (4),[4] strychnine (5),[5] vinblastine (6)[6] and vincristine (7).[7] Thus lysergic acid diethylamide (8), is a powerful hallucinogen possessing remarkable physiological properties of inducing symptoms similar to schizophrenia and reserpine is effective in the treatment of high blood pressure. One of the most important contributions of the 19th century chemists was the isolation of several active principles, from a number of plant sources which had long been known for their medicinal properties.

The investigation of indole alkaloids as well as other components of the family *Apocynaceae*, received a boost in the early 1960's by the discovery of two *Catharanthus* alkaloids vinblastine and vincristine which have been used in the treatment of a number of malignant diseases including Hodgkins disease and acute leukaemia in children. These alkaloids are among the most potent



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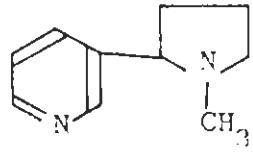

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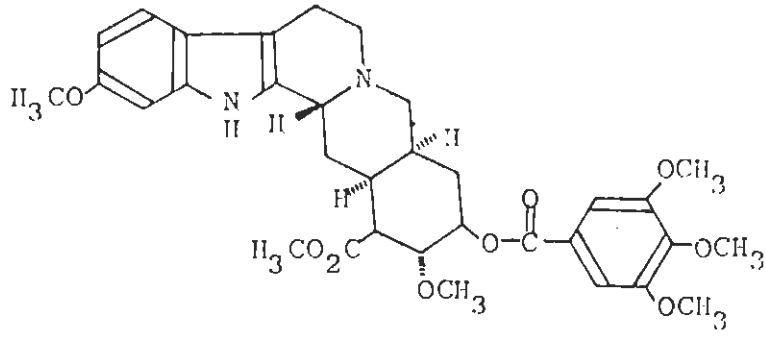

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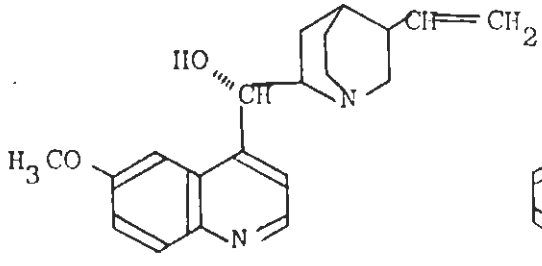

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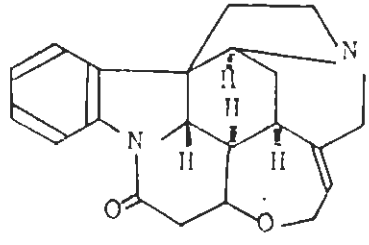

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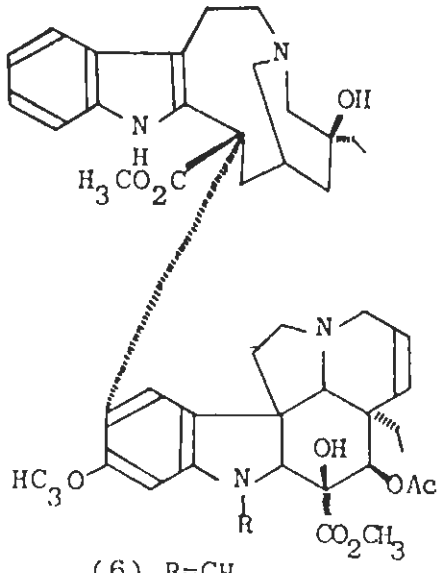
chemotherapeutic agents known to man, but these are present in minute traces in the leaves of *Catharanthus roseus*. Their isolation on a commercial scale is cumbersome, involving separation on the basis of their differential basicities and elaborate column chromatographies, which raises their price to several thousand dollars per gram. This has attracted the attention of a number of groups towards their synthesis. In the syntheses of these binary antitumour alkaloids achieved so far vindoline and catharanthine have been used as starting points.[8-10] It was therefore important to develop an isolation procedure which could afford these alkaloids in bulk without having to resort to extensive chromatographic separations.

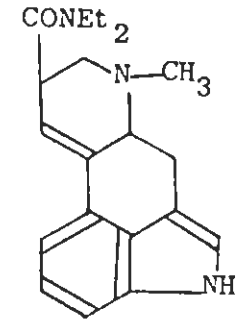
The present work was directed to develop and optimise procedures for the isolation of catharanthine, vindoline and vinblastine as well as to investigate new alkaloids isolable from the plant.

Work was also directed by us aimed at developing new synthetic approaches to the ß-carboline nucleus,



(6) R=CH_3

(7) R=CHO



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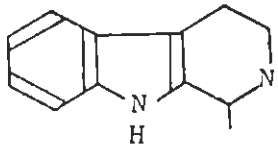

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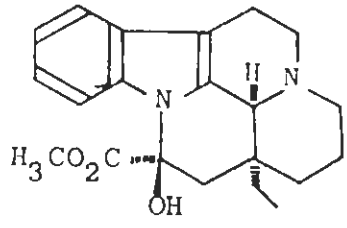

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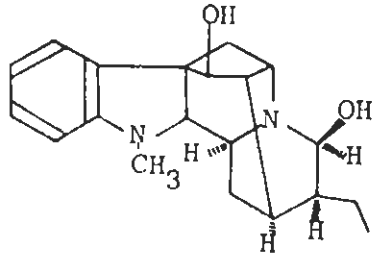


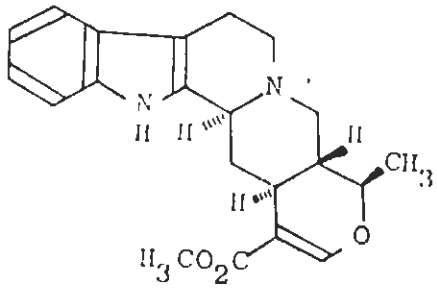
(11)

because many indole alkaloids, particularly of the *Corynanthe* and *Yohimbe* series contain a β-carboline nucleus (9), and some of these are physiologically important. Thus vincamine (10)[11] is a cereberal vasodilator while ajmaline (11)[12] is used in cardiac arrhythmias. β-carboline alkaloids, such as ajmalicine (12) and corynantheine (13) have also been recognised as important biosynthetic intermediates, which are later transformed to the *Strychnos*, *Aspidosperma* and *Iboga* alkaloidal systems through certain fascinating rearrangements.[13]

1.2 BIOSYNTHESIS

The 1200 or so indole alkaloids[14] show a wide variety of skeletal types, but three of the most important of these are those belonging to the *Corynanthe*, *Aspidosperma* and *Iboga* classes. It has been established that these three major classes of alkaloids arise in nature by the combination of secologanin with tryptophan.

Intensive investigations mainly by three research groups[15-17] have established the monoterpenoid



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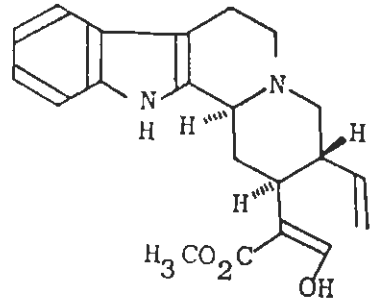

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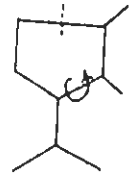
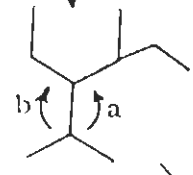
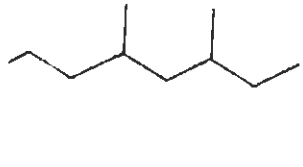




Yohimbe-Strychnos



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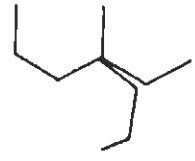
Iboga

(15)



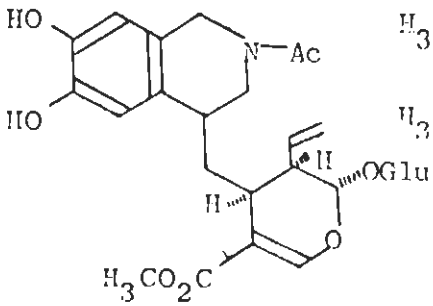
(17)

Aspidosperma

Scheme-1

origin of all the three types of alkaloids by labelling experiments using C_{14} labelled compounds. These results were in complete agreement with the earlier proposal of Thomas[18] and Wenkert[19] that the skeletal system (15) is generated by fission of a cyclopentane monoterpene (14) with rotation about the single bond as indicated in Scheme (1). It has been shown that unit (15) is structurally related to (16) and (17), and may be transformed into the other type of bond fission either at (a) or (b) (Scheme-1).

The postulated fission occurs after combination with tryptophan and leads to the *Strychnos* skeleton, while further rearrangement generates the *Aspidosperma* and *Iboga* skeleta from it. This was indicated by the isolation of nitrogenous glucoside i.e. ipecoside (18) from *Cephaelis ipecacuanha*.[20] The correlation of ipecoside (18) with dihydroprotoemetine (19)[21] led to the proposal[22] that the glucoside loganin (20) first affords the aldehyde (21) which reacts with tryptamine (22) to eventually produce the diverse indole alkaloid skeleta (Scheme-2). This has been confirmed by labelling experiments The presence of loganin in



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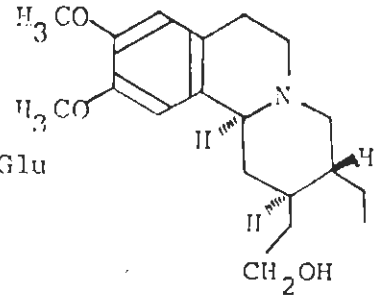

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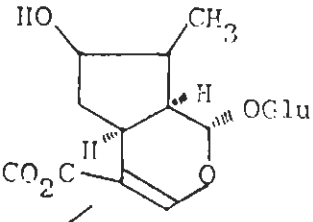

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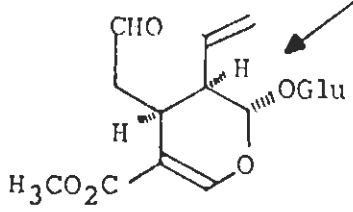

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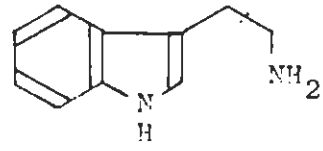

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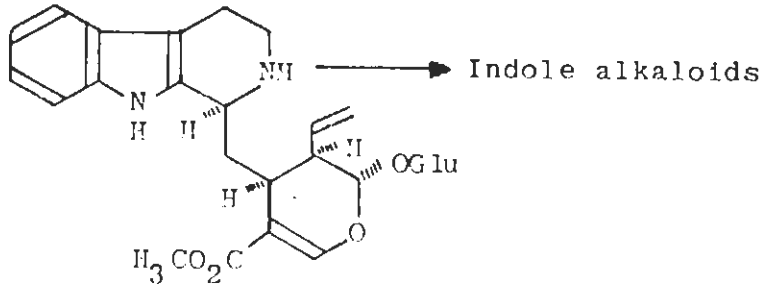
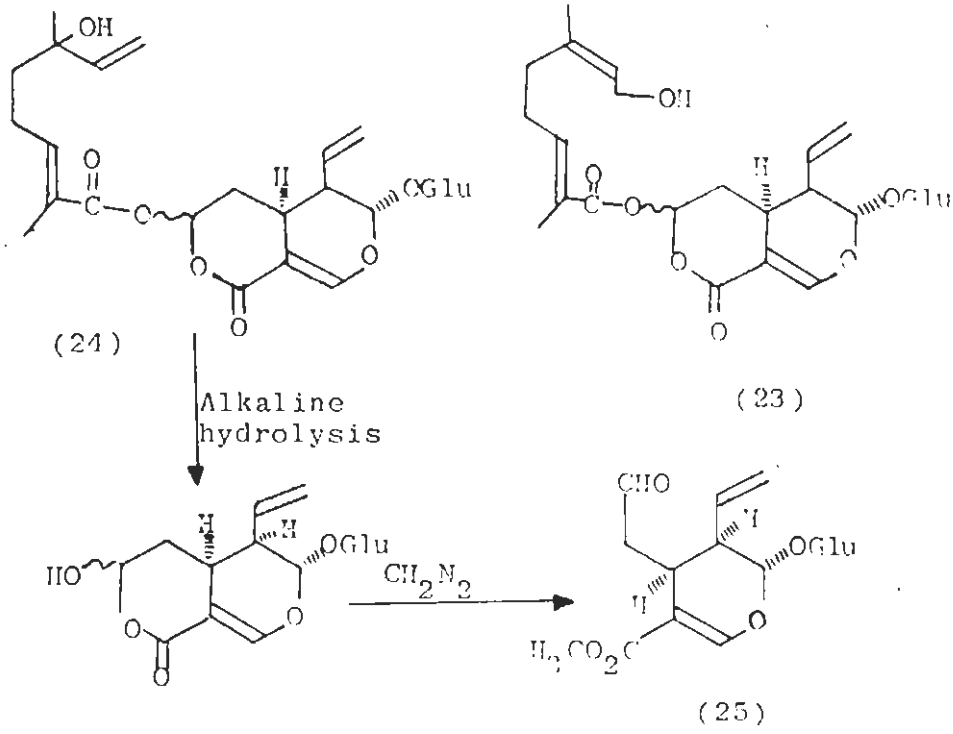


Scheme-2

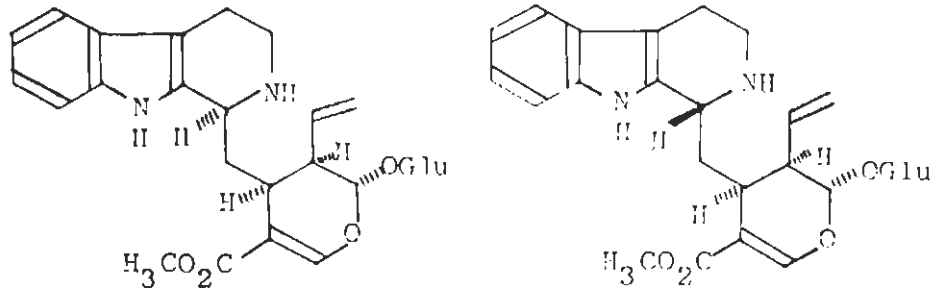
Catharanthus roseus was also proved by dilution analysis,[23,24] and by direct isolation from different plant species.[25]

Later the isolation of two new glucosides foliamenthin (23) and menthiofolin (24) from *Menyanthes trifoliata*,[26,27] led to the suggestion of a possible intermediate beyond loganin (20) i.e. secologanin (25).[28,29] The aldehyde secologanin was obtained after alkaline hydrolysis followed by diazomethane treatment of the glucoside foliamenthin and menthiofolin (Scheme-3). Formation of secologanin (25) during the biosynthetic pathway was further confirmed by its isolation from *C.roseus* by radiochemical dilution analysis and by macro-isolation as crystalline derivatives.[28,29]

Investigation into later stages of the biosynthesis suggested that two basic glucosides, vincoside (26) and isovincoside (27), play a key role. Over the years this has been an area of considerable controversy, particularly with regard to the C-3 stereochemistry of the nitrogenous glucoside


OH
OGlu
(24)
Alkaline
hydrolysis
(23)
HO
CH_2N_2
CHO
H_3CO_2C
(25)

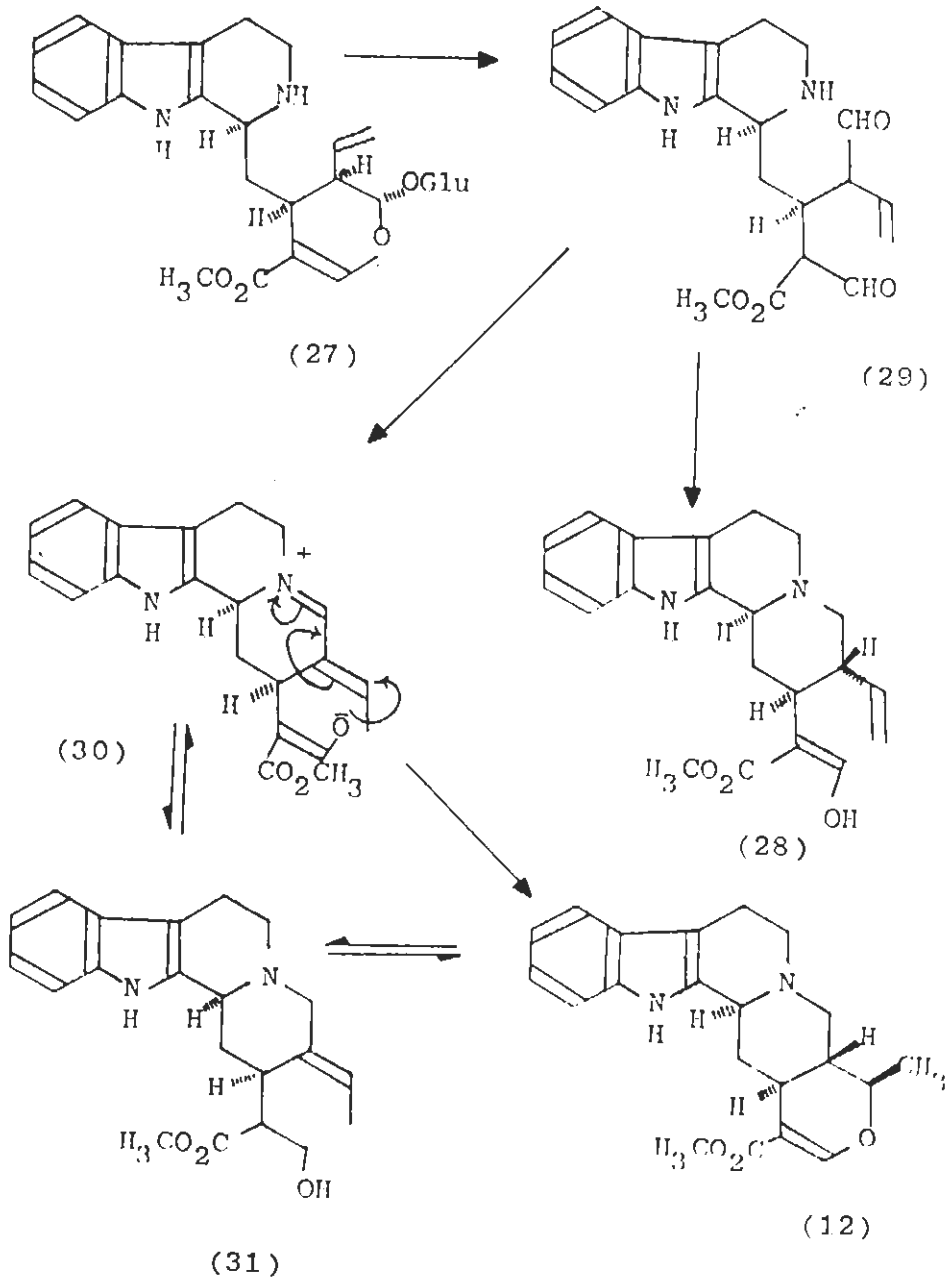
Scheme-3


NH
OGlu
H_3CO_2C
(26)
(27)

precursors. Zenk and coworkers[30] have established that it is the C-3α H isomer strictosidine (isovincoside) which is the precursor of indole alkaloids and have also isolated the enzyme system strictosidine synthase,[31] which produces this intermediate.

However, it remained to be proved whether a generalisation of the precursor function of strictosidine (27) was applicable to the alkaloids with C-3β stereochemistry. This was established by labelling experiments, when it was shown that conversion of strictosidine to C-3β compounds proceeded with the loss of hydrogen at C-3, while it was retained in the formation of the C-3α series. Thus the universal role of strictosidine (27) as the common biogenetic precursor for alkaloids with both 3-α and 3-β configuration was confirmed.[32]

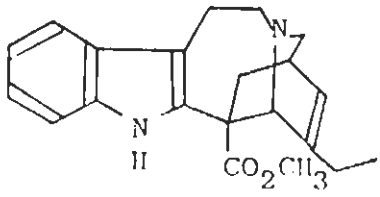
The biological conversion of strictosidine (27) into corynantheine aldehyde (28) and geissoschizine (31) may take place by enzymatic hydrolysis of the glucoside residue (27) followed by reductive condensation of the nascent aldehyde (29) to



Scheme-1

corynantheine aldehyde (28), while ajmalicine (12) could be obtained by cyclization of (30) or geissoschizine (31), (Scheme-4). Earlier experiments had shown that both chemical entities, play a significant role in the biosynthesis of *Catharanthus* alkaloids.[33-36]

The subsequent stages in the formation of indole alkaloids involve the formation of the various *Corynanthe* alkaloids and their conversions through certain key link alkaloids into the *Yohimbe*, *Strychnos*, *Aspidosperma* and *Iboga* bases. Results obtained from labelling experiments have provided considerable proof of the importance of corynantheine aldehyde (28) and geissoschizine (31) during the course of biosynthesis. When (O-methyl-^{3}H) corynantheine aldehyde was fed into *C.roseus* seedlings, radioactive catharanthine (32) and vindoline (33) were produced. However low incorporation into the alkaloids was recorded, when the mature plant was supplied with (O-methyl-^{3}H) and ring C-^{3}H-corynantheine aldehyde.[37,27] Deuterated akuammicine (34) and coronaridine (35) were obtained when (ar-^{2}H)-geissoschizine was administered to *C.roseus* seedlings,[22]



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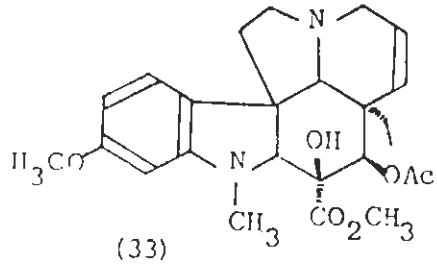

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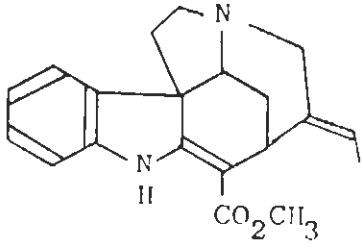

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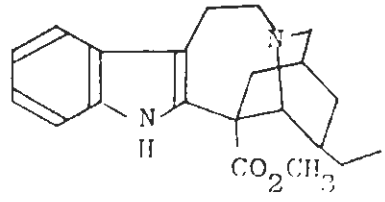

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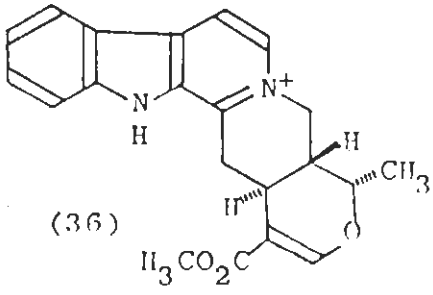

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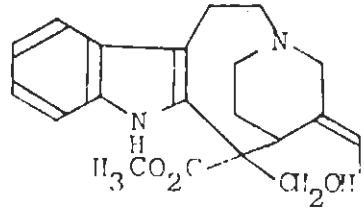

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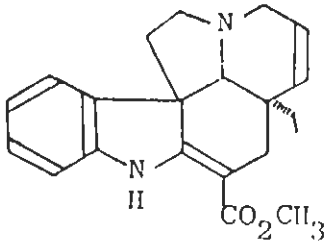

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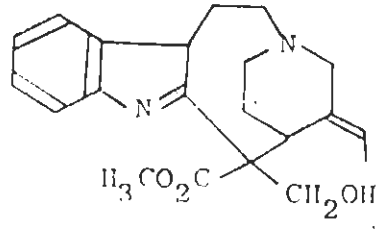


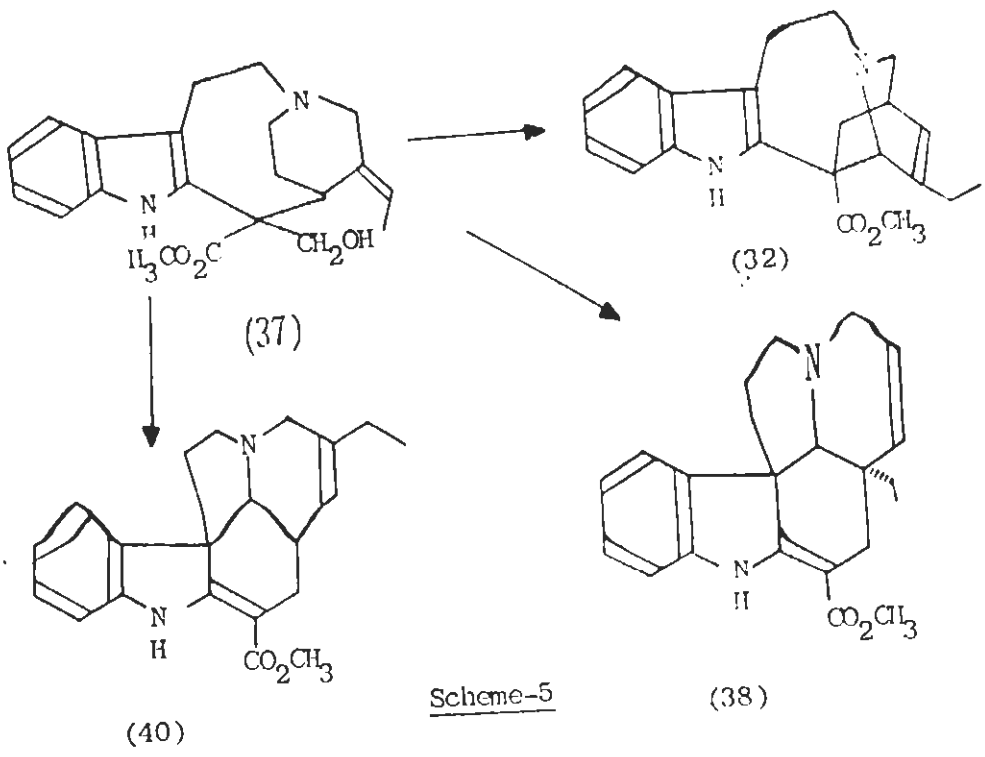
(39)

whereas radioactive ajmalicine (12), serpentine (36), akuammicine (34), vindoline and catharanthine were isolated when the mature plant[23] was supplied with both (ar-^{3}H) and (O-methyl-^{3}H) geissoschizine. The presence of geissoschizine in *C. roseus* was established by its direct isolation from the plant.[27,35,36] These result provided evidence for the biological conversion of the *Corynanthe* system of geissoschizine into the rearranged *Aspidosperma* and *Iboga* systems. The efficient incorporation of geissoschizine and akuammicine suggest an α-β rearrangement of geissoschizine to generate the *Strychnos* skeleton of akuammicine (34).

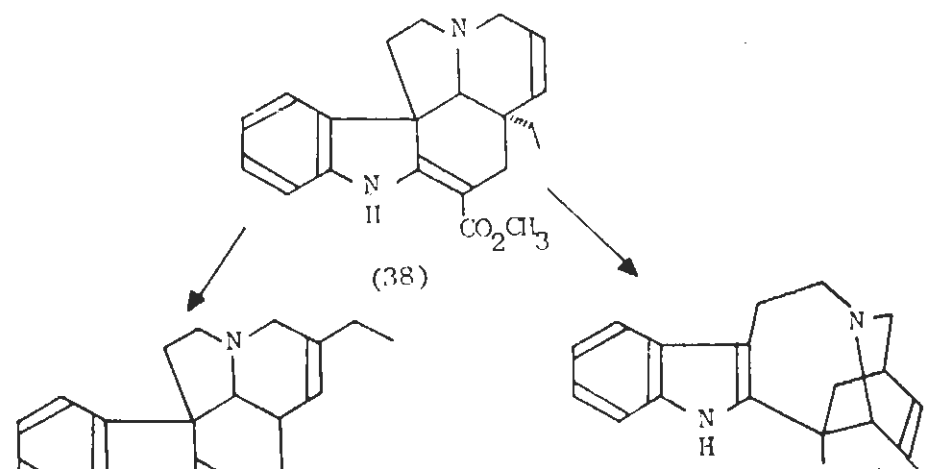
The transformation of the *Corynanthe-Strychnos* unit (15) to the *Aspidosperma* (17) and *Iboga* (16) systems was confirmed, after the isolation of three important alkaloids stemmadenine (37) tabersonine (38) and preakuammicine (39) from young *C.roseus* seedlings.[34,35] Later feeding of (O-methyl-^{3}H)-and (11-^{14}C)-stemmadenine in *C.roseus* and isolation of radio-labelled tabersonine, vindoline and catharanthine, established the formation of stemmadenine and tabersonine as late

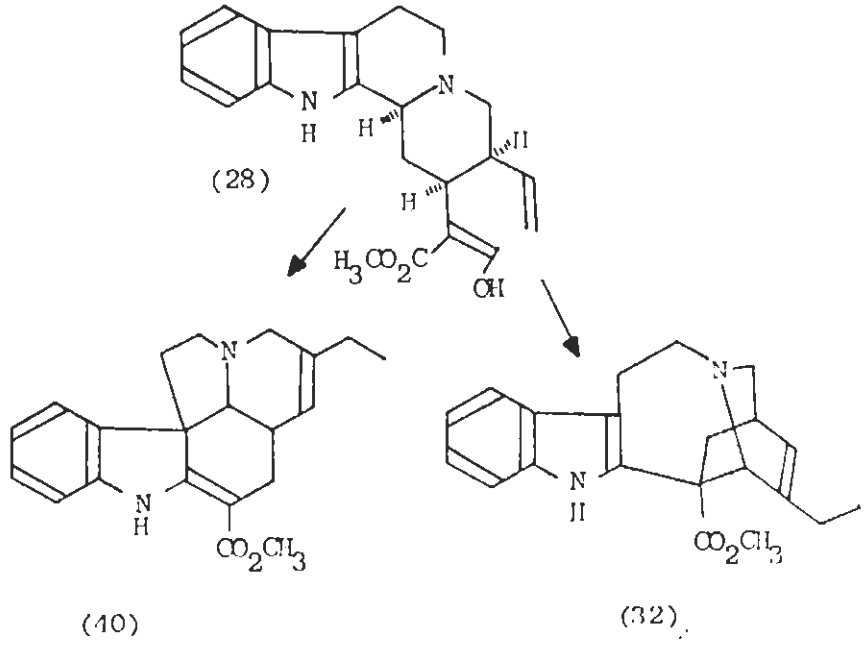
intermediates in the biosynthetic pathway of indole alkaloids.[34] (O-methyl-^{3}H) and (11-^{14}C)-tabersonine were similarly incorporated into vindoline and catharanthine. This was further confirmed by the isolation of radio labelled vindoline and catharanthine by feeding (aryl-H^{3})-tabersonine to *C. roseus* plants.[38] These results support the sequence[34] stemmadenine (37)→ tabersonine (38)→ catharanthine (32).

In vitro evidence for these transformations, was provided by Scott in 1968 when he reported that stemmadenine upon refluxing in glacial acetic acid can be converted into tabersonine (38) and catharanthine alongwith pseudocatharanthine (40)[34] (Scheme-5). It was further claimed that refluxing tabersonine for 10 hrs., resulted in its transformation to catharanthine and pseudocatharanthine (Scheme-6). He also claimed that corynantheine aldehyde (28) after refluxing in acetic acid for 72 hours yielded a mixture of pseudocatharanthine and catharanthine (Scheme-7). Unfortunately these results could not be repeated. Smith[39] reported his failure to isolate or detect any catharanthine, pseudocatharanthine or tabersonine upon



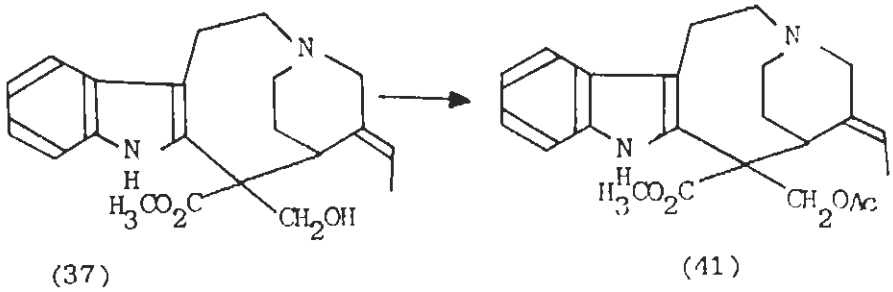
Scheme-5





(28) (10) (32)

Scheme-7

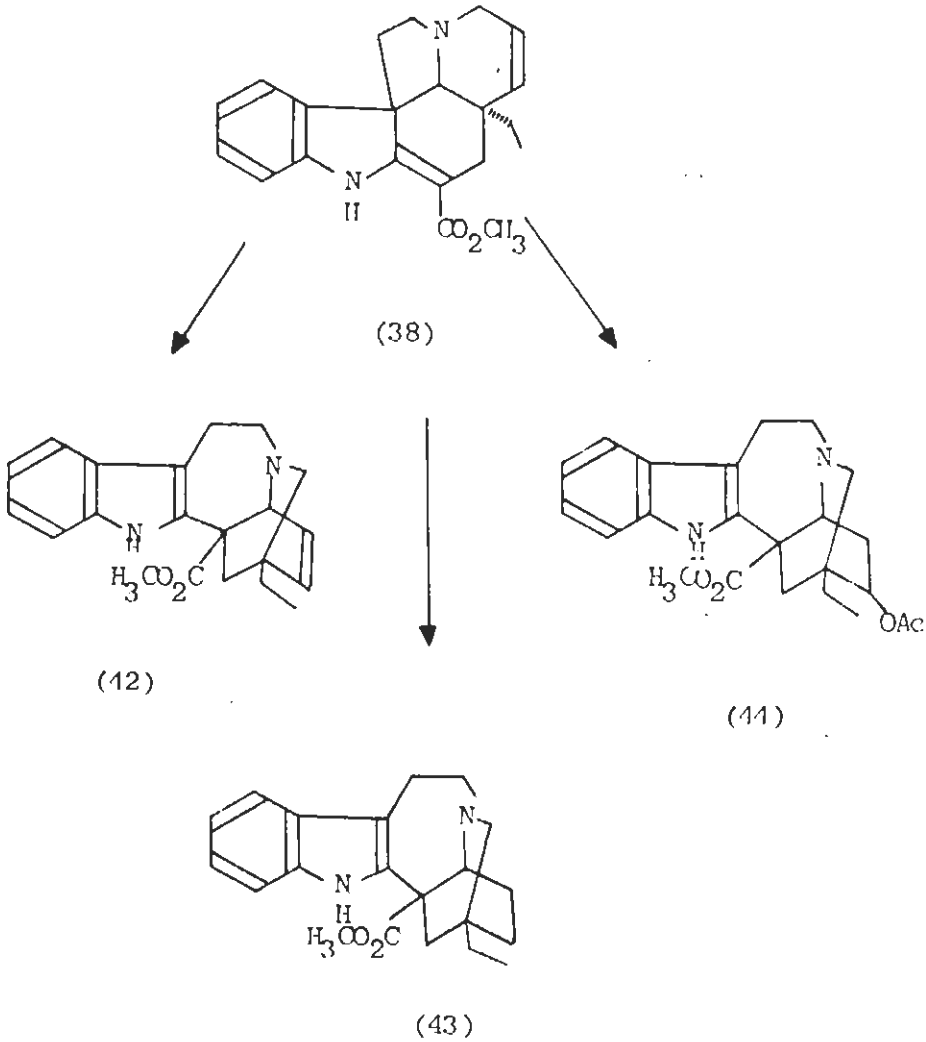


(37) (41)

Scheme-8

heating stemmadenine in acetic acid (Scheme-5). After 16 hrs., the reaction mixture consisted of unreacted stemmadenine and O-acetyl stemmadenine (41) (Scheme-8). Tabersonine under similar conditions, did not give any catharanthine or pseudocatharanthine, but three new bases, namely allocatharanthine (42), dihydro-allocatharanthine (43) and acetoallocatharanthine (44) were isolated (Scheme-9). With the exception of allo-catharanthine, the formation of which does not require the intermediacy of the acrylic ester (45), the for-mation of the other two bases does proceed via the acrylic ester.

In vivo evidence for the occurrence of an acrylic ester type of intermediate at some stages in the biosynthetic sequence was first obtained by the isolation from *Rhazya* species of the dimeric indole alkaloids, secamines.[41] Structure (46)[42,43] of the parent alkaloids secamine clearly indicates the pre-sence of two complete seco units. The acrylic ester derivatives (47) has been synthesized and detected in *Rhazya orientalis* after administration of (O-methyl-^{3}H)-loganin to the plant.[44] Completely reduced acrylic



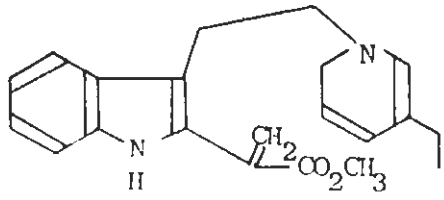
(38)

(42)

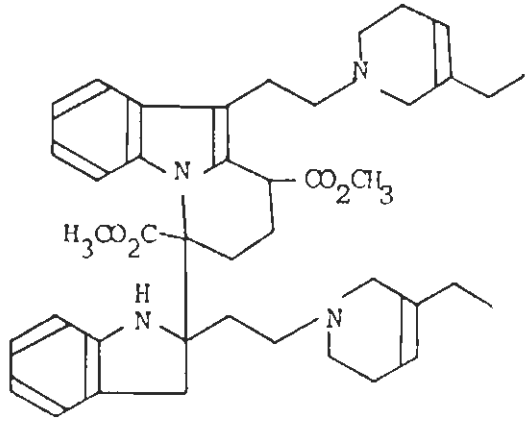
(44)

(43)

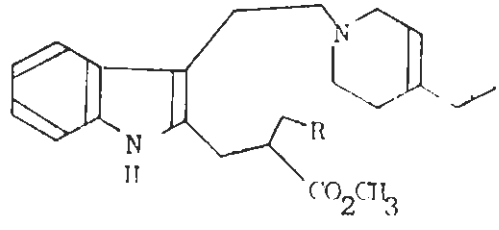
Scheme-9



(45)



(46)



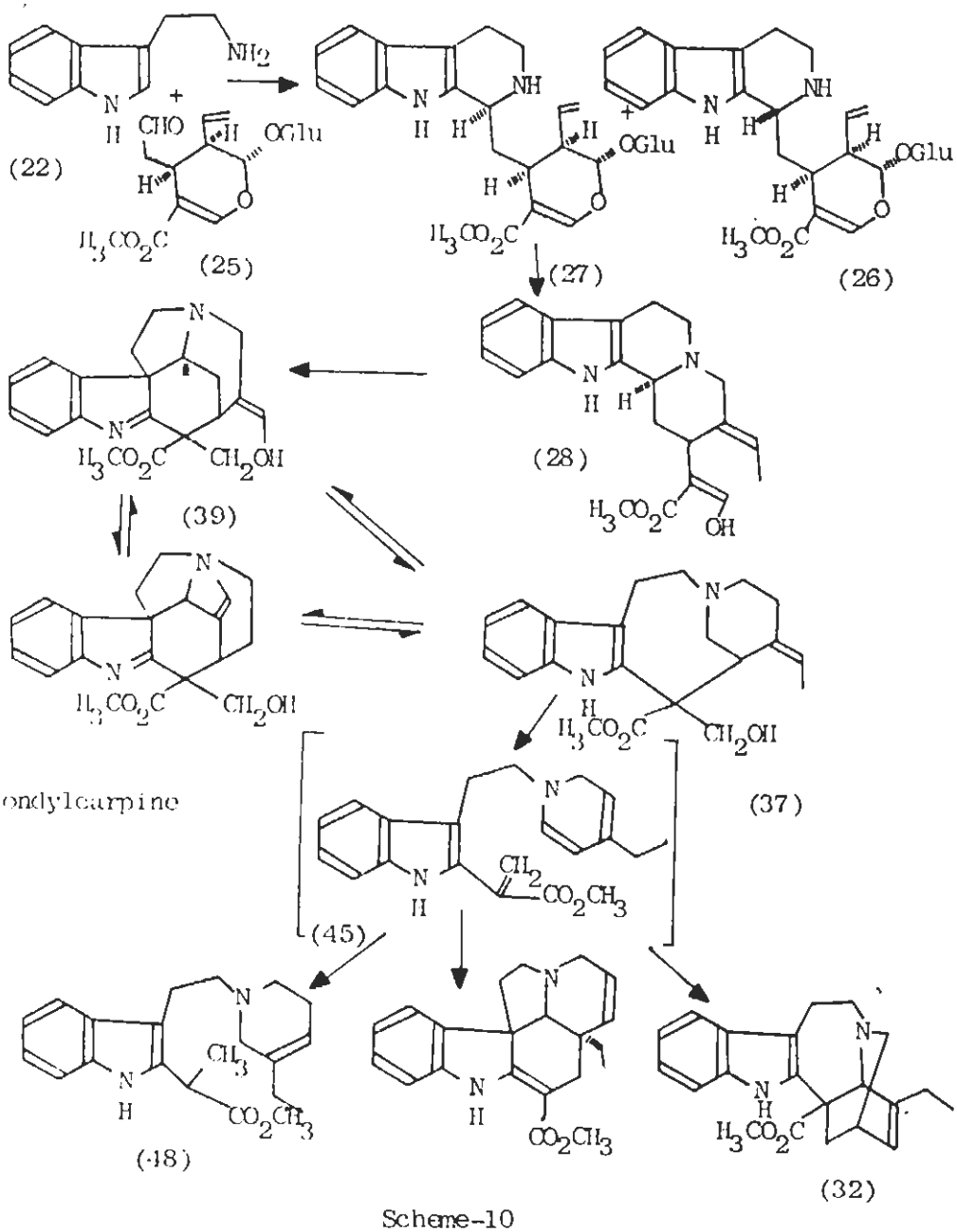
(47) R=OH

(48) R=H

ester derivatives (48) has been detected in *R.orientalis* after feeding (2-^{14}C)-tryptophan.[45] The presence of these derivatives provides strong indirect evidence in the favour of acrylic ester as an intermediates.

Scott and coworkers[13] by an examination of the sequential appearance of the various alkaloidal types in the germinating seeds of *Catharanthus roseus*, were able to gather considerable information on the various stages of the alkaloidal biosynthesis, which lie beyond vincoside. These sequential experiments, clearly favour the following order of alkaloid biosynthesis (Scheme-10) *Corynanthe*----→ *Corynanthe—Strychnos*---→ *Aspidosperma*----→ *Iboga*.

Recently Kutney et.al. have demonstrated and confirmed the postulated intermediacy of acrylic ester (45) in vitro transformation to *Iboga* and *Aspidosperma* alkaloidal systems.[46]



Scheme-10

2.0 CATHARANTHUS ALKALOIDS

One of the most thoroughly investigated plant species in the family Apocynaceae is *Catharanthus roseus G.Don*, also called *Vinca rosea* Linn. or *Lochnera rosea*.[47] More commonly the plant is known as periwinkle. This plant has enjoyed the reputation in folklore in various parts of the world of being an oral hypoglycaemic agent. Infusions prepared from the leaves have been reported as being used in Brazil against hemorrhage and scurvy, as a mouth-wash, for tooth ache and for the healing and cleaning of chronic wounds.[48] A limited antibiotic activity of the total alkaloids against *V.cholera* and *M.pyrogenes var-aureus*, as well as a significant and sustained hypotensive action was reported by Chopra and co-workers.[49] In vitro activity of selected alkaloids from *Catharanthus roseus* against vaccina and polio type III viruses has been reported by Farnsworth and co-workers.[50] In the Philippines and the British West Indies the plant was commonly used as an oral hypoglycaemic agent,[51] and a "tea" made from its leaves is prescribed by local "witch doctors" in Jamaica for the treatment of diabetes.

The hypoglycaemic properties were found to be associated with a number of alkaloids[52] but what proved more significant was the independent discovery by a Canadian group at the University of Western Ontario and scientists at the Lilly Research Laboratories in Indianapolis that some of the alkaloidal fractions from the leaves of this plant possessed marked oncolytic properties.[53,54] Subsequent efforts by the two groups led to the isolation of active principles which were found to be binary indole alkaloids, vinblastine (6), vincristine (7), vinrosidine (49) and vinleurosine (50).[55-62,6,7]

Vinblastine became a prescription product in 1961 and vincristine in 1963, and these highly profitable products are coming into increasingly wider use. Vinblastine has been used successfully in the treatment of Hodgkin's disease,[63] epidermoid carcinoma of the head and neck[64] and ovarian and breast cancer.[65] In combination vinblastine and chorambucil are more effective in the treatment of Hodgkin's disease than either agent alone[66] and the combination of vinblastine and bleomycin has proved quite effective for the treatment of testicular cancer.

Vincristine has a quite different and wider spectrum of activity. In combination with prednisone it is used in acute childhood lymphocytic leukaemia[67] and with other agents it is effective for the treatment of Wilm's tumor, breast cancer, Hodgkin's disease and large bowel cancer.[68]

The investigation on *Catharanthus roseus* plant by the Lilly group and others has led to the isolation of over ninety alkaloids, and new alkaloids continue to be isolated[69,70] from this plant. Our present efforts have resulted in the isolation and structure elucidation of further new alkaloids from *Catharanthus roseus*.

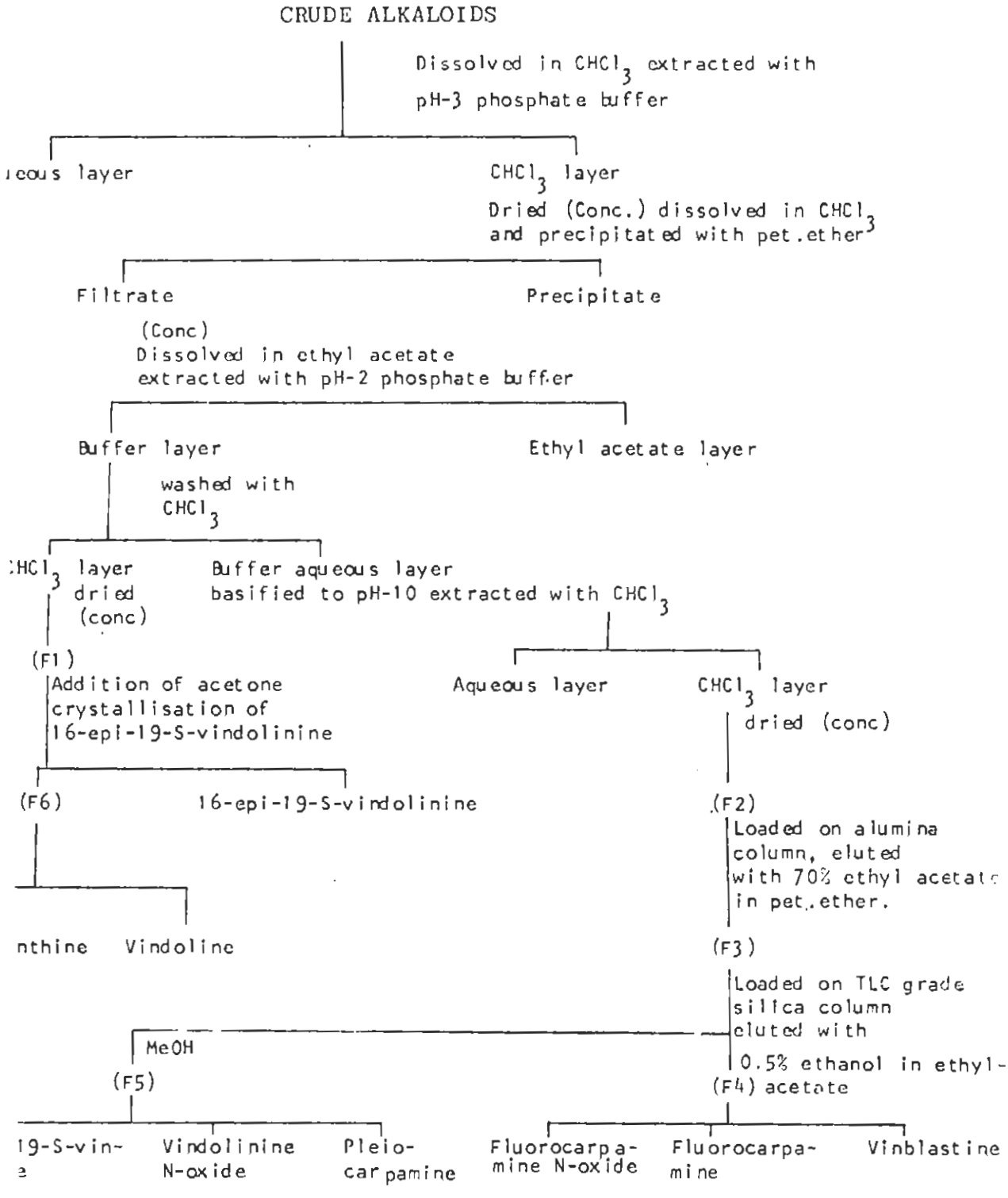
2.1 NEW ALKALOIDS FROM C.ROSEUS

I) 16-EPI-19-S-VINDOLININE- A NEW ALKALOID FROM C.ROSEUS

The procedure adopted for the isolation of alkaloids from the leaves of *Catharanthus roseus* is shown in Fig.(1). The air-dried leaves of the plant were finely crushed with an ultra-turrax in ethanol. The crushed material was filtered and washed with ethanol. The ethanolic filtrates were combined and evaporated under vacuum to a gum. The gum was acidified with 5% HCl and washed with chloroform. The ice cold solution was then basified with ammonia solution and extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulphate and concentrated to afford the crude alkaloidal gum.

The alkaloids were dissolved in chloroform and extracted with pH3 phosphate buffer. The chloroform layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to afford the residual alkaloidal mixture. This was dissolved in chloroform and then hexane added which caused selective precipitation of some alkaloids. The precipitates were

FIGURE-I


CRUDE ALKALOIDS
Dissolved in $CHCl_3$ extracted with pH-3 phosphate buffer
eous layer
$CHCl_3$ layer
Dried (Conc.) dissolved in $CHCl_3$ and precipitated with pet.ether
Filtrate
Precipitate
(Conc)
Dissolved in ethyl acetate extracted with pH-2 phosphate buffer
Buffer layer
Ethyl acetate layer
washed with $CHCl_3$
$CHCl_3$ layer dried (conc)
Buffer aqueous layer basified to pH-10 extracted with $CHCl_3$
(F1)
Addition of acetone crystallisation of 16-epi-19-S-vindolinine
Aqueous layer
$CHCl_3$ layer dried (conc)
(F6)
16-epi-19-S-vindolinine
(F2)
Loaded on alumina column, eluted with 70% ethyl acetate in pet.ether.
nthine
Vindoline
(F3)
Loaded on TLC grade silica column eluted with 0.5% ethanol in ethyl-acetate
MeOH
(F5)
(F4)
19-S-vin-e
Vindolinine N-oxide
Pleio-carpamine
Fluorocarpa-mine N-oxide
Fluorocarpa-mine
Vinblastine

filtered off and the filtrate was again concentrated to a gum. The gum was dissolved in ethyl acetate and extracted with pH2 phosphate buffer. The aqueous layer was separated, extracted with chloroform and the organic layer concentrated to a gum to afford a fraction (F_1). The aqueous acidic layer was basified with ammonia solution to pH-10 and extracted with chloroform. The chloroform layer was dried and concentrated to a gum to afford another fraction (F_2).

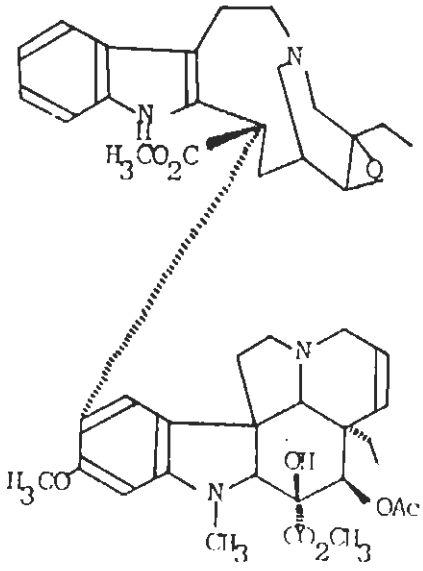
The addition of acetone to fraction (F_1) resulted in the crystallization of a substance which afforded positive test for an alkaloid with Dragendorff's reagent and which was recrystallized from ethanol as a colourless crystalline solid, m.p. 200^{o}C. In order to establish the structure of this alkaloid, its spectral data were recorded. The u.v. spectrum in methanol showed the presence of a dihydroindole chromophore with absorptions at λ_{max} 212, 246 and 303 nm. The I.R. spectrum showed the presence of an ester carbonyl with an absorption at 1730 cm^{-1}.

The substance afforded a mass spectrum which was very similar to that reported for vindolinine,[72] a major alkaloid isolated from *Catharanthus roseus* and to epi-vindolinine isolated by Potier and co-workers[73] from *Melodinus balansae*. A high resolution mass measurement on the molecular ion afforded the exact mass to be m/z 336.1837 in agreement with the formula $C_{21}H_{24}N_2O_2$ (calcd. 336.1837).

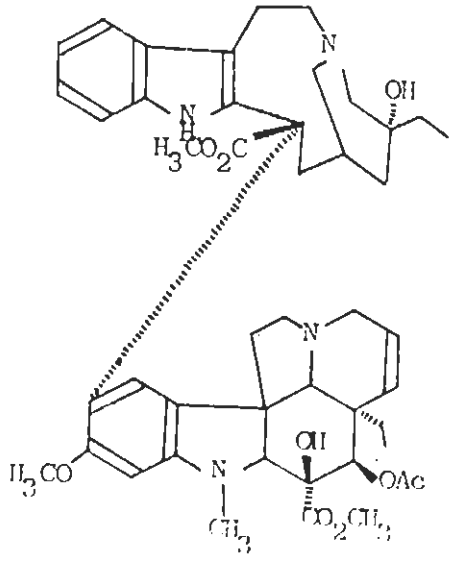
The ^{13}C-NMR spectrum of the alkaloid (broad band and off-resonance) showed interesting similarities to the ^{13}C-NMR spectra reported for 19-R-vindolinine (51)[74] 19-S-vindolinine (52)[74] and 16-epi-19-R-vindolinine (53)[74] (Table I). The ester carbonyl carbon resonated at δ 173.47 whereas the methyl of the ester group resonated at δ 52.6 (quartet). The substance afforded four doublets for the tertiary aromatic carbon atoms and two singlets for the two quaternary aromatic carbon atoms. A characteristic singlet appeared at δ 81.36 corresponding to the quaternary carbon atom α to the indoline nitrogen.

T A B L E - I

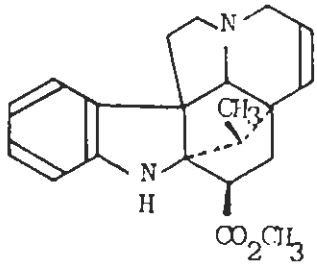
Carbon	19-R-VINDOLININE Chemical shift*	19-S-VINDOLININE Chemical shift*	16-epi-19-R-VINDOLININE Chemical shift*	16-epi-19-S-VINDOLININE (56) Chemical shift*	Multiplicities
2	81.4	80.4	80.5	81.36	singlet
3	58.0	57.5	57.4	58.14	triplet
5	50.3	49.8	50.1	48.9	triplet
6	36.3	37.2	35.0	34.66	triplet
7	59.8	58.4	60.7	59.98	singlet
8	139.8	139.0	135.7	135.30	singlet
9	123.6	123.1	123.1	123.70	doublet
10	121.0	121.2	118.9	122.74	doublet
11	127.2	127.0	126.9	125.9	doublet
12	112.0	111.8	109.0	112.71	doublet
13	149.4	149.2	148.7	148.46	singlet
14	128.5	128.1	128.2	128.61	doublet
15	130.7	131.0	130.6	132.56	doublet
16	39.2	42.7	39.4	38.96	doublet
17	29.1	31.4	31.9	28.73	triplet
18	7.4	10.1	7.8	7.15	quartet
19	48.4	51.2	44.8	48.46	doublet
20	46.2	44.2	47.8	44.58	singlet
21	78.0	74.2	76.4	74.19	doublet
$-\overset{O}{\overset{\|}{C}}-O$	174.2	172.8	174.5	173.47	singlet
$-OCH_3$	51.8	51.2	51.7	52.6	quartet



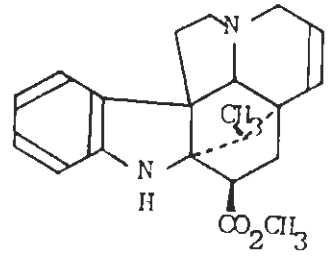
(49)



(50)



(51)



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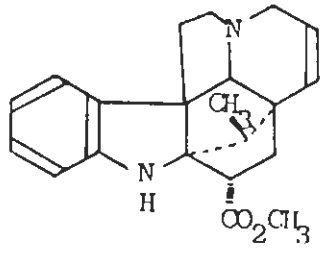

(53)

The H-NMR spectrum of the alkaloid in hand recorded on a 200-MHz instrument showed the presence of a doublet at δ 0.62 (J=7.4Hz) which is assigned to the C-18 methyl protons. The proton adjacent to the carbomethoxy function resonated as double doublet at δ 3.18(J_1=12.2Hz, J_2=5.8Hz). A quartet at δ 2.26 (J=7.4Hz) was assigned to the C-19 bridge-head proton. A double doublet at δ 6.41 was assigned to the olefinic proton at C-15, showing coupling with the vicinal olefinic proton and allylic coupling with the C-3 proton (J_1=10Hz, J_2=2.8Hz). The other olefinic proton at C-14 resonated as a doublet of double doublets at δ 5.48 (J_1=10Hz, J_2=5.2Hz, J_3=1.8Hz). The chemical shift of δ 0.62 for the methyl group is consistent with a 19-S-configuration as the methyl group of 19-S-vindolinine resonates at δ 0.57 while the methyl group in 19-R-vindolinine resonates further downfield at δ 0.95.

Direct t.l.c. comparison with authentic samples of vindolinine and 19-epi-vindolinine* showed that the substance could be just separated from these two materials in 25% ethanol in ethyl acetate on silica gel plates.

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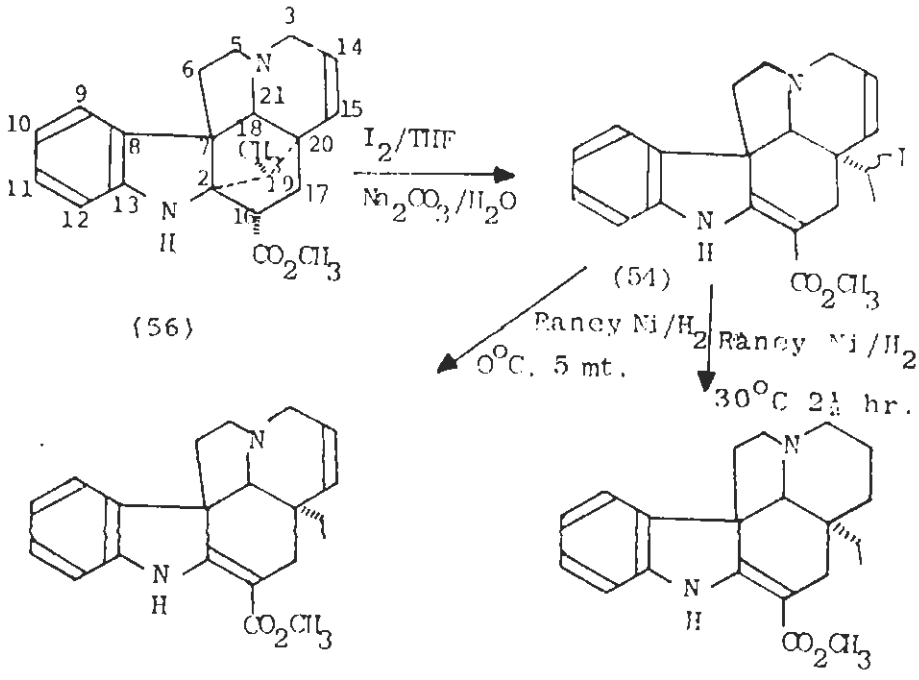
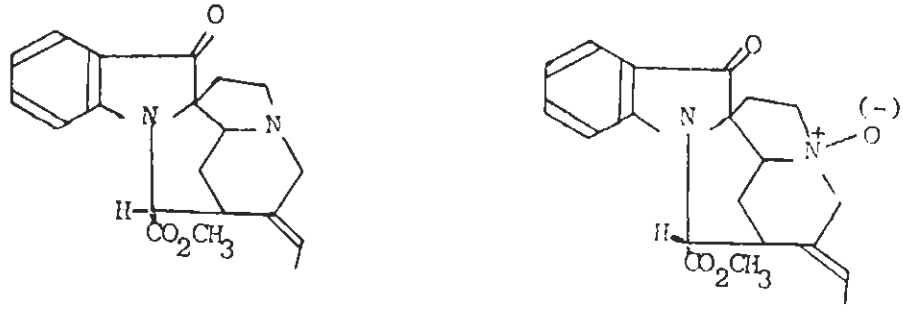
*Kindly supplied by Professor P.Potier.

In order to confirm the structure, the new alkaloid was subjected to an oxidative cleavage reaction[75,76] with iodine/THF/H_2O/Na_2CO_3 when it was found to be smoothly converted to the iodo-compound (54). The u.v. showed an anilinoacrylate chromophore at λ_{max} 228, 298, 330 nm. The i.r. spectrum showed absorptions at 1680 cm^{-1} consistent with the presence of an α,β-unsaturated ester carbonyl group. The mass spectrum afforded the molecular ion peak at m/z =462 while the base peak appeared at 335. The nmr spectrum in ($CDCl_3$) showed a doublet for the methyl group at δ 1.63 and a quartet at δ 3.92 due to the C-19 proton. These spectral data were consistent with the structure of the iodo-compound (54). On hydrogenolysis with Raney Ni at 30°C for two hours, the iodo-compound was found to be transformed to (-)-vincadifformine (55). When the same hydrogenolysis experiment was repeated at 0°C for 5 mts., quantitative conversion to tabersonine (38) was observed (Scheme 11). The identity of the synthetic hydrogenolysis products was established by direct chromatographic and spectroscopic comparisons with authentic samples of tabersonine and vincadifformine.


I2/THF
Na2CO3/H2O
(56)
(54)
Raney Ni/H2
0°C, 5 mt.
Raney Ni/H2
30°C 2½ hr.
(38)

Scheme-11


(57)
(58)

The above sequence of experiments conclusively establish that the new alkaloid has the same stereochemistry at C-7, C-20 and C-21 as vindolinine. Furthermore, since it can be converted to (-)-vincadifformine, $[\alpha]_D^{\pm}$-542° (EtOH), it must have the same absolute configuration at C-7, C-20 and C-21, as (-)-vincadifformine and hence belong to the same enantiomeric series. As the proton nmr had indicated a 19-S-configuration and as the alkaloid differed in its H and C-13 nmr from the three known diastereoisomers of vindolinine (19-R-vindolinine, 19-S-vindolinine, 16-epi-19-S-vindolinine), it is assigned to be 16-epi-19-S-vindolinine (56).[77]

II) *ISOLATION OF OTHER NEW ALKALOIDS FROM C.ROSEUS*

In continuation of further work on isolation and structure elucidation of alkaloids of *Catharanthus roseus*, other basic constituents have been isolated from the leaves of the plant. The fraction (F_2) as shown in Fig.(1) was loaded on a flash chromatography column packed with alumina (neutral activity I). The column was eluted with ethyl-acetate and the eluates were concentrated to afford a fraction (F_3) which

was again loaded on a similar flash chromatographic column packed with t.l.c. grade silica. Elution with 50% ethylacetate in pet.ether removed faster moving substances. Subsequent elution with 0.5% ethanol in ethyl acetate afforded a fraction (F_4). Finally the column was washed with MeOH to afford yet another fraction (F_5).

The fraction (F_4) was loaded on a preparative high pressure liquid chromatograph (column diameter 2.2 cm, column length 50 cm. column packing "Lichroprep" 15-25 um silica, solvent pressure 1000-1200 lb/sq inch, rate of flow 13 ml/minute) and eluted with 50% ethylacetate in pet.ether. The eluates were concentrated and subjected to preparative t.l.c. in 25% ethanol in ethyl acetate. Two basic components A and B with bright yellow colour and intense fluoroscence were isolated.

In order to elucidate the structure of the faster moving substance A, its spectral data were recorded. The u.v. spectrum exhibited characteristic absorption typical of the 3-acylindoles at λ_{max} (MeOH): 235, 257 and 398 nm. The i.r. spectrum showed the

presence of an ester carbonyl group by the presence of an absorption at 1740 cm^{-1}, and the presence of a keto function by the absorption at 1685 cm^{-1}. The mass spectrum afforded M^+ at 338.1611 (calc: for $C_{20}H_{22}N_2O_3$, 338.1630) while the other major peaks were present at 279, 265, 231, 193, 172, 160 and 121. The nmr spectrum showed a doublet at δ 1.63 indicating the presence of a methyl group attached to an olefinic carbon. The ester methyl group resonated at δ 3.7 (J=6.8Hz) as a singlet. A doublet resonated at δ 4.55 (J=9.3Hz) which was assigned to the C-16 proton. Four aromatic protons resonated between the region δ 6.62-7.6 while a one-proton doublet appeared at δ 5.5 (J=6.8Hz) which was assigned to the olefinic proton. The spectral data of this base were found to be identical with those of fluorocarpamine, isolated previously by R.Raschnitz and G.Spiteller;[78] the substance A was therefore identified to be fluorocarpamine (57).

The slower moving substance B which remained virtually on the base line showed the M^+ peak in its mass spectrum at m/z = 354 with characteristic loss of

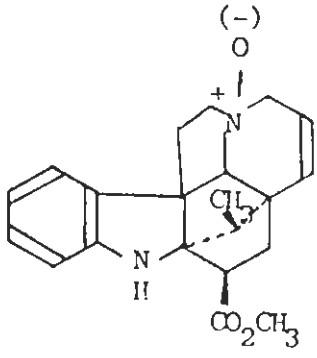
oxygen (m/z = 338) as encountered in N-oxides. Other major peaks were present at 279, 265, 231, 192, 160 and 121, these fragments being similar to those of fluorocarpamine. The u.v. spectrum (MeOH) showed absorptions at λ_{max} 235, 257 and 398 nm, which were characteristic of the fluorocarpamine type system. The i.r. spectrum showed absorptions at 1740 cm^{-1} (ester carbonyl) and 1685 cm^{-1} (ketonic carbonyl). From the mass spectrum and other data, it was apparent that the substance was the N-oxide of fluorocarpamine.

To confirm its structure the substance was treated with PCl_3 in chloroform and the mixture stirred for 15 minutes at room temperature, when it was found to be smoothly converted to a faster moving material, which was chromatographically and spectroscopically identical with fluorocarpamine. The polar nature of the material, its characteristic mass spectrum and its ready deoxygenation with PCl_3 to fluorocarpamine unambiguously established it to be fluorocarpamine-N-oxide (58), a new alkaloid.

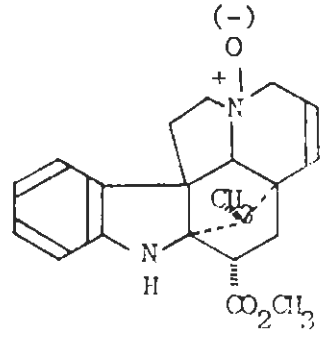
Additional alkaloids were isolated from the fraction (F_5) as shown in Fig.(1). Preparative t.l.c. of this fraction in pure ethanol allowed the separation of a slower moving band. A second preparative t.l.c. in 50% ethylacetate -50% ethanol afforded two components C and D. In order to elucidate the structure of component D, its spectral data were recorded. The u.v. spectrum,with absorptions at (MeOH). λ_{max} 215, 247 and 300 nm,was found to be characteristic of the dihydroindole system. The i.r. spectrum ($CHCl_3$) with an absorption at 1730 cm^{-1} showed the presence of an ester carbonyl. An accurate mass measurement afforded the exact mass to be 352.1786 which was identical with the mass calculated for the formula $C_{21}H_{24}N_2O_3$ (352.1786). Other major peaks occurred at 336, 229, 170, 154 and 135. The characteristic loss of oxygen, the polar nature of the material and the similarity of the mass spectrum with that of vindolinine,[72] suggested that the material in hand was the N-oxide of vindolinine or one of its diastereoisomers. However, the nmr spectrum showed the presence of two doublets at δ 0.62 and δ 0.95, which suggested that the substance was a mixture of two diastereoisomeric N-oxides.

Different solvents systems were investigated in attempts to separate the two components without success. However, when the material was treated with PCl_3 in chloroform at 30°C, it was found to be smoothly transformed to vindolinine and 16-epi-19-S-vindolinine, which were identified by spectroscopic and chromatographic comparisons with authentic samples. The N-oxides isolated were therefore identified to be 16-epi-19-S-vindolinine N-oxide (59) and vindolinine-N-oxide (60),[73] the former being a new dihydroindole alkaloid.

A high resolution mass measurement of the M^+ of the faster moving substance C isolated from F_5 afforded the exact mass to be 322.1645 which corresponded to the formula $C_{20}H_{22}N_2O_2$ (322.1660), while other major peaks were present at 263, 234, 180, 154 and 108. The u.v. spectrum (MeOH) showed absorption maxima at λ_{max} 265 and 230 nm. The i.r. spectrum ($CHCl_3$) showed an absorption at 1730 cm^{-1} indicating the presence of an ester carbonyl. The mass spectrum showed M^+ at m/z 322 and other major peaks at 263, 234, 180, 154 and 108. Comparison of the spectral data



(59)



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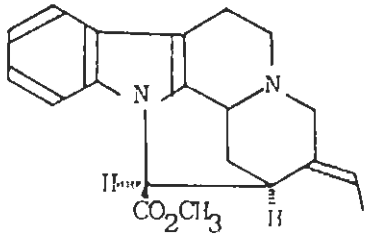

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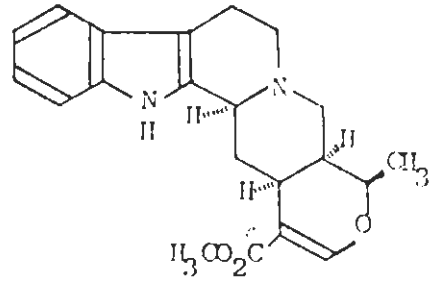
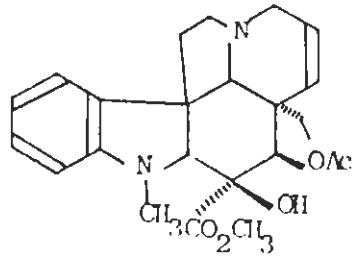


(62)



(63)

of the material with those reported for pleiocarpamine (61)[79] established the two to be identical.

16-Epi-19-S-vindolinine, fluorocarpamine-N-oxide and 16-epi-19-S-vindolinine-N-oxide are new alkaloids while pleiocarpamine, vindolinine-N-oxide and fluorocarpamine have not previously been reported from *C. roseus*.

In the course of the above studies several other known alkaloids were also isolated from *C. roseus* i.e. ajmalicine, tetrahydroalstonine, vindorosine and tabersonine. These were obtained from different fractions (obtained from *C. roseus* leaves by the procedure described previously). Chromatography of fraction F_6 on silica (70-230 mesh) afforded tetrahydroalstonine (62) on elution with 28% ethylacetate in pet.ether. It was identified by comparison of spectral data.[80] Further elution of the same column with 35% ethyl acetate in pet.ether yielded ajmalicine (12) as a crystalline substance. It was identified by spectral and t.l.c. comparison with an authentic sample.[81]

When fraction F_6 was loaded on a flash silica (70-230 m) column and eluted with 20% ethyl acetate in pet.ether vindorosine (63), was obtained which was identified by comparison of spectral data.[82] Another alkaloid, tabersonine, was isolated when fraction F_4 was chromatographed on a preparative high pressure liquid chromatograph. Elution with 60% chloroform in pet.ether, yielded tabersonine (38). It was purified by preparative t.l.c. and identified by spectral and t.l.c. comparison with an authentic sample.[83]

2.2 ISOLATION OF VINDOLINE, CATHARANTHINE AND VINBLASTINE

The binary indole alkaloids, vinblastine (6) and vincristine (7) are among the most potent chemotherapeutic agents known to man and are being used for the treatment of several different types of cancers, including Hodgkin's disease, acute leukaemia in children and chariocarcinoma. As the alkaloids occur only in minute trace in the leaves of *Catharanthus roseus*, their isolation on a commercial scale poses a serious problem to the pharmaceutical industry, and raises their price to several thousand dollars per gram, thus limiting their general use. For these reasons their synthesis (partial or total) has been the subject of a considerable amount of work in the past 20 years.[84-91] All the earlier attempts were however unsuccessful due to the complexity of the structure of these alkaloids and led to compounds having an unnatural configuration at C-16 which were consequently biologically inactive.

Vinblastine consists essentially of a tetracyclic indole portion, bonded to a pentacyclic dihydroindole moiety by a C-C bond. The latter is in fact

the *Aspidosperma*-type alkaloid vindoline. It was interesting to note that the indole moiety of the binary alkaloids, vinblastine, vincristine, vinleusosine (49) and vinrosidine (50) is tetracyclic. In spite of intensive investigation of the *C. roseus* plants no such iboga-type tetracyclic compound have ever been isolated from this plant, the known iboga alkaloids being pentacyclic in origin.

This led Atta-ur-Rahman to propose a novel biogenetic hypothesis[92-94] that such tetracyclic systems may have been biosynthesized within the plant by a direct nucleophilic attack of vindoline on some common, suitable pentacyclic derivative of an iboga alkaloid such as catharanthine, the structure of which is similar to the tetracyclic indole moiety of vinblastine. This attack could simultaneously cleave the C_{16}-C_{21} bond and consequently result in the formation of the tetracyclic indole portion. Further, on the basis of model studies, he predicted that such an attack would afford significant quantities of the binary product bearing the natural configuration at C-16. The biosynthetic hypothesis was

later verified in vivo by tritium labelling experiments carried out by Daddona and Hutchinson[95] and by the work of Potier[96] who reported a new C_{16}-C_{21} skeletal fragmentation of ibogaine derivative, induced by the modified Polonovski reaction which in the presence of vindoline afforded a vinblastine-type compound with a natural C-16 configuration.

The first partial synthesis of the binary alkaloids vinblastine and vincristine based on biosynthetic hypothesis[92] was reported in 1976, due to work of Atta-ur-Rahman and co-workers[8] and two improved syntheses have been reported by the same group in 1978[9,97,98] and 1980.[99,100] Potier and co-workers have subsequently reported an identical route in 1979.[101]

All the syntheses of these binary antitumour alkaloids achieved so far are based on the biosynthetic hypothesis, vindoline and catharanthine being used as starting points. It was therefore important to develop rapid procedures for the isolation of these alkaloids.

The previous procedure by which these alkaloids have been isoalted is involved, cumbersome and time consuming. The earlier procedure involves defatting of the alcoholic crude extract of *Catharanthus roseus* plant by extraction with petroleum ether, after which the drug is intimately mixed with petroleum ether. The drug is then intimatelv mixed with a 2% aqueous solution of tartaric acid. The alkaloids are extracted from the acidic solution into organic solvents at varying pH values. The fractionated alkaloids are then subjected to successive column chromatographies in order to isolate catharanthine, vindoline and vinblastine in a pure state.

The procedure described below which has been developed by us is rapid, and avoids any extensive chromatographies for the isolation of catharanthine and vindoline from *Catharanthus roseus*.

The fraction F_6 (the mother liquor of 16-epi-19-S-vindolinine as shown in Fig.1) was subjected to flash chromatography. This consists of dry packing silica gel (70-230 mesh) in a sintered column fitted with a

ground-glass bottom joint and tap. This column is fitted on top of a Buchner flask. The substance is loaded on the column and the eluting solvent forced down the column by means of applied vacuum on the collecting Buchner flask. The substance was adsorbed on silica and loaded on such a column packed with dry silica. The column was eluted with increasing polarities of pet.ether and ethyl acetate mixtures. The catharanthine containing fraction was obtained with 70:30 (petroleum ether-ethyl acetate). When no more catharanthine was detectable on t.l.c. the eluent was changed to 45:55 (pet.ether/ethyl acetate) and the vindoline-containing fraction was collected. Crystallisation of catharanthine was effected in MeOH-ether while vindoline was crystallized in ether. The identity of catharanthine (32) and vindoline (33) was confirmed by spectroscopic comparisons with authentic samples.[102,103]

As the isolation procedure described previously for the isolation of vinblastine is very cumbersome and time consuming, we have also developed a rapid procedure for the isolation of vinblastine from the leaves of *Catharanthus roseus*.

The vinblastine containing fraction F_2 (obtained as shown in Fig.1) was loaded on a flash chromatography column packed with alumina (neutral,activity I). The column was eluted with 70% ethyl acetate in pet-ether. The eluates containing vinblastine were concentrated to a gum and again loaded on a similar flash chromatography column packed with t.l.c. grade silica. Elution with 50% ethyl acetate in pet-ether removed the faster moving substances. Subsequent elution with 0.5% ethanol in ethyl acetate afforded a further enriched vinblastine-containing fraction. The vinblastine containing fraction was loaded on a preparative high pressure liquid chromatograph and eluted first with 50% ethyl acetate in pet-ether, and then successively 60% ethyl acetate in pet-ether, 65% ethyl acetate in pet.ether and finally with 70% ethyl acetate in pet.ether to afford pure vinblastine from the last eluates.

The identity of vinblastine (6) was confirmed by spectroscopic comparison with an authentic sample,[103,104] as well as by chromatographic comparisons on t.l.c.

2.3 A REMARKABLE OXIDATIVE FRAGMENTATION OF 16-EPI-19-S-VINDOLININE

We have described the isolation and structure of 16-epi-19-S-vindolinine (56), a new dihydroindole alkaloid from the leaves of *Catharanthus roseus*.[77] in an earlier section of this thesis. Vindolinine (52) is a diastereoisomer of the new alkaloid, isolated by us. Its structure has been revised mainly on the basis of C-13 NMR.[74] It is one of the major monomeric alkaloids isolated from the Madagascan *Catharanthus* species and particularly from the most common and thoroughly studied of them, i.e., *C.roseus*. The structure of vindolinine was further confirmed chemically by Potier and co-workers,[76] by an oxidative cleavage reaction with iodine/THF/H_2O/Na_2CO_3 which cleaved the C_2-C_{19} bond of (52). This reaction was also used by us, while establishing the structure of 16-epi-19-S-vindolinine.

Our objective to investigate procedures for the cleavage of C_2-C_{19} bond of (56) resulted in the discovery of a remarkable reaction which not only cleaves the C_2-C_{19} bond but also causes a fragmentation

of the piperidine ring resulting in the facile transformation of the hexacyclic (56) to the tricyclic product (68) in one step on oxidation with lead tetra-acetate.[105]

16-epi-19-S-vindolinine when refluxed in benzene for 3 hrs. in the presence of an equimolar amount of lead tetra-acetate, under nitrogen was found to be smoothly transformed to two faster moving products. The major product formed in 70% yield afforded a normal indolic u.v. spectrum. The i.r. spectrum (KBr) showed bands at 1655 cm^{-1} and 1730 cm^{-1}, which are assigned to N_b-CHO and ester carbonyl groups respectively. The mass spectrum showed M^+ at 352.1783 (calc. for $C_{21}H_{24}N_2O_3$, 352.1786) and other major peaks were present at 320, 293, 214, 169 and 154. The p.m.r. spectrum ($CDCl_3$) showed resonances at δ 1.23 (J=5.6Hz) which is assigned to the C-18 methyl protons. A singlet δ 3.67 showed the presence of the ester methyl group. A quartet at δ 5.46 (J=5.6Hz) was assigned to the ethyledine C-19 proton. A multiplet at δ 4.7-6.1 was assigned to two olefinic protons at C-14 and C-15. The four aromatic protons resonated at δ 6.9-7.6 as complex multiplets. A

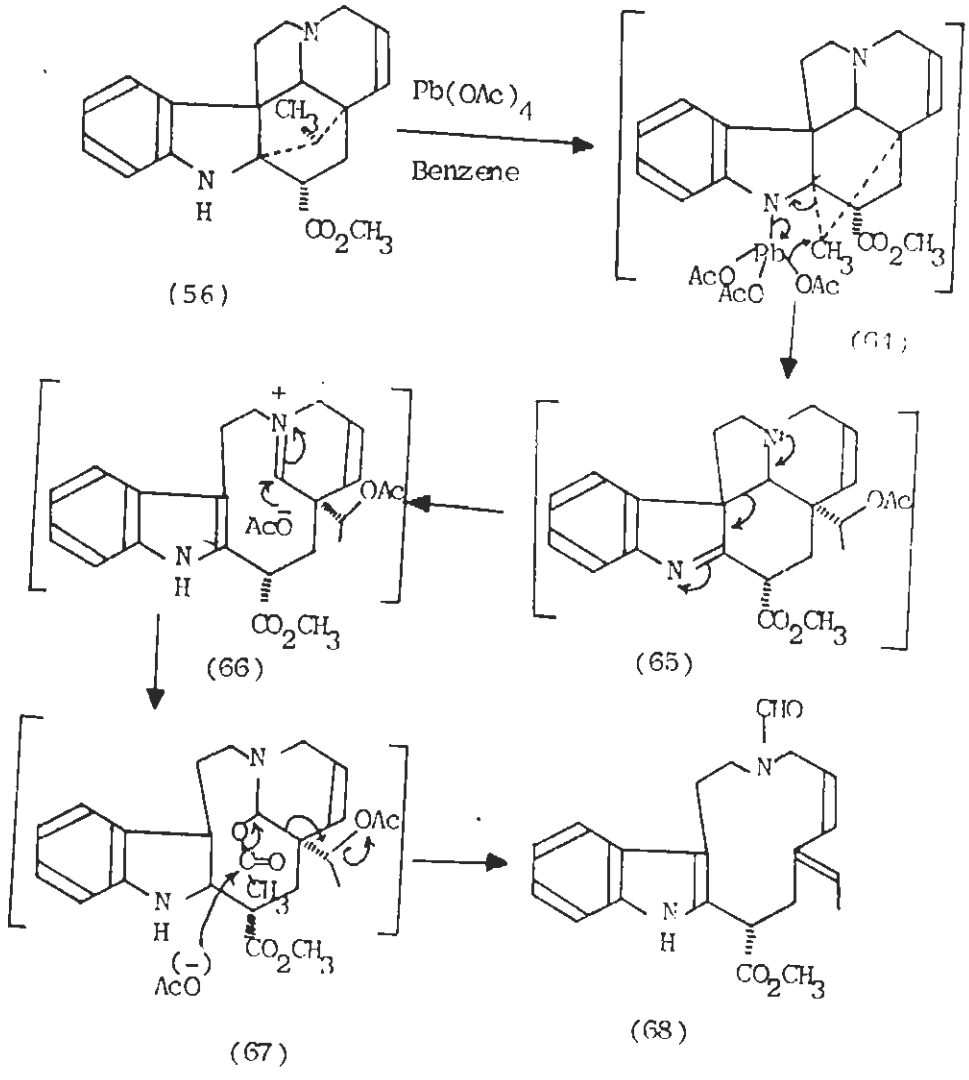
singlet at δ 8.00 was assigned to the N-formyl proton while another singlet at δ 8.35 was ascribed to the NH proton. Irradiation at δ 5.46 resulted in the collapse of the methyl group at δ 1.23 to a singlet, confirming the presence of the C-19 ethylidene proton.

The above spectroscopic data were identical with those for (68), a product previously reported to be formed from 19-iodo-tabersonine on heating with sodium acetate in DMF.[106] In order to confirm the structure of the oxidation product, 16-epi-19-S-vindolinine (56) was oxidized with iodine under conditions previously reported for the oxidation of its diastereoisomer. This afforded the corresponding 19-iodo-tabersonine in quantitative yields. Treatment of the latter with sodium acetate in hot DMF afforded (68), A direct spectroscopic and chromatographic comparison of the product formed by lead tetra-acetate oxidation with that prepared from 19-iodo-tabersonine unambiguously established its structure.

A plausible mechanism for the formation of (68) is presented (Scheme 12). The compound (56) reacts with lead tetra-acetate by the formation of N_a nitrogen Pb bond to afford (64). Intramolecular rearrangement can then result in the formation of the indolenine (65) with cleavage of the C_2-C_{19} bond. The intermediate (65) rearranges to the immonium species (66) with the participation of N_b nitrogen lone pair. The immonium species is then attacked by the acetate ion at C-21 and thus converted to the species (67). Subsequent attack of another acetate ion on to the carbonyl carbon of the bonded acetate is accompanied by the fragmentation of the piperidine moiety and the departure of the tertiary acetate function to afford (68) bearing a N-CHO grouping (Scheme 12).

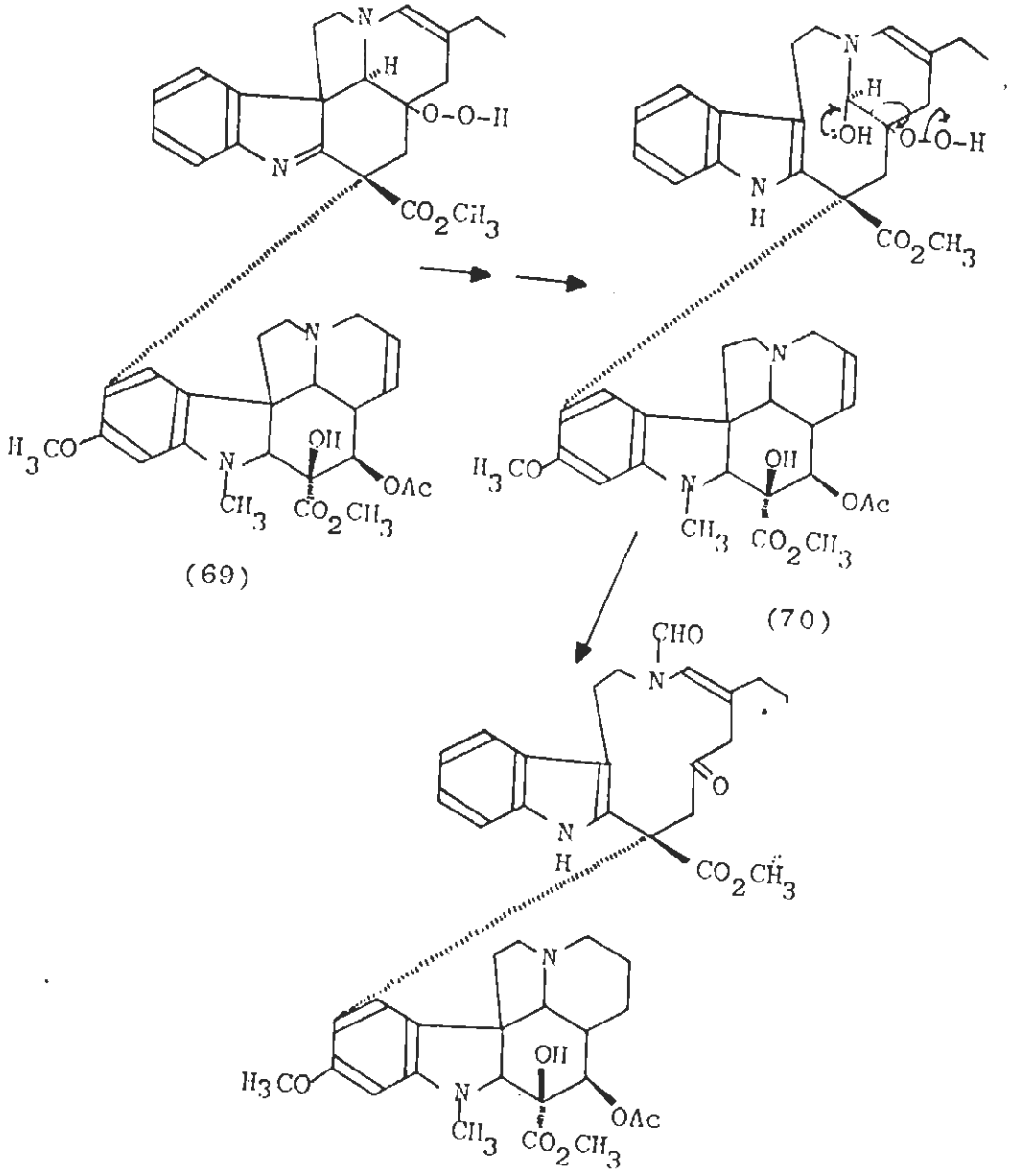
The second minor product formed in the lead tetra-acetate oxidation possessed a u.v. characteristic for the dihydroindole system. Further work on the structure of this material is under progress.

The facile formation of (68) from (56) is biogenetically interesting particularly in view of the



Scheme-12

occurrence of the binary indole alkaloids such as catharine (71)[107] in which one of the moieties bears a distinct resemblance to (68). This raises the interesting possibility that the indole moiety of catharine may arise in nature by a parallel process occurring in a binary precursor alkaloid such as (69) (Scheme No.13).



(69)

(70)

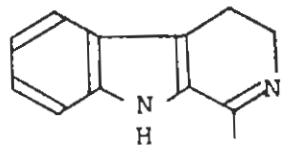
(71)

Schome-13

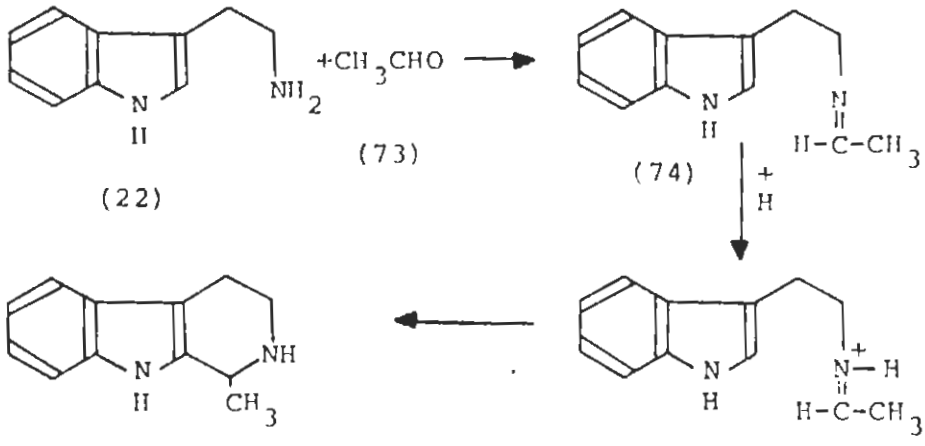
3.0 NEW METHODS FOR THE SYNTHESIS OF β-CARBOLINES

Many indole alkaloids, particularly of the *Corynanthe* and *Yohimbe* series contain the β-carboline nucleus (72). Many of these are physiologically important, e.g. reserpine and ajmaline, which are employed in the treatment of high blood pressure and cardiac arrhythmias respectively.[108,109] β-Carboline alkaloids such as cathenamine and geissoschizine have been recognised as important biosynthetic intermediates, which are transformed to *Strychnos*, *Aspidosperma* and *Iboga* alkaloidal systems through certain rearrangements. Considerable attention has therefore been paid to the synthesis of these alkaloids by a number of research groups. The most frequently employed methods for the generation of β-carboline nucleus are the Pictet-Spengler[110] and Bischler-Napieralski cyclisations.[111]

The Pictet and Spengler cyclisation was originally employed for the [illegible]tion of tetrahydroisoquinoline derivatives. Later it was extended to the synthesis of β-carboline systems by Ta[illegible]sui,[112,113] and by

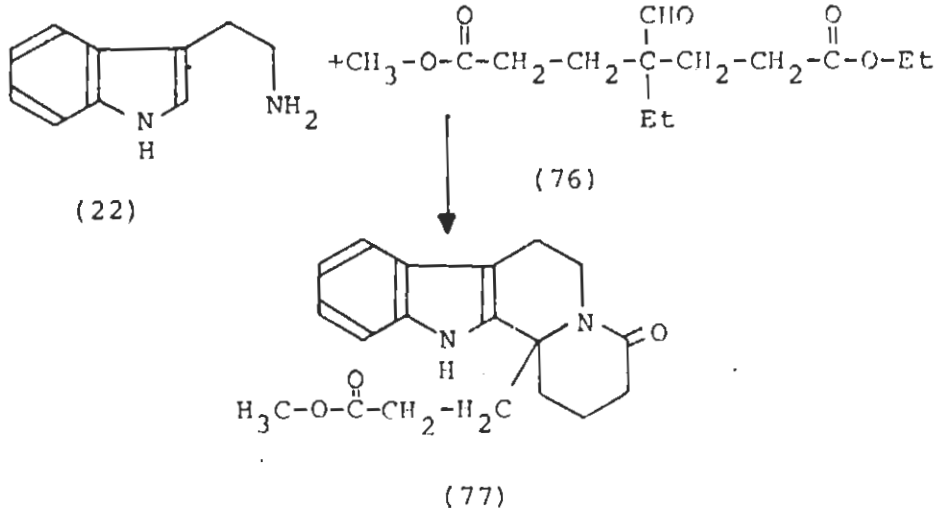


(72)



(22) (73) (74) (75) (9)

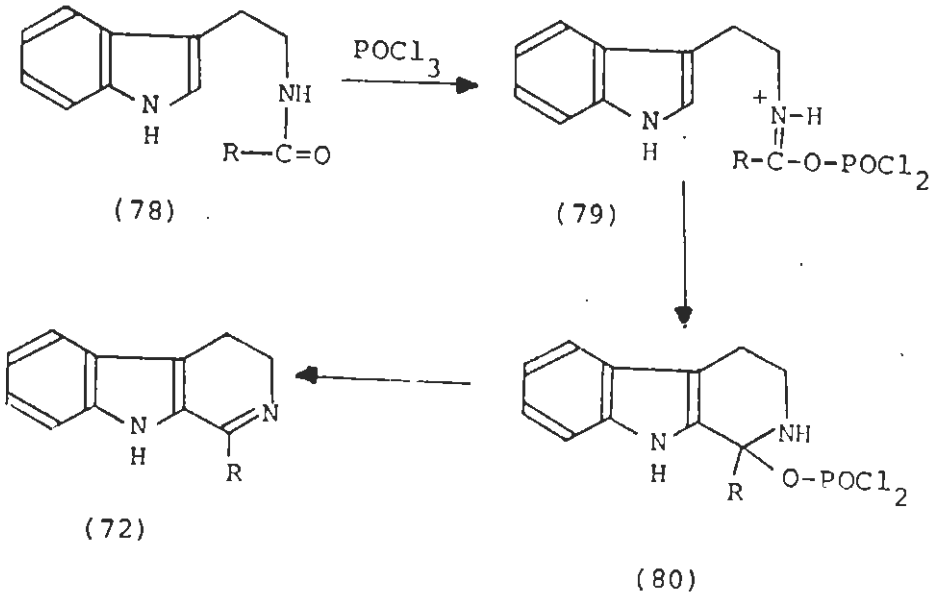
Scheme-14



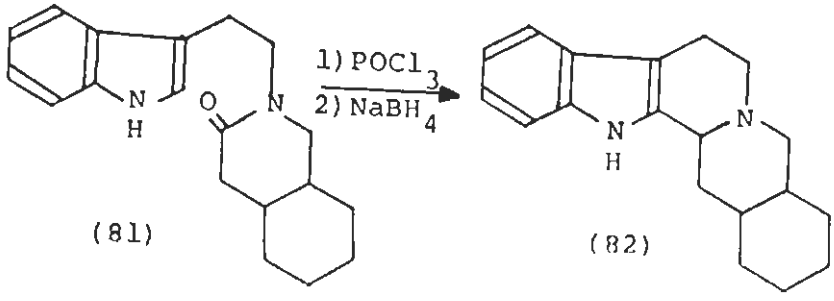
(22) (76) (77)

Akabori and Saito.[114] In its simple form, the reaction is exemplified by the acid-catalyzed Mannich reaction of acetaldehyde (73) with tryptamine (22) to generate the ß-carboline skeleton (72) (Scheme 14). The reaction could be extended to other synthetic objectives, by varying the substituents on the aldehydic moiety. Thus the compound (77) obtained by this reaction serves as a key intermediate in the total synthesis of the indole alkaloid vincamine (10).[115] A number of indole alkaloids synthesis have been reported by Van Tamelen using this method.[116-118]

Another widely used method for the synthesis of ß-carbolines is the Bischler-Napieralski reaction,[111] which involves the formation of amide complexes (79) with phosphorous oxychloride or phosphorous pentoxide. The complex undergoes an intramolecular attack to afford the cyclised dihydro ß-carboline (72) which can be further reduced to the tetrahydro ß-carboline (Scheme 15). This cyclisation has been extensively utilised in the synthesis of a number of indole alkaloids of medicinal repute e.g. ajmalicine (12)[119,120] and reserpine (4).[121] The synthesis of stereoisomers of


POCl3
NH
R—C=O
(78)
+N-H
R-C-O-POCl2
(79)
NH
O-POCl2
(80)
(72)

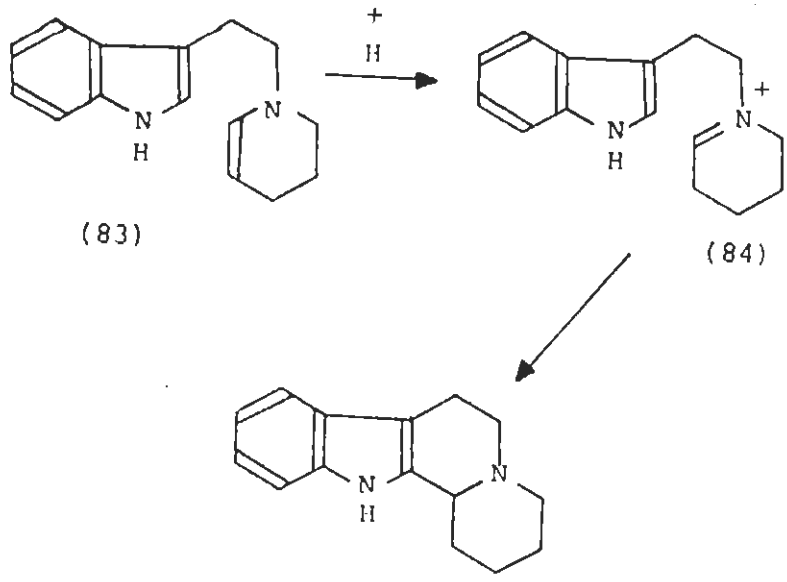
Scheme-15


1) POCl3
2) NaBH4
(81)
(82)

yohimbane (82)[122,123] is a typical example of this cyclisation.

The cyclisation of 1-[2-(3-indolyl) ethyl] tetrahydropyridines is another approach for the synthesis of β-carbolines. The 1-[2-(3-indolyl) ethyl] tetrahydropyridines (83) on protonation in acidic medium generates an iminium bond (84), which is followed by cyclization to (85). Synthesis of the pentacyclic compound (87)[124] and d-l-epialloyohimbane (90) reported by Wenkert[125,126] was also achieved by this approach. Later Wenkert and Wickberg[127] reported the oxidative cyclisation of 1[2-(3-indolyl) ethyl] piperidines with mercuric acetate. The oxidation of (91) to give (92) and (93), is an example of oxidation followed by cyclisation.

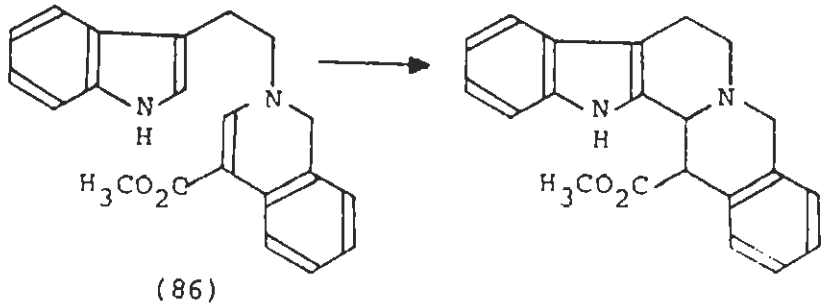
The β-carboline nucleus has also been synthesised by using different N-imidotryptamines. The first direct approach was introduced by Clemo and Swan,[128,129] to a pentacyclic system based on the condensation product (94) of tryptamine and homphthalic acid or anhydride. Esterification of the homophthalamic



(83)

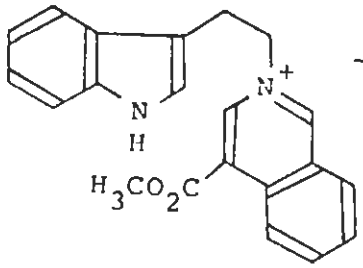
(84)

(85)

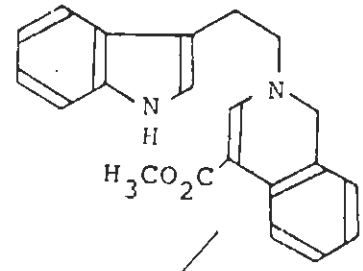


(86)

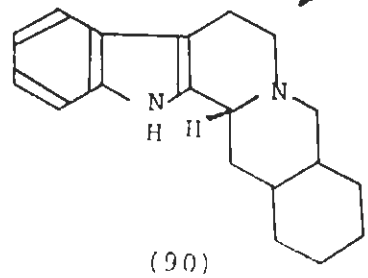
(87)



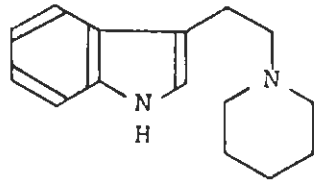
(88)



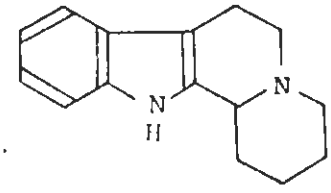
(89)



(90)



(91)



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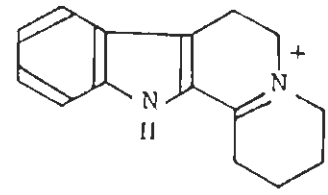
(92)

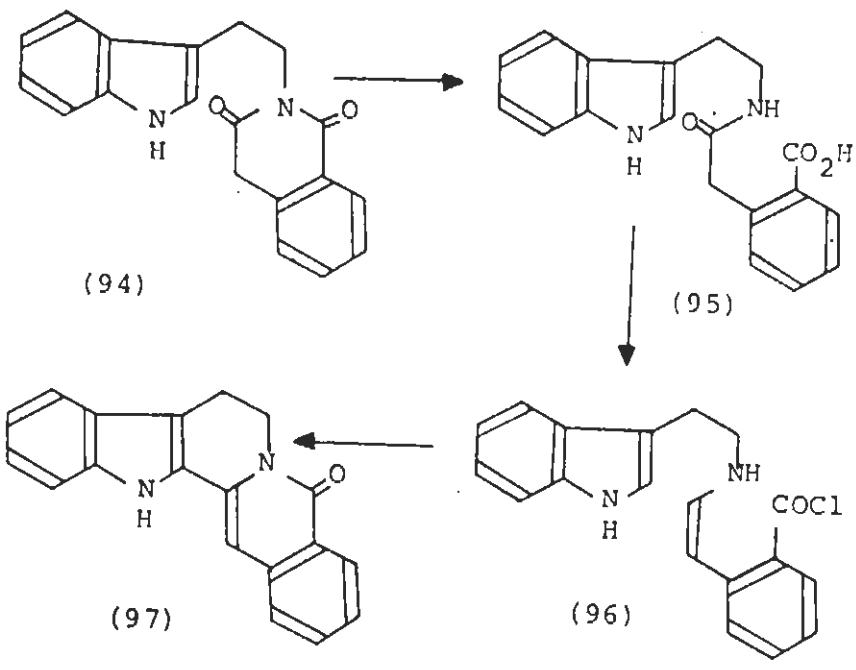


(93)

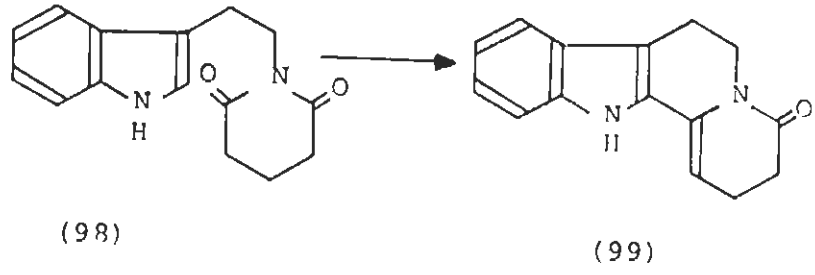
acid followed by ring closure with $POCl_3$ yielded the ß-carboline derivatives (97) (Scheme 16). Morrison[130] has also reported the cyclisation of N-glutarimidotryptamine (98) to the enamide (99) with phosphorus pentoxide in refluxing xylene.

In 1964 Wenkert[131] reported his failure to cyclize N-succinimidotryptamine (100) under Bischler-Napieralski conditions. Later this cyclisation was achieved under very mild conditions by Atta-ur-Rahman[132] by increasing the electrophilic character of the imide carbonyl by alkylating it with Meerwein's reagent.[133] An intermediate immonium species (101) was generated, which on treatment with $NaBH_4$ gave the cyclized ß-carboline lactam (102) (Scheme 17). Bocchi and co-workers[134] have also reported the formation of this lactam by a condensation of tryptamine with 2-pyrrolinone (103).

In 1977 Atta-ur-Rahman[135] reported another facile procedure for the synthesis of ß-carbolines, which involves the generation of Vielsmeir complexes of a number of indolic imides (98), (100) and (104) with phosphorus oxychloride in refluxing benzene. These



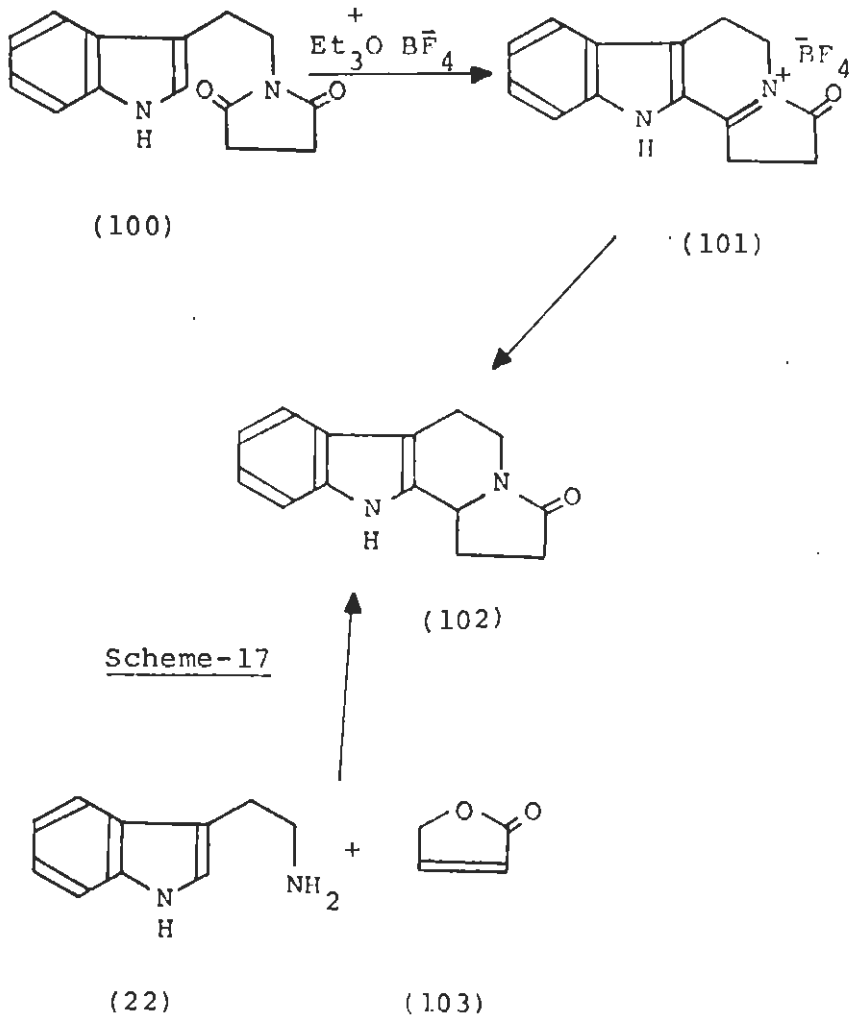
Scheme-16



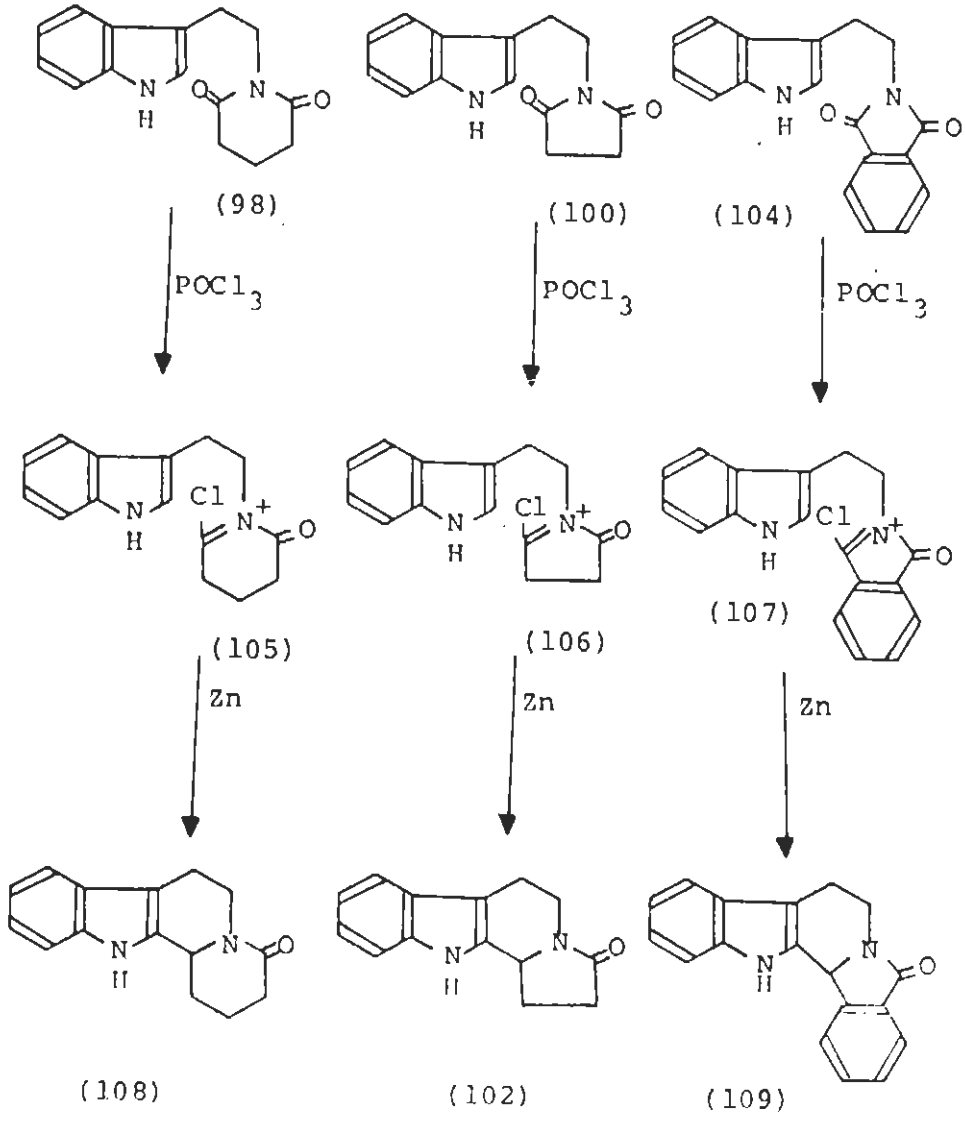
complexes were reductively cyclised by Zn dust under mild conditions to afford the corresponding β-carbolines (108), (102) and (109) respectively (Scheme 18). This procedure avoids the difficulties posed by previous procedures due to the intermediate generation of the unstable conjugated enamides in the synthesis of β-carbolines from N-imidotryptamines.

The indolic imides have been reductively cyclised in the presence of sodium borohydride-hydrochloric acid to generate the β-carboline nucleus. In 1954 E.Tagman,[137] achieved the partial reduction of 3,3-disubstituted 2,5-dioxypyrrolidine (110) and 2,6-dioxopiperidine (111) with lithium aluminium hydride to the corresponding hydroxy lactams (112) and (113) respectively. In 1975 Speckamp and coworkers[138,139] claimed the selective reduction of one of the carbonyl function of succinimide and glutarimide by sodium borohydride in the presence of hydrochloric acid to afford the carbinol lactam

It has been reported that reductions of cyclic imides with sodium borohydride under acidic



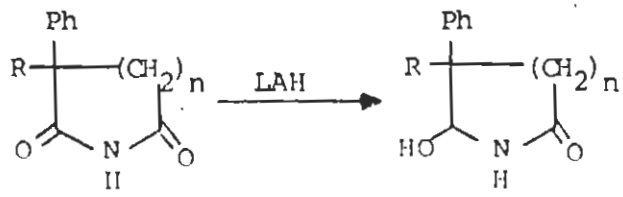
Scheme-17

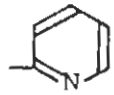
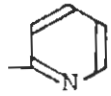


Scheme-18

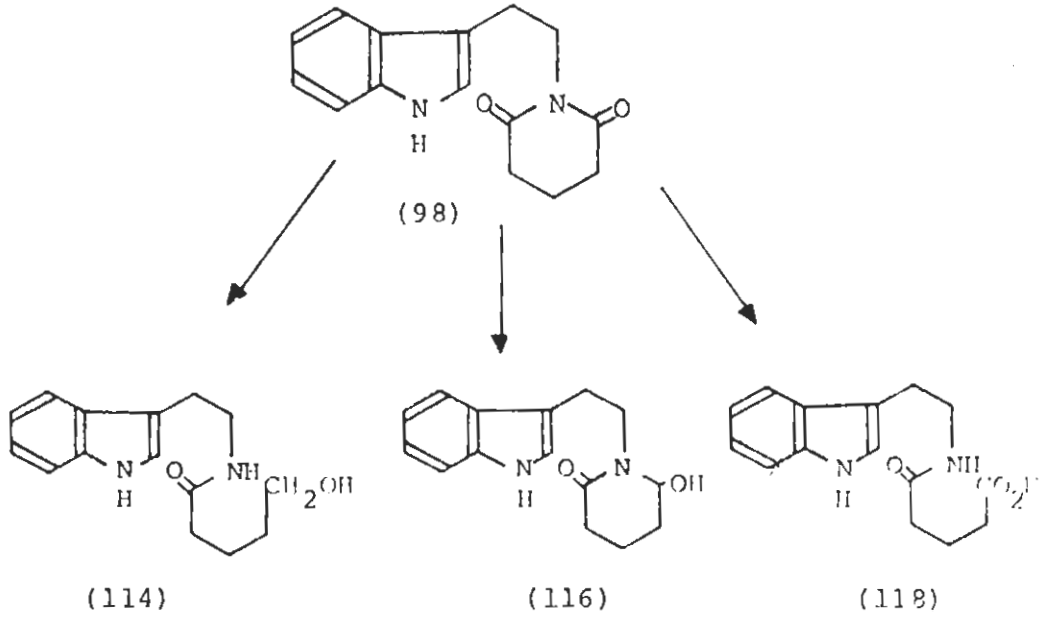
conditions gives the corresponding cyclised product in good yields.[140] Literature search showed the formation of ring cleaved product of the respective imides in low yields. In 1975 Speckamp had reported the conversion of imides into the hydroxy lactam in acidic media,[138,141] as well as buffered controlled conditions.[142] As this appeared to contradict results earlier reported in the literature, Atta-ur-Rahman and co-workers,[143] reinvestigated the reductions described by Speckamp. It was found that the indolic imides under conditions similar to those applied by Speckamp, gave mainly the secoamide alcohols (114,115) rather than the previously reported hydroxy lactam (116,117). In addition to the amide alcohols, the reaction of N—succinimidotryptamine and glutarimidotryptamine with sodium borohydride in absolute ethanol afforded low yields of the corresponding amide esters (118) and (199) respectively.

Atta-ur-Rahman and co-workers,[140] have independently reported new procedures for the building up of the pharmacologically important ß-carboline nucleus. The starting materials used were the



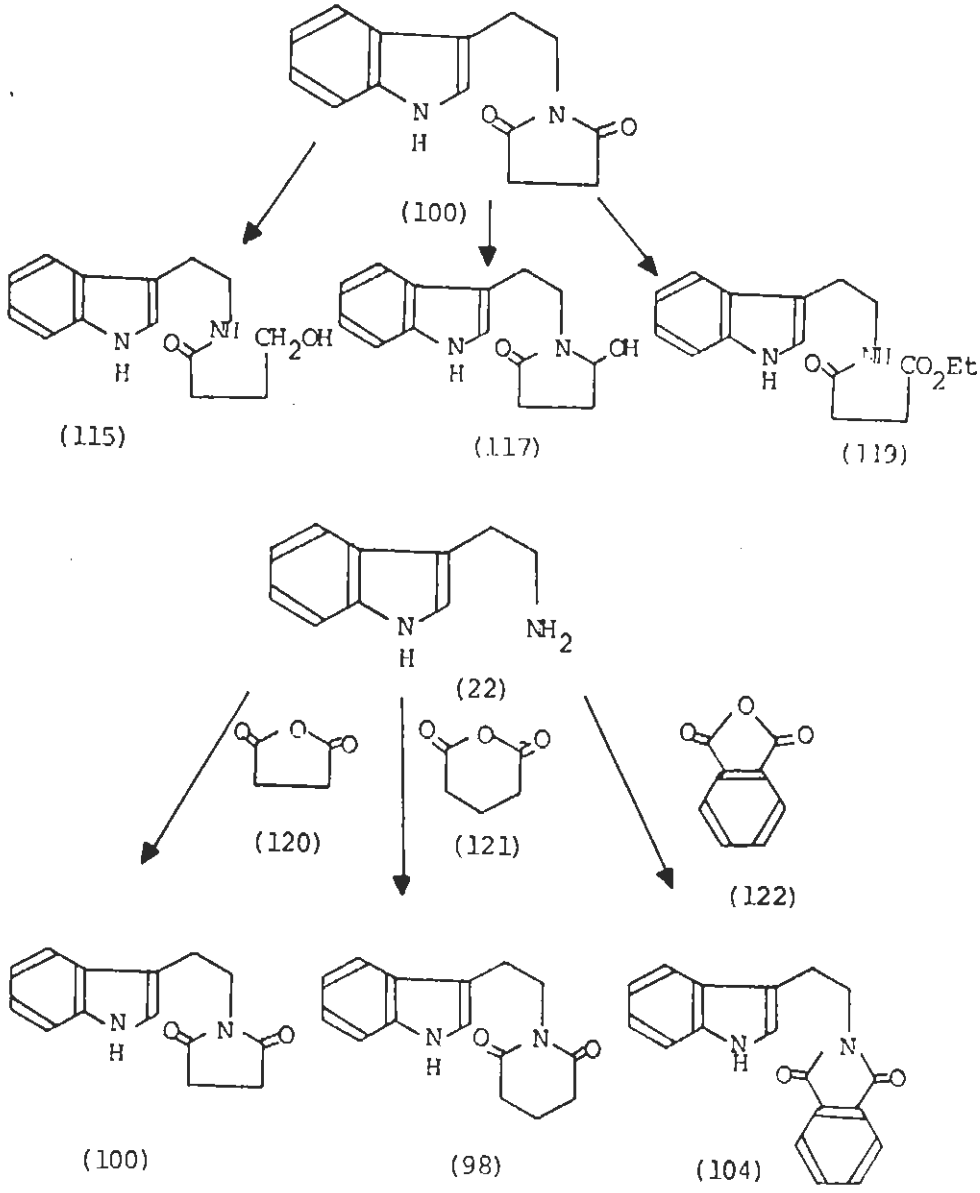
(110) n=1, R= 
(112) n=1, R= 

(111) n=2, R=$(CH_2)_2N(C_2H_5)$ (113) n=2, R=$(CH_2)_2N(C_2H_5)_2$

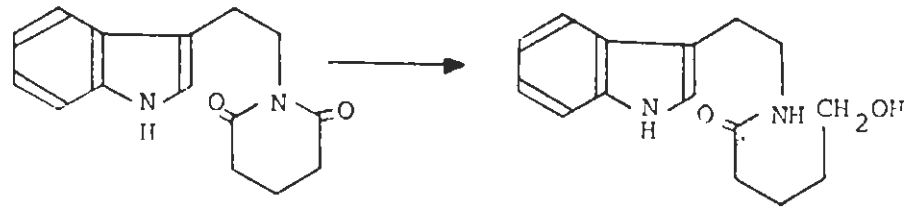


(114) (116) (118)

N-imidotryptamines, obtained by condensation of tryptamine (22) with succinic (120) glutaric (121) and phthalic (122) anhydrides respectively (Scheme 19). The N-imidotryptamines (98), (100) and (104) when treated with sodium borohydride and 2 N HCl were found to be converted to the corresponding ß-carboline lactams (108), (102) and (109) in excellent yields at temperatures between 0-6°C. However at 24°C the main products were the corresponding ring-cleaved amide alcohols (114), (115) and (123) respectively (Scheme 20). With phthalimidotryptamine, the hydroxy lactam (124) was obtained both at 0°C and 24°C, which was later converted to the cyclised ß-carboline (109) on aqueous work up. A similar approach has been independently developed by Speckamp and co-workers.[144]

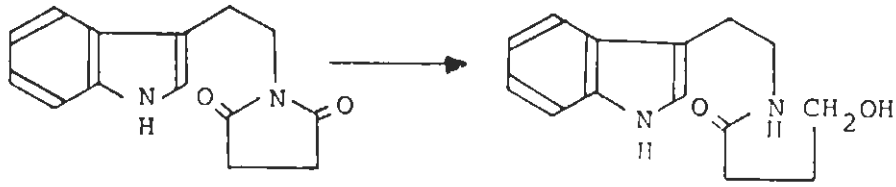


Scheme-19



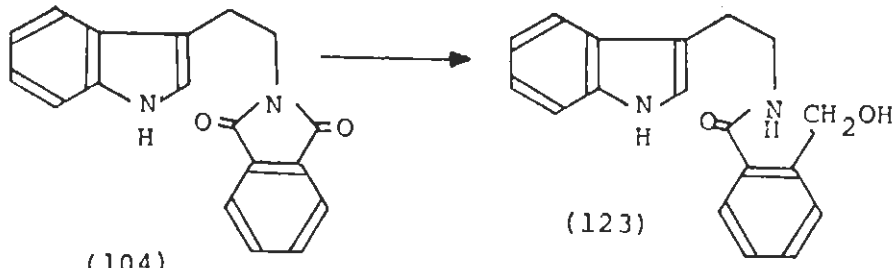
(98)

(114)



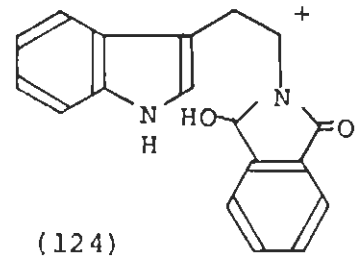
(100)

(115)



(104)

(123)



(124)

Scheme-20

3.1 A NEW SYNTHESIS OF β-CARBOLINES BY METAL-ION CATALYSED REDUCTION OF N-IMIDOTRYPTAMINES.

Various new procedures for building up of the pharmacologically important β-carboline nucleus have been reported by Atta-ur-Rahman and co-workers. The procedure based on reduction of imides with sodium borohydride to the corresponding α-hydroxylactams followed by cyclisation with hydrochloric acid suffered from the drawback that there was a tendency of the cleavage of the rings to afford the corresponding seco-amide alcohols, particularly at higher temperatures.[140] It was felt necessary to develop an efficient procedure for the synthesis of α-hydroxylactams from N-imidotryptamines, which could be subsequently cyclised to β-carbolines. Here we report an improved method for the synthesis of β-carbolines, involving the neat conversion of N-imidotryptamines to the corresponding α-hydroxylactams with sodium borohydride in the presence of various metal halides.

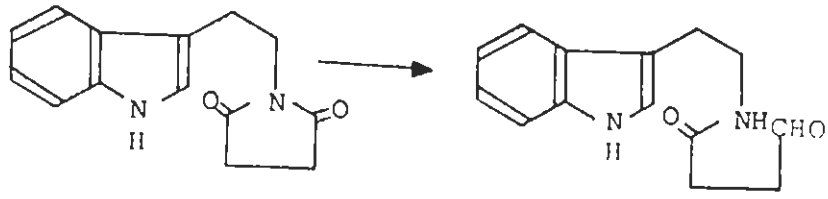
N-Succinimidotryptamine was prepared by the condensation of tryptamine with succinic anhydride (120) in refluxing dry toluene (Scheme 19). N-succinimidotryptamine (100) was allowed to react with one equivalent of

metal halide and one equivalent of sodium borohydride in ethanol at $0^{o}C$. T.l.c. of reaction mixture after intervals of 10, 30, 60, 120 minutes, showed gradual formation of one main product. The product was compared with authentic samples of the amide alcohol (115), amide ester (119) and the cyclised β-carboline lactam (102) and it was found to be different.

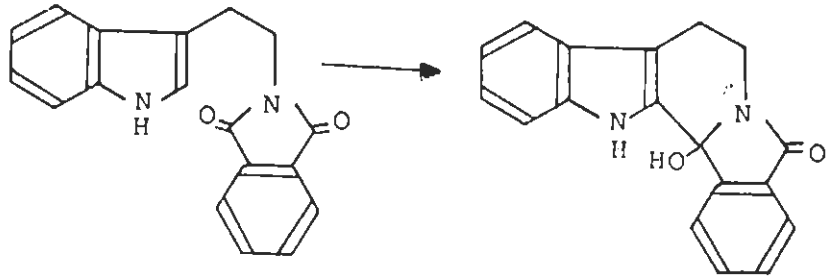
Preparative t.l.c. enabled the separation of the new product. The ultra-violet spectrum (MeOH) showed that it had absorptions at λ_{max} 274 and 293 nm characteristic for the indolic chromophore. The infrared spectrum ($CHCl_3$) showed the presence of O-H and N-H stretching vibrations at 3400 cm^{-1} and 3280 cm^{-1}. The n.m.r. (d_6-DMSO) exhibited a resonance at δ 5.0 as a broad singlet which was assigned to the hydroxyl proton. A triplet resonated at δ 3.7 due to the proton attached to the carbon bearing the hydroxyl function. The 4 aromatic protons resonated in the region δ 6.7-7.8 while a one-proton singlet appeared at δ 8.4 indicative of the presence of the indolic NH proton. The mass spectrum of the compound showed the molecular ion peak at m/z 244, which was consistent with that expected for the

hydroxylactam (117) or for the cleaved aldehyde (125). However the formation of the latter product was excluded by nmr studies, which showed no downfield signal for the aldehydic proton. From these spectral data, the compound in hand was identified as the hydroxylactam (117).[146,147]

The role of metal halides was observed, by conducting parallel blank reactions in identical conditions without the metal halides, and it was found that presence of certain metal halides produced a significant increase in the yield and rate of formation of the hydroxy lactam. The presence of $SnCl_2$ afforded 60-65% of the hydroxylactam after two hours at 0^oC while 20% of hydroxylactam formation was observed in a parallel blank reaction at the end of this time period. A moderate catalytic effect was observed when $CuCl_2.2H_2O$ and $CdCl_2$ were used. The effect of $HgCl_2$, $MnCl_2.4H_2O$, $CeCl_2$, $SbCl_2$, $BaCl_2$ and $CoCl_2$ was not significant as compared to blank reaction, which afforded the hydroxylactam in 20% yield. When the same sets of reactions were repeated at room temperature (30^oC), in the presence of $CuCl_2.2H_2O$, $CoCl_2$ and $MnCl_2.4H_2O$ the



(100) (125)



(104) (126)

hydroxylactam was formed in 40-50% yield, whereas greater tendency for the formation of the amide alcohol was observed in reactions with $SnCl_2$, $SrCl_2$, $SbCl_3$ and $BaCl_2$. The results are tabulated in Table II.

When the concentration of sodium borohydride was increased from one equivalent to five equivalents, the temperature maintained at 0^oC and the reactions carried out in the presence of $CuCl_2.2H_2O$, $CoCl_2$, $CrCl_3.6H_2O$, $FeCl_3$, $SnCl_2$, $SrCl_2$, $SbCl_3$, $NiCl_2.6H_2O$ and $CeCl_3$, a clean conversion to the hydroxy lactam (117) in 85-98% yield was observed, while the reaction in the presence of $CdCl_2$ afforded only 40% of the hydroxylactam and traces of amide alcohol (115) as compared to 60% of (117) formed in the blank reaction. The same reductions were then repeated at 30^oC, and it was observed that a quicker reduction to the hydroxy lactam was observed in a majority of the reactions as compared to a blank reaction (carried out without the metal halides), however there was an increased tendency for the formation of the undesired cleaved amide alcohol (115). As the reaction progressed

the concentration of the hydroxy lactam and the unconverted imide decreased while that of the amide alcohol increased (70-90% of amide alcohol (115) formation in the presence of $CoCl_2$, $HgCl_2$, $SnCl_2$, and $NiCl_2.6H_2O$). However, in reactions with $CrCl_3.6H_2O$, $SrCl_2$, $ShCl_3$, $CeCl_3$ and $BaCl_2$ gradual increase in concentration of both the hydroxylactam (117) and amide alcohol (115) was observed. Interestingly $MnCl_2.4H_2O$ was the only halide which was observed to form barely 5% of the amide alcohol and which specifically promoted the formation of the hydroxy lactam over two hours at this temperature. Reactions in presence of $CdCl_2$ were similar to the blank reactions. The results are given in Table III.

A complete conversion of the starting imide into the products was observed, when the concentration of sodium borohydride was increased from five equivalents to ten equivalents and the temperature was kept constant at $0^{\circ}C$. Further reduction of the hydroxylactam to the amide alcohol was prevented if the low temperature was carefully maintained, while increase in the reaction temperature resulted in the increased formation

of the amide alcohol. However the reaction carried out in the presence of $CdCl_2$ or $MnCl_2.4H_2O$ afforded barely 40-50% hydroxylactam formation at 30°C after two hours, while the blank reaction showed the quantitative formation of amide alcohol at this temperature. The results are recorded in the Table IV.

From the above series of reactions, it can be concluded that the formation of the hydroxylactam is enhanced in the presence of metal halides at low temperatures, whereas at higher temperatures and with higher concentration of sodium borohydride it is the formation of the amide alcohol which predominates.

Similar reductions were carried out with N-glutarimidotryptamine (98) (N-glutarimidotryptamine was prepared by condensation of tryptamine with glutaric anhydride (Scheme 19))[130] in the presence of various metal halides. A new product was observed to be formed which was different from authentic samples of amide ester (118), ß-carboline (108) and the amide alcohol (114) on t.l.c. Preparative t.l.c. of the reaction

mixture afforded the pure substance. The u.v. spectrum in methanol indicated the indolic nature of the product. The i.r. spectrum showed the presence of the amide carbonyl stretching vibration at 1658 cm^{-1}. The nmr spectrum indicated the hydroxyl proton at δ 4.74 as a broad hump while the indolic NH proton appeared at δ 8.2 as a singlet. The aromatic protons resonated as multiplets in the region δ 6.9-7.8. The mass spectrum of the product showed the molecular ion peak at m/z 258 which corresponded to that expected for the hydroxylactam. On the basis of the above spectral data, the product was identified as the hydroxylactam (116).

It was observed that in reductions with one equivalent each of N-glutarimidotryptamine (98), $NaBH_4$ and metal halides at $0^{o}C$ only $SnCl_2$ and $SrCl_2$ promoted the formation of the hydroxylactam affording 60% of the product (116) after two hours. Reaction with $CoCl_2$, $CrCl_2.6H_2O$, $FeCl_3$, $SbCl_3$ and $NiCl_2.6H_2O$ showed moderate catalytic effect. The remaining reactions did not give sufficiently improved yields of the product as compared to that obtained from the blank

reaction. The halide of cadmium was observed to retard the formation of the hydroxylactam. When the same set of reductions was carried out at 30°C the amide alcohol (114) was obtained in addition to amide ester (118). The results are tabulated in Table V.

When the concentration of sodium borohydride was increased from one equivalent to five equivalents and the reaction conducted at 0°C, higher yield conversions to the corresponding hydroxylactam was observed with all metal halides except with $CdCl_2$. Reactions employing $CuCl_2$, $CrCl_2.6H_2O$, $FeCl_3$, $HgCl_2$, $SrCl_2$, $SnCl_2$, $SbCl_2$, $NiCl_2.6H_2O$, and $MnCl_2.4H_2O$ all gave 70-85% yields of the hydroxylactam (116) whereas the reaction with $BaCl_2$ and the blank reaction afforded the hydroxylactam only in 45% yield. When the same reductions were repeated at 30°C, an increased formation of the undesired amide alcohol was observed after two hours. $MnCl_2.4H_2O$ was the only one which afforded the hydroxylactam in 80% yield at the end of two hours at this higher reaction temperature. Reactions with $FeCl_3$, $HgCl_2$, $SnCl_2$, $SbCl_2$ and $CeCl_2$ afforded 70-90% of the undesired amide

alcohol. $CdCl_2$ was observed to give 15% of the hydroxylactam. The results are given in Table VI.

Reduction of N-phthalimidotryptamine (104)

(N-phthalimidotryptamine was prepared by condensation of tryptamine with phthalic anhydride (Scheme 19))[148] with 5 equivalents of sodium borohydride and one equivalent of metal halides at $0^{o}C$, resulted in the quantitative conversion to the corresponding hydroxylactam (124) alongwith some amide alcohol (123), and cyclised β-carboline (109) while in the presence of $CuCl_2$, $FeCl_3$ and $NiCl_2.4H_2O$, the formation of a new product was observed; the results are recorded in Table VII.

The product obtained was isolated by preparative t.l.c. The u.v. spectrum of the product showed an indolic chromophore, while the i.r. spectrum indicated the presence of amide carbonyl at 1670 cm^{-1} and the presence of OH and NH stretching vibrations at 3280 cm^{-1} and 3320 cm^{-1}. The nmr spectrum showed the indolic NH proton at δ 10.89 as a singlet, while the four aromatic protons resonated in the region δ 6.8-7.8. The mass spectrum of the substance showed the molecular

ion peak at m/z 290 with other major peaks at 160, 148, 105, 85, 77 and 69. From these spectral data the compound was identified as the cyclised β-carboline (126) formed with the retention of the hydroxyl function.[146,147]

The exact mechanism of catalysis exhibited by metal halides, which was observed in above series of reactions, cannot be advanced at this stage due to lack of kinetic data. It is however known that metal ions have the tendency to form complexes with electron donating species due to availability of empty d-orbitals. Thus a possible mechanistic pathway may involve the complexation of the metal ion with the lone pair of electrons on the imide nitrogen or with the carbonyl oxygen atom. In either case the electrophilicity of the carbonyl carbon will be enhanced and this will facilitate the attack of the hydride ion and thus expedite the formation of hydroxy lactam.

Another possibility for the increased formation of the hydroxylactams in the presence of metal halides, is that once the hydroxylactam is formed, it

would be stabilized by complexing with the metal ion and the further reduction of the hydroxylactam to the cleaved amide alcohol would be prevented on account of the reduced tendency of ring opening of the hydroxylactam to the corresponding aldehyde. It has also been reported that reducing power of sodium borohydride is enhanced by addition of certain metal halides.[149]

4.0 STRUCTURE ELUCIDATION OF ALKALOIDS FROM THE SEEDS OF *RHAZYA STRICTA*

4.1 INTRODUCTION

The genus *Rhazya* belongs to the indole alkaloids-rich family *Apocynaceae*, and it comprises two species.[150] *Rhazya stricta Decaisne* is abundantly distributed in various parts of Pakistan. It is a small glabrous erect shrub and it also grows profusely in the north western regions of the Indian subcontinent.[151,152] The second species *Rhazya orientalis* A Dc., occurs more commonly in north western Turkey and Western Thrace. No medicinal uses of *R.orientalis* have been reported, but *R.stricta* is reputed in the indigenous medical system as a bitter tonic and as a curative for chronic rheumatism.[153-156]

The presence of alkaloids in *R.stricta* was first detected by Hooper[157] and by 1945 its rich alkaloid content was established. Since then the total number of indole alkaloids isolated from *Rhazya* species has risen to more than fifty.[158-161]

Indian workers in 1972, demonstrated that extracts of *R. stricta* showed marked leucopenic effect in rats when given orally (20 mg/kg) and that a single i.p. injection (15 mg/kg) significantly reduced the white blood cell count for 7-10 days.[162] Recently Cordell and co-workers[163] have reported the isolation of biologically active alkaloids from *R. stricta*.

Keeping in view the reputed anti-cancer activity of *Rhazya* alkaloids it was considered interesting to investigate the seeds of the plant, particularly since much work has been done on the alkaloids of *Rhazya stricta* but the seeds have been little investigated.

4.2 RESULTS AND DISCUSSION

The procedure adopted for the isolation of alkaloids is as follows:

Powdered *Rhazya stricta* seeds were percolated with hot ethanol. The ethanolic extracts were concentrated to a dark brown gum. The crude extract was successively washed with petroleum ether to remove fatty substances. The deffated acidic solution was basified with ammonia solution and extracted with chloroform. The chloroform extracts were dried and evaporated to afford the crude alkaloids, which were subjected to column chromatography over neutral alumina (activity II-III). Elution with 3:1 benzene: chloroform mixtures afforded a fraction containing two alkaloids, one of which could be crystallised.

In order to elucidate its structure it spectral data were recorded. Its u.v. spectrum (MeOH) exhibited characteristic indole chromophore: λ_{max} at 230 and 293 nm. The i.r. spectrum (KBr) showed absorption at 3360 cm^{-1}, indicating the presence of C-H stretching. High resolution mass spectrometry afforded the exact

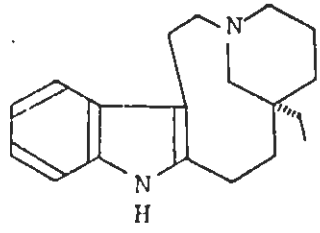
mass to be 282.2075 which corresponded closely with the mass calculated for the formula $C_{19}H_{26}N_2$ (282.2095) the other major peaks were present at 253, 157, 143, 125, 110 and 96. The nmr spectrum ($CDCl_3$) showed an absorption at δ 0.82 (J = 6.2Hz) as a triplet indicating the presence of a methyl group. The four aromatic protons resonated in the regions at δ 7.0-5.57.. A broad singlet at δ 7.6 indicated the presence of indole N-H proton. From a comparison of these spectral data[164] with those reported in the literature,[165-168] the substance was identified to be quebrachamine (127).

Another alkaloid was obtained from the same mother liquors which afforded quebrachamine. In order to elucidate its structure the spectral data were studied. Its u.v. spectrum (MeOH) showed the presence of the indolenine chromophore, affording absorptions at λ_{max} 214, 236 and 293 nm. The i.r. spectrum showed the presence of a hydroxyl group with absorption at 3400 cm^{-1}. The nmr spectrum in deutrochloroform showed a triplet at δ 0.87 (J = 6.2Hz) indicating the presence of a methyl group. A quartet appeared at δ 1.25 (J = 6.2Hz) due to the methylene protons. A complex

multiplet between the regions at δ 7.1-7.8 were assigned to the four aromatic protons. The hydroxyl proton resonated at δ 1.6 as a broad singlet. An accurate mass measurement on the molecular ion peak showed the exact mass to be 314.199, which was in agreement with the formula $C_{19}H_{26}N_2O_2$ (314.1994) while other major peaks appeared at 297, 281, 172, 146, 130, 124 and 83. From a comparison of these spectral data[164] with those reported in the literature[169] the alkaloid was identified as rhazidigenine-N-oxide(128). This alkaloid has not previously been reported from *Rhazya stricta*.

Further elution of the same column with benzene: chloroform (3:1), afforded another crystalline base. Its u.v. spectrum (MeOH) showed absorptions at λ_{max} 227, 283 and 293 nm, typical of the indole chromophore. The i.r. spectrum (KBr) showed peaks at 3340 cm^{-1} indicating the presence of a hydroxyl group and a strong absorption at 1715 cm^{-1} supporting the presence of an ester group. The nmr spectrum in deuterated chloroform showed complex multiplets between the regions at δ 6.97-7.55 integrating for 4 aromatic protons. One

proton resonated as a quartet at δ 5.47 showing the presence of an olefinic proton adjacent to a methyl group while a three-proton doublet at δ 1.75 was assigned to the ethylidene methyl group. The mass spectrum showed M^+ at 352; accurate mass measurement afforded the exact mass to be 352.1779, which corresponded closely with the mass calculated for the formula $C_{21}H_{24}N_2O_3$ (352.1786); other major peaks were present at 351, 321, 293, 249, 169 and 115. On the basis of a comparison of these spectral data with those reported in literature[170-171] the alkaloid was identified as akuammidine (129).



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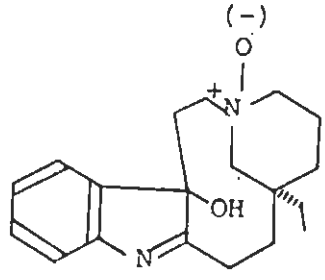

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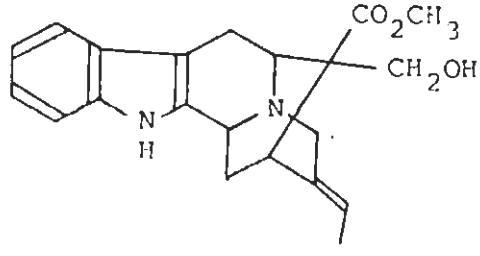

(129)

5.0 EXPERIMENTAL

5.1 GENERAL NOTES

Infra-red spectra were recorded on a Jasco IRA-1 infra-red spectrophotometer. Ultra violet spectra were recorded on Pye Unicam SP.800A and Shimadzu UV-240 spectrophotometers. Nuclear magnetic resonance spectra were recorded on a Jeol JNM-PMX 60 and Bruker WP-100-SY FT-NMR spectrometers using tetramethylsilane as an internal standard in $CDCl_3$ unless otherwise stated. Mass spectra were measured on Finnigan MAT 112 and Finnigan MAT 312 double focussing mass spectrometers connected to PDP 11/34 computer system. High resolution mass measurements were carried by peak matchings and by measurements on Spectrosystem 188 computer linked to Finnigan MAT 312 mass spectrometer. For preparative high pressure liquid chromatography an Altex preparative HPLC system with UV detector was used. Thin layer chromatography was carried out on silica gel GF-254 precoated plates of E.Merck which were viewed in UV light or developed with iodine vapours. Column chromatography was generally carried out using silica gel type-60 (70-230 mesh). Compounds were checked for purity by h.p.l.c. on a Jasco (Familic 100, II spectrometer) high performance liquid chromatograph. Melting points were determined with a Gallenkamp apparatus. All melting points were uncorrected.

5.2 CATHARANTHUS ALKALOIDS

5.2.1 *ISOLATION OF 16-EPI-19-S-VINDOLININE*

Air dried leaves of *Catharanthus roseus* (16 kg) were finely crushed with an ultra-turrax in ethanol (32 litres). The crushed material was filtered and washed thoroughly with ethanol (7 litres). The ethanolic filtrates were combined and evaporated under vacuum to a gum. The gum was acidified with 5% HCl (4 litres) and washed with chloroform (3 litres). The ice-cold solution was then basified (700ml, 33% NH_3 solution), and extracted with chloroform (4 litres). The chloroform extracts were dried over anhydrous sodium sulphate and concentrated to the crude alkaloidal gum (100 gms).

The alkaloids (100 gms) were dissolved in chloroform (300 ml) and extracted with pH-3 phosphate buffer (one litre). The chloroform layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to afford the alkaloidal mixture (50 gm). This was dissolved in chloroform (200 ml) and hexane (400 ml) was added which caused selective precipitation of some alkaloids. The precipitate

were filtered off and the filtrate was again concentrated to a gum (30g). The gum was dissolved in ethyl acetate (200 ml) and extracted with pH-2 phosphate buffer (one litre) and concentrated to a gum (12.5g). Addition of acetone resulted in the crystallisation of (56) which was recrystallised from ethanol as a white crystalline solid (0.5gm) m.p. 200°C; $(\alpha)_D$=+40° (MeOH); U.V.(MeOH): λ_{max} 212, 246, 303 nm (ε, 9341, 5913, 2150); λ_{min} 226, 276 nm (ε, 1831, 537); I.R. (KBr): 1730 cm^{-1} (ester carbonyl); NMR ($CDCl_3$): δ=0.62 (d, 3H, J=7.4 Hz, CH-$C\underline{H}_3$), 2.26 (q, 1H, J=7.4Hz, $C\underline{H}$-CH_3), 3.18 (dd, 1H, J_1=12.2 Hz, J_2=5.8Hz, C-16H), 3.76 (s, 3H, $OC\underline{H}_3$), 5.84 (ddd, 1H, J_1=10Hz, J_2=5.2Hz, J_3=1.8Hz, C-14H), 6.41 (dd, 1H, J_1=10Hz, J_2=2.8Hz, C-15H), 6.7-7.1 (m, 4 aromatic protons); C-13 NMR ($CDCl_3$): δ =173.47 (CO), 148.46 (C-13), 135.3 (C-8), 132.6 (C-15), 128.61 (C-14), 125.9 (C-11), 123.7 (C-9), 122.74 (C-10), 112.71 (C-12), 81.36 (C-2), 74.19 (C-21), 59.98 (C-7), 58.14 (C-3), 52.6 (OCH_3), 48.9 (C-5), 48.46 (C-19), 44.58 (C-20), 38.96 (C-16), 34.66 (C-6), 28.73 (C-17), 7.14 (C-18). M.S: m/z 336 (M^+, 57%), 230 (31%), 229 (28%), 216 (23%), 170 (86%), 135 (94%), 134 (100%), 122 (23%), 121 (24%), 93 (20%), 77 (21%); exact mass measurement: 336.1837 ($C_{21}H_{24}N_2O_2$, requires 336.1837), 230.1155 ($C_{14}H_{16}NO_2$, requires 230.1180), 170.0957 ($C_{12}H_{12}N$, requires 170.0969), 135.1039 ($C_9H_{13}N$, requires 135.1047), 134.0965 ($C_9H_{12}N$, requires 134.0969).

Cleavage of 16-epi-19-S-vindolinine (56) to 19-iodo-tabersonine (54)

The alkaloid (56), (200mg. 0.59 m.mole) was dissolved in THF (20ml) and 10% Na_2CO_3 solution (5ml) was added. A solution of iodine (175mg, 1.09 m.mole) dissolved in THF (20ml) was added dropwise at room temperature with constant stirring. The solution was stirred for one hour, diluted with water and extracted with chloroform. The chloroform layer was successively washed with 10% $Na_2S_2O_3$ solution, dried over anhydrous sodium sulphate and concentrated under vacuum to afford a gummy mass. T.l.c. (80% hexane, 20% EtOAc) showed the formation of a faster running product and some unreacted starting material. This was purified by flash chromatography through a small silica column. Elution with 5% ethyl acetate in pet.ether, afforded the pure compound (54) (70% yield). U.V. (MeOH): λ_{max}228, 298, 330; λ_{min}265, 310 nm; I.R. ($CHCl_3$): 1680 cm^{-1}; NMR ($CDCl_3$):δ=1.63 (d, 3H, J=6.4Hz, -HCl-C$\underline{H}_3$), 3.92 (q, 1H, J=6.4, -C$\underline{H}$I-CH_3); M.S: m/z = 452 (M^+, 7%), 335 (100%), 303 (18%), 275 (5%), 228 (50%), 197 (25%), 168 (50%), 121 (20%). This spectral data was identical with those reported in literature for (54).[76]

Reduction of 19-iodo-tabersonine (54) to vincadifformine (55)

The compound (54) (20mg, 0.043 m.mole) was dissolved in MeOH (2ml) and added to a suspension of Raney Nickel

(30mg) saturated with hydrogen in MeOH (5ml) containing NaOH (10mg). The solution was stirred for 2½ hour at 30°C, filtered, concentrated and fractionated between chloroform (10ml) and water (10ml). The chloroform layer was separated, dried over anhydrous Na_2SO_4 and concentrated to afford a gum which on t.l.c. showed complete conversion to a slower moving substance. The substance was purified by preparative t.l.c. to afford pure vincadifformine (55) (13mg, 86%), m.p. 125°C; optical rotation $(\alpha)_D$=-542° (EtOH); U.V.(MeOH): λ_{max}328, 295, 230nm; λ_{min}305, 265 nm; I.R.($CHCl_3$): 1670 cm^{-1} (ester); N.M.R. ($CDCl_3$): δ=0.60 (t, 3H, J=7.4Hz, CH_2-$\underline{CH_3}$), 3.76 (s, $OC\underline{H_3}$), 7.3-6.7 (m, 4H, aromatic), 8.9 (s, 1H, NH); MS: m/z = 338 (M^+, 20%), 307 (6%), 279 (5%), 214 (5%), 124 (100%); exact mass measurement: 338.1989 ($C_{21}H_{26}N_2O_2$ requires 338.1994).

Reduction of 19-iodo-tabersonine (54) to tabersonine (38)

To 30mg of a Raney Nickel suspension in MeOH (5ml) saturated with hydrogen was added the compound (54) (20mg 0.043 m.mole) dissolved in 2ml of MeOH containing NaOH (10mg). The solution was stirred for 5 min. at 0°C, filtered, concentrated and fractionated between chloroform and water. The chloroform layer was separated, dried (anhydrous Na_2SO_4) and concentrated to afford a gummy product, which on t.l.c. showed complete conversion to a slower moving

material. The substance was purified by preparative, t.l.c. and identified as (38) (14mg, 90%). U.V. (MeOH): λ_{max}328, 298, 230 nm; λ_{min}305, 265 nm; I.R. ($CHCl_3$): 1670 cm^{-1} (ester carbonyl); N.M.R. ($CDCl_3$): δ=0.62 (t, 3H, J=6.8Hz, CH_2-$\underline{CH_3}$), 3.75 (s, $OC\underline{H_3}$), 6.5 - 5.7 (m, 2H, $\underline{HC}$=$C\underline{H}$), 7.3 - 6.7 (m, 4H, aromatic protons), 9.0 (s, 1H, NH); M.S: m/z = 336 (M^+, 30%), 305 (5%), 229 (22%), 214 (10%), 168 (28%), 135 (100%), 122 (35%), 107 (45%); exact mass measurement: 336.1837 ($C_{21}H_{24}N_2O_2$, requires 336.1837).

5.2.2 *ISOLATION OF FLUOROCARPAMINE (57) AND FLUOROCARPAMINE N-OXIDE (58)*

The fraction (F 3) (obtained as shown in Fig.1) (0.6gm) was loaded on a preparative high pressure liquid chromatograph (column diameter 2.2 cm, column length 50 cm. column packing "Lichroprep" 15-25 µm silica, solvent pressure 1000-1200 lb/sq inch, rate of flow 13 ml/minute) and eluted with 50% ethyl acetate in pet.ether (2 litre). The eluates were concentrated and subjected to preparative layer chromatography (E.Merck silica gel, 2mm thick plates) in 75% ethyl acetate - 25% ethanol. Two components A and B with bright yellow fluoroescense were isolated. The faster moving substance A afforded the following spectral data:- U.V.(MeOH): λ_{max} 235, 257, 398 nm; λ_{min} 245, 272, 355nm; I.R. ($CHCl_3$): 1740 cm^{-1} (ester carbonyl) 1685 cm^{-1} (ketonic carbonyl); NMR ($CDCl_3$); δ = 1.63 (d, 3H, J=6.8Hz, =CH-C$\underline{H}_3$), 3.7 (s, 3H, OC$\underline{H}_3$), 4.55 (d, 1H, J=9.3Hz, C-16H), 5.5 (q, 1H, J=6.8Hz, =C$\underline{H}$-CH$_3$), 6.62-7.6 (m, 4H, aromatic protons); M.S: m/z = 338 (M^+, 23%), 279 (11%), 265 (14%), 231 (6%), 193 (6%), 160 (10%), 121 (100%); exact mass measurement: 338.1611 ($C_{20}H_{22}N_2O_3$, requires 338.1630). This spectral data was consistent with that reported for fluorocarpamine (57) in the literature.

The slower moving component B which remained virtually on the base line afforded the following spectral data:- U.V. (MeOH): λ_{max} 235, 257, 398 nm; λ_{min} 245, 272, 355 nm; I.R. ($CDCl_3$): 1740 cm^{-1} (ester carbonyl), 1685 cm^{-1} ketonic carbonyl; M.S: m/z = 354 (M^+, /6%), 338 (26%), 279 (15%), 265 (15%), 231 (11%), 193 (8%), 160 (8%), 126 (55%), 121 (100%); exact mass measurement: 354.1569 ($C_{20}H_{22}N_2O_4$, requires, 354.1579). From its characteristic spectral data the substance was identified as fluorocarpamine N-oxide (58). It was further confirmed by its deoxygenation to fluorocarpamine.

The substance (58) (1mg., .0029 m.mole) was dissolved in chloroform (2ml) and (0.05ml) PCl_3 was added. The mixture was stirred for 15 min. at room temperature and then fractionated between water (5ml) and chloroform (5ml). The chloroform layer was washed with 10% Na_2CO_3 solution, separated and concentrated under vacuum. The reaction product formed was compared with an authentic sample of fluorocarpamine and the two were found to be identical on t.l.c. in 25% ethanol in ethyl acetate as well as on comparison of spectral data (I.R., U.V., NMR, mass). This confirmed the identity of fluorocarpamine N-oxide.

5.2.3 *ISOLATION OF 16-EPI-19-S-VINDOLININE-N-OXIDE (59), VINDOLININE-N-OXIDE (60) AND PLEIOCARPAMINE (61).*

Preparative t.l.c. (silica gel E.Merck) of fraction F_3 (obtained as shown in Fig.1) in pure ethanol allowed the separation of the slower moving band. A second preparative t.l.c. of this band in 50% ethyl acetate-50% ethanol afforded two components A and B. The slower moving component B, had the following spectra:- U.V. (MeOH): λ_{max} 215, 247, 300nm λ_{min} 230, 278nm; I.R. ($CHCl_3$): 1730 cm^{-1} (ester carbonyl); N.M.R. ($CDCl_3$): δ = 0.62 (d, 3H, J = 7.4Hz, CH-CH_3), 1.02 (d, 3H, J=7.4Hz, CH-CH_3), 3.75 (s, 3H, OCH_3) 5.84 (ddd, 1H, J_1=10Hz, J_2=5.4 Hz, J_3=2Hz, C-14 olefinic proton), 6.35 (dd, 1H, J_1=10Hz, J_2=2.8Hz, C-15, olefinic proton), 6.4 ~ 5.8 (m, 4H, aromatic); M.S: m/z = 352 (M^+, 17%), 336 (65%), 230 (31%), 229 (35%), 216 (27%), 170 (96%), 135 (100%), 134 (89%), 122 (32%), 121 (35%), 93 (33%), 77 (39%); exact mass measurement: 352.1786 ($C_{21}H_{24}N_2O_3$, requires 352.1786).

The material was treated with PCl_3 (0.01ml) in chloroform at 30°C for 10 mts. when it was found to be smoothly transformed to vindolinine (51) and 16-epi-19-S-vindolinine (56) which were identified by spectroscopic and chromatographic comparison with authentic samples. The N-oxides isolated were thus identified as vindolinine N-oxide (60) and 16-epi-19-S-vindolinine N-oxide (59).

The substance C isolated from fraction F_3 (2mg), U.V. (MeOH): λ_{max}230, 285nm; λ_{min}250 nm; I.R. ($CHCl_3$): 1730 cm^{-1} (ester carbonyl); M.S.: m/z = 322 (M^+, 87%), 293 (6%), 263 (100%) 234 (51%), 180 (100%), 168 (20%), 154 (26%), 108 (26%); exact mass measurement: 322.1645 (calcd. for $C_{20}H_{22}N_2O_2$, 322.1660). Comparison of the spectral data of the material with those reported for pleiocarpamine (61) established the two to be identical.

5.2.4. *ISOLATION OF TETRAHYDROALSTONINE (62), AJMALICINE (12), VINDOROSINE (63), AND TABERSONINE (38).*

The fraction F_6 (12gms) was loaded on dry flash silica column (120gm) (E.Merck, 70-230 mesh) subsequent elution with 28% ethyl acetate-72% pet.ether, afforded a light brown crystalline substance, m.p. 230°C, which was identified as tetrahydroalstonine on the basis of spectral data recorded. U.V. (MeOH): λ_{max} 226, 283, 290nm; λ_{min} 265, 287 nm; I.R. (KBr): 1698 cm^{-1} (ester carbonyl) 2800-2780 cm^{-1} (Bohlmans bands); N.M.R. ($CDCl_3$): δ =1.38 (d, 3H, J=6Hz, CH-CH_3), 3.73 (s, 3H, OCH_3), 4.48 (m, 1H), 7.5-7.0 (m, 4H, aromatic protons), 7.54 (s, 1H), 8.0 (bs, 1H, indolic NH); M.S: m/z = 352 (M^+, 100%), 351 (90%), 337 (35%), 321 (5%), 293 (8%), 223 (35%), 169 (55%), 156 (100%).

Another crystalline base was obtained in the form of light yellow microneedles, m.p. 258°C, on elution of the same column with pet.ether containing 35% ethyl acetate. This was identified as ajmalicine by spectral and t.l.c. comparison with an authentic sample.

When the same fraction (F_6) was loaded on another dry column of silica (ratio of material loaded: silica, 1:15) and eluted with 80% pet.ether containing 20% ethyl

acetate, it afforded vindorosine which was identifed by comparison of spectral data with those of authentic sample. U,V. (MeOH): λ_{max} 214, 250, 302 nm; λ_{min} 230, 270nm; I.R. ($CHCl_3$): 1745 cm^{-1} (ester carbonyl); N.M.R. ($CDCl_3$): δ =0.45 (t, 3H, J = 6Hz, $-CH_2-\underline{CH_3}$), 2.67 (s, 3H, $-CO-\underline{CH_3}$), 3.76 (s, 3H, $N-\underline{CH_3}$), 3.70(s, 3H, $O\underline{CH_3}$), 5.88-6.33 (m, 2H, olefinic protons), 7.34-6.44 (m, 4H, aromatic protons); M.S: m/z = 426 (M^+, 5%), 367 (3%), 339 (2%), 282 (10%), 266 (30%), 222 (5%), 158 (50%), 135 (100%), 121 (30%).

Fraction F_4 (0.6gm) was loaded on a preparative high pressure liquid chromatograph (column diameter, 2.2cm, column length 50cm, column packing "Lichroprep" 15-25µm silica, solvent pressure 1000-1200 lb/sq inch, rate of flow 13 ml/min.) and eluted with 60% chloroform in pet.ether. The eluates were concentrated to a gummy substance. This was further purified by preparative t.l.c. on silica gel 60 PF-254 plates using 9:1 ethyl acetate - pet.ether to afford a substance which was identified as tabersonine on t.l.c. and spectral comparison with an authentic sample.

5.2.5. *ISOLATION OF VINDOLINE (33) AND CATHARANTHINE (32)*

A flash (dry) column chromatographic procedure developed by us was applied to isolate catharanthine and vindoline. Silica gel (120gm., 70-230 mesh) was packed in a sintered column fitted with a ground glass bottom joint and tap. This column was fitted on a Buchner flask. The substance was loaded on the column and the eluting solvent forced down the column with the help of vacuum applied on the collecting Buchner flask.

The vindoline and catharanthine rich fraction F_6 (obtained as shown in Fig.I) (12gm) was loaded on the column. The column was eluted first with 28% ethyl acetate in pet.ether (2 litre) and then with 35% ethyl acetate in pet.ether (4 litre) which afforded pure catharanthine (0.5 gm). Elution with 45% ethyl acetate in pet. ether (2 litre) removed the other alkaloids. Subsequent elution with 55% ethyl acetate in pet.ether (5 litre) then afforded pure vindoline (2 gms). The identity of catharanthine and vindoline was confirmed by chromatographic and spectroscopic comparisons with authentic samples.[172,173]

5.2.6. ISOLATION OF VINBLASTINE (6)

The vinblastine containing fraction F_2 (10 gms) (Fig.I) was loaded on a flash chromatography column packed with 160 grams of alumina (neutral activity I). The column was eluted with 70% ethyl acetate in pet.ether (6 litre). The eluates were concentrated to a gum (8gms) and again loaded on the same type of column packed with 24 grams of TLC grade silica. Subsequent elution with 0.5% ethanol in ethyl acetate (2 litre) afforded a vinblastine rich fraction (0.5 gm).

The vinblastine containing fraction (0.5 gm) was loaded on a preparative HPLC (column diameter 2.2 cm, column length 50 cm, column packing "Lichroprep" 15-25 µm silica, solvent pressure 1000-1200 16/Sq inch, rate of flow 13ml/minute) and eluted successively with 50% ethyl acetate in pet.ether (2 litre), 60% ethyl acetate in pet.ether (4 litre), 65% ethyl acetate in pet.ether (2 litre). The last elution afforded pure vinblastine (50mg) as the slowest moving material. The identity of vinblastine was confirmed by spectroscopic and chromatographic comparison with an authentic sample.[7]

5.2.7. *OXIDATIVE FRAGMENTATION OF 16-EPI-19-S-VINDOLININE (56) TO (68)*

Lead tetraacetate (0.04gm, 0.09 m.mole) was added to a solution of 16-epi-19-S-vindolinine (0.1 gm., 0.29 m.mole) in benzene (40 ml). The reaction mixture was refluxed for 3 hrs. under a nitrogen atmosphere with constant stirring, the benzene was evaporated under vacuum and the reaction product was fractionated between ethyl acetate (30ml) and water (30ml). The ethyl acetate layer was separated, dried over Na_2SO_4 and concentrated under vacuum to a gummy mass. T.l.c. in chloroform showed the formation of two faster moving spots. The major product was purified by passing through a small silica (70-230 mesh) flash chromatography column. The column was eluted with increasingly polar mixtures of pet.ether and chloroform. The fraction obtained on elution with 40% chloroform - 60% pet. ether (100ml) contained the desired product (68), which was crystallised from ether as a white solid (70mg, 70% yield)m.p. 115°C; U.V.(MeOH): λ_{max}220, 285, 293 nm; λ_{min}255, 290 nm; I.R. ($CHCl_3$): 1655 cm^{-1} (N_b-CHO), 1730 cm^{-1} (ester carbonyl); NMR ($CDCl_3$): δ =1.63 (d, 3H, J=5.6Hz, >C=CH-C$\underline{H}_3$), 3.67 (s, 3H, OC$\underline{H}_3$), 5.46 (q, 1H, J=5.6Hz, >C=C$\underline{H}$-CH_3), 5.7-6.1 (m, 2H, $\underline{H}$C=C$\underline{H}$), 7.6-6.9 (m, 4H, aromatic protons), 8.00 (s, 1H, N_b-C$\underline{H}$O), 8.35 (s, 1H, N$\underline{H}$); M.S: m/z = 352 (M^+, 50%), 320 (60%), 293 (15%), 214 (100%), 169 (95%), 154 (70%), 108 (35%), 77 (34%); exact mass measurement: 352.1772 ($C_{21}H_{24}N_2O_3$ requires 352.1786).

5.3 NEW METHODS FOR THE SYNTHESIS OF β-CARBOLINES

5.3.1 *PREPARATION OF N-[2-(3-INDOLYL) ETHYL]SUCCINIMIDE (100)*

A solution of tryptamine (2.0 gm., 12.5 m.mole) and succinic anhydride (0.95 gm., 12.5 m.mole) in anhydrous toluene was refluxed for 12 hours in a Dean and Starks apparatus. T.l.c. showed conversion of the starting material to a major faster running product. The solution was evaporated under reduced pressure and the crude mixture after crystallisation from ethanol afforded a light brown crystalline product which was further purified by recrystallisation, yield: 2.8gm., (95%); m.p. 166-168°C (lit. 166-167); U.V. (MeOH): λ_{max}225, 274, 281, 290 nm; λ_{min}245, 270, 288 nm; I.R (Nujol); λ_{max}3250 cm^{-1} (indole N-H), 1762 cm^{-1} (C=O), 1692 cm^{-1} (C=O); N.M.R. (d_6-DMSO): δ =6.96-7.66 (m, 4H, aromatic protons), 10.83 (1H, s, indole NH); M.S: m/z = 242 (M^+, 74%), 149 (20%), 143 (66%), 143 (66%), 130 (100%), 85 (40%), 78 (15%), 69 (15%), 58 (64%).

5.3.2. *REDUCTION OF N-SUCCINIMIDOTRYPTAMINE WITH $NaBH_4$ IN PRESENCE OF METAL HALIDES*

$CuCl_2.2H_2O$ (1.7 mg., 0.01 m.mole); $CoCl_2$ (1.2 mg, 0.01 m.mole); $CrCl_3.6H_2O$ (2mg, 0.01 m.mole); $FeCl_3$ (1.6 mg, 0.01 m.mole); $HgCl_2$ (2mg., 0.01 m.mole); $CdCl_2$ (1.8mg., 0.01 m.mole); $SbCl_3$ (2mg., 0.01 m.mole); $NiCl_2.6H_2O$ (2mg, 0.01 m.mole); $MnCl_2.4H_2O$ (1.9mg, 0.01 m.mole); $CeCl_3$ (2mg, 0.01 m.mole); and $BaCl_2$ (2mg, 0.01 m.mole) were dissolved in 13 different tubes each containing absolute ethanol (1 ml). To each tube was added N-succinimidotryptamine (2.4mg, 0.01 m.mole) and the contents of the test tubes were gently warmed to make a homogeneous solution. The resulting solutions were then cooled to ice-salt temperature (0 to $-6^{\circ}C$) and $NaBH_4$ (0.3mg, 0.01 m.mole) added to each test tube, followed by thorough mixing and plugging of the test tubes. A blank reaction was run at the same time with the imide (2.4 mg, 0.01 m.mole) in absolute ethanol (1 ml) and $NaBH_4$ (0.3 mg., 0.01 m.mole). The reactions were followed by t.l.c. (on 20 x 20 cm Merck plates precoated with silica gel PF_{254} in 95% chloroform - 5% methanol). Aliquots were drawn at intervals of 10, 30, 60 and 120 min. respectively. The chromatographic plates were developed with iodine vapours, and the results are

tabulated in Table II. The same set of reactions was repeated at (30°C) and the results are given in Table II.

In a second set of reactions, the above reductions were repeated under identical conditions but with one equivalent each of succinimidotryptamine and metal halides and five equivalent of sodiumborohydride (1.5 mg, 0.05 m. mole). The results are tabulated in Table III.

All the reductions were also repeated at the two temperatures with concentrations of $NaBH_4$ increased to 10 equivalents and the results are tabulated in Table IV.

TABLE-II

REACTION OF N-SUCCINIMIDOTRYPTAMINE WITH ONE EQUIVALENT OF METAL HALIDE AND ONE EQUIVALENT OF $NaBH_4$

METAL HALIDE	TIME IN MIN.	REACTION TEMPERATURE (0°C)					REACTION TEMPERATURE (30°C)				
		a	b	c	d	e	a	b	c	d	e
"A"	10	T	0	0	0	M	5	0	T	0	95
	30	10	"	"	"	90	20	"	10	"	70
	60	15	"	"	"	85	25	"	25	"	50
	120	20	"	"	"	80	30	"	35	"	35
$CuCl_2 2H_2O$	20	5	0	0	0	95	20	0	0	0	80
	30	15	"	"	"	85	30	"	"	"	70
	60	30	"	"	"	70	40	"	"	"	60
	120	40	"	"	"	60	40	"	10	"	50
$CoCl_2$	10	10	0	0	0	90	30	0	0	0	70
	30	15	"	"	"	85	35	"	"	"	65
	60	25	"	"	"	75	40	"	"	"	60
	120	30	"	"	"	70	45	"	T	"	55
$CrCl_2 6H_2O$	10	T	0	0	0	M	5	0	0	0	95
	30	10	"	"	"	90	15	"	"	"	85
	60	20	"	"	"	80	25	"	"	"	75
	120	25	"	"	"	75	30	"	"	"	70
$FeCl_3$	10	T	0	0	0	M	5	0	0	0	95
	30	10	"	"	"	90	15	"	"	"	85
	60	15	"	"	"	85	20	"	"	"	80
	120	25	"	"	"	75	30	"	"	"	70

Contd.

$HgCl_2$	10	T	0	0	0	M	T	0	0	0	M
	30	T	"	"	"	M	15	"	"	"	85
	60	20	"	"	"	80	20	"	"	"	80
	120	20	"	"	"	80	25	"	"	"	75
$SnCl_2$	10	10	0	0	0	90	5	0	5	0	90
	30	25	"	"	"	75	20	"	20	"	60
	60	50	"	"	"	50	15	"	40	"	45
	120	60	"	T	"	40	10	"	60	"	30
$SrCl_2$	10	20	0	0	0	80	10	0	5	0	85
	30	30	"	"	"	70	20	"	10	"	70
	60	50	"	"	"	50	20	"	45	"	35
	120	60	"	5	"	35	10	"	60	"	30
$CdCl_2$	10	0	0	0	0	M	0	0	0	0	M
	30	"	"	"	"	M	"	"	"	"	M
	60	"	"	"	"	M	"	"	"	"	M
	120	10	"	"	"	90	15	"	T	"	85
$SbCl_3$	10	T	0	0	0	M	5	0	T	0	95
	30	10	"	"	"	90	20	"	10	"	70
	60	20	"	"	"	80	25	"	25	"	50
	120	25	"	"	"	75	30	"	35	"	35
$NiCl_2 6H_2O$	10	T	0	0	0	M	T	0	0	0	M
	30	5	"	"	"	95	T	"	"	"	M
	60	15	"	"	"	85	20	"	"	"	80
	120	20	"	"	"	80	30	"	"	"	70

Contd.

$MnCl_2 4H_2O$	10	T	0	0	0	M	10	0	0	0	90
	30	10	"	"	"	90	20	"	"	"	80
	60	15	"	"	"	85	25	"	"	"	75
	120	25	"	"	"	75	40	"	"	"	60
$CeCl_3$	10	5	0	0	0	95	5	0	0	0	95
	30	10	"	"	"	90	10	"	"	"	90
	60	15	"	"	"	85	20	"	"	"	80
	120	20	"	"	"	80	25	"	T	"	75
$BaCl_2$	10	T	0	0	0	M	5	0	T	0	95
	30	10	"	"	"	90	15	"	10	"	75
	60	15	"	"	"	85	20	"	30	"	50
	120	20	"	"	"	80	30	"	35	"	35

TABLE-III

REACTION OF N-SUCCINIMIDOTRYPTAMINE WITH ONE EQUIVALENT OF METAL HALIDE AND FIVE EQUIVALENT OF $NaBH_4$

METAL HALIDE	TIME IN MIN.	REACTION TEMPERATURE (0^oC)					REACTION TEMPERATURE (30^oC)				
		a	b	c	d	e	a	b	c	d	e
"A"	10	T	0	0	0	M	10	0	0	0	90
	30	10	"	"	"	90	25	"	5	"	70
	60	40	"	"	"	60	60	"	20	"	20
	120	60	"	"	"	40	40	"	50	"	10
$CuCl_2 2H_2O$	10	25	0	0	0	75	60	0	0	0	40
	30	55	"	"	"	45	80	"	"	"	20
	60	80	"	"	"	20	M	"	T	"	T
	120	M	"	"	"	T	60	"	40	"	T
$CoCl_2$	10	T	0	0	0	M	60	0	20	0	20
	30	20	"	"	"	80	65	"	20	"	15
	60	60	"	T	"	40		"	40	"	0
	120	95	"	T	"	5	30	"	70	"	"
$CrCl_3 6H_2O$	10	40	0	0	0	60	90	0	10	0	0
	30	60	"	"	"	40	60	"	40	"	"
	60	85	"	"	"	15	50	"	50	"	"
	120	M	"	"	"	T	30	"	70	"	"
$FeCl_3$	10	40	0	0	0	60	85	0	5	0	10
	30	60	"	"	"	40	60	"	35	"	5
	60	90	"	"	"	10	55	"	45	"	0
	120	M	"	"	"	T	40	"	60	"	"

Contd.

$HgCl_2$	10	25	0	0	0	75	80	0	10	0	10
	30	50	"	"	"	50	60	"	40	0	0
	60	60	"	"	"	40	20	"	80	"	"
	120	60	"	"	"	40	T	"	M	"	"
$SnCl_2$	10	50	0	0	0	50	60	0	10	0	30
	30	80	"	"	"	20	60	"	20	"	20
	60	100	"	"	"	T	50	"	40	"	10
	120	90	"	10	"	T	25	"	70	"	5
$SrCl_2$	10	40	0	0	0	60	20	0	20	0	60
	30	70	"	"	"	30	30	"	20	"	50
	60	80	"	"	"	20	40	"	40	"	20
	120	85	"	5	"	10	10	"	50	"	10
$CdCl_2$	10	T	0	0	0	M	T	0	0	0	M
	30	10	"	"	"	90	10	"	"	"	90
	60	30	"	T	"	70	30	"	"	"	70
	120	40	"	T	"	60	40	"	T	"	60
$SbCl_3$	10	5	0	0	0	95	20	0	0	0	80
	30	20	"	"	"	80	30	"	5	"	65
	60	45	"	"	"	55	45	"	25	"	30
	120	60	"	"	"	40	40	"	50	"	10
$NiCl_2 6H_2O$	10	30	0	0	0	70	60	0	20	0	20
	30	60	"	"	"	40	65	"	20	"	15
	60	80	"	"	"	20	65	"	35	"	T
	120	90	"	T	"	10	30	"	70	"	T

Contd.

$MnCl_2 4H_2O$	10	45	0	0	0	55	40	0	0	0	60
	30	80	"	"	"	20	70	"	T	"	30
	60	95	"	"	"	5	80	"	T	"	20
	120	M	"	"	"	T	85	"	5	"	10
$CeCl_3$	10	40	0	0	0	60	20		20		60
	30	50	"	"	"	50	30		20		50
	60	70	"	"	"	30	40		35		25
	120	90	T	T	"	10	40		50		10
$BaCl_2$	10	T	0	0	0	M	10	0	0	0	90
	30	5	"	"	"	M	25	"	5	"	70
	60	10	"	"	"	90	60	"	20	"	20
	120	30	"	"	"	70	50	"	40	"	10

TABLE-IV

REACTION OF N-SUCCINIMIDOTRYPTAMINE WITH ONE EQUIVALENT OF METAL HALIDE AND TEN EQUIVALENTS OF $NaBH_4$

METAL HALIDE	TIME IN MIN.	REACTION TEMPERATURE (0^oC)					REACTION TEMPERATURE (30^oC)				
		a	b	c	d	e	a	b	c	d	e
"A"	10	20	O	O	O	80	40	O	30	O	30
	30	35	"	"	"	65	25	"	65	"	10
	60	40	"	"	"	60	T	"	M	"	O
	120	60	"	"	"	40	T	"	M	"	O
$CuCl_2 2H_2O$	10	40	O	O	O	60	70	O	20	O	10
	30	85	"	"	"	15	40	"	60	"	O
	60	95	"	5	"	O	35	"	65	"	O
	120	90	"	10	"	O	30	"	70	"	O
$CoCl_2$	10	30	O	O	O	70	50	O	30	O	20
	30	80	"	"	"	20	40	"	45	"	15
	60	90	"	10	"	O	35	"	65	"	O
	120	80	"	20	"	O	30	"	70	"	"
$CrCl_3 6H_2O$	10	35	O	O	O	65	90	O	10	O	O
	30	85	"	"	"	15	60	"	40	"	"
	60	95	"	5	"	O	50	"	50	"	"
	120	90	"	10	"	O	10	"	90	"	"
$FeCl_3$	10	30	O	O	O	70	85	O	5	O	10
	30	75	"	"	"	25	60	"	35	"	5
	60	90	"	10	"	O	55	"	45	"	O
	120	80	"	20	"	"	20	"	80	"	"

Contd.

$HgCl_2$	10	70	O	O	O	30	80	O	10	O	10
	30	90	"	"	"	10	60	"	40	"	O
	60	M	"	"	"	T	20	"	80	"	"
	120	M	"	"	"	O	T	"	M	"	"
$SnCl_2$	10	60	O	O	O	40	60	O	10	O	30
	30	90	"	"	"	10	60	"	20	"	20
	60	M	"	T	"	O	50	"	40	"	10
	120	90	"	10	"	T	40	"	60	"	O
$SrCl_2$	10	55	O	O	O	45	40	O	20	O	40
	30	60	"	"	"	40	50	"	40	"	10
	60	85	"	5	"	5	T	"	M	"	O
	120	90	"	10	"	O	T	"	M	"	"
$CdCl_2$	10	10	O	O	O	90	60	O	10	O	30
	30	15	"	"	"	85	45	"	30	"	25
	60	15	"	T	"	85	40	"	40	"	20
	120	40	"	T	"	60	35	"	50	"	15
$SbCl_3$	10	60	O	O	O	40	50	O	45	O	5
	30	90	"	"	"	10	20	"	80	"	O
	60	M	"	T	"	O	O	"	M	"	"
	120	M	"	T	"	"	"	"	M	"	"
$NiCl_2 6H_2O$	10	50	O	O	O	50	60	O	20	O	20
	30	80	"	"	"	20	45	T	40	T	15
	60	90	"	T	"	10	40	"	60	"	T
	120	M	"	T	"	O	35	"	65	"	O

Contd.

$MnCl_2 4H_2O$	10	60	O	O	O	40	40	O	O	O	60
	30	80	"	"	"	20	70	"	20	"	10
	60	95	"	"	"	5	65	"	30	"	5
	120	M	"	"	"	T	45	"	50	"	5
$CeCl_3$	10	50	O	O	O	50	50	O	10	O	40
	30	50	"	"	"	50	50	"	30	"	20
	60	70	"	"	"	30	40	"	50	"	10
	120	M	T	T	"	O	30	"	60	"	10
$BaCl_2$	10	20	O	O	O	80	50	O	10	O	40
	30	25	"	"	"	75	30	"	55	T	15
	60	50	"	"	"	50	T	"	M	O	O
	120	80	"	"	"	20	T	"	M	"	"

"A" = Blank reaction

a = Estimated yield of the hydroxy lactam (%)

b = Estimated yield of the β-carboline lactam (%)

c = Estimated yield of the amide alcohol (%)

d = Estimated yield of the amide ester (%)

e = Estimated yield of the unreacted N-Succinimidotryptamine (%)

T = Trace formation of the products

M = Major formation of the products (above 95% estimated yield).

NOTE: *Yields were found to vary with the activity of sodium borohydride.*

5.3.3. PREPARATION OF HYDROXY LACTAM (117)

To a solution of N-succinimidotryptamine (100 mg, 0.413 m.mole) and $MnCl_2.4H_2O$ (80mg., 0.413 m.mole) in absolute ethanol (25ml) was added finely crushed $NaBH_4$ (78 mg., 2 m.mole) at 0-6°C. The reaction was magnetically stirred for 2 hours at 0°C. T.l.c. (95% chloroform - 5% ethanol) at the end of this period showed the conversion of the starting imide to mainly a slower running product and traces of the alcohol (115). Excess of $NaBH_4$ was filtered off and the solvent evaporated under reduced pressure. The reaction mixture was fractionated between chloroform and water. The chloroform layer was separated, dried (anhydrous Na_2SO_4) and concentrated under reduced pressure. Preparative t.l.c. on silica gel PF_{254} coated plates afforded a major new product. The compound was eluted off with ethyl acetate. Filteration and evaporation of the filtrate afforded the new product (117), yield: 80mg (80%); U.V.(MeOH): λ_{max} 224, 274, 292 nm; λ_{min} 247, 279, 289 nm; I.R.(KBr) 1680 cm^{-1} (amide C=O), 3300-3440 (broad, indole NH and OH); N.M.R. ($CDCl_3$): δ =3.7 (t, 1H, J = 7H, CHOH), 5.0 (broad s, 1H, CHOH), 6.7-7.8 (4H, m, aromatic protons), 8.4 (s, 1H, indole NH); M.S: m/z = 244 (M^+, 25%), 266 (7%), 143 (100%), 130 (90%), 115 (7%), 103 (22%), 85 (9%), 77 (30%), 68 (18%).

5.3.4. PREPARATION OF N-[2-(3-INDOLYL) ETHYL] GLUTARIMIDE (98)

Tryptamine (3g., 18.75 m.mole) and glutaric anhydride (4.27g., 37.45 m.mole) were refluxed in anhydrous toluene for 6 hours. T.l.c. then showed complete conversion of tryptamine to a faster running product. The solvent was removed on a rotary evaporator and the product crystallised from ethanol as a white crystalline solid: yield: 3.7gm (78%); m.p. 172-173°C; U.V.(MeOH): λ_{max} 221, 273, 283, 290 nm; λ_{min} 245, 276, 288 nm; I.R.(KBr): 3302 cm^{-1} (indole NH); 1722 cm^{-1} (C=O), 1662 cm^{-1} (C=O); N.M.R. δ =6.9-7 (m, 4H, aromatic protons), 10.8 (s, 1H, indole NH); M.S: m/z = 256 (M^+, 90%), 242, (6%), 144 (49%), 143 (100%), 130 (99%), 117 (22%), 103 (83%), 95 (96%), 84 (26%), 76 (27%), 63 (26%), 55 (81%).

5.3.5. *SODIUM BOROHYDRIDE REDUCTION OF N-GLUTARIMIDOTRYPTAMINE IN PRESENCE OF METAL HALIDES.*

$CuCl_2.2H_2O$ (1.7 mg., 0.01 m.mole); $CoCl_2$ (1.2 mg., 0.01 m.mole); $CrCl_3.6H_2O$ (2mg., 0.01 m.mole); $FeCl_3$ (1.6 mg., 0.01 m.mole); $HgCl_2$ (2 mg., 0.01 m.mole); $SnCl_2$ (1.8 mg., 0.01 m.mole); $SrCl_2$ (1.2mg., 0.01 m.mole); $CdCl_2$ (1.8mg., 0.01 m.mole); $SbCl_3$ (2 mg., 0.01 m.mole); $NiCl_2.6H_2O$ (2mg., 0.01 m.mole); $MnCl_2.4H_2O$ (1.9 mg., 0.01 m.mole); $CeCl_3$ (2mg., 0.01 m.mole) and $BaCl_2$ (2mg., 0.01 m.mole), were taken in different tubes and each dissolved in anhydrous alcohol (0.5 ml). To each test tube was added N-glutarimidotryptamine (2mg., 0.01m.mole). The resulting solutions were cooled to $0^{\circ}C$. To each tube was added $NaBH_4$ (0.3 mg., 0.01 m.mole) at the same temperature. A blank reaction was simultaneously run without the metal halide. The reactions were followed by t.l.c. and aliquots drawn after 10, 30, 60 and 120 min. The plates were developed with iodine vapours. The reactions were repeated at room temperature (28-30$^{\circ}C$). The observations are recorded in Table V.

In the next sets of experiments the concentration of $NaBH_4$ was increased to 5 equivalents and the reductions performed as above were carried out at 0 to $-6^{\circ}C$ and at $30^{\circ}C$. The observations are tabulated in Table VI.

TABLE-V

REACTION OF N-GLUTARIMIDOTRYPTAMINE WITH ONE EQUIVALENT OF METAL HALIDE AND ONE EQUIVALENT OF $NaBH_4$

METAL HALIDE	TIME IN MIN.	REACTION TEMPERATURE (0°C)					REACTION TEMPERATURE (30°C)				
		a	b	c	d	e	a	b	c	d	e
"A"	10	0	0	0	0	M	10	0	0	0	90
	30	"	"	"	"	M	15	"	T	"	85
	60	T	"	"	"	M	20	"	T	"	80
	120	20	"	"	"	80	20	"	T	T	80
$CuCl_2 2H_2O$	10	0	0	0	0	M	T	0	0	0	M
	30	"	"	"	"	M	15	"	"	"	85
	60	T	"	"	"	M	20	"	"	"	80
	120	20	"	"	"	80	25	"	"	"	75
$CoCl_2$	10	0	0	0	0	M	T	0	0	0	M
	30	"	"	"	"	M	10	"	"	"	90
	60	T	"	"	"	M	15	"	"	"	85
	120	50	"	T	"	50	20	"	"	"	80
$CrCl_3 6H_2O$	10	T	0	0	0	M	10	0	0	0	90
	30	5	"	"	"	95	10	"	"	"	90
	60	10	"	"	"	90	15	"	"	"	85
	120	40	"	T	"	60	20	"	5	"	75
$FeCl_3$	10	5	0	0	0	95	T	0	0	0	M
	30	20	"	"	"	80	10	"	"	"	90
	60	30	"	T	"	70	20	"	"	"	80
	120	50	"	5	"	45	20	"	"	"	80

Contd.

$SnCl_2$	10	T	0	0	0	M	5	0	0	0
	30	10	"	"	"	90	10	"	"	"
	60	30	"	T	"	70	15	"	"	"
	120	60	"	5	"	35	20	"	"	"
$SrCl_2$	10	10	0	0	0	90	10	0	0	0
	30	10	"	"	"	90	10	"	"	T
	60	30	"	T	"	70	10	"	"	10
	120	65	"	T	"	35	10	"	"	15
$CdCl_2$	10	0	0	0	0	M	T	0	0	0
	30	"	"	"	"	M	T	"	"	"
	60	"	"	"	"	M	T	"	"	"
	120	T	"	"	"	M	T	"	T	T
$SbCl_3$	10	T	0	0	0	M	5	0	0	0
	30	10	"	"	"	90	10	"	"	"
	60	20	"	"	"	80	10	"	T	"
	120	40	"	"	"	60	20	"	T	"
$NiCl_2 6H_2O$	10	T	0	0	0	M	5	0	0	0
	30	10	"	"	"	90	5	"	"	"
	60	25	"	"	"	75	10	"	"	"
	120	35	"	T	"	65	20	"	"	"
$MnCl_2 4H_2O$	10	0	0	0	0	M	10	0	0	0
	30	"	"	"	"	M	15	"	"	"
	60	T	"	"	"	M	20	"	"	"
	120	20	"	"	"	80	25	"	T	T

cd.

$CeCl_3$	10	0	0	0	0	M	10	0	0	0
	30	"	"	"	"	M	15	"	"	"
	60	T	"	"	"	M	20	"	"	T
	120	20	"	"	"	80	20	"	"	T
$BaCl_2$	10	0	0	0	0	M	10	0	0	0
	30	"	"	"	"	M	15	"	"	"
	60	T	"	"	"	M	20	"	T	T
	120	20	"	"	"	80	20	"	T	T

TABLE-VI

REACTION OF N-GLUTARIMIDOTRYPTAMINE WITH ONE EQUIVALENT OF METAL HALIDE AND FIVE EQUIVALENT OF $NaBH_4$

METAL HALIDE	TIME IN MIN.	REACTION TEMPERATURE (0°C)					REACTION TEMPERATURE (30°C)				
		a	b	c	d	e	a	b	c	d	e
"A"	10	5	0	0	0	95	10	0	T	T	90
	30	30	"	"	"	70	25	"	25	20	30
	60	45	"	5	"	50	10	"	45	20	25
	120	45	"	5	"	50	T	"	60	20	20
$CuCl_2 2H_2O$	10	T	0	0	0	M	10	0	T	0	90
	30	60	"	"	"	40	25	"	T	"	75
	60	60	"	"	"	40	35	"	10	"	55
	120	80	"	"	"	20	55	"	30	"	15
$CoCl_2$	10	5	0	0	0	95	40	0	T	0	60
	30	50	"	"	"	50	40	"	20	"	40
	60	50	"	"	"	50	35	"	40	5	20
	120	60	"	10	"	30	30	"	50	5	15
$CrCl_3 6H_2O$	10	20	0	0	0	80	55	0	5	0	40
	30	60	"	T	"	40	60	"	10	"	30
	60	80	"	T	"	20	55	"	15	"	30
	120	85	"	T	"	15	45	"	45	"	10
$FeCl_3$	10	40	0	T	0	60	55	0	5	0	40
	30	70	"	T	"	30	20	"	60	T	20
	60	75	"	T	"	25	15	"	85	T	T
	120	80	"	10	"	10	0	"	M	T	0

Contd.

$HgCl_2$	10	50	0	0	0	50	75	0	5	0	20
	30	70	"	"	"	30	50	"	50	"	T
	60	70	"	"	"	30	10	"	90	"	T
	120	80	"	"	"	20	10	"	90	"	T
$SnCl_2$	10	30	0	0	0	70	60	0	T	0	40
	30	70	"	"	"	30	50	"	40	"	10
	60	70	"	T	"	30	30	"	60	T	10
	120	75	"	10	"	15	20	"	70	T	10
$SrCl_2$	10	50	0	0	0	50	50	0	25	0	25
	30	70	"	"	"	30	10	"	60	10	20
	60	70	"	"	"	30	5	"	60	20	15
	120	75	"	"	"	25	T	"	70	30	0
$CdCl_2$	10	5	0	0	0	95	T	0	0	0	M
	30	5	"	"	"	95	T	"	"	"	M
	60	10	"	"	"	90	10	"	T	T	90
	120	10	"	5	"	85	15	"	T	5	80
$SbCl_3$	10	20	0	0	0	80	60	0	T	0	40
	30	20	"	"	"	80	40	"	35	T	25
	60	50	"	T	"	50	10	"	70	T	20
	120	70	"	5	"	25	T	"	70	10	20
$NiCl_2 6H_2O$	10	60	0	0	0	40	60	0	[illegible]	[illegible]	40
	30	60	"	T	"	40	30	"	[illegible]	[illegible]	[illegible]
	60	70	"	T	"	30	0	"	60	20	[illegible]
	120	70	"	5	"	25	"	"	[illegible]	30	30

$MnCl_2 \cdot 4H_2O$	10	45	c	0	0	35	50	c	0	0	50
	30	70	"	"	"	30	55	"	"	"	30
	60	70	"	"	"	30	30	"	"	10	20
	120	75	"	15	"	10	80	"	"	15	10
$CoCl_2$	10	60	c	0	0	30	50	"	10		50
	30	70	"	"	"	40	40	"	35	5	30
	60	80	"	5	"	20	5	"	80	5	7
	120	80	"	5	"	20	0	"	90	"	6
$NaCl_2$	10	5	0	0	0	M	15	0	5	5	95
	30	25	"	"	"	75	25	"	25	20	30
	60	45	"	5	"	50	10	"	55	20	15
	120	45	"	5	"	50	5	"	70	20	10

"A" = Blank reaction

a = Estimated yield of the hydroxy lactam (%)

b = Estimated yield of the 2-carboline lactam (%)

c = Estimated yield of the amide alcohol (%)

d = Estimated yield of the amide ester (%)

e = Estimated yield of the unreacted glutarimidoxytyptophane (%)

T = Trace formation of the products

M = Major formation of the products (above 95% estimate yield).

NOTE: Yields were based on the yield of the mixture of the two isomers.

5.3.6. *PREPARATION OF HYDROXY LACTAM [116]*

Finely powdered $NaBH_4$ (73.8mg., 1.98 m.mole) was added to a magnetically stirred solution of $CrCl_3.6H_2O$ (104 mg., 0.39 m.mole) and glutarimidotryptamine (100mg., 0.39 m.mole) in anhydrous ethanol (20ml) at 0^oC to -6^oC. T.l.c. showed the conversion of the starting imide to a major and some minor products after 2.5 hours. Excess of $NaBH_4$ was filtered off and the solvent evaporated under reduced pressure. The new major product was isolated by preparative t.l.c. as a white crystalline porduct; 75mg. (75% yield), U.V.(MeOH): λ_{max} 222, 275, 282, 291 nm; λ_{min} 246, 278, 288 nm; I.R.(KBr): 3280-3420 cm^{-1} (broad, indolic NH and OH), 1628 cm^{-1} (C=O, amide); N.M.R.($CDCl_3$): δ =3.08 (t, 2H, J=7Hz, $C\underline{H}_2CH_2N$), 3.75 (t, 2H,J=10Hz, $CH_2-C\underline{H}_2-N$), 4.74 (broad s, 1H,CHO$\underline{H}$), 6.9-7.8 (m, 4H, aromatic protons), 8.2 (1H, s, NH indole); M.S: m/z = 258 = (M^+, 48%), 238 (22%), 183 (14%), 167 (10%), 157 (16%), 142 (80%), 141 (100%), 117 (24%), 103 (36%), 83 (82%), 77 (12%), 58 (9%).

5.3.7. *PREPARATION OF N-[2-(3-INDOLYL) ETHYL] PHTHALIMIDE (104)*

Tryptamine (5.20g., 32.5 m.mole) and phthalic anhydride (9.62g., 60 m.mole) were heated in refluxing dry toluene (150 ml) for 12 hours in a Dean and Starks apparatus. The toluene was evaporated under vacuum and the crystallization of the crude mixture in hot ethanol gave a fine white crystalline product (104) (8.9gm., 95% yield), m.p. 164-166°C. (Lit.164°C); U.V.(MeOH): λ_{max}225, 282, 290nm, $_{min}$245, 288 nm; I.R.(KBr): 1712 cm^{-1} (C=O), 1762 cm^{-1} (C=O), 3345 cm^{-1} (NH); N.M.R. (d_6-DMSO): =6.96-8.03 (8 aromatic protons), 10.81 (indole N-H); M.S: m/z = 290 (M^+, 67%), 185 (8%), 160 (12%), 149 (40%), 130 (87%), 105 (30%), 104 (100%), 85 (50%), 76 (82%), 59 (65%).

5.3.8. *SODIUM BOROHYDRIDE REDUCTION OF N-PHTHALIMIDOTRYPTAMINE IN THE PRESENCE OF METAL HALIDES*

$CuCl_2.2H_2O$ (1.7 mg., 0.01 m.mole), $CoCl_2$ (1.3 mg., 0.01 m.mole), $CrCl_3.6H_2O$ (2.6mg., 0.01 m.mole), $HgCl_2$ (2.7mg., 0.01 m.mole) $SnCl_2$ (1.9 mg., 0.01 m.mole), $SrCl_2$ (1.6mg., 0.01 m.mole), $CdCl_2$ (1.8mg., 0.01 m.mole), $SbCl_3$ (2.3mg., 0.01 m.mole), $NiCl_2.6H_2O$ (2.4mg., 0.01 m.mole), $MnCl_2.4H_2O$ (2mg., 0.01 m.mole), $CeCl_3$ (2.5mg., 0.01 m.mole) and $BaCl_2$ (2mg., 0.01 m.mole), were taken in different flasks and each dissolved in anhydrous ethanol (0.5 ml). To each flask was added N-phthalimidotryptamine (2.9mg., 0.01 m.mole). The resulting solutions were cooled to $-5^{o}C$. To each tube was added $NaBH_4$ (1.9mg., 0.05 m.mole). A blank reaction was monitored by t.l.c. for comparison purposes. Aliquots drawn after 10, 30, 60 and 120 min. The plates were developed with iodine. The same set of reactions was repeated at room temperature ($28-30^{o}C$). The observations are recorded in Table VII.

TABLE-VII

REACTION OF N-PHTHALIMIDOTRYPTAMINE WITH ONE EQUIVALENT OF METAL HALIDE AND FIVE EQUIVALENT OF $NaBH_4$

METAL HALIDE	TIME IN MIN.	REACTION TEMPERATURE (0^oC)				REACTION TEMPERATURE (30^oC)			
		a	b	c	d	a	b	c	d
"A"	10	80	O	O	20	O	O	60	40
	30	80	"	"	20	"	"	M	T
	60	90	"	"	10	"	"	M	O
	120	M	"	T	T	"	"	100	"
$CuCl_2 2H_2O$	10	40	60	O	O	O	O	100	O
	30	40	60	"	"	"	"	100	"
	60	40	60	"	"	"	"	100	"
	120	40	60	"	"	"	"	100	"
$CoCl_2$	10	40	40	O	20	O	O	80	20
	30	50	40	"	10	"	"	M	T
	60	70	20	10	O	"	"	100	O
	120	80	10	10	"	"	"	100	"
$CrCl_3$	10	100	O	O	T	O	O	100	O
	30	100	"	"	O	"	"	100	"
	60	M	T	"	"	"	"	100	"
	120	M	T	T	"	"	"	100	"
$FeCl_3$	10	40	60	O	O	O	O	80	20
	30	40	60	T	"	"	"	100	O
	60	40	60	T	"	"	"	100	"
	120	40	60	T	"	"	"	100	"

Contd.

$HgCl_2$	10	M	O	O	T	O	O	100	O
	30	M	"	"	O	"	"	100	"
	60	100	"	"	"	"	"	100	"
	120	90	"	10	"	"	"	100	"
$SnCl_2$	10	M	O	O	T	O	O	100	O
	30	M	"	"	T	"	"	100	"
	60	M	"	T	O	"	"	100	"
	120	90	"	10	"	"	"	100	"
$SrCl_2$	10	80	O	O	20	O	O	80	20
	30	90	"	"	10	"	"	100	O
	60	M	"	T	T	"	"	100	"
	120	90	"	10	O	"	"	100	"
$CdCl_2$	10	70	O	O	30	O	O	40	60
	30	80	"	"	20	"	"	60	40
	60	85	"	"	15	"	"	80	20
	120	90	"	"	10	"	"	100	O
$SbCl_3$	10	M	O	O	T	O	O	100	O
	30	M	"	T	O	"	"	100	"
	60	95	"	5	"	"	"	100	"
	120	95	"	5	"	"	"	100	"
$NiCl_2 6H_2O$	10	40	60	T	O	O	O	90	10
	30	40	60	O	"	5	"	95	O
	60	40	50	10	"	O	"	O	"
	120	40	50	10	"	"	"	"	"

Contd.

$MnCl_2 4H_2O$	10	100	O	T	O	100	O	O	O
	30	100	"	T	"	100	"	"	"
	60	100	"	T	"	100	"	"	"
	120	95	"	5	"	100	"	"	"
$CeCl_3$	10	100	O	O	O	O	O	100	O
	30	100	"	T	"	"	"	"	"
	60	95	"	5	"	"	"	"	"
	120	95	"	5	"	"	"	"	"
$BaCl_2$	10	80	O	O	20	O	O	60	40
	30	80	"	"	20	"	"	M	T
	60	90	"	T	10	"	"	100	O
	120	M	"	T	T	"	"	100	"

"A" = Blank reaction

a = Estimated yield of the hydroxy lactam (%)

b = Estimated yield of the alcohol (%)

c = Estimated yield of the amide alcohol (%)

d = Estimated yield of the unreacted N-Phthalimidotryptamine (%)

T = Trace formation of the products

M = Major formation of the products (above 95% estimated yield)

NOTE: *Yields were found to vary with the activity of sodium borohydride.*

5.3.9. PREPARATION OF ALCOHOL (126)

To a solution of N-phthalimidotryptamine (116mg., 0.40 m.mole) and $NiCl_2.6H_2O$ (96.0mg., 0.40 m.mole) in absolute ethanol (25ml) was added finely crushed $NaBH_4$ (78mg., 2 m.mole) at 0^oC. The reaction was magnetically stirred at 0^oC. T.l.c. after 7 hours showed the formation of a slower running product and traces of unreacted N-phthalimidotryptamine. Excess $NaBH_4$ was filtered and the solvent evaporated under reduced pressure. The new product was isolated by preparative t.l.c. U.V.(MeOH): λ_{max} 225, 282, 292 nm; λ_{min} 245, 288nm; I.R.($CHCl_3$): 1670 cm^{-1} (C=O), 3350 - 3200 cm^{-1} (broad, OH, NH); N.M.R. (d_6-DMSO): δ =6.8-7.8 (8 aromatic protons), 10.89 (s, indole NH); M.S: m/z = 290 (M^+, 50%), 185 (5%), 160 (10%), 143 (20%), 104 (100%), 83 (80%), 74 (28%), 69 (12%), 59 (65%).

5.4 STRUCTURE ELUCIDATION OF ALKALOIDS FROM THE SEEDS OF *RHAZYA STRICTA*.

5.4.1. *ISOLATION OF QUEBRACHAMINE (127), RHAZIDIGENINE N-OXIDE (128) AND AKUAMMIDINE (129)*

Powdered *Rhazya stricta* seeds (1 Kg) were extracted with ethanol (6 lit.) in a soxhlet extractor for 12 hours. The dark brown extract was concentrated under vacuum on a rotary evaporator to a gum. The crude extract was acidified and the acidic solution was successively washed with petroleum ether (2 lit.) to remove the fatty substances. The defatted acidic solution was basified and extracted with chloroform (1.5 lit.). The chloroform extracts were dried and evaporated to afford the crude alkaloidal mixture (6gm.).

Column chromatography of the mixture was carried out on neutral alumina (activity II - III, 240gm). Elution with a 3:1 benzene: chloroform mixture afforded a fraction containing two alkaloids, from which one was crystallised with petroleum ether (60-80^{o}C) and identified as quebrachamine (127) m.p. 141-142^{o}C; U.V. (MeOH): λ_{max}230, 293 nm; λ_{min}257 nm; I.R.(KBr): 3360 cm^{-1} (N-H stretching);

N.M.R.($CDCl_3$): δ =0.82 (t, 3H, J=6.2Hz, CH_2-$C\underline{H}_3$), 7.0-7.57 (m, 4H, aromatic proton) 7.6 (br, s, 1H, NH); M.S: m/z = 282 (M^+, 62%), 253 (18%), 157 (45%), 143 (60%), 125 (82%), 110 (100%), 96 (42%); exact mass measurement: 282.2095 ($C_{19}H_{26}N_2$) required 282.2095).

The second alkaloid was isolated from the mother liquors of the quebrachamine containing fractions and was identified as rhazidigenine N-oxide. m.p. 20[illegible]10°C; I.R.(KBr): 3400-3160 cm^{-1} (-OH), 1610 cm^{-1}, 670 cm^{-1}, U.V.(MeOH): λ_{max}214, 236, 293, nm, λ_{min}264 nm; N.M.R. ($CDCl_3$): δ =0.87 (t, 3H, J=6.2Hz, CH_2-$C\underline{H}_3$), 1.25 (q, 2H, J=6.2Hz, -$C\underline{H}$-CH_3), 1.6 (brs, 1H, OH, O$\underline{H}$); M.S: m/z = 314 (M^+, 8%), 297 (40%), 281 (100%), 241 (5%), 210 (4%), 172 (20%), 124 (25%), 96 (20%); exact mass measurement: 314.1990 ($C_{19}H_{26}N_2O_2$ requires, 314.1994).

Akuammidine was obtained from the later eluates of the same column with the same eluting solvent (3:1 benzene:chloroform) m.p. 240-245°C (decomposes); I.R.(KBr): 1710 cm^{-1} (ester carbonyl), 3340 cm^{-1} (-OH); U.V.(MeOH): λ_{max}227, 283, 292 nm; λ_{min}252 nm; N.M.R. ($CDCl_3$): δ =1.62 (d, 3H, J=2.5 Hz, $C\underline{H}$-$C\underline{H}_3$), 5.47 (q, 1H, J = 2.5Hz, C=$C\underline{H}$-CH_3), 2.95 (s, 3H, O$C\underline{H}_3$), 6.97-7.55 (m, 4H, aromatic protons); M.S: m/z = 352 (M^+, 58%), 321 (40%), 293 (15%), 249 (70%), 169 (100%), 115 (30%), 77 (30%); exact mass measurement: 352.1779 ($C_{21}H_{24}N_2O_3$, requires 352.1786).

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7.0 PUBLICATIONS

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