CHAPTER 1

REVIEW OF LITERATURE

INFORMATION ABOUT NOMENCLATURE OF *Rauvolfia serpentina*

The dried root of *Rauvolfia serpentina* Benth. ex Kurz, commonly known as serpentina root, in Sanskrit as Sarpandha and in Hindi Chandrabhaga is one of the most important drugs used in modern medicine.

The genus *Rauwolfia* was named in honour of a sixteenth century traveller and botanist Leenard Rauwolf; serpentina refers to long tapering snake like roots. There have been various orthographies of the genus. It was Plumier who in 1703, named the genus *Rauvolfia.* However, Burmann in subsequent years revised the Plumier's work in 1760 and changed the spelling of the genus to *Rauwolfia* and that form prevailed untill very recent times. The original spelling *Rauvolfia* was ultimately restored following the provisions of Article 82 of the Botanical Nomenclature (1952) which conserves original spelling. (Wealth of India, 1969; Datta *et al.*, 1963).

MEDICINAL AND PHARMACEUTICAL USE OF *Rauvolfia serpentina*

R. serpentina is among the most important medicinal plants native to India. The roots of the plant have been used in the indigenous system of medicine from ancient times. The importance of the root drug and the alkaloids obtained from it has been recognized in the allopathic system in the treatment of hypertension or as a sedative and tranquillizing agent (Akram *et al.*, 1993; Roy *et al.*, 1996; Roja and Heble, 1996). A large number of alkaloids have been isolated

from the roots of this plant. The important among these are ajmalicine, ajmaline, ajmalinine, rescinamine, reserpine, reserpinine, serpentine, serpentinene and yohimbine. Detailed studies have been carried out on the chemistry of these alkaloids, their pharmacodynamics and their varying roles in essential hypertension and neuropsychiatric conditions. The findings especially those relating to the therapeutic action of reserpine attracted world wide attention and large quantities of roots were exported to USA and countries in Western Europe. As almost whole of the material comes from wild sources, the supplies declined sharply by 1952. In 1955, Government of India put a ban on the export of the raw drug and attempts to cultivate the plant were taken up at a number of places. A reassessment of the resources both from wild and cultivated sources has, therefore, been done and prospects for augmenting the supplies, have been examined in detail (Biswas, 1956; Chandra, 1956; Mukharjee, 1959, Rajgopalan, 1959). Rath *et al.* (1999) observed that the plant has got some effect similar to that of attenonol in allopathic medicine. According to Pravathi Devi (2000) the usage of the plant is safe without side effect like hormones and keeps the woman as feminine forever.

ECOLOGICAL ADAPTATION OF *R. serpentina* IN INDIA.

R. serpentina grows wild under a wide range of climatic conditions. It, however, prefers a tropical or sub-tropical belt having the benefit of monsoon rains averaging between 250 cm and 500 cm and the annual temperature ranging between 10°C and 38°C. The major soil types under the natural growth of the plant are sandy alluvial loam, red lateritic loam and in some places the stiff dark loam. A large percentage of humus, ensuring uniform moisture level is always associated with a good growth. The soils in most of the places are acidic having a pH ranging between 4 and 5.

In the sub-Himalayan zone, the areas lying to the west of Sirmor district are poor in the natural occurrence of the plant. In the central region, i.e., between Sirmor and Gorakhpur district of Uttar Pradesh, the plant is frequently noticed in shady moist or sometimes swampy localities. In the eastward localities in Bihar, north Bengal and Assam as well as in Khasi Jaintia and Garohills the plant is encountered more numerously on the forest margins of mixed deciduous forests. In the Western ghats, *R. serpentina* occurs more frequently in Goa, Coorg, North Canara and Shimoga Districts of Karnataka and Palghat, Calicut and Trichur Districts of Kerala. In Orissa, Andhra and Madhya Pradesh, the areas comprising the catchment of river Godavary are the richest. The plant is chiefly associated with Sal (*Shorea robusta*) forests as well as bamboo brakes (Raghavan Nair, 1955; Rajkhovas, 1967). Ecophysiological study of *R. serpentina* has been done by Barnah and Nath (2000).

TRADE AND COMMERCE OF *R. serpentina* IN INDIA

The major trade centres dealing with *Rauvolfia* roots are Kolkata, Mumbai and Patna which in turn are fed by a number of primary trade centres, viz. Dehra Dun, Ramnagar and Tanakpur in U.P., Hazari Bagh and Patna in Bihar, Coochbehar in West Bengal, Gauhati in Assam, Tura in Meghalaya, Bhavanipatna in Orissa, Vishakhapatnam in Andhra, Sirsi in Maharashtra and Palghat in Kerala where material from a particular growing area are received. The drug in commerce is sold under various trade names, viz., Assam, Malabar, Canara, Orissa, Bihar, Himalayan and Dehra Dun varieties. The materials from Bihar, Canara and Dehra Dun fetch a higher price (Sulochna, 1959; Vardvajaw, 1963).

CHEMISTRY OF *Rauvolfia* ALKALOIDS

The number and quantity of alkaloids isolated from *R. serpentina* and numerous other species vary widely even in the same species. More than 20 indole

alkaloids have been isolated so far from *R. seprentina* and most of the alkaloids are confined to the roots of the plant. The total alkaloid contents in the root range from 1.7 to 3.0% which are mostly concentrated in the bark (about 90%). Alkaloids are also present in leaves, stems and seeds, but not in significant amount as compared to roots.

The first report concerning hypotensive properties of *Rauvolfia* extracts was made by Chopra *et al.* in 1933. This observation together with the increasing use of the drug as a sedative, stimulated much interest in solving alkaloid from *Rauvolfia*. Although several bases were isolated and investigated, none of them appeared to possess the therapeutic value of the crude extracts. It was later shown that the sedative principle of *R. seprentina* resided in the 'oleoresin' fraction and was presumed to be due to a non-alkaloidal component (Dutta *et al.*, 1947). Investigation on this oleoresin fraction was initiated by Muller and coworkers, who succeeded in isolating the indole alkaloid reserpine (Muller *et al.*, 1952), which was shown to be the principal hypotensive and sedative ingredient of *R. serpentina* (Akram *et al.*, 1993; Roy *et al.*, 1995; Roja and Heble, 1996).

SPECIALITY OF CHEMICAL STRUCTURE OF INDOLE ALKALOIDS

With a few minor exceptions, tryptophan and its decarboxylation product, tryptomine, give rise to the large class of indole alkaloids. These bases usually contain two nitrogen atoms of which one is indolic in nature. Of the several alkaloid groups within the indole class, two may be produced depending on the type of concentration occurring between tryptomine and an aldehyde or ketoacid. A Mannich reaction involving the alpha carbon atom of the indole nucleus affords a beta-carboline derivative; reaction involving the beta position gives rise to an indolenine.

Thus, out of the two nitrogen atoms in the indole alkaloids, one is secondary in nature (R_1NH) and the other is in the form of tertiary amine (R_2N). Since the nitrogen atom bears an unshared pair of electrons, such compounds are basic and resemble ammonia in chemical property. The degree of basicity varies greatly depending upon the structure of the molecule and presence and location of other functional groups. Like ammonia, the alkaloids are converted into their salts by aqueous mineral acids and when the salt of an alkaloid is treated with hydroxide ion, nitrogen gives up a hydrogen ion and the free amine is liberated. The positive charge of the nitrogen ion depends on the presence of how many organic groups are covalently bonded to nitrogen and positive charge of this ion is balanced by some negative ion [$R_3N^+X^-$]. If the nature of ammonium ion is such that there is no proton to give up, it will not be affected by hydroxide ion. Consequently, the compounds will have chemical properties quite different from those of the amines. For the most part of the alkaloids are insoluble or sparingly so in water but the salts formed upon reacting with acids are usually freely soluble. The free alkaloids are usually soluble in ether or chloroform or other relatively nonpolar, immisible solvents in which, however, the alkaloidal salts are insoluble. This permits a ready means for the isolation and purification of indole alkaloids.

ALKALOIDS ISOLATED FROM DIFFERENT SPECIES OF *Rauvolfia* WITH SPECIAL INTEREST ON *R. serpentina*

Siddiqui and Siddiqui (1931) were able to isolate five different types of alkaloids from the root of *Rauvolfia serpentina* Benth. They were able to determine their molecular formulae and few other characteristics as (i) ajmaline ($C_{20}H_{26}O_2N_2$. $3H_2O$). m.p.159°–160°, (ii) ajmalinine ($C_{20}H_{26}O_3N_2$. $1.5H_2O$). m.p.180°–181°, (iii) ajmalicine m.p.250°–252°, (iv) serpentine ($C_{20}H_{20}O_3N_2$. $1.5H_2O$). m.p.263°–264° and (v) serpentinine ($C_{20}H_{20}O_5N_2$. $1.5H_2O$). m.p.263°–265°C and their contents in the plant root were 0.1%, 0.25%, 0.02%, 0.08% and 0.8% respectively.

Siddiqui and Siddiqui (1935) studied the ajmaline series of alkaloids of *R. serpentina*. They showed that ajmaline takes up 2 atoms of bromine in the cold yielding a crystalline dibromo derivative, which indicates the presence of an olefine double bond in its molecule. It also forms a sulphonic acid, characterized through its salts, and a tri-nitro derivative. From the crystalline product obtained on heating ajmaline to 200°C and provisionally named in its crude and undefined condition as pyroajmaline, it was possible to isolate after repeated crystallisation from ethyl acetate some unchanged ajmaline (m.p. 158–160°C) but mainly a product which melts at 265–266°C, agreeing with ajmaline in its analysis, colour reactions and NH and N-Me groups.

Siddiqui (1939), working on the roots and root bark of *R. serpentina* collected from the more temperate climate of the Dun Valley, reported the presence of two isomers of ajmaline, namely isoajmaline and neoajmaline.

Schlitter and Schwarz (1950) however, were able to isolate only two alkaloids viz., ajmaline and serpentine from the roots of *Rauwolfia serpentina* Benth.

Bose (1956) isolated a new alkaloid, serpinine, $C_{20}H_{24}ON_2$ from *R. serpentina*. Its colour reactions, properties, UV and IR spectra were examined. He carried out several degradative experiments. From the available experimental data, a hexahydro-*ß*-Carboline structure of serpentine was suggested. It was found to be closely related to ajmaline in properties and constitution. The U.V. curve of serpinine, which was studied in ethanol, resembled very closely those of the indoline. It showed λ_{max} at 250 mμ and 293mμ and λ_{min} at 227 and 272mμ respectively. The infrared (IR) spectrum of serpinine exhibited absorption bands at 6.25μ (indoline nucleus), 6.84μ (phenyl nucleus), 7.24μ (C-Me), 7.4μ (N-Me) and 13.55μ (o-distributed phenyl).

Bose *et al.* (1956) studied the alkaloid contents of *R. beddomei*, a plant indigenous to South India. They isolated sarpagine from the roots of *R. beddomei*.

Alkaloids were drained from the powdered roots by cold percolation with ethyl alcohol. Chromatographic resolution of a benzine solution of this crude base over Merck's alumina furnished an alkaloid, $C_{22}H_{24}O_3N_2$, m.p.257°C. From the infra-red (peak absorption at 5.87 and 6.16μ) and ultraviolate data (increased absorption at 250mμ) of this alkaloid, the existence of a *ß*-alkoxy-cyclic ester group has been concluded. The infra-red spectrum also exhibited bands at 2.85μ (>NH), 7.25μ (C–CH_2) and 9.05μ (ether bridge). The alkaloid was shown to be a tetrahydro-*ß*-carboline derivative from evidence of colour reactions.

Datta (1956) in a pharmacognostic investigation on the following species - *Rauwolfia serpentina, R. canescens, R. heterophylla, R. hirsuta, R. densiflora, R. decurva and R. perakensis* found positive test for the presence of reserpine in the extract of root powder in all the species except *R. densiflora, R. decurva* and *R. perakensis.*

Siddiqui (1958) studied the alkaloids of *R. serpentina* and the mode of their occurrence. The procedure adopted for the isolation of therapeutically active constituents in their naturally occurring complex form offered a new approach to studies in medicinal plant materials. The substances isolated were petroleum ether soluble oleo-alkaloid fraction—resajmaline—greenish, viscous oily liquid containing fatty matter serposterol, and unsaturated higher alcohols along with around 2.3% reserpine and 0.5% rescinnamine and traces of ajmaline. Ethyl acetate–benzene soluble alkaloidal complex–ajmalexine–forming a cream coloured powder with a concentration of the weaker *Rauwolfia* bases including 5.5% of rescinnamine and some unknown substances. Serpajmaline fraction, soluble in water, mainly contained the stronger bases serpentine, serpentinine, ajmaline and two unknown substances.

Siddiqui *et al.* (1959) studied the action of bromine on ajmaline and its various derivatives to elucidate the mechanism of this reaction and earlier points of disagreement in respect of the chemical characteristics of ajmaline had been experimentally checked up for clarification. Further, on the basis of studies in the

action of cyanogen bromide on the diacetyl derivatives of ajmaline and hexahydroajmaline, the antifibrillant cardiac action of ajmaline was correlated with the N-stability of the carbinolamine structure, which appeared to function as a cardiophore grouping in the ajmaline molecule.

Timmins and Court (1976) isolated indole alkaloids alstonine, 10-methoxygeissoschizol, tetrahydroalstonine, vomalidine, α-yohimbine and 19, 20–dehydroyohimbine, an unidentified anhydronium-like base and choline from *R. obscura* stems. The diester alkaloids reserpine and rescinnamine, which occur in roots, were not detected.

Iwu and Court (1978a) reported that the leaves of *R. cumminsii* yielded at least 12 indole alkaloids. Two E-seco indole alkaloids (corynantheol and corynantheal), 7 ajmalan type dihydroindole alkaloids (endolobine, norpurpeline, dihydronorpurpeline, normitordine, norseredamine, nortetraphyllicine and seredamine–17–0–(3', 4', 5') trimethony benzoate), 2 sarpagan alkaloids (normacusine β–0–methyl and an incompletely characterized compound) and the indoline alkaloid picrinine were isolated. The apparent scarcity of Na^- methylated indoline alkaloids in the plant may prove to be significant.

Iwu and Court (1978b) isolated 18 indole alkaloids from *R. cumminsii* roots and 17 were characterized. The alkaloids comprised sarpagan, yohimbine, 18-hydroxy-yohimbine, heteroyohimbine, anhydronium, α-acyl indole and dihydroindole types. Dihydroindoles were not previously reported in *R. cumminsii* roots.

Iwu and Court (1978c) isolated 24 indole alkaloids from the stem bark of *Rauwolfia comminsii* and among these 21 were identified. The alkaloids comprised E-seco, sarpagan, dihydroindole, yohimbine, heteroyohimbine, 18-hydroxyyohimbine ester and anhydronium types together with peraksine and deacetylpicraline. The probable biosynthesis of alkaloids was discussed.

Sabri and Court (1978) isolated 22 indole alkaloids from the stem bark of Nigerian *R. vomitoria* and 20 of them were characterized. The alkaloids comprised E-seco heteroyohimbine, sarpagan, dihydroindole, yohimbine and heteroyohimbine types.

Amer and Court (1980) isolated nineteen alkaloids from Ghanaian *R. vomitoria* leaves. The alkaloids comprised E-seco indole, sarpagan, picrinine, akuammiline, heteroyohimbine, oxindole, yohimbine and indolenine types.

Akinloye and Court (1980a) isolated and identified twenty-one indole alkaloids from the leaves of *R. oreogiton*. The alkaloids comprised E-seco heteroyohimbine, heteroyohimbine, akuammiline, akuammicine, pleiocarpamine, picraline, picrinine, dihydroindoline and sarpagan types. No chemical differentiation between the leaves of *R. oreogiton* and *R. volkensii* could be established.

Akinloye and Court (1980b) isolated and identified thirteen alkaloids from the leaves of *R. volkensii*. The alkaloids included E-seco heteroyohimbine, heteroyohimbine, sarpagan, dihydroindoline, pleiocarpamine, picrinine and akuammicine types together with peraksine.

Lastra *et al.* (1982) isolated three alkaloids, viz., aricine, tetrahydroalstonine and vellosinine from the leaves of *R. cubana*. These alkaloids were also identified. No reserpine was detected in the roots of *R. tetraphylla*, whereas ajmaline was found in the roots. Reserpine (0.04%) and ajmaline (0.07%) were isolated from the roots of *R. cubana*. Roots of *R. cubana* showed pharmacological activity attributed to Rauvolfia alkaloids.

Nasser and Court (1983) isolated eighteen alkaloids from South African *Rauwolfia caffra* and these were corynane, sarpagan, peraksine, akuammicine, macroline, indolenine and harman types. Heteroyohimbines and dihydroindoles were not detected. The principal alkaloids were the indolenine compounds

raucaffrinoline, perakine and vomilenine and the indole alkaloids peraksine and dihydroperaksine.

Court (1983) reported the distribution of indole alkaloids in the leaves, stem and roots of 10 African mainland *Rauwolfia* species such as *R. oreogiton, R. volkensii, R. affroa, R. macrophylla, R. mannii, R. obscura, R. rosea, R. cumminsii, R. mombasiana and R. vomitoria.* He also discussed the interrelationships of the alkaloid types.

Nasser and Court (1983) reported the alkaloids *R. caffra* seeds. The results indicated that seeds yielded 0.012% total alkaloids comprising the sarpagan compound normacusine β, α-yohimbine, alloyohimbine, an incompletely characterized yohimbine. Chromatographic evidence indicated traces of nonajmalan and ajmalan compounds.

Siddique *et al.* (1985) isolated a new dihydroindole alkaloid sandwicoline from undried winter roots of *R. serpentina* of Nepalese origin. Its structure was determined as 21-monohydro-N-methyl-sandwicine through chemical and spectral studies.

Kan *et al.* (1986) isolated four monomeric indole alkaloids from the bark of *Rauvolfia media.* Three of them were the known cabucine, reserpiline and mauiencine and the fourth was a new alkaloid 12-hydroxymauiensine.

Siddique *et al.* (1986) determined the structure of a new yohambanoide, rescinnaminol ($C_{32}H_{42}N_2O_6$ m.p. 241–43°C). This alkaloid was isolated from *R. serpentina* roots and was elucidated through spectroseopic methods.

Siddique *et al.* (1987a) reported that the alcoholic extract of the roots of *R. serpentina* had been elucidated as cyclonexy ester of indolepropionic acid by spectral and chemical studies.

Siddique *et al.* (1987b) enlisted two hundred and four alkaloids isolated from various *Rauwolfia* species and their molecular formulae, melting points and specific rotation were presented.

Siddique *et al.* (1987c) isolated a new alkaloid yohambinine from the alcoholic extract of *R. serpentina* roots and was identified as 5 beta–methylpseudoyhimbane by spectroscopic studies. It is the first 5–methyl yohimbanoid isolated from any plant.

Siddique *et al.* (1987d) elucidated the structure of a new base, rescinnamidine ($C_{35}H_{44}N_2O_9$, m.p. 260–61°C) isolated from the roots of *R. serpentina* as 2', 3'–dihydrorescinnamidine.

Siddique *et al.* (1987e) isolated a new dihydroindole alkaloid ajmalinimine from the roots of *R. serpentina* collected from Thailand. Its structure was determined as 10–C, 17–O–diacetylajmaline on the basis of chemical and spectroscopic studies.

Siddique *et al.* (1987f) were able to identify a new heteroyohimban alkaloid-ajmalicidine, from the roots of *Rauwolfia serpentina* of Thi-origin. Its structure was elucidated as 1–carbomethoxy–17 α–hydroxy–16–decarbomethoxy 16, 17–dihydro-ajmalicine through chemical and spectral studies.

Intipoua *et al.* (1988) studied alkaloid composition in the roots, stem and leaves of *Rauwolfia caffra, R. cansescens, R. heterophylla, R. serpentina* and *R. verticilata.* Ajmaline alkaloids were accummulated in the roots but reserpine alkaloids were found in all parts of the plants. Recommendations were made for using *R. cansescens* for commercial growing as a species with the highest alkaloid content.

Schuibel *et al.* (1989) observed the production of gluco-alkaloid raucaffricine in cell suspension culture of *Rauwolfia serpentina.* The alkaloid production medium was modified by adding 100 g sucrose and 2.5 g

$MgSO_4.7H_2O$/1 of the medium. The culture produced after 18 days upto 1.6 g raucaffricine when the medium was inoculated with 200 g of cell grown for 10 days in Linsmaier–Skoog medium. This yield exceeded that was known from intact plants (*Rauwolfia caffra*) by factor of 12.

Nikolaeva *et al.* (1990) presented data on ajmaline content in various organs of the following *Rauwolfia* spp. Grown in the Transcaucasus Georgian SSR, USSR: *R. caffra, R. canescens, R. heterophylla* and *R. serpentina*. Data were also given on ajmaline content in the root bark of the following species from the flora of Vietnam : *R. cambodiana, R. cansescens, R. serpentina, R. verticillata, R. vomitoria and R. littoralis, R. vomitoria* and , *R. cansescens* were the most valuable species with respect to ajmaline content.

Falkenhagen *et al.* (1992) cultured the hairy roots of *R. vomitoria* in hormone-free B5 medium. The six weeks old cultures were used for phytochemical analysis. The major alkaloids detected were vinerine, perakine, ajmaline, asmalinol and a yohimbine-isomer. Structures of all the alkaloids were detected, except ajmalinol, which was reexamined.

Endresse *et al.* (1992) observed that when ajmaline, one of the major alkaloids of *R. serpentina* was added to *R. serpentina* cell suspension culture, a new series of alkaloids was formed. Five novel alkaloids were isolated from these ajmaline-fed cell cultures and their structures were deduced. Four of the novel products belonged to the raumacline group: 6α–hydroxy aumacline, 6α–methoxy-raumacline, 19–hydroxy–N–methylraumacline and 20–isoraumacline. The structure of a further new alkaloid was different from the raumaclines and belonged to the sarpagine alkaloids.

Endresse *et al.* (1993) isolated a group of new alkaloids, the raumaclines, and some related alkaloids from *R. serpentina* cell suspensions fed with high levels of ajmaline; their structures were determined and sysnthesis developed

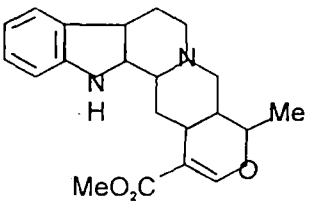
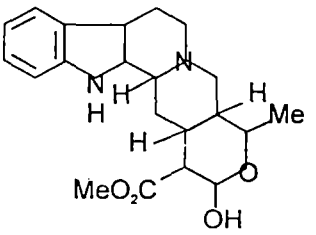
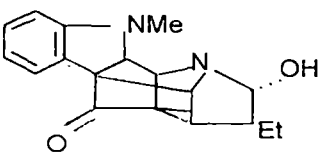
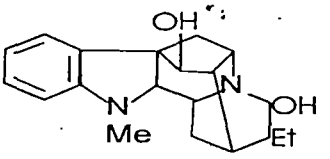
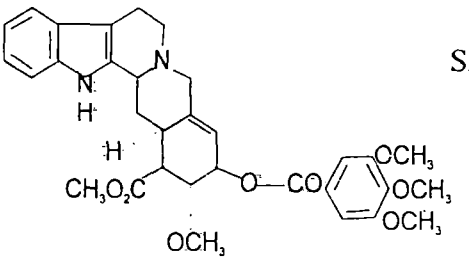
providing an essential prerequisite to the further study of their biosynthesis at the enzymatic level.

Bianco *et al.* (1994) isolated from the bark of *Rauwolfia grandiflora* a new monoterpenoid δ–lactone, isoboonein, together with boonein, loganin and loganic acid. The structure of isoboonein, established by spectroscopical methods, was confirmed by partial synthesis from loganic acid.

Ferreira Batista *et al.* (1996) obtained a new alkaloid, sellowiine (N-demethyl-20-deethyl suaveoline), from leaves of *R. sellowii*, collected at two different locations in Southern Brazil. They also obtained the known alkaloids, perakine, raucffrinoline, vomilenine, 19α, 20α–epoxy–akuammicine, picrinine and 12–demethoxytabernulisine. The NMR spectra of the alkaloids were assigned completely.

Some important alkaloids in different species of *Rauvolfia* including *R. serpentina* have been presented in Table 1.

Table 1 : Some important alkaloids isolated from *Rauvolfia* with special interest on *R. serpentina*.

Name of alkaloid, Name of the plant' Empirical formula & Melting point (°C)	Chemical structure	Reference
1 Ajmalicine *R. serpentina* $C_{21}H_{24}N_2O_3$ 253-4		Janot & Le Men, 1956 and Sharma & Moss, 1961
2 Ajmalicinine *R. serpentina* $C_{21}H_{26}N_2O_4$ 245-6		Bombardelli *et al.*,1974
3 Ajmalidine *R. sellowii* $C_{20}H_{24}N_2O_2$ 241-2		Prakash *et al.*,1955; Bartlett *et al.*, 1962
4 Ajmaline *R. serpentina* *R. vomitoria* $C_{20}H_{26}O_2N_2$ 205-7		Siddiqui and Siddiqui, 1931; Mukherji *et al.*, 1949; Chatterjee & Bose, 1954
5 Ajmalinine *R. serpentina* *R. vomitoria* $C_{20}H_{26}O_3N_2$ 180-1		Siddiqui and Siddiqui, 1931; 1932, 1935
6 Deserpideine *R. nitida* $C_{32}H_{36}O_8N_2$ 149-52		Smith *et al.*, 1964, 1967

Contd.

Table 1 (Contd)

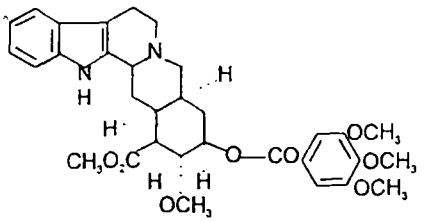
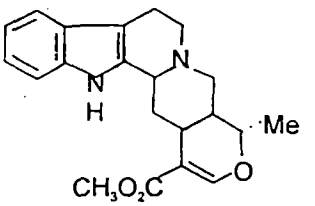
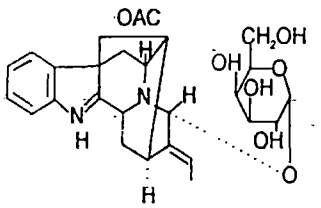
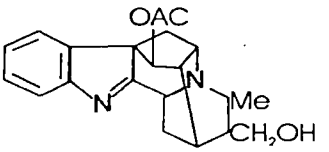
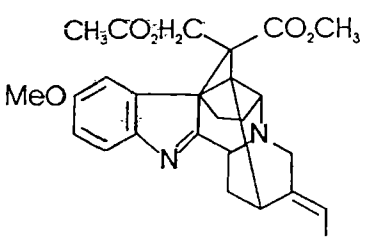
7	Deserpidine *R. canescence* $C_{32}H_{38}O_8N_2$ 228-32		MacPhillamy *et al*, 1953; Neuss *et al.*, 1955
8	Raubasine *R. serpentina* $C_{21}H_{24}O_3N_2$ 256-7		Klohs *et al.*, 1954; Shamma and Richey, 1963; Finch *et al.*, 1966
9	Raucaffricine *R. caffra* $C_{27}H_{32}O_8N_2$ 220		Khan *et al.*, 1965
10	Raucaffridine *R. caffra* $C_{21}H_{24}O_3N_2$ 221		Khan *et al.*, 1965
11	Raucaffriline *R. caffra* $C_{21}H_{22}O_3N_2$ 200-01		Khan *et al.*, 1965
12	Raucaffrinoline *R. caffra* $C_{21}H_{24}O_3N_2$ 236		Khan and Siddiqui, 1972
13	Raufloricine *R. confertiflora* $C_{24}H_{28}O_5N_2$ 190-2		Danieli *et al.*, 1972

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Table 1 (Contd)

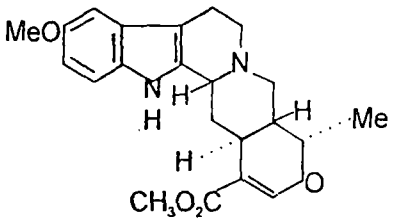
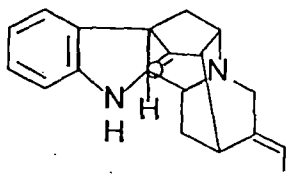
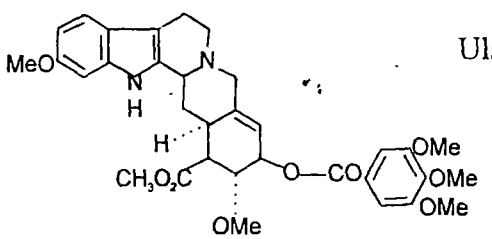
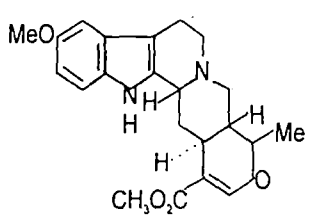
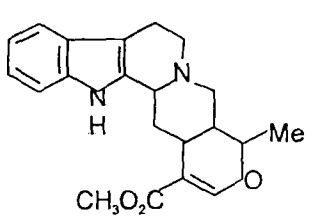
14	Raufloridine *R. confertiflora* $C_{22}H_{26}O_4N_2$ Indefinite		Danieli *et al.*, 1971
15	Rauflorine *R. confertiflora* $C_{19}H_{20}ON_2$ 221		Danieli *et al.*, 1971
16	Raugalline *R. serpentina* $C_{21}H_{28}O_3N_2$ 185		Le Gall, 1960
17	Rauhimbine *R. serpentina* $C_{21}H_{26}O_3N_2$ 218-25		Hoffmann and Helv, 1954
18	Raujemidine *R. canescens* $C_{33}H_{38}O_9N_2$ 144-5		Ulshafer *et al.*, 1956
19	Raumitorine *R. vomitora* $C_{22}H_{26}O_4N_2$ 138		Poisson *et al.*, 1954; Shamma and Richey, 1963; Finch *et al.*, 1966
20	Rauniticine *R. nitida* $C_{21}H_{24}O_3N_2$ 233-5		Salkin *et al.*, 1961; Shamma and Richey, 1963; Finch *et al.*, 1966

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Table 1 (Contd)

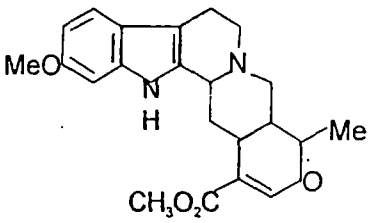
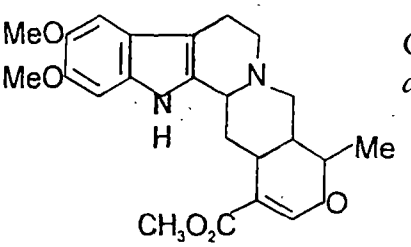
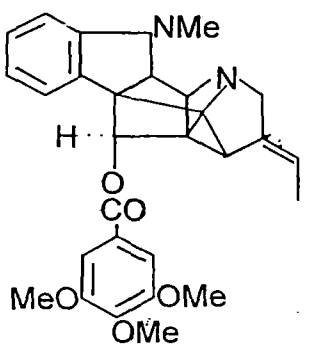
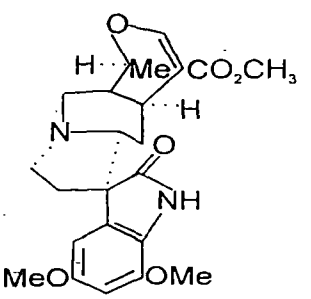
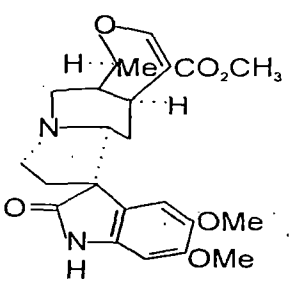
21 Raunitidine *R. serpentina* $C_{22}H_{26}O_4N_2$ 276-8		Salkin *et al.*, 1961; Shamma and Richey, 1963
22 Raupine *R. serpentina* $C_{20}H_{26}O_3N_2$ 325		Bodendorf and Eder, 1953
23 Rauvanine *R. vomitora* $C_{23}H_{28}O_5N_2$ 135		Goutarel *et al.*, 1961; Finch *et al.*, 1966
24 Rauvomitine *R. vomitora* $C_{30}H_{34}O_6N_2$ 115-7		Haack *et al.*, 1955; Poisson *et al.*, 1955; Bartlett *et al.*, 1962
25 Rauvoxine *R. vomitora* $C_{23}H_{28}O_6N_2$ 210-1		Patel *et al.*, 1964; Pousset and Poisson, 1964
26 Rauvoxinine *R. vomitora* $C_{23}H_{28}O_6N_2$ 203		Patel *et al.*, 1964; Pousset and Poisson, 1964

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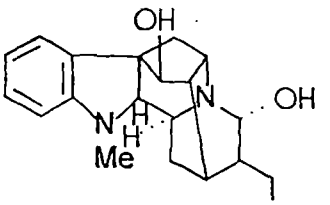
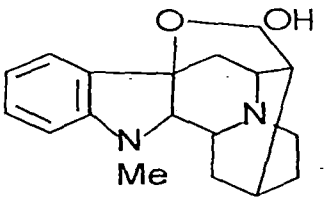
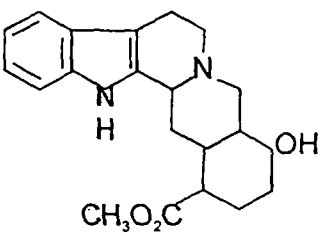
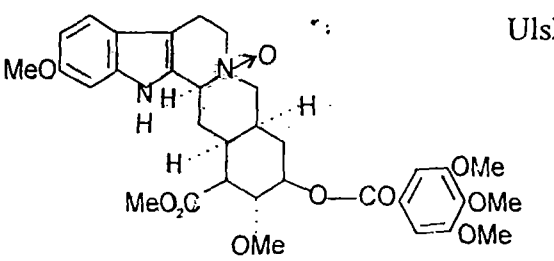
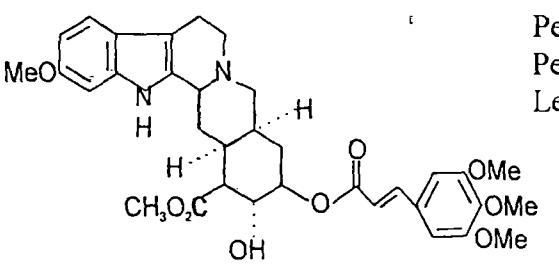
Table 1 (Contd)

27 Rauwolfine *R. serpentina* *R. caffra* $C_{20}H_{26}O_2N_2$ 235-8		Itallie and Steenhauer, 1932; Koepfli, 1932.
28 Rauwolfinine *R. serpentina* $C_{19}H_{26}O_2N_2$ 231-3		Bose, 1954
29 Rauwolscine *R. canescens* $C_{21}H_{26}O_3N_2$ 231-2		Mukherjee, 1941, 1946
30 Renoxydine *R. vomitora* $C_{33}H_{40}O_{10}N_2$ 238-41		Ulshafer *et al.*, 1957
31 Rescidine *R. vomitora* $C_{34}H_{40}O_9N_2$ 183-6		Peopelak *et al.*, 1961; Peopelak and Lettenbauer, 1963

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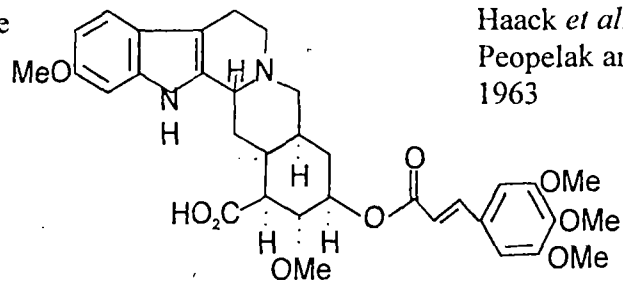
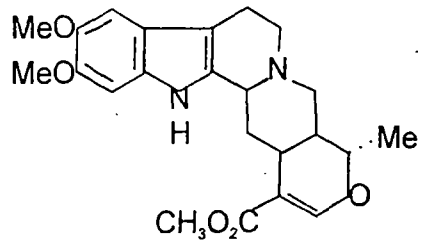
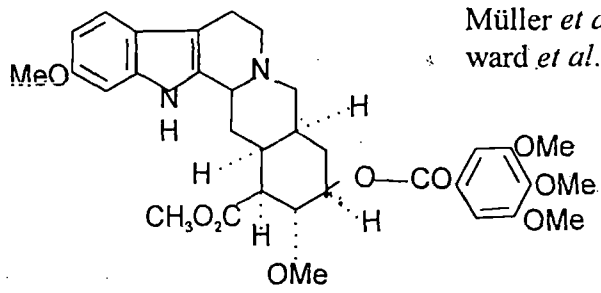
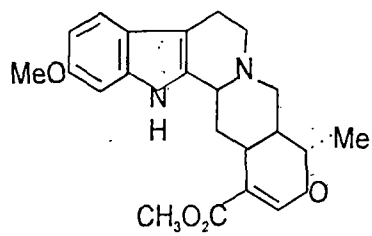
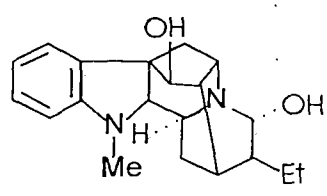
32	Rescinnamine *R. serpentina* *R. vomitora* $C_{34}H_{42}O_9N_2$ 238-9		Haack *et al.*, 1954; Peopelak and Lettenbauer, 1963
33	Reserpiline *R. serpentina* *R. canescens* $C_{23}H_{28}O_5N_2$ Indefinite		Klohs *et al.*, 1954; Stoll *et al.*, 1955; Shamma and Richey, 1963
34	Reserpine *R. serpentina* $C_{33}H_{40}O_9N_2$ 262-63		Müller *et al.*, 1952; Woodward *et al.*, 1958
35	Reserpinine *R. serpentina* $C_{22}H_{26}O_4N_2$ 243-4		Janot and Le Men, 1954; Schlittler *et al.*, 1954; Shamma and Richey, 1963.
36	Sandwicensine *R. sandwicensis* $C_{19}H_{22}ON_2$ 260-2		Gorman *et al.*, 1957
37	Sandwicine *R. sandwicensis* *R. mauiensis* $C_{20}H_{26}O_2N_2$ 220-2		Gorman *et al.*, 1957; Ronchetti *et al.*, 1971

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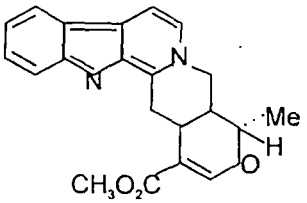
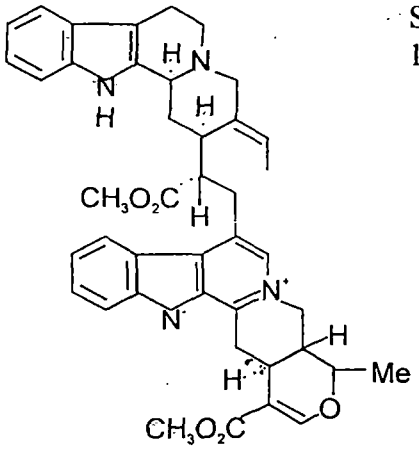
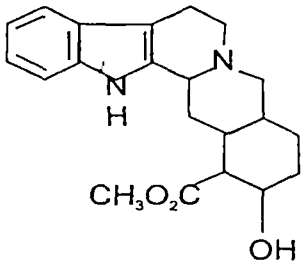
Table 1 (Contd)

38	Seredine *R. vomitora* $C_{23}H_{30}O_5N$ 291		Poisson *et al.*, 1954; Goutarel *et al.*, 1954
39	Serpentine *R. serpentina* $C_{21}H_{20}O_3N_2$ 158		Siddiqui and Siddiqui, 1931, 32, 35
40	Serpentinine *R. serpentina* $C_{42}H_{44}O_5N_4$ 263-5		Siddiqui and Siddiqui, 1931, 32, 35
41	Serpine *R. serpentina* $C_{21}H_{26}O_3N_2$ 215		Chatterjee and Bose, 1954
42	Serpinine *R. serpentina* $C_{20}H_{24}ON_2$ 315-7		Bose, 1954, 1955
43	Serpagine *R. beddomei* $C_{22}H_{24}O_3N_2$ 257		Bose, et al., 1956

METHODOLOGY FOR QUANTITATIVE ESTIMATION OF INDOLE ALKALOIDS IN *Rauvolfia* SPECIES

Shah and Hossain (1965) described a spectrophotometric method for the determination of the amount of serpentine in serpajmaline. The absorbance of serpentine in 5N acetic acid at 307mμ was used for the submicro spectrophotometric determination of this substance in serpajmaline which is predominantly a mixture of serpentine, serpentinine and ajmaline. Serpentinine which also absorbed at 307mμ was separated from the complex through electrophoresis. The extent of interference due to ajmaline in the UV absorbance measurements of serpentine was determined. The method was accurate within ± 1–2%.

Cieri (1983) presented for the identification estimation of some of the alkaloids of *R. serpentine* by high performance liquid chromatography (HPLC) and TLC. Rescinnamine was detected at 330 nm, at which wavelength reserpine fluorescence was negligible. Reserpine was detected at 280 nm, where rescinnamine fluorescence was small. Other alkaloids detected were raubasinine, ajmalicine, yohimbine, ajmaline and serpentine. For TLC, $CHCl_3$–CH_3OH (97+3) and $CHCl_3$–CH_3OH (80+20) were used as developing solvents and spots were detected under long and short wave UV light. A semiquantitative TLC procedure was also developed for serpentine, the content of which was in the 0.2–0.25% range.

Shimolina *et al.* (1984a) reported the quantitative determination of total alkaloids in *R. serpentina* tissue culture. Comparative data were presented on the weight, volumetric and extraction–photometric methods in the determination of total alkaloids. The volumetric method was recommended as the most objective and the least time–consuming method.

Shimolina *et al.* (1984b) developed a method for the determination of vomilenine and a closely related alkaloid on thin layer of silica gel with subsequent determination by elution spectrophotometric method. Maximum vomilenine content observed was 0.50–0.65%.

Nguyen *et al.* (1989) developed spectrophotometric and extraction photometric methods for determining ajmaline, reserpine and serpentine in the root bark of *R. serpentina, R. littoralia, R. cambodiana, R. verticillata, R. vomitoria and R. canescens*. The methods were simple, accurate and reproducible. High contents of the alkaloids was established in all samples. Recommendations were made for using *Rauwolfia spp.* growing in Vietnam as sources of raw materials in reserpine and ajmaline production.

Gubar *et al.* (1993) developed a rapid method for estimation of submicroquantities of alkaloids in cultured cells and studied alkaloid accumulation in *Rauwolfia serpentina* tissue culture. Quantitative method for estimation of indole alkaloids was elaborated by microcolumn chromatograph. The developed method permitted carrying out simultaneous determination of ajmalicine, reserpine, vomilenine, ajmaline and serpentine in the tissue culture extracts. Various *Rauwolfia* cell lines were analysed. About 30 different alkaloids were detected and contents of some alkaloids were measured in the cultured cells including alkaloids used in medicine, such as ajmalicine, reserpine, ajmaline and serpentine.

Cieri *et al.* (1987) established a method for the determination of reserpine and rescinnamine in *R. serpentina* powder or tablets by liquid chromatography (LC) with fluorescence detection. The sample was dispersed in CH_3OH, 0.5N and H_2SO_4 was added and the mixture was extracted with chloroform. The extracts were separated from interfering materials on a celite–0.1N NaOH column and the elutes were collected in 50 ml CH_3OH. After complete removal of the $CHCl_3$, reserpine and rescinnamine were determined by liquid chromatography on a normal phase column with CH_3OH as mobile phase.

A simple sensitive and highly specific method involving fluorescence analysis of reserpiline in highly acidic solutions was described by Balon-Almeda *et al.* (1986). The method could be employed for determination of reserpiline in commercial reserpine-rescinnamine preparations.

A radioimmuno assay (RIA) was developed for the individual measurement of serpentine in plant extracts. Each RIA was assessed for its sensitivity, specificity and accuracy. The method involving preparation of antigens and antibodies specific for these alkaloids as well as the radiochemical preparation of serpentine was successfully applied in the selection of individual plants with higher than average contents of these alkaloids for breeding and tissue culture purposes (Arens *et al.*, 1978).

BIOSYNTHETIC PROCESSES OF INDOLE ALKALOIDS

Any approach to improve indole alkaloid production requires through knowledge of the alkaloid biosynthetic pathway, enzymology and genetic regulation. The biosynthesis of indole alkaloids has been intensively studied especially during the last four decades. Although progress has been achieved in understanding the chemical make up of different indole alkaloids, but the accumulation of knowledge in connection with biosynthesis of these types of alkaloids not satisfactory, though certain main intermediates are well known. As there are certain problems in understanding the process of biosynthesis and their relationship between various groups of alkaloids, further work in this line is very much needed as complete picture is still not available.

Most of the work has been done with *Catharanthus roseus*, because the species has many advantages for biosynthetic studies. The incorporation of tryptophan into all major classes of indole alkaloids was shown by Leete's group (Leete, 1961; Yamazaki and Leete, 1965). Following tracer technology with the help of [2-C^{14}]–tryptophan specially lebelled reserpine and serpentine in *R.*

serpenitina and vindoline in *Catharanthus roseus* could be possible. The label form [3-C^{14}]–tryptophan was located at the predicted site in ibagaine (*Tabernanthe ibago*). But to find out the pathway, which is operative in nature apparently, require studies at the enzymatic level.

SOME RECENT OBSERVATIONS ON ENZYME ACTIVITY IN BIOSYNTHETIC PROCESS OF INDOLE ALKALOIDS AND THE ROLE OF PLANT TISSUE CULTURE

It is now possible to understand that vast majority of indole alkaloids are biosyntherised in plants follow shikimate and mevalonate pathways (Fig-1). Indole alkaloids consist of tryptamine provided by tryptophan (from shikimate pathway) and a terpenoid part provided by the iridoid glucoside secologanin (from mevalonic acid pathway). The first enzyme involved in undole alkaloid biosynthesis is tryptophan decarboxylase (TDC), which converts amino acid tryptophan in to tryptamine. The biosynthesis of secologanin requires a number of enzymatic reactions of which the first step of reaction is the hydroxylation of geraniol to 10-hydroxy geraniol catalysed by the enzyme geraniol 10-hydroxylase ($G_{10}H$). Tryptamine and secologanin are condensed by the enzyme strictosidine synthase (SS) to form strictosidine, which is the common precursor of all indole alkaloids. This enzyme might be considered a logical point for limiting flux in the biosynthetic pathway.

The enzyme catalysing the condensation reaction was discovered and named by Stockigt and Zenk (1977). It was purified from *Catharanthus roseus* cell culture and characterized by Treimer and Zenk (1979). But the enzyme from *C. roseus* cell culture was found to split into four distinct multiple forms, which were difficult to characterize individually (Pfitzner and Zenk, 1988). Because of high yield of Strictosidine Synthase in *R. serpentine* as compared to *C. roseus* culture and the stability of soluble *Rauvolfia* enzyme (besides, the yield is also

considerably higher than that of *C. roseus*), the enzyme of *Rauvolfia* has advantage for its utilization in biotechnology. The time course of enzyme formation and growth parameters of cell in culture have been worked out. The enzyme is present with inoculum (day 6) in only small amount. The activity increases slowly to peak at day 12. In contrast, dry weight increase peaked at day 10 and maximal activity is therefore reached in the stationary growth phase.

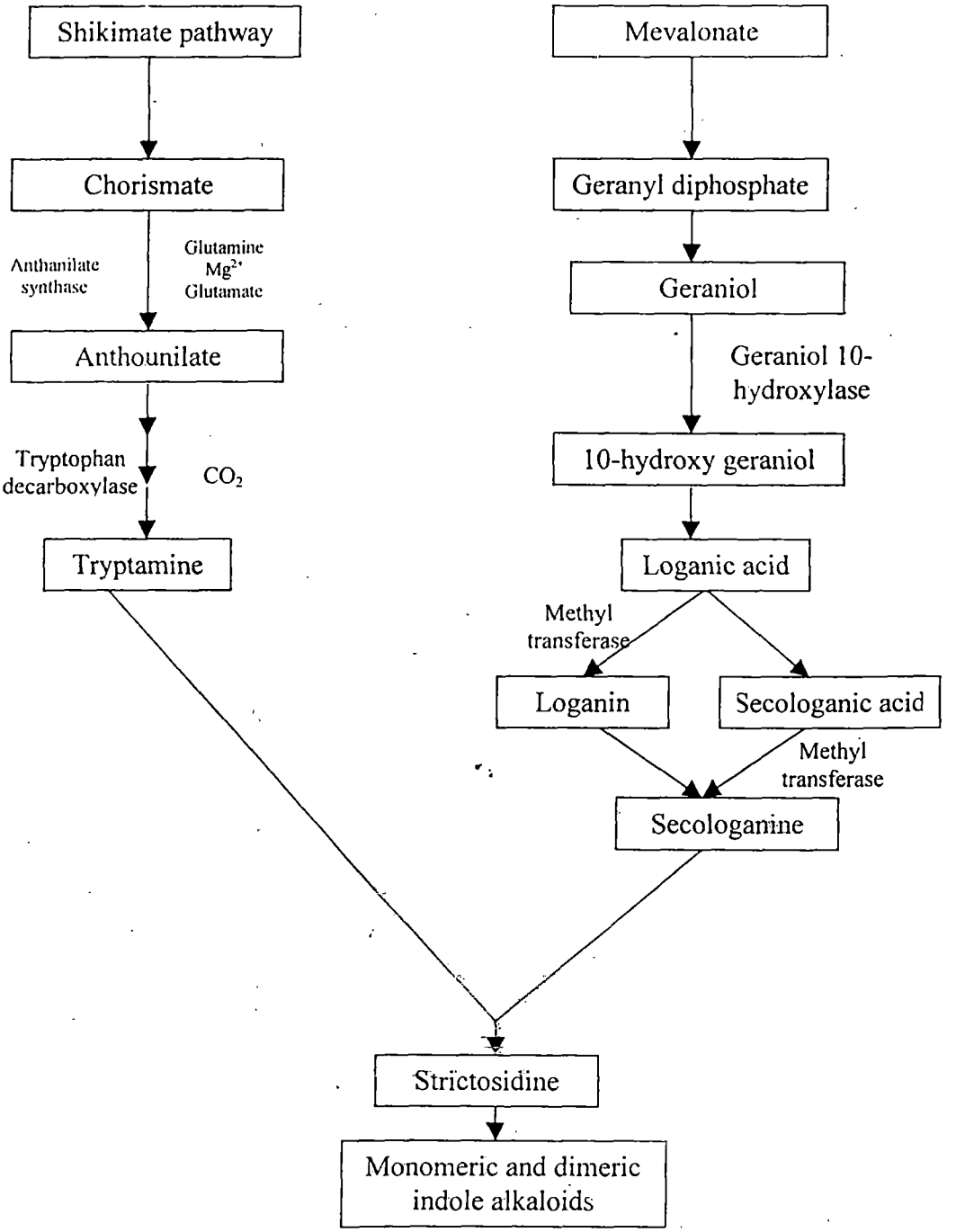


Fig. 1 : Biosynthesis of indole alkaloids (Misra *et al.*, 1996)

The availability of homogenous strictosidine synthase from *Rauvolfia* should facilitate basic studies regarding the structure and the application of the polypeptide.

In an attempt to study molecular biology of alkaloid biosynthesis and to learn about the organization of genes involved in secondary metabolism, it is necessary as a first step to purify strictosidine synthase to homogeneity. Because of the occurrence of multiple forms of synthase in *C. roseus*, the enzyme from *R. serpentina* is advantageous for its utilization.

Strictosidine was initially named as isovincoside. As an intermediate between isovincoside and cathenamine, 4,2,1-dehydrocornanthinealdehyde might be involved (Stockigt *et al.*, 1978)

Ajmaline synthase from *C. roseus* culture extract was made by Scott *et al.* (1977) but the enzyme rapidly lost its ability to synthesize ajmaline but retained glucosidase activity.

Meehan and Coscia (1973) isolated from *C. roseus* a microsomal mixed function oxidase, which convert monoterpene alcohol geraniol to their corresponding 10-hydroxy derivatives. This membrane bound cytochrome P-450 dependent monooxygenase is inhibited by an end product Catharanthine-an indole alkaloid suggesting feed back control of the pathway (Mc Farlane *et al.*, 1975), because both 10-hydroxy monoterpenes are good precursors for loganine. Stockigt *et al.* (1976) prepared an enzyme extract from cell suspension culture of *C. roseus* and could show the conversion of tryptamine plus secologanine in presence of NADPH into ajmaciline. If the incubation was performed in the absence of pyridine nucleotide, a new alkaloid cathenamine is accumulated and which is apparently an obligate intermediate in the biosynthesis of a ajmalacine and related alkaloids (Stockigt *et al.*, 1977).

Recently a new indole alkaloid of heteroyohimbine type was isolated from the roots of *R. serpentina* as ajmalicidine (Siddiqui *et al.*, 1987), but according to Lonnasmaa and Tolvanen (1994), it should be named as ajmaline and which was based on ^{13}C-NMR value. According to them, the methoxy-carbonyl group attached to the indole nitrogen is a very rare structural feature that was almost exclusively been found in certain *Kopsia* type alkaloid. The majority of the tryptophan derived alkaloid belongs to the class complex indole alkaloid which are built up from tryptamine and C_9-C_{10} unit.

It is now established that three general monoterpenoid skeletons are recognized as giving rise to most of the complex indole alkaloids; these skeletons are designed as the Aspidosperma, Corynanrhea and Ibago types taking the name of the plants which are rich in alkaloids with the respective monoterpenoid nuclei.

SUBCELLULAR COMPARTMENTATION OF INDOLE ALKALOID METABOLISM

The subcellular compartmentation of secondary metabolites is believed to play an important role in the regulation of their metabolism. Only a few enzymes in the pathway have been clearly localized. The subcellular localization of the enzymes involved in mevalonate pathway has two models. According to the first model, the mevalonate pathway occurs at three different sites, viz., cytosol, mitochondria and plastids. The second model suggests that the isopentenyl diphosphate is formed mainly in the cytosol and subsequently transferred to other compartments for synthesis of isoprenoids.

The shikimate pathway for biosynthesis of tryptophan is cytosolic. The conversion of tryptophan into tryptamine by tryptophan decarboxylase is also shown to occur in the cytosol. The product tryptamine is channelized into vacuole where strictosidine synthase enzyme is localized and, therefore, coupling of secologanin with tryptamine to form strictosidine occurs in vacuole.

The geraniol-10-hydroxylase is found to be associated with tonoplast (provacuolar membranes). It might be possible that the synthesis of secologanin occurs only in provacuole, while alkaloids are stored in mature vacuoles.

Ajmalicine is accumulated inside the vacuole by an ion trap mechanism. Ajmalicine freely diffuses across the tonoplast in neutral form and accumulates in its charged protonated form. Ajmalicine accumulation can be increased by acidification of the vacuole or by the proton pump activity. However, when there is a higher level of ajmalicine in the vacuole, it can convert into its tetra dehydro derivative serpentine by vacuolar peroxidases. The charged *serpentine* molecules are trapped inside the tonoplast. For ion trapping, energy flow comes from Mg^{2+} ATPase.

TISSUE CULTURE IN *Rauvolfia serpentina*

Mitra and Kaul (1964) grew excised embryos of *R. serpentina* in modified White's medium supplemented with IAA, 2,4-D, coconut water, yeast extract, adenine and adenine sulfate, either singly or in combination. Of the auxins tested for effectiveness in establishing root callus tissue in cultures, 2,4-D was found to be the most effective. Of different concentrations used 2,4-D at 1 ppm gave the best result. Excised embryos placed on a basal medium containing IAA (1 ppm) and coconut water (10%) responded in a different manner. From their hypocotyl and radical regions many roots were formed and their epicotyledonary apices developed into callus tissue. The three types of callus tissue isolated from the initial callus mass during successive transfers differed in their external and internal morphology, nutritional requirements, potentiality for differentiation and ability to synthosize reserpine. Callus tissue developed from the sides of young stem segments placed on the basal medium supplemented with IAA (1 ppm) and coconut water (10%), whereas 2,4-D and coconut water together failed to induce callus formation in stem segments.

Mitra *et al.* (1965) reported that callus formation was localized to the exposed cells at the cut faces of excised roots, stems and leaves and to the injured regions of cotyledons of *Rauvolfia serpentina*. During callus formation in excised root tips or radicale tips, the growth of the apical meristem was inhibited but the tissues of the subapical region continued to divide resulting in its swelling. The pith, phloem and cortex were induced to divide to form callus in radicale tips, excised roots and stems. The phloem and parenchyma around the vascular regions of the leaf lamina were activated to form callus in excised leaf segments, whereas palisade and spongy mesophyll remained inactive in the nutrient media used. Suitable media for rapid and continuous growth of these callus tissues were established either using modified nutrient solutions of White or Murashige and Skoog as basal media. Coconut water and 2,4-D were essential supplements to these media.

Mitra (1968) reported that growth of isolated roots of *R. serpentine* in a modified White's nutrient solution was enhanced when supplemented with α –NAA (0.001mg / l) and β-IBA (0.01mg/l); these factors did not prevent, after 21 days of culture, a decline in growth rate. By successive transfer of 21 days old culture, isolated roots continued their growth through 4 passages. Kinetin (0.001 mg /l) slightly enhanced the level and duration of growth of excised roots. The roots in light grown cultures receiving kinetin became pale green. Gibberellic acid at a concentration of 0.01 mg /l enhanced linear growth of main axis in the first passage only in dark cultures. Casein hydrolysate (vitamin free, DIFCO) and ammonium nitrate were not beneficial to growth of isolated roots.

Nikolaeva *et al.* (1978) achieved the production of a finely dispersed suspension culture of *R. serpentina* tissue by collection and transplantation of only fine aggregates and isolated cells. Examination of the characteristics of the aggregated and accumulated alkaloids in the suspension culture revealed no differences in content of alkaloids in aggregates of different dimensions. The method of collection and transplantation of only fine aggregates and individual

cells of suspension cultures made it possible to produce suspension cultures relatively homologous in their morphology.

Vollosovich and Vollosovich (1982) studied the possibility of cultivating *R. serpentina* on microbiological waste, such as molasses with 50% sucrose, hydrols from lignin and corn with 40% glucose, as well as syrup and green syrup, was studied. Molasses and hydrols could not be used to cultivate *R. serpentina* because of their toxicity. The green syrup was promising for the cultivation of isolated plant tissues. The syrup could be used in industry to obtain *R. serpentina* biomass.

Vollosovich *et al.* (1982) reported that the optimum of alkaloids in the tissue culture of *R. serpentina* was obtained at 1:60 ratio of ammonium nitrate and sucrose in the nutrient medium. Increase in ammonium nitrate concentration decreased tissue weight, enhanced alkaloid and protein synthesis and suppressed starch accumulation. Higher concentrations of sucrose acted in the opposite direction.

Pfitzner *et al.* (1984) isolated a plant enzyme vellosimine reductase from *Rauvolfia* cell suspension cultures. This new enzyme was purified (110-fold) and characterized. The reductase is a specific enzyme of the sarpagine pathway catalyzing the NADPH dependent conversion of vellosimine into 10-deoxysarpagine. The latter alkaloid is the immediate biogenatic precursor of sarpagine as shown by its high in vivo incorporation rate (86%) into sarpagine.

Uesato *et al.* (1984) administered 2H and 13C labelled compounds to *R. serpentina* suspension cultures and the results indicated that ajmaline and vomilenine produced by these cultures were biosynthesized via 10-hydroxygeraniol, 10- hydroxynerol and iridodial in the same way as secologanin, vindoline etc. in *Catharanthus roseus* and *Lonicera morrowii.* Therefore, this cyclization mechanism appeared to be common in plants containing secoiridoids and indole alkaloids.

Akram and Ilahi (1985) reported that callus cultures of *Rauvolfia serpentina* were initiated on MS medium supplemented with 1% casein hydrolysate, 1mg/l NAA and 0.5 mg/l kinetin. Stem callus, which regenerated buds, was heterogeneous in texture, friable at the periphery and top but compact and hard at the base and core. Bud regeneration was noticed after 20 weeks culture of callus on White's root culture medium containing 100 ml/l coconut milk, 10 ml/l biotin, 250 mg/l sodium diethyl-dithiocarbamate and 0.8 mg/l NAA. The plantlets formed roots both with 0.8 mg/l NAA given continuously or with 24 h. treatment of 3 mg /l each of IAA and IBA. The plantlets established themselves quite easily in soil. For the first few weeks in soil they were nourished with half strength Knop's solution. Afterwards they became completely autotrophic.

Roja *et al.* (1985) isolated and characterized some indole alkaloids from multiple shoot cultures of *R. serpentina*. Shoot cultures were established from the axillary meristem of the field –grown plants. The cultures were maintained on MS agar medium supplemented with 0.1 ppm of NAA and 1.0 ppm of BA. The shoot cultures were dried and extracted with hexane followed by ammonia / methanol. Identification of the alkaloids was achieved through TLC, mass spectral analysis and HPLC. The alkaloid pattern (TLC) of the shoot cultures, leaves and roots differed considerably. The shoot cultures contained alkaloids of the roots (ajmaline, yohimbine) as well as the alkaloids of the leaves (ajmalidine). Colorimetric estimation of the total alkaloids indicated that the shoot cultures contained 0.71%, the leaves 0.54% and the roots 2.64%.

Akram and Ilahi (1986) reported that using benzyl aminopurine (BAP) (2 mg/l) and NAA (0.8 mg/l) root callus of *Rauwolfia serpentina* was induced to bud formation which further developed into shoots. The isolated shoots rooted with 24 h treatment of 3mg/l IAA and 3 mg/l IBA. The plantlets transferred to soil were initially watered with half strength Knop's solution until they became autotrophic and were found to grow well in open field conditions. The root callus is, therefore, suggested as a potential tissue for the formation of plantlets in *R. serpentina.*

Schubel *et al.* (1986) isolated a novel highly substrate specific *Rauwolfia* enzyme, raucaffricine beta-D–glucosidase, from cell suspension cultures of *R. serpentina*. The enzyme was purified and its major characteristics were investigated. Its limited distribution in different cell cultures and differentiated plant revealed that the enzyme was present in significant amounts exclusively in cultured Rauwolfia cells.

Yamamato and Yamada (1986) maintained cultured *R. serpentina* cells on a modified MS medium for 13 years and these cells produced much more of the pharmecologically important alkaloids-ajmalicine (0.005-0.12%) and reserpine (0-0.003%)

Ilahi and Akram (1987) observed that young leaves of *Rauwolfia serpentina* inoculated on MS medium supplemented with NAA (1 mg/l) + Kinetin (K) (10 mg/l) and 10% coconut milk under 24 h. light induced callus, while 2,4-D induced callus under 16 h. light. Callus induction and its growth was more on explants taken from *in vitro* raised seedling on 2,4-D (1mg /l) or NAA (1mg/l) +K (10 mg/l) and 10% coconut milk under 24 h light. On sub-culturing the callus exhibited good growth. Similarly, leaf callus growth on Abou Mandour (AM) medium containing NAA, BAP, K, adenine sulphate and 2,4-D with 1g/l of casein hydrolysate showed excellent growth. The cultures analyzed showed 0.00686, 0.00489 and 0.00735% serpentine in calli growing on MS medium with NAA + K, 2,4-D and AM medium respectively. In the same cultures ajmaline was found at 0.0249, 0.0281 and 0.040%, respectively.

Yamamoto and Yamada (1987) reported that the cultured *R. serpentina* calluses consisted of cell colonies that had different fluorescences under 365 nm UV- light. These were divisible into two main categories, yellow-green and blue-white. The HPLC analysis had shown that the yellow-green fluorescent strains produced much reserpine, while the other variety produced much of 3,4,5-trimethoxybenzoic acid. A combination of 10M BA and 10M NAA enhanced production of reserpine in the yellow-green fluorescent variety.

Mathur *et al.* (1987) developed a tissue culture procedure for the establishment and propagation of a colchi-autotetraploid of *Rauvolfia serpentina* for possible commercial exploitation. Multiplication of autotetraploid shoots was obtained either through axillary bud elongation on MS medium containing 0.5 mg l^{-1} NAA and 0.05 mg l^{-1} kinetin, via multiple shoot formation on MS medium supplemented with 1.0 mg l^{-1} 6-benzyl amino purine and 0.1mg l^{-1} NAA. Rooting was induced by transferring the shoots to MS medium containing 1.5 mg l^{-1} NAA alone. The plantlets, thus formed, were tetraploid in nature by cytological observations of the root tips. They exhibited 80-90% success in establishment under glasshouse and field conditions.

Hampp and Zenk (1988) purified strictosidine synthase to homogeneity from suspension cells of *Rauvolfia serpentina* (920-fold purification, 35% yield). This enzyme which catalyses the stereospecific condensation of secologanin and tryptamine to H-3α (S)-strictosidine, is the key intermediate in monoterpenoid indole alkaloid biosynthesis. The specific activity was184 nkat/mg. The isolated enzyme was a single polypeptide, Mr 30000, possessing a 5.3% carbohydrate content. The enzyme had a pH-optimum at 6.5, a temperature optimum at 45°C, isoelectric point at pH 4.5, and apparent K_m values both for tryptamine and secologanin of 4 mM. The enzyme was immobilized and had, in this form, a half-life of 100 days at 37°C.

Ilahi *et al.* (1988) used various organs such as root, leaf and stem of *Rauwolfia serpentina* to induce callus formation and then organogenesis. Plantlet regeneration was achieved in root and stem calli by a combination of different growth hormones viz., NAA, IAA, IBA, BAP, kinetin. For this different media viz., White's Root Culture (WRC), Murashige and Skoog (MS) and Abou Mandour (AM) were utilized. Hormonal requirement differed with the explant source. A large number of plantlets was produced *in vitro* for further establishment under the natural conditions. Root, stem and leaf calli were analyzed for alkaloids. Ajmaline was the major alkaloids produced by cultures.

Alkaloids in such plants were higher in leaf and stem cultures than the parent plant.

Ilahi and Khan (1988) obtained root explants from mature plants and sub cultured juvenile aseptic seedlings for callus formation with 2,4-D, NAA and kinetin. Explants taken from seedling roots gave the best response for callus formation or WRC medium containing 1 mg/l 2,4-D and 100 ml/l coconut milk. Callus propagation in subcultures was found good both on initiation medium and on AM medium with BAP, K, AS, NAA, 2,4-D and caesine hydrolysate (CH) at 0.1, 0.3, 0.4, 1.0, 6.0 and 1000 mg/l. The alkaloids screened were serpentine, ajmaline, raubasine, raupine and reserpine. The major alkaloid present in cultures was ajmaline. Its maximum percentage was 0.0573% in the cultures grown under dark on AM medium, corresponding to an increase of 94.61% over cultures kept for 16 hr. in light.

Akram *et al.* (1990) observed the behavior on of field trials of root regenerated plants of *R. serpentina*. Root callus was induced to differentiate buds with 0.8 mg l^{-1}NAA and 2 mg l^{-1} benzyl adenine purine (BAP). Buds were rooted with 24 h. treatment of 1AA + 1BA (both 3 mg l^{-1}). Rooted buds were differentiated into autotrophic plantlets. The plants on transfer to soil thrived well and matured successfully to produce flowers and fruits. Karyotype analysis of flower buds showed pollen mother cells as having 22 chromosomes (2n), as in cultivated *Rauwolfia* plants.

Roja *et al.* (1990) reported that the multiple shoot cultures of *R. serpentina* were established from axillary meristems of the field grown plants on MS medium supplemented with BA (1.0 ppm) and NAA (0.1 ppm). Growth hormones influenced the morphogenetic events of the shoot cultures, such as root initiation in 1AA combinations, stunted shoot formation in 2,4-D and slender shoot formation in kinetin + NAA combinations The morphogenetic responses were associated with marginal changes in alkaloidal production. The cultures produced high levels of alkaloids including ajmaline (0.15%), ajmalidine and 3-epi-α-

yohimbine. The alkaloid concentrations in the cultures were comparable to the alkaloids in the roots of the intact plant.

Jocelyne and Cheniux (1991) cultured *R. vomitoria* mesophyll protoplasts in Murashige and Tucker liquid medium containing growth regulators. Calli produced shoots, however, rooting did not occur. Somatic embryos achieved different patterns of development.

Ruyter *et al.* (1991) compared the indole alkaloid content of *Rauwolfia serpentina* roots from regenerated plants (from stem and root callus) with the parental stock. Although the total alkaloid content appeared to be slightly higher in the roots from the regenerated plants, HPLC- analysis of individual alkaloids indicated that the contents of the alkaloids ajmaline, serpentine and reserpine were lower than in the roots of the parental stock. The glucoalkaloid raucaffricine was identified as a constituent of all samples, thus providing the first evidence for its occurrence in roots of *R. serpentina.*

Sharma and Chandel (1992) reported that on a standard shoot culture medium, nodal cultures of *R. serpentina* could be maintained for nine months at 25°C. Low temperature incubation of *in vitro* cultures appeared highly promising as cultures exhibited normal health even after 15 months of storage at 15°C. On the other hand, 10°C and 5°C were found deleterious to growth of *the R. serpentina* cultures.

Roy *et al.* (1995) studied *in vitro* culture method for the clonal propagation of *Rauvolfia serpentina.* They used shoot tips and lateral buds from field-grown plants as explants. When the explants were cultured on MS medium with 1.5 mg/l BAP + 0.5 mg/l NAA, multiple shoot buds were formed. Subculture in the same nutrient medium gave a higher number of shoots. When the regenerated shoots were excised and subcultured individually in the same nutrient medium, they also produced multiple shoots. The shoots had continued to proliferate through ten subcultures with average 25 shoots per transfer. For rooting, the shoots were

excised from the culture flask and implanted individually on root induction medium consisting of half strength MS salts supplemented with 1.0 mg/l each of IBA and IAA. Within 3 weeks of transfer 100% rooting was achieved on this medium. They were transferred to a tray containing soil and compost and covered by polyethylene sheets. After 2 weeks they were transferred to the open field where 95% of the plantlets survived.

Sharma *et al.* (1995) reported procedure for in vitro multiplication and *in vitro* conservation of six threatened endangered medicinal plants viz., *Colcus forskohilii, Gentiana kurroo, Picrorhiza kurroa, Rauvolfia serpentian, Saussurea lappa* and *Tylophora indica*, Various combinations of growth regulators were tested to select optimal medium for initiation and further shoot multiplication. Slow growth experiments were performed and the shelf-life of shoot cultures in multiplication and/or modified medium could be extended for 11-20 months depending on the species.

Sarker *et al.* (1996) reported that multiple shoots were induced from nodal segments and shoot apices *of Rauvolfia serpentina.* MS medium containing 1.0 mg/l BA and 0.1 mg/l NAA was found to give the best shoot proliferation rate. Callus formed at cut bases of the explants which produced shoots when sub cultured on media containing low concentration of BA (0.5 or 0.1 mg/l) and NAA (0.1 mg/l). The regenerated shoots were treated for 10 days in NAA or IBA supplemented media and then transferred to auxin omitted media for root initiation. Of the two auxins tested, NAA was found to be more effective than IBA and maximum rooting (83%) with 4-8 roots per shoot was recorded on the medium containing 1.0 mg/l NAA. The plants with well-developed root systems were transferred to pots containing soil and sand mixture and nearly 60% of the plant survived.

Ilahi *et al.* (1997) conducted tissue culture experiments using nodal explants on MS medium supplemented with various phytohormones. A pinkish-yellow callus appeared on nodal segments cultured on MS fortified with 1.5 mg/l

BAP and 10 mg/l 2,4-D. The induced callus could be easily proliferated on the same medium. Excellent callus was also induced when MS was supplemented with 0.5 mg/l each of IAA and BAP. Prolific shoot formation occurred on this callus when BAP and IAA were added at 1.0 mg/l each along with 5.0 mg/l adenine sulphate (AS). Shoot regeneration frequency could be further increased by culturing this organogenic callus on MS medium fortified with 1.0 mg/l BAP and 5.0 or 10.0 mg/l AS. This callus could be maintained on medium of the same hormonal concentration for an indefinite culture period without any obvious loss in its vigour. Roots could also be regenerated on the organogenic callus cultured on BM fortified with 1.5 mg/l Kn and 1.0 mg/l 2,4-D. Rooting of the young shoots occurred on a medium containing 1.0 mg/l each of IBA and NAA and 2.0 mg/l 2,4-D. These plantlets were hardened and transferred to field condition. All the important *Rauwolfia* alkaloids have been isolated from the callus, regenerated shoots and roots using various techniques. The alkaloid concentrations in the cultures were compared to those in the root of intact plant.

Recently Akhtar *et al.* (2001) has reviewed the hairy root culture of medicinal plants including *R. serpentina.*

SEED GERMINATION, VEGETATIVE PROPAGATION AND CULTURE OF DIFFERENT SPECIES OF *Rauvolfia* WITH SPECIAL INTEREST ON *R. serpentina.*

Abrol *et al.* (1956) carried out an experiment on the method of propagation of *R. serpentina* in Jammu and Kashmir state of India in March-April period. Seeds were procured from South India and were pretreated with different concentrations of H_2SO4 before sowing in the months of June and July. It was found that, treatment by 90% H_2SO4 for about 1 min gave as high as 60%-70% germination and seeds were germinated in about 3 weeks time.

Biswas (1956) made an effort for the experimental cultivation of *Rauwolfia serpentina* and *R. canescens* in the lower hill-ranges of Rongoalong, the border of Bhutan. The average rainfall at the place was 273 mm at an elevation between 1500 ft. and 38000 ft., temperature varied from maximum 87°F to minimum 46°F with high humidity. The highest percentage of rooting of cuttings after one month was 81.25 in June and 62.5 in May. The average percentage of rooting of cuttings planted from March to June was 58. The germination of the seed, however, varied from March to July was March-45 days 10 to 15%, April-34 days 15 to 20%, May-21 days, 25 to 30%, June-38 days 10 to 15% and July-48 days 5 to 10%.

Badhwar *et al.* (1956a) found a wide difference in percentage of germination of *Rauwolfia serpentina* Benth-seeds, and to find out the optimum temperature and humidity for their germination. They already collected some data on temperature and rainfall in Dehra Dun, India from March to July and their effect of raising the crop through root cuttings and stem cuttings during this period.

Badhwar *et al.* (1956b) studied the methods of propagation and their effect on root production in *R. serpentina.* The comparative performance of plants raised by different methods indicated that the sub-aerial portion and the root were the best in those raised from seeds. It was about 6.5 times that of roots produced by plants raised from stem cuttings and 3.5 times that of roots obtainable from plants grown from root cuttings. By planting small stumps of roots with a portion of the stem above the collar resulted in 100% success.

Sobti *et al.* (1956a) worked on the cultivation of *R. serpentina* in an experimental scale in different states of India. Classified seeds obtained from 8 sources by the 'Float and Sink' method into (1) light seeds-floating on water, (2) medium seeds-sinking in water but floating on 10% NaCl solution and (3) heavy seeds; sinking in 10% NaCl solution. It was found that germination was 4% in case of seeds floating on water, the percentages of germination was greatest

(about 20%) in seeds which sink in 10% saline solution, and floated on saline but sinking water was nearly 12%.

Sobi *et al.* (1956b) reported about the analysis of roots of one year, two years and three years old plants for their total alkaloids in *R. serpentina* was found that 1 year old plants had 1.6% which was slightly less than that of two and three years old plants had 1.7%. These results indicated that *R. serpentina* can be grown in Jammu and Kashmir state in India and its large scale propagation from seeds is possible provided the seeds are properly selected.

Hedayatullah (1959) reported a detailed study regarding the culture and propagation of *R. serpentina.* For the vegetative propagation of the plants, stem, root and leaf cutting were experimented with. It was found that both stem and root cuttings could be used successfully for the vegetative propagation, but leaf cutting had no propagative value. However, with seeds, among the freshly ripened fruits, dried fruits and peeled seeds, the peeled seeds were most suitable for propagation. Higher average percentage germination was obtained during the four months of the monsoon, i.e., May, June, July and August. The spacing of 1½' X 1' was most suitable for cultivation of *R. serpentina.*

Dutta *et al.* (1963) reported the different aspects of cultivation of *R. serpentina.* This plant could be propagated by seeds, root cuttings, stem cuttings and root stumps. A success of 51 to 82% was observed with root cuttings. 40 to 65% success was obtained by stem cuttings. However, better results were obtained after applying 40 ppm solution of IAA. Outstanding success of 90 to 95% survivals was met with root stumps. The limitation of this method in extensive plantations was that it required a large number of root stumps. Among these methods of propagation, optimum yield was obtained when propagation was by seeds. It was found that there was no significant increase in the yield of roots as a result of the application of fertilizers. In some cases, slight increases were recorded with superphosphate. Spacing of 45 cm between the rows and 30 cm

between the plants was recommended. It was most economical to harvest roots after 15 months of planting.

Mitra (1976) studied the factors responsible for the formation of non-viable and viable seeds of *R. serpentina*. Inflorescenes were ontogenetically axillary. Flower primordia developed up to the stage of opening of flowers in 30-35 days and into mature fruits in another 40-45 days. Pollen grains were highly fertile which could not be a cause for formation of non-viable seeds; pollen and stigma were found compatible as shown by high percentage of fruit setting in self-pollinated flowers. Hardly any seed without embryo and endosperm was found, seeds of fully mature black fruits were of 2 kinds, one with mature embryos enclosed in solid endosperm and the other with immature embryos within shriveled and desiccated endosperm. Non-viability of seeds was due to shriveling up of endosperms, which in turn arrested the development of immature embryos to become mature. Degree of temperature and percentage of humidity prevailing in different months caused shriveling up of endosperm at its milk stage to form varying number of non-viable seeds in different months. Harvesting of ripe black fruits in May gave the highest percent (85) of viable seeds and the lowest percent (15) of non-viable seeds.

Maheswari *et al.* (1982) tested germination of two varieties of *R. serpentina* seed, collected from Regional Research Laboratory Jammu-Tawi (JT) and Araku Valley (AV) of Vishakhapatham. Out of 128 seeds of JT variety, 110 seeds (86%) germinated, whereas out of 120 seeds of AV variety, 64 seeds (53.3%) germinated. Use of fresh seeds in place of stem/root cuttings for viable propagation was suggested.

Mohammad and Shukla (1986) reported that the use of pesticides was not suitable for maximal sprouting from roots cuttings of *R. serpentina*. Nutrient carriers combined with growth hormones were useful in hastening up the sprouting and emergence of healthy plants.

Chandra (1956a) took hand wood cuttings of *Rauwolfia canescens* L, 12 to 15 cm in length and varying diameter (0.4 to 1.0 cm) and treated them with 30 ppm solution of Indole acetic acid and naphthalene acetic acid for 12 hours. Controls were similarly treated with water only and all were planted in pots with sand under irrigation. It was observed that in the beginning all the cuttings gave out new leaves. For more than one month all sets including controls had green leaves, but later the leaves of controls started wilting. The leaves of treated ones continued to be green. After one month and a half the treated cuttings started production of callus. The observations were continued further till no more mortality was observed.

Chandra (1956b) further reported that *R. serpentina* preferred clayey soils. In the neighbourhood of Lucknow, *R. serpentina* plants were found to flourish well under mango trees. In view of this, it was suggested that cultivation could profitably to be carried in mango orchards, which provide sufficient shade and other suitable condition for the best growth of *R. serpentina*.

GROWTH PHYSIOLOGY OF *Rauvolfia serpentina* UNDER VARIOUS TREATMENTS AND CONDITIONS WITH SPECIAL INTEREST ON THEIR PRODUCTIVITY OF ALKALOID CONTENT

Hedayatullah (1959) reported that the economic harvesting age of *R. serpentina* in respect of yield per acre and alkaloids content was between 12 months and 18 months after transplantation. It was observed that the yield of root varied between 1500 lbs and 300 lbs per acre.

Siddiqui *et al.* (1959) reported that different plant growth factors–both external and internal may affect the alkaloids content of the plant. Age of the routine of *Rauwolfia serpentina* had influence on the alkaloidal content. The young roots had relatively lower alkaloids content than that of two years old plant.

Dhar (1965) studied the variation in the alkaloid content and morphology of four geographical races of *R. serpentina*. These races were collected from Rangu, Rishikesh, Calcutta and Dehra Dun. The plant collected from Rishikesh was morphologically different from all other races. The alkaloid content was highest in Rishikesh race (2.66%) being double than that of the Calcutta race which contained 1.35 %, Rangu and Dehra Dun races were intermediate. The results suggested that it would be possible to select high alkaloid-containing races of *R. serpentina* for large scale cultivation.

Biswas (1969) studied the growth and changes in the alkaloidal contents of *R. serpentina* under complete defloration treatment. Plants under complete defloration were bushy in form as a result of intensive vegetative growth, both in terminal and lateral shoots, which caused proportionate increase in root growth. The roots were more branched with profuse production of fibrous roots compared to control. Complete defloration resulted in large increase (62.9% in fresh and 56.50% in moisture free root, respectively) of root weight. An appreciable increase (18.00% based on moisture free root) of total alkaloid content per unit weight of the moisture free root was also observed.

Saini and Mukherjee (1970) reported the effect of complete and partial defloration on the root yield of *R.serpentina* under low and high levels of nitrogen fertilization. Reproductive growth, even when partially reduced, limited root growth. Extra application of nitrogen resulted in an increase in reproductive growth but was without any effect on root yield. A complete defloration increased root weight and also caused the plants to respond to extra application of nitrogen. The onset of dormant period was delayed by about a month under complete defloration. The increase in root weight was due to a proportionate increase in stem and leaf growth caused by defloration treatments. The alkaloid content per unit weight of dry root did not change with complete defloration.

Biswas (1970) also reported the results of four treatments of defloration (including control) with two nitrogen levels on total yield of root and alkaloid

content of *R.serpentina*. Defloration treatment consisted of partial defloration in which each of the inflorescences was cut retaining 10 or 5 flowers. The results revealed that a substantial increase in the moisture free weight of the root was due to complete defloration. The increase was more marked at high nitrogen level. In the partially deflorated plants, the effect of defloration was non-significant even at high nitrogen level. There was also a substantial increase in the alkaloid extent with complete defloration, at both levels of nitrogen. No such differences were noticed for partial deflorations.

Biswas (1971) observed the effect of complete defloration treatment on the differentiation of wood and bark of the root and total alkaloidal contents of *R. serpentina*. Complete defloration resulted in increased production of moisture-free intact root, root wood and root bark to the extent of 28.20, 20.26 and 38.80% over those of the control. An increase in the total alkaloid contents (0.87, 0.01 and 0.80% of moisture-free intact roots, root woods and barks, respectively) was also observed in the roots of plants under complete defloration. The root wood and bark ratio was noticed to be lowered in completely deflorated plants, which produced much thicker barks as compared to the root wood.

Later Biswas (1973) made an investigation to study the effect of complete defloration on the growth and development, yield of moisture-free root and shoot and changes in the alkaloidal (total, reserpinoid and residual) mineral (N, P and K), sugar (total, glucose and sucrose) and ash contents of roots of *R. serpentina*. Complete defloration had a profound influence on the growth and development. The increase in the total yield of moisture-free roots per plant under complete defloration was intimately associated with the appreciable increase in the total, reserpinoid and residual alkaloids per unit of moisture-free root. The increased sugar and mineral contents had direct bearings of the production of the alkaloids.

Nandi and Chatterjee (1975) made an attempt to increase the total alkaloid yield of *R. serpentina* by N:P:K trials either singly or in combination. It was found that alkaloid biogenesis in root remained inversely correlated with laminar area.

Those treatments, which distinctly reduced lamina area, enhanced the total alkaloid content. Maximum alkaloid synthesis was noticed in combination with N:P:K and this increase in the content of total alkaloid, gained momentum with the advancement of age.

Nandi and Chatterjee (1978) also studied some of the very important areas of growth and physiology of eleven medicinal plants including *Rauvolfia serpentina* with special reference to synthesis of active principles in relation to light and growth hormones. In *R. serpentina,* enhancement of alkaloid synthesis had been revealed in higher photoperiodic cycles only. In the species, post-reproductive stage showed enhanced aklaloid synthesis and increase was about 7% over the vegetative stage. GA_3 treatment also promoted the synthesis of alkaloid, suggesting that the mechanisms of biogenesis of alkaloid were equally sensitive towards GA_3 as well as light.

Biswas (1982) evaluated correlation at the genotypic level between some characters and root yield and alkaloid content in two populations of *R. serpentina.* The root yield showed highly positive correlation with branch per plant in the clonal population, serving as a good indicator of root production. A significantly positive correlation was observed between root yield and alkaloid content. Clonal population showed better association between different characters than the seedling population.

Maheshwari *et al.* (1985) reported that at both Indore and Akola, the net returns were higher when *Rauvolfia* was intercropped than when grown alone. At Indore, soybean in the wet season and garlic in winter were most suitable and remunerative intercrops followed either by chilli in the wet season and onion in winter or soybean in the wet season and onion in winter. *Rauvolfia,* soybean and garlic in association gave the highest total net returns per hectare (Rs. 37,952), followed by *Rauvolfia,* chilli and onion association (Rs. 36, 538) and *Rauvolfia,* soybean and onion association (Rs. 35,917). At Akola, soybean in the wet season followed by onion in winter was the most remunerative intercrop (Rs. 28,630/ha),

followed by *Rauvolfia*, soybean and garlic combination (Rs. 26,360/ha) and *Rauvolfia*, chilli and onion association (Rs. 26,011/ha). The quality of the roots of intercropped *Rauvolfia* was comparable to pharmacopoeial standard.

Haqh *et al.* (1986) treated *Rauvolfia serpentina* plants with 500 and 1000 ppm potassium napthenate (KNap) to study the effects on a number of vegetative, reproductive and biochemical parameters. Treatment with 500 ppm KNap caused little or no effect on any of the parameters studied. However, treatment with 1000 ppm KNap resulted in significant increase in plant height (26%), number of leaves (36%), leaf area (29%), number of branches (63%), number of inflorescences (84%), number of fruits (75%), total alkaloid content of roots (23%), total alkaloid content of leaves (5%) and reserpine content (11%), ajmalicine content (6%) and sterol content (3%) of the roots.

Maheshwari *et al.* (1988) studied the effect of N and P fertilizers on the yield of roots, its alkaloid content and alkaloid yield of *R. serpentina*. An increase in the level of N greatly increased dried root yield (maximum at 45 kg N/ha). The total alkaloid content in root and its yield remained higher at 30 kg N/ha followed by 45 kg N/ha compared with the control. An increase of the level of P appreciably increased the dried root yield. The maximum was recorded at 60 kg P/ha, beyond which it declined. The total alkaloids and their yield were higher at 60 kg P/ha, the biometric traits such as plant height, dried root weight, dried root: shoot ratio and diameter of root increased with N and P by 45 and 60 kg/ha, respectively. These two levels also gave higher net monetary return.

Maheshwari *et al.* (1991) conducted an experiment to schedule the irrigation for *R. serpentina* on a shallow black soil. The irrigations were given on the basis of cumulative pan evaporation (50 mm) at irrigation water: cumulative pan evaporation ratio of 0, 0.15, 0.30, 0.45, 0.60, 0.75 and 0.90. The dry root and alkaloid yield and water use efficiency were increased upto an IW: CPE ratio of 0.75. The alkaloid content was not affected by irrigation schedules. The maximum net returns/ha were obtained at an IW: CPE ratio of 0.75.

Sethi *et al.* (1991) studied the variation of chemo-botanical characters in the indigenous collections of *R. serpentina.* The highest range of variations for total root weight/ plant and number of secondary roots/ plant was observed in the collections from Coondapur and Conacana regions of Karnataka and Goa, respectively. These two characters contributed to high alkaloid recovery. The Coondapur region gave the highest range (1.58–2.03) of total alkaloids with of mean of 1.81. The same pattern was observed for reserpine content with the range of 0.07-0.24 and mean value of 0.16%. Such chemo-botanical variations in these materials were natural due to their geographic, ecological and topographic variation.