Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 93, No. 4, May 1984, pp. 661–676.

Alkaloids from Indian medicinal plants and their biosynthesis

D S BHAKUNI
Central Drug Research Institute, Lucknow 226001, India

Abstract. The chemical, pharmacological and biosynthetic status of alkaloids from plants which either have a reputation in folklore medicine or the extracts of which have shown consistent biological activity in a broad biological screen has been reviewed.

Keywords. Medicinal plants; alkaloids; biological activity; synthesis; biosynthesis.

1. Introduction

Alkaloids occupy a unique place in the family of natural products and provide challenging problems for structural elucidation, synthetic and biosynthetic studies. Recognising the importance of alkaloids and alkaloid biosynthesis coupled with our interest in biologically active natural products, we initiated some 15 years ago, at the Central Drug Research Institute, Lucknow, a study of various aspects of alkaloids of Indian plants which either show a consistent biological activity when screened or have a reputation in folklore medicine. The results of chemical, pharmacological and biosynthetic studies carried out on alkaloids from these plants have been reviewed.

2. Alkaloids from medicinal plants

A search for hypotensive agents in the active alkaloidal fraction of the alcoholic extract of the leaves and stems of *C. sparsiflorus* (Euphorbiaceae) (Bhakuni *et al* 1969), a South American plant which has now completely naturalised and grows as a weed in the plains of India, led to the isolation of proaporphines, crotsparine (1) (Bhakuni and Dhar 1968), N-methylcrotsparine (2), N,O-dimethylcrotsparine (3), dihydroproaporphine, crotsparinine (4) (Bhakuni and Dhar 1969) and the known aporphine, sparsiflorine (6) (Chatterjee *et al* 1965). The structures and stereochemistries of the new bases have been assigned (Bhakuni *et al* 1970b).

Proaporphines (Stuart and Cava 1968) are important biogenetic precursors of aporphines and the latter are also obtained chemically from proaporphines by the dienone-phenol and dienone-benzene rearrangements (Barton and Cohen 1957). The absolute configuration of proaporphines has been assigned with the aid of CD data (Snatzke and Wollenberg 1966) and by comparison of the optical rotation of dienone bases and the corresponding aporphines and also by chemical degradation procedure (Cava *et al* 1964a/b). The absolute configurations of the proaporphine alkaloids of *C. sparsiflorus* have been determined by direct synthesis from 1-benzyltetrahydroisoquinolines (Bhakuni *et al* 1972a/b).

Crotoflorine (Chatterjee and Majumdar 1968) and jacularine (Stuart *et al* 1968) which have been isolated as their acetates from *C. sparsiflorus* and *C. linearis*

respectively, are most probably identical with the acetate of crotsparine and crotsparinine respectively.

Crotsparine (1), N-methylcrotsparine and N-methylcrotsparinine are hypotensive agents (Dubey *et al* 1969). N-Methylcrotsparinine (5) produces a sharp but shortlived fall in blood pressure which could be antagonised by atropine to a large extent suggesting thus a cholinergic mechanism of action. N-Methylsparsiflorine methoiodide is a potent neuromuscular blocking agent. At 5 to 10 mg/kg (i.v.) in cats it produces 100% blockage of neuromuscular transmission for 30 to 60 min. Glaziovine, an enantiomer of (S)-N-methylcrotsparine, is a potent tranquillizer devoid of depressent effects.

In a routine biological screening a 50% ethanolic extract of *Cocculus pendulus* Diels. (Menispermaceae), a shrub that grows in dry parts of North and Western India, showed hypotensive and anticancer activities (Bhakuni *et al* 1969). A detailed chemical investigation of the active fraction of the ethanolic extract furnished six bisbenzylisoquinolines, pendulin (Gupta *et al* 1970), cocsolin (Joshi *et al* 1974), cocsulin (Bhakuni *et al* 1970), cocsulinin (Joshi *et al* 1974), pendulinin and pendin. Of these bases pendulin (7) showed hypotensive activity and cocsulinin (8) was found active against cells derived from human carcinoma of the nasopharynx (9 KB). An account of chemical work leading to the assignment of the structures and stereochemistries of new bases of *C. pendulus* has been reported (Bhakuni and Joshi 1975).

Cocculus laurifolius DC. (Menispermaceae), an ever green shrub grows in tropical and subtropical regions of the world. A 50% ethanolic extract of the leaves when tested hypotensive and neuromuscular activities were confirmed (Bhakuni and Dhar 1969). A systematic study of the active fraction of the alcoholic extract led to the isolation of ten new abnormal *Erythrina* alkaloids, isococculidine (12) (Bhakuni *et al* 1976), isococculine (13) (Singh *et al* 1978), coccuvine (14) (Singh *et al* 1976), coccuvinine (15) (Singh and Bhakuni 1977), cocculitine (16) (Singh *et al* 1977), cocculitinine (17), cocculidinone (18), cocculimine (20), coccudienone (19), coccoline (21) and coccolinine (22) (Pande *et al* 1976) and three new dibenz[*d,f*] azonines, laurifonine (24), laurifine (25) and laurifinine (26) (Pande and Bhakuni 1976). In addition to these new bases known morphinan-dienone alkaloid, sebiferine (23) (Sivakumaran and Gopinath 1976); two proaporphines, stepharine (27) and N-methylstepharine (28) (Cava *et al* 1964); five quaternary aporphines, magnoflorine, laurifoline, chlorides, isocorydine, O-methylisocorydine and boldine methochlorides and benzylisoquinolines, coclaurine, N-methylcoclaurine, reticuline, and laudanidine have also been isolated. The structures and stereochemistries of the new abnormal *Erythrina* alkaloids and dibenz [*d,f*] azonine bases have been determined (Bhakuni and Jain 1980).

Isococculidine (12), the major alkaloid of the leaves of *C. laurifolius* has been found to have neuromuscular blocking and hypotensive activities (Kar *et al* 1976), cocculidine (10) and cocculine (9) exhibit hypotensive activity. This activity in these alkaloids has been found due to ganglionic blocking action. The quaternary aporphines, O-methylisocorydine, isocorydine, and boldine methochlorides exhibit *d*-tubocurarine like curarising action on sciatic skeletal muscles. These quaternary bases also induce hypotensive effects in dogs, cats and rabbits. This activity has been found due to considerable ganglionic blocking action on various sympathetic and para-sympathetic ganglia.

Extracts of the roots of *Thalictrum foliolosum* DC (Rannunculaceae), a tall perennial herb, are used by the natives for the treatment of many ailments. A 50% ethanolic extract of the plant when screened, spasmolytic activity was confirmed (Dhawan *et al*

1980). A systematic chemical study of the active fraction of the alcoholic extract gave a new aporphine, N,O,O-trimethylsparsiflorine and known bisbenzylisoquinolines, (Chatterjee *et al* 1952; Gopinath *et al* 1959; Vashishtha *et al* 1941) thalidasine and thalrugosidine, benzylisoquinoline-aporphine base, thalicarpine, benzylisoquinoline, reticuline, berberine bases, berberine and palmatine and quaternary aporphine, magnoflorine (Bhakuni and Singh 1982). Of these bases thalicarpine (29) (Shamma and Rothenberg 1978) produces transient hypotensive effects in cats and exhibits significant inhibitory activity against Walker carcinosarcoma 256 in rats. Thalrugosidine (31) (Shamma *et al* 1967) exhibits antimicrobial activity. Thalidasine (30) (Kupchan *et al* 1967) has significant inhibitory activity against Walker intramuscular carcinosarcoma 256 in rats. The base also shows hypotensive and antimicrobial activities. Berberine and palmatine exhibit antimicrobial activity against a wide variety of micro-organisms including fungi and protozoa (Amin *et al* 1969). Berberine in addition shows cytotoxic and neoplasm inhibitory effects against tumor cells. The base markedly inhibits acetylcholinesterase both *in vivo* and *in vitro* thereby causing temporary decrease of blood pressure. Magnoflorine exhibits marked hypotensive activity. Thalidasine, thalicarpine, thalrugosidine, and N,O,O-trimethylsparsiflorine have been screened for antibacterial and antifungal activities and found to be inactive (Srivastava 1982).

About 250 *Corydalis* species occur in the Himalayan region and Khasi hills. *C. meifolia*, a perennial herb with underground tubers, is one of these species and grows at an altitude of 12000 to 15000 feet. Extracts of many *Corydalis* species are reported to be efficacious in many ailments in the Ayurvedic system of medicine. Ochotensine, an alkaloid that occurs in many of these species, has been reported to stimulate isolated guinea pig and rabbit uterus and induces a fall in blood pressure in anaesthetised cats.

Although a number of *Corydalis* species have been investigated and a variety of 1-benzylisoquinoline derived alkaloids have been isolated, *C. meifolia* wall appeared to have escaped the attention of chemists and pharmacologists. The plant was, therefore, collected from Kedarnath, UP, India. A 50% ethanolic extract of the plant material was prepared, screened and spasmolytic activity was confirmed in the extract (Dhawan *et al* 1980). Careful chromatography of the active fraction on silica gel gave six tetrahydroprotoberberines, (+)-sinactine, apocavidine, stylopine, (+)-cavidine, cheilanthifoline, and dehydrocavidine, two spirobenzylisoquinolines, yenhusomine and yenhusomidine, one phthalideisoquinoline, corlumine, one benzophenanthridine, dihydrosanguinarine and protopine (Bhakuni and Chaturvedi 1983). Of the isolated bases, dehydrocavidine is a new base. The remaining alkaloids, although known, have been isolated for the first time from the plant. A number of alkaloids isolated from *C. meifolia* exhibit biological activity. Corlumine is used as convulsant. Subconvulsive doses of the base increase the frequency and depth of respiration. The base has little effect on profused frog heart. Its effects on isolated intestine and uterus are irregular. Protopine has an inhibiting action on isolated frog heart muscles or nerve and a stimulating action on guinea pig intestine. The base has been screened against transplant hard mouse tumor sarcoma 180 and Ehrlish mouse carcinoma. Inhibition of tumor induced by protopine was significant and associated with considerable cytotoxic side effects. Protopine when injected intravenously in rabbits and guinea pigs, exerts a moderate pressor effects and sensitized the animal to induction of cardiac arrythmias by adrenaline. In small doses the base has a narcotic action in frog, while large doses abolish reflex activity and show a curare like action. Large doses (18 mg to 200 mg/kg) when given parenteraly to

experimental animals, induce excitement or convulsions. Small doses, slow the heart, lower blood pressure and has quietening effect. Spirobenzylisoquinoline, yenhusomine, is an analgesic and hypotensive. Cavidine, protopine, corlumine, yenhusomine and dehydrocavidine have shown moderate to high order of spasmolytic activity (Patnaik 1983).

C. cornuta and *Dicentra macrocapnos* Prain. (Papaveraceae) have good reputation in folklore medicine. These plants were, however, not investigated chemically. These were, therefore, collected from the Himalayan region from an altitude of 7000 to 8000 feet. Chemical examination of these furnished (−)-stylopine, protopine and (−)-coreximine (Jain and Bhakuni 1977). *C. govaniana* wall., a perennial herb with bright yellow flowers, grows in the Western Himalayas at an altitude of 2500 to 4000 metres, was yet another important medicinal plant. Systematic chemical investigation of the plant has given three new protoberberines, govadine (Mehra *et al* 1976), govanine (Mehra *et al* 1976), corygovanine and a known phthalideisoquinoline base, bicucculine. The structures of the new bases have been assigned (Mehra *et al* 1976).

Extracts of the rhizomes of *Stephania glabra* Miers. (Menispermaceae), a glabrous climber indigenous to the lower Himalayas (5000 to 6000 feet) have long been used by the natives as antidysenteric, antipyretic and antiasthmatic. A 50% ethanolic extract of the rhizome when screened, hypotensive and spasmolytic activities were confirmed in the extract (Dhar *et al* 1968). A search for active principles in the active fraction led to the isolation of two bisbenzylisoquinolines, cycleanine and N-desmethylcycleanine, five tetrahydroprotoberberines, capaurine, corynoxidine, tetrahydropalmatine, corydalmine and stepholidine, two proaporphines, stepharine and pronuciferine and four quaternary protoberberine salts, palmatine, dehydrocorydalmine, jatrorrhizine and stepharanine (Bhakuni and Gupta 1982). Many of these alkaloids exhibit biological activity. Tetrahydropalmatine hydrochloride causes hyperthermia in rats. Palmatine has been found to possess ACTH-like bactericidal and anticholinesterase effects. It has been concluded that palmatine, dl-tetrahydropalmatine and ergot alkaloids have an analogous pharmacological mechanism. Stepharine has been reported to have antihypertensive properties. Cycleanine is an antitumor agent. Structure activity relationship has been studied in capaurine for emetine type activity. pronuciferine hydrochloride shows good spasmolytic activity. There was 75% blockage at a dose of 25 microgram when tested on the tissue ileum of guinea pig.

S. elegans is yet another interesting medicinal plant which has not been investigated chemically. The plant was, therefore, collected from Dehradun and systematic investigation resulted in the isolation of epihernandolinol, N-methylcorydalmine, hasubanonin, aknadinin, cyclanoline, magnoflorine, isotetrandrine, isochondodendrine and cycleanine (Singh *et al* 1981). Many of these alkaloids are well known for their biological activities.

Several species of *Litsea* (Lauraceae) are found in India. Extracts of many of these species are reported efficacious in the treatment of various ailments. A 50% ethanolic extract of *L. glutinosa* var. *glabraria* exhibited spasmolytic activity (Bhakuni *et al* 1969). The extract of *L. wightiana* showed significant spasmolytic, hypothermic and blood pressure lowering activities (Dhar *et al* 1974). Investigations of the biologically active fraction of the alcoholic extract have yielded six aporphines, norboldine, boldine, laurotetanine, N-methyllaurotetanine, actinodaphnine, and N-methylactinodaphnine (Tewari *et al* 1972). Glaucine, boldine, norboldine, isoboldine, norcorydine and laurotetanine have been obtained from *L. wightiana* (Bhakuni and Gupta 1983). From

L. sebifera, boldine, laurotetanine and actinodaphnine have been isolated (Uprety *et al* 1972). The alkaloidal constituents of *Actinodaphne obovata* Bl., collected from Eastern Himalayas and Assam have also been investigated and aporphines, laurotetanine, N-methyllaurotetanine, and actinodaphnine have been isolated (Bhakuni *et al* 1972).

A 50% ethanolic extract of the leaves of *Annona squamosa* L. (Annonaceae), a small evergreen tree which grows wild and is also cultivated throughout India for its fruits, exhibited anticancer activity. Search for active principles led to the isolation of corydine, a base reported to have anticancer activity and six other aporphines, anonaine, roemerine, norcorydine, norisocorydine, isocorydine and glaucine (Bhakuni *et al* 1972). Of these alkaloids glaucine is reported to cause narcosis in animals. Boldine has sedative properties. It is a cardiotonic and its toxicity is quite low, Glaucine and laurotetanine increase antimitotic effects of colchicine. The occurrence of anonaine, norlaureline, glaucine, corydine and isocorydine in *A. squamosa* is of biosynthetic interest.

Alangium lamarckii Thw. (Alangiaceae), *Senecio tenuifolius* Burm. (Compositae), *Retanilla ephedra* Brogn. (Rhamnaceae), *Aristotelia chilensis* (Eleocarpaceae) and *Discaria crenata* (Rhamnaceae) all are respectable plants in folklore medicine. The alkaloidal constituents of these plants have been investigated. Two new indole alkaloids, alangimarckine and ankorine have been isolated from *A. lamarckii* (Battersby *et al* 1966). The occurrence of dihydroprotoemetine with tubulosine and deoxytubulosine in *A. lamarckii* is of considerable biosynthetic interest and this holds also for ankorine and alangimarckine. Four macrocyclic diester pyrrolizidine alkaloids, sencionine, senkirkine, O-acetylsenkirkine, and integerrimine have been obtained from the leaves and stems of *S. tenuifolius* (Bhakuni and Gupta 1982). Senecionine and integerrimine generally co-occur in *Senecio* species. These bases are, however, present in *S. tenuifolius* as minor constituents only. Integerrimine is an ester of integerrinecic acid whereas senecionine is the ester of the *trans*- isomer, senecic acid. From the roots of *R. ephedra*, the peptide alkaloids, integerresine and crenatine A, the aporphines, boldine and norboldine and the benzylisoquinolines, armepavine, norarmepavine, coclaurine and N-methylcoclaurine have been isolated (Bhakuni *et al* 1974). The unusual indole alkaloids, aristoteline and aristotelone have been obtained from *Aristotelia chilensis* (Bhakuni *et al* 1970).

3. Synthesis of alkaloids

(+)-Ophiocarpine, a minor alkaloid of the leaves and stems of *Cocculus pendulus* Diels, has been assigned the structure and configuration as shown in 32. A synthesis of (±)-ophiocarpine from berberine has been achieved (Bhakuni *et al* 1982). Antofine (33) and alkaloid C (34) are important phenanthroindolizidine alkaloids. These bases have been synthesized from 2-(3-hydroxy-4-methoxyphenyl)-1,6,7,8,8a-pentahydro-3-(4-hydroxyphenyl)-indolizin-4-one (Bhakuni *et al* 1982). Laurifonine (24), a dibenz [*d,f*]azonine alkaloid isolated from *Cocculus laurifolius* has been prepared from 4,4',5-trimethoxy-2,2'-biphenylaldehyde (Bhakuni and Mangla 1981). A biogenetic type synthesis of the phenanthroindolizidine alkaloid, tylophorine, has been reported (Mangla and Bhakuni 1980).

Tetrahydroprotoberberines, scoulerine, coreximine, tetrahydropalmatine and related compounds are of considerable biosynthetic interest. 1-Bromoscoulerine, 1,12-

dibromo scoulerine, 1-bromotetrahydropalmatine and 1,12-dibromotetrahydropalmatine and the corresponding mono- and dibromoreticuline derivatives could be utilised for 'aberrant biosynthesis'. (±)-Scoulerine, tetrahydropalmatine, coreximine and their 1-bromo- and 1,12-dibromo- derivatives have been synthesized by Mannich condensation of the 1-benzyltetrahydroisoquinolines with formaldehyde, using bromine as a protective group (Bhakuni and Kumar 1983). A convenient synthesis of septicine, a secophenanthroindolizidine alkaloid, has been developed (Mangla and Bhakuni 1980).

4. Biosynthesis of alkaloids

Reticuline (35), the putative precursor of a large number of 1-benzylisoquinoline derived alkaloids (Bhakuni 1976) is a good model to study some of the aspects of early stages of the biosynthesis of 1-benzylisoquinoline derived alkaloids. Since 1910 it has been believed that dopa gives rise to both the 'halves' of norlaudanosoline from which reticuline could be derived in Nature. Feeding experiments revealed that dopa in fact contributes only to the formation of the phenethylamine part of reticuline in *Litsea glutinosa* and the benzylic portion is biosynthesized from 3,4-dihydroxyphenylpyruvic acid not derived from dopa. This is a most surprising result (Tewari *et al* 1975). Other aspects of the biosynthesis of reticuline have also been studied and it has been demonstrated that trihydroxylated 1-benzyltetrahydroisoquinolines are not the precursor of reticuline. There is no specificity of O-methylation in the biosynthesis of reticuline. However, N-methylation of norreticuline is a specific process. Feeding experiments suggest that O-methylation precedes N-methylation in the biosynthesis of reticuline (Bhakuni *et al* 1977).

Coclaurine (36) is an established precursor of proaporphine, aporphine and bisbenzylisoquinoline alkaloids (Bhakuni 1976). Feedings of tyrosine, tyramine, dopa, dopamine and 4-hydroxyphenylpyruvic acid in *Annona reticulata* revealed that while tyramine, dopa and dopamine contribute to the formation of the phenethylamine portion of coclaurine, tyrosine and 4-hydroxypyruvic acid are incorporated into both halves. Tracer experiments have shown that coclaurine is biosynthesized *via* the intermediacy of nor-coclaurine-1-carboxylic acid, 1,2-didehydronorcoclaurine and norcoclaurine (Prakash *et al* 1979).

Papaverine (37), one of the major 1-benzylisoquinoline alkaloids of *Papaver somniferum* is clinically used as an antispasmolytic agent. The main effect of papaverine hydrochloride is relaxation of smooth muscles. Papaverine has been shown to be biosynthesized in *P. somniferum* from two units of tyrosine *via* norlaudanosoline and ror-reticuline. The dehydrogenation of the 1-benzyltetrahydroisoquinoline precursor is an important step in the biosynthesis of papaverine. The mechanism of this reaction could be step-wise or it could proceed in a concerted manner. Further, this process could occur in a partially methylated 1-benzyltetrahydroisoquinoline precursor, such as nor-reticuline or it could take place in a completely methylated derivative such as norlaudanosine. The efficient incorporation of (±)-norlaudanosine into papaverine when coupled with the earlier data that 1,2-dehydroreticuline did not participate in the biosynthesis of papaverine clearly demonstrated that the dehydrogenation step occurs after complete methylation, presumably at the norlaudanosine level and probably in a concerted manner. Since biological methylation normally occurs in a definite sequence,

partial O-methylation of nor-reticuline at C-7 or C3′ could give rise to norlaudanidine or norcodamine respectively. Norlaudanosine can then of course be reached from both of these isomers by further O-methylation. However, when norcodamine and norlaudanidine were fed in parallel experiments to *P. somniferum*, the former was incorporated 10 times less efficiently than the latter, suggesting strongly that norlaudanidine is an intermediate between nor-reticuline and norlaudanosine. Parallel feeding experiments with reticuline and norreticuline demonstrated that N-demethylation of reticuline is not a favoured process in the biosynthesis. Although papaverine does not possess an asymmetric centre, yet enzymatic reactions are generally stereospecific and one can expect that either of the enantiomers of norreticuline would be the biological precursor of papaverine. (−)- and (+)-, norreticulines when fed, papaverine was exclusively biosynthesized from (−)-norreticuline (Uprety *et al* 1975).

According to the most accepted biogenetic theory (Barton and Cohen 1957), oxidative cyclisation of coclaurine (36) could give rise to proaporphine alkaloid, crotsparine (1). The dihydroproaporphine, crotsparinine (4) could be formed by reduction of one of the double bond of crotsparine type intermediate and the aporphine alkaloid, sparsiflorine (6) would arise from crotsparine by dienone- phenol rearrangement. It has been demonstrated by feeding labelled (±)-coclaurine, (±)-isococlaurine and (±)-norcoclaurine to *Croton sparsiflorus* plants that crotsparine, dihydrocrotsparine and sparsiflorine are specifically biosynthesized from (±)-coclaurine. The presence of this key intermediate in *C. sparsiflorus* has been confirmed by trapping experiments (Bhakuni *et al* 1974). Crotsparine and crotsparinine have opposite configuration while sparsiflorine and crotsparine have the same configuration at the corresponding asymmetric centres. If crotsparine is a biological precursor of crotsparinine, a change in configuration should occur at position C-6a in the isoquinoline moiety of the proaporphine during the course of biochemical transformations. Alternately crotsparine and crotsparinine could be biosynthesized by independent routes. Tracer experiments have confirmed that the latter route is followed in the biosynthesis of these bases. The biosynthesis of N-methylcrotsparine, N-methylcrotsparinine and N-methylsparsiflorine have been studied in detail and results have been reported (Bhakuni and Jain 1981).

The biosynthesis of yet another proaporphine alkaloid (Haynes *et al* 1965), crotonosine (45) has also been studied. Tracer experiments with (±)-, (+)- and (−)-, coclaurines demonstrated specific incorporation of (+)-isomer into crotonosine, isomeric (±)-isococlaurine was not a precursor of the base although (±)-norcoclaurine was incorporated. The evidence obtained has supported the view that oxidative cyclisation of (+)-coclaurine to a dienone is involved. Double labelling experiments involving the methoxy group of (±)-coclaurine showed that most but not all, the methoxy activity was lost in conversion into crotonosine (Barton *et al* 1967).

Boldine (38), the choleretic principle of *Peumus boldus* Molina, is an attractive model to verify some of the current views on the biosynthesis of aporphine alkaloids. The incorporation of (±)-norprotosinomenine, (±)-nororientaline, (±)-norreticuline, (±)-, (−)-, and (+)-, reticulines into boldine in *Litsea glutinosa* have been studied. Specific utilization of (+)-reticuline into boldine has been demonstrated (Tewari *et al* 1974). The evidence supports the direct oxidative coupling of (+)-reticuline to isoboldine which in turn is shown to be a specific precursor of boldine. Double labelling experiments involing the methoxy group of (±)-reticuline showed that most but not all

of the methoxy activity is lost in conversion into boldine (Bhakuni *et al* 1977).

Two biosynthetic pathways have been reported for the formation of 1,2,10,11-tetrasubstituted aporphine alkaloids. In one of the pathways direct *ortho-ortho* oxidative coupling of reticuline is involved to provide bulbocapnine and magnoflorine types of aporphine bases, while the second pathway makes use of neoproaporphine intermediate to furnish corydine types of compounds. The biosynthesis of isocorydine (39) could be envisaged both from reticuline and protosinomenine. In the third scheme the required isocorydine skeleton could also be generated from orientaline *via* a dienone. Feeding of labelled (±)-nororientaline in parallel with (±)- norprotosinomenine and (±)-reticuline established that isocorydine (39) is exclusively and specifically biosynthesized in *Annona squamosa* Linn. plants from reticuline. Feeding with (±)-norlaudanidine demonstrated that it is a poor precursor of isocorydine. Feeding with labelled (+)- and (−)-norreticulines showed that stereospecificity is maintained in the conversion of 1-benzyltetrahydroisoquinoline into isocorydine (Prakash *et al* 1978).

The quaternary aporphine alkaloids, magnoflorine (41) and laurifoline (40), well known for their curarizing and hypotensive properties, could form in Nature from 1-benzyltetrahydroisoquinoline precursors by alternate biosynthetic routes. Feeding of labelled 1-benzyl-tetrahydroisoquinoline precursors revealed that magnoflorine and laurifoline in *Cocculus laurifolius* are biosynthesized from reticuline.

Parallel feedings with (S)-, and (R)-, reticulines demonstrated that the bases are stereospecifically formed in the plants from (S)-reticuline (Bhakuni *et al* 1980).

Biogenetic theory (Barton and Cohen 1957) suggests that the so-called 'abnormal aporphine alkaloids' lacking an oxygen substituent in ring D are derived from 1-benzylisoquinoline by oxidation to a dienone, reduction to dienol and dehydration with rearrangement to the fully aromatic compound. Using labelled precursors the relationship between the 1-benzylisoquinoline alkaloid, coclaurine and the aporphine alkaloid, roemerine (44) in *Papaver dubium* L. has been studied (Barton *et al* 1966). Various congeners of coclaurine have been used in the investigation of this biosynthesis. The corresponding biosynthesis of anonaine (43) in *Annona reticulata* L. has also been studied. The biosynthesis of mecambrine (42) has been more briefly investigated. The relationship between mecambrine and roemerine has been demonstrated. The results as a whole provide good support for the hypothesis that aporphine alkaloids of the anonaine-roemerine type are derived from coclaurine type precursors through phenol oxidation. Dienone of the crotonosine (45), mecambrine (42) types are intermediate (Barton *et al* 1967).

According to biogenetic theory (Barton and Cohen 1957), the abnormal aporphine alkaloid, nornuciferine-1 (46) could be biosynthesized from coclaurine derivatives. Tracer experiments in *Croton sparsiflorus* with (±)-norcoclaurine, coclaurine and N-methylcoclaurine demonstrated that N-methylcoclaurine is specifically incorporated into nornuciferine-1. The evidence supports direct oxidative coupling of (+)-(S)-N-methylcoclaurine to give N-methylcrotsparine which in turn is shown to be a specific precursor of nornuciferine-1. The experiments also showed that N-methylcrotsparine is reduced to N-methylcrotsparinol which is then preferentially dehydrated and rearranged to nornuciferine-1 (Bhakuni *et al* 1979).

Sebiferine (23), a morphinandienone alkaloid could be biosynthesized from suitably substituted 1-benzyltetrahydroisoquinoline precursors through alternate biosynthetic routes. Tracer experiments established that sebiferine in *Cocculus laurifolius* is

specifically biosynthesized from reticuline. A double labelling experiment involving the 4′-O-methyl group of (±)-norreticuline showed that the methoxy group is retained in the bioconversion of the precursor into sebiferine. There was, however, considerable loss of the tritium at C-1. Parallel experiments with (+)- and (−)-reticulines showed that the stereospecificity is not maintained in the biosynthesis of sebiferine from 1-benzylisoquinoline precursors. Feeding experiments also demonstrated that the enzyme system present in plants can efficiently convert flavinantine into sebiferine (Bhakuni *et al* 1978).

Stereochemical aspects related to the biosynthesis of the morphine alkaloids have been studied. Ozonolysis of tritiated salutaridiols-I and II, the biosynthetic intermediates of morphine alkaloids, has afforded glyceric acids whose absolute configurations have been determined by the isotope dilution method. The stereochemistry of salutaridinol-I, thus has been defined unambiguously. Oxidation of tritiated (+)- and (−)-reticulines followed by isotopic dilution analysis, has confirmed the configurational relationship between morphine and benzylisoquinoline alkaloids. Further support for this has been obtained by the reductive fission of salutaridine to give after appropriate methylation, (−)-laudanosine. The bond cleaved in this reduction is the bond formed in the biogenetic oxidation of (−)-reticuline (Barton *et al* 1967).

Phenanthroindolizidine alkaloids tylophorine (47) and tylophorinine (48) (Govindachari *et al* 1954) exhibit wide range of biological activities and provide fascinating problem for biosynthetic studies. Tracer experiments (Mulchandani *et al* 1969, 1971) revealed that ring A and carbon atoms C_{10} and C_6' and ring B and carbon atoms C_9 and C_7, of tylophorine in *Tylophora asthmatica* are derived from phenylalanine and tyrosine respectively. Ornithine is also incorporated, thus suggesting its participation in tylophorine biosynthesis *via* Δ′-pyrrole. Late stages of biosynthesis of tylophorine and tylophorinine have also been studied with labelled substituted 6,7-diphenylhexahydroindolizines and a trisubstituted compound has been shown as an intermediate (Herbert and Jackson 1977). We disagreed with this and re-examined the biosynthesis of these alkaloids. Initially administration of 3,4-dihydroxyphenylalanine (dopa) to young *T. asthematica* plants showed that ring B and carbon atoms C_9 and C_7' of tylophorine and tylophorinine are derived specifically from dopa. Experiments with ^{14}C and ^{3}H labelled 6,7-diphenylhexahydroindolizines demonstrated that both the alkaloids in *T. asthmatica* are biosynthesized from 6-(3-hydroxy-4-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,2,3,5,7,8a-hexahydroindolizine (Bhakuni and Mangla 1981).

Aristolochic acid (49), a representative of the substituted 10-nitrophenanthrene-1-acids which occurs in many species of the genus *Aristolochia* and several other members of the family Aristolochiaceae, is a potent tumor inhibitor. Biogenetically it is considered to be derived from 1-benzyltetrahydroisoquinoline precursors *via* aporphine intermediates. The incorporation of tyrosine, dopa, nororientaline, orientaline, prestepharine and stepharine into aristolochic acid in *Aristolochia bracteata* has been studied. Specific incorporation of nororientaline has been demonstrated. The evidence strongly supports the hypothesis that the oxidative coupling of orientaline gives prestepharine which is converted into stepharine. Oxidative cleavage of stepharine then furnishes aristolochic acid. An experiment with doubly labelled nororientaline showed its incorporation intact into the product and confirmed the view that the methylenedioxy group in aristolochic acid originates from an O-methoxyphenol precursor. Parallel feedings with (−)- and (+)-, orientalines confirmed that stereospecificity is

maintained in the biosynthesis of aristolochic acid from the 1-benzyltetrahydroisoquinoline precursors (Sharma *et al* 1982).

Laurifinine (26), a typical representative of trisubstituted dibenz [*d,f*] azonine alkaloids isolated by us from the leaves of *Cocculus laurifolius* could be formed in nature from N-norprotosinomenine, an established precursor of *Erythrina* alkaloids (Barton *et al* 1968). *Para-Para* oxidative coupling of norprotosinomenine could give neoproaporphine, which on reduction followed by elimination and rearrangement would furnish dibenz [*d,f*] azonine skeleton. Reduction of imine followed by N-methylation could finally yield laurifinine. Labelled tyrosine was initially fed to young cut branches of *C. laurifolius* and it was found that the plants were actively biosynthesising laurifinine. In subsequent experiments the theoretical precursors of laurifinine such as nor protosinomenine, nororientaline, norreticuline and N-[2-(3-hydroxy-4-methoxyphenyl)-ethyl]-2-(4′-hydroxyphenyl) ethylamine when were fed, it was found that only norprotosinomeine was efficiently metabolized by the plants to form laurifinine. Intact incorporation of norprotosinomenine into laufifinine was demonstrated by double labelling experiments with (±)-[1-^{3}H, 4′-methoxy-^{14}C], and [1-^{3}H, 7-methoxy-^{14}C]N-norprotosinomenines. In both the experiments ^{3}H:^{14}C ratios in the precursors and the product were essentially the same. The experiments further showed that 4′ and 7- OMe groups and the hydrogen atom at the asymmetric centre of the precursor are retained in the bioconversion into laurifinine. Parallel experiments with (+)- and (−)-N-norprotosinomenines demonstrated specific utilisation of (+)-isomer into laurifinine (Bhakuni and Jain 1981).

Cocculidine (10) and cocculine (9), the hypotensive principles of *Cocculus laurifolius* and are representative of abnormal *Erythrina* alkaloids. These bases could be formed in nature from norprotosinomenine. In the bioconversion, one of the oxygen function of the precursor could be eliminated by dienone-benzene rearrangement. However, the possibilities of the formation of these abnormal *Erythrina* alkaloids from other 1-benzyltetrahydroisoquinolines such as norreticuline and nororientaline and from the suitably substituted N-phenethylamine could not be ruled out (Barton *et al* 1974). The incorporation of labelled (±)-, N-norprotosinomeine, N-norreticuline, N-nororientaline and N-[2-(3-hydroxy-4-methoxyphenyl) ethyl]-2-(4′-hydroxyphenyl) ethylamine into coculidine and cocculine in *C. laurifolius* were studied and specific utilisation of N-norprotosinomenine into the alkaloids demonstrated. Feeding of protosinomenine revealed that the plants do not have the ability to carry out selective N-demethylation to give N-norprotosinomenine. A double labelling experiment with (±)-[1-^{3}H, 4′-methoxy-^{14}C]-N-norprotosinomenine showed that the 4′-O-methoxy group of the precursor is retained in the bioconversion and the erythrinan ring system is not formed by addition of the secondary amino function onto an orthoquinone system. Parallel experiments with (+)- and (−)-N-norprotosinomenines demonstrated specific incorporation of (+)-isomer into cocculidine. High incorporation of cocculidine into cocculine revealed that O-demethylation is the terminal step in the biosynthesis of cocculine. Feeding experiments also showed that the plants can convert isococculidine into cocculidine with very high efficiency. N-norprotosinomenine has been specifically incorporated into cocculidine and cocculine, its presence in *C. laurifolius* has been shown by trapping experiments. N-Norprotosinomenine is thus a true biological precursor of the abnormal *Erythrina* alkaloids (Bhakuni and Singh 1978).

Tetrahydropalmatine and palmatine, isolated from *Stephania glabra* are representatives of protoberberine alkaloids. These bases occur in nature either as tetrahydropro-

toberberines or quaternary protoberberine salts. Tetrahydroprotoberberines are also important intermediates in the biosynthesis of a large number of 1-benzyltetrahydroisoquinoline derived alkaloids (Bhakuni 1976). Recent tracer experiments have shown that tetrahydroprotoberberine alkaloids give rise in nature to benzophenanthridine, spirobenzylisoquinoline, protopine, phthalideisoquinoline, rhoeadine and retroprotoberberine alkaloids. A biogenetic connection between the benzylisoquinoline and berberine group of alkaloids (Robinson 1955) recognised quite early has been firmly confirmed by tracer experiments (Gear and Spenser 1963; Batterby *et al* 1963; Gupta and Spenser 1965 and Skerl and Gros 1971). Further it has been demonstrated that C atom 8 of berberine group of alkaloids is derived from the N-Me group of 1-benzylisoquinoline precursors (Barton *et al* 1965 and Battersby *et al* 1965). Negligible incorporation of reticuline into tetrahydropalmatine in *Papaver somniferum* has been recorded (Brochman Hanssen *et al* 1971).

We re-examined the biosynthesis of tetrahydropalmatine and palmatine. Tyrosine was initially fed to young cut branches of *C. laurifolius* and young plants of *Cissampelos pariera* and it was found that plants in both cases were biosynthesising tetrahydropalmatine and palmatine. Incorporation of tyrosine into protoberberine alkaloids was, however, slightly higher in *C. laurifolius*. In subsequent experiments labelled hypothetical precursors were fed to young cut branches of *C. laurifolius* plants. Feeding of tyrosine in parallel with (±)-nororientaline, norprotosinomenine and norlaudanidine revealed that these 1-benzyltetrahydroisoquinoline derivatives are very poorly metabolised by the plants. Feeding with (±)-norlaudanosoline and reticuline showed that these are efficient precursors of the bases. The completely methylated 1-benzyltetrahydroisoquinoline, (±)-laudanosine was not incorporated. Biosynthetic tetrahydropalmatine derived from (±)-[3-^{14}C] reticuline was treated with methyl iodide which had essentially the same radioactivity as the parent base. The methiodide was converted into methohydroxide and then subjected to Hofmann degradation to give the methine-I with essentially no loss of radio activity. Ozonolysis of the methine-I gave radioactive formaldehyde (dimedone derivative, 98 % of original activity). Biosynthetic palmatine derived from (±)-[3-^{14}C] reticuline was reduced with Sn/HCl to give tetrahydropalmatine which had essentially the same radio activity as the parent base. It was then subjected to Hofmann degradation as above to give the corresponding methine which on ozonolysis afforded radio active formaldehyde (dimedone derivative, 97 % of the original activity). The results thus established specific incorporation of reticuline into tetrahydropalmatine and palmatine. Reticuline was incorporated intact into tetrahydropalmatine was shown by double labelling experiment as follows: (+)-[1-^{3}H, 4′-Methoxy-^{14}C] Reticuline was fed to the young cut branches of *C. laurifolius* and biosynthetic tetrahydropalmatine was isolated. The ratios of ^{14}C : ^{3}H was 1 : 38 and in the biosynthetic base 1 : 37. The C atoms 8 in tetrahydropalmatine and palmatine are formed by oxidative cyclisation of N-Me group of reticuline have been shown as follows. (±)-N-Methyl-[^{14}C] Reticuline was fed and biosynthetic bases of interest were isolated. Labelled palmatine was treated with phenylmagnesium bromide to give 8-phenyldihydropalmatine. Chromic acid oxidation of the radioactive compound in the usual way (Kuhn–Roth) gave radioactive benzoic acid (102 % original activity). Biosynthetic tetrahydropalmatine derived from (±)-N-methyl-[^{14}C] reticuline was dehydrogenated to give radioactive palmatine which was degraded as above to give radioactive benzoic acid (98 % original activity). The foregoing experiments established that reticuline is a specific precursor of tetrahydropalmatine and palmatine in *C.*

laurifolius. The precursors used, however, were racemic. It would be expected that in the biotransformation only one of the two optical isomers should act as a direct substrate. Parallel feedings with (+)- and (−)- reticulines demonstrated that stereospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline precursors into tetrahydropalmatine and palmatine. (+)-Reticuline was incorporated about 70 times more efficiently than the (−)-enantiomer. Feeding of labelled tetrahydropalmatine and palmatine showed that the former was very efficiently incorporated into the latter whereas the incorporation of the latter into former was practically negligible. (+)-Reticuline has been isolated from *C. laurifolius*. Its presence in the plants was again confirmed by feeding tyrosine. (+)-Reticuline is, thus, a true precursor of tetrahydropalmatine and palmatine. The foregoing experiments strongly support the following sequence for the biosynthesis of tetrahydropalmatine and palmatine in *C. laurifolius*. Tyrosine → norlaudanosoline → (+)-reticuline → tetrahydropalmatine → palmatine (Bhakuni *et al* 1980).

Several abnormal *Erythrina* alkaloids have been isolated by us from the leaves of *Cocculus laurifolius* (Bhakuni and Jain 1980). We have studied the early stages of biosynthesis of cocculidine, cocculine (Bhakuni and Singh 1978) and isococculidine (Bhakuni *et al* 1977) and have shown that these alkaloids are stereospecifically biosynthesized from (+)-(S)-norprotosinomenine. We have also examined the late stages of biosynthesis of these alkaloids and found that isococculidine in the plants is converted into coccoline *via* coccuvinine and isococculine into coccolinine *via* coccuvine (Bhakuni and Jain 1980).

5. Bisbenzylisoquinoline alkaloids

Bisbenzylisoquinoline alkaloids constitute the largest group of isoquinoline alkaloids and are distributed mostly among the members of Menispermaceae and Rannunculaceae families (Shamma 1972). Biogenetically bisbenzylisoquinoline alkaloids are considered to be formed in Nature by oxidative dimerisation of simple 1-benzyltetrahydroisoquinoline bases. Different sub-groups of bisbenzylisoquinoline alkaloids have been recognised due to availability of different sites for oxidative dimerisation. The members of the sub-group differ in the nature of the oxygenated substituents or the oxidation state or degree of substitution at the two nitrogen atoms or in stereochemistry at the two asymmetric centres. Based on these differences bisbenzylisoquinolines have been classified recently into twenty-six types (Shamma and Moniot (1976). We have studied the biosynthesis of representatives of several groups of bisbenzylisoquinoline alkaloids.

The simple bisbenzylisoquinoline, tetrandrine which affects the central nervous system, respiratory and skeletal muscles and is also an effective tumor inhibitor, has been shown to be formed in Nature by oxidative dimerisation of S-(+)-N-methylcoclaurine (Bhakuni *et al* 1980). The biosynthetic sequence of cocsulinin, an anticancer agent of *Cocculus pendulus* (Bhakuni and Joshi 1975) have been traced (Bhakuni *et al* 1978). Oxyacanthine, the hypotensive principle of *Berberis vulgaris* Linn. and a representative of bisbenzylisoquinoline alkaloids containing two phenyl ether linkages have been shown to be formed in Nature by inter- and intra-molecular oxidative couplings of (S)- and (R)-,N-methyl-coclaurines (Bhakuni *et al* 1978). Isotetrandrine which exhibits anti-inflammatory, analgesic and hypothermic effects

have also been demonstrated to be biosynthesised from (S)- and (R)-,N-methyl-coclaurines (Bhakuni *et al* 1980).

6. Biosynthesis, a tool for determining the structure and absolute configuration of alkaloids

Biosynthesis has been used as a tool for determining the structures and absolute configurations of alkaloids. Isomeric bases nortiliacorinine A and nortiliacorinine B have been isolated from *Tiliacora* species (Anjaneyulu *et al* 1969). The position of N-methyl group and the stereo-chemistries at the two asymmetric centres in these bases remained undefined. By using coclaurine derivatives the structure and absolute configuration of nortiliacorinine A has been defined (Bhakuni *et al* 1981).

The absolute configuration at the asymmetric centres C-1 and C-1′ in the diastereoisomeric alkaloids tiliacorine and tiliacorinine cannot be determined by the usual sodium-ammonia cleavage method because the two lower rings of these bases are linked through a direct carbon-to-carbon bond, rather than through the much more common ether bridge. By tracer experiments it has been shown that tiliacorine has the S- and R-configuration at the asymmetric centres C-1 and C-1′ respectively and tiliacorinine has the 'SS-' configuration at both centres (Bhakuni and Jain 1981; Bhakuni *et al* 1978). Absolute configuration of bisphenylbisbenzylisoquinoline alkaloid, tiliageine has also been similarly determined (Bhakuni and Singh 1978). Tumor inhibitory base, thalicarpine (29) which also possesses hypotensive activity, is a representative of aporphine-1-benzylisoquinoline alkaloids. Biogenetically thalicarpine is unique. It is one of the rare dimeric alkaloid which could derive in Nature from two reticuline units. The base could also be formed by oxidative coupling of reticuline and preformed aporphine of N-methyllaurotetanine type. Using norlaudanosoline derivatives it has been established that thalicarpine is stereospecifically biosynthesised by oxidative coupling of (S)-reticuline. Feeding experiments also showed that the plants can convert (S)-boldine and (S)-isoboldine into thalicarpine (Bhakuni and Jain 1982).

(+)-Sinactine is a tetrahydroprotoberberine alkaloid. The absolute configuration of the base has been determined by biosynthetic method (Bhakuni *et al* 1983).

7. Aberrant biosynthesis of unnatural alkaloids

We know very little about the potentiality of higher plants to carry out transformations on organic molecules which they do not normally produce or contain. These types of transformation where an 'unnatural precursor' is converted into an 'unnatural product' in living system is called 'abberrant biosynthesis'. This area of research is relatively unexplored in higher plants. A few reported examples of aberrant biosyntheses are the conversion of 5-fluoronicotinic acid into 5-fluoronictonine in the tobacco plant (*Nicotiana tabacum* (Leete *et al* 1971); and conversion of N-methyl-Δ′-piperideinium chloride into higher homologue of nicotine (Leete and Chedekel 1972).

We have demonstrated bioconversion of 8-bromo- and 8,2′-dibromo- reticulines in *Cocculus laurifolius* into 1-bromo- and 1,12-dibromotetrahydropalmatine and also of 2′-nitro and 2′-aminoreticulines into 12-nitro and 12-amino-tetrahydropalmatines. The experiments suggest that many of the enzymes in plants are non-specific and are able to catalyse the biosynthesis of 'unnatural alkaloids' from 'unnatural precursors'.

Acknowledgements

The experimental part of this work reviewed in this paper is mainly done by the students of the present author. Their names have come in the references cited. He is grateful to all of them for their sincere and active participation in the programme.

References

Amin A H, Subhaiah T V and Abbasi K M 1969 *Can. J. Microbiol.* **15** 1067
Anjaneyulu B, Govindachari T R, Sathe S S, Viswanathan N, Gopinath K W and Pai B R 1969 *Tetrahedron* **25** 3091
Barton D H R, Bhakuni D S, Chapman G M and Kirby G W 1966 *Chem. comm.* 176
Barton D H R, Bhakuni D S, Chapman G M and Kirby G W 1967a *J. Chem. Soc.* (C) 1295
Barton D H R, Bhakuni D S, Chapman G M and Kirby G W 1967b *J. Chem. Soc.* (C) 2134
Barton D H R, Bhakuni D S, James R and Kirby G W 1967c *J. Chem. Soc.* (C) 129
Barton D H R and Cohen T 1957 *Festschrift A Stoll.* Birkhauser A G Basel p. 117
Barton D H R, Hesse R H, and Kirvy G W 1965 *J. Chem. Soc.* (C) 6379
Barton D H R, James R, Kirby G. W, Turner D W and Widdowson D A 1968 *J. Chem. Soc.* (C) 1529
Barton D H R, Potter C J and Widdowson D A 1974 *J. Chem. Soc. Perkin I* 346
Battersby A R, Francis R J, Hirst M and Staunton J 1963 *Proc. Chem. Soc.* 268
Battersby A R, Francis R J, Ruveda B A and Staunton J 1965 *Chem. Comm.* 89
Battersby A R, Kapil R S, Bhakuni D S, Popli S P, Merchant J R and Salgar S S 1966 *Tetrahedron Lett.* 4965
Bhakuni D S 1976 *J. Sci. Ind. Res.* **35** 461
Bhankuni D S and Chaturvedi R 1983 *J. Nat. Prod.* **46** (in press)
Bhakuni D S and Dhar M M 1968 *Experientia* **24** 1026
Bhakuni D S and Dhar M M 1969 *Experientia* **25** 354
Bhakuni D S, Dhar M L, Dhar M M, Dhawan B N and Mehrotra B N 1969 *Indian J. Exp. Biol.* 7 250
Bhakuni D S, Gonzalez C, Sammes P G and Silva M 1974a *Rev. Latinoamer. Quin.* **5** 158
Bhakuni D S, Gupta N C and Dhar M M 1970a *Experientia* **26** 241
Bhakuni D S and Gupta P K 1982a *Indian J. Chem.* **B21** 393
Bhakuni D S, Gupta P K, Joshi P P and Gupta S 1982 *Indian J. Chem.* **B21** 389
Bhakuni D S and Gupta S 1982a *Planta Med.* **46** 193
Bhakuni D S and Gupta S 1982b *J. Nat. Prod.* **45** 407
Bhakuni D S and Gupta S 1983 *Planta Med.* **47** in Prwss
Bhakuni D S and Jain S 1980a *Tetrahedron* **36** 2153
Bhakuni D S and Jain S 1980b *Tetrahedron* **36** 3107
Bhakuni D S and Jain S 1981a *J. Chem. Soc. Perkin I* 2598
Bhakuni D S and Jain S 1981b *Tetrahedron* **37** 3171
Bhakuni D S and Jain S 1981c *Tetrahedron* **37** 3175
Bhakuni D S and Jain S 1982 *Tetrahedron* **38** 266
Bhakuni D S, Jain S and Chaturvedi R 1979 *Tetrahedron* **35** 2323
Bhakuni D S Jain S and Gupta S 1980c *Tetrahedron* **36** 2491
Bhakuni D S, Jain S and Gupta S 1983 *Tetrahedron* **39** 3455
Bhakuni D S, Jain S and Singh A N 1978a *J. Chem. Soc. Perkin I* 380
Bhakuni D S, Jain S and Singh A N 1980a *Phytochem.* **19** 2347
Bhakuni D S, Jain S and Singh R S 1980d *Tetrahedron* **36** 2525
Bhakuni D S and Joshi P P 1975 *Tetrahedron* **31** 2575
Bhakuni D S and Kumar P 1983 *Indian J. Chem.* **B22** 5
Bhakuni D S and Mangla V K 1981 *Tetrahedron* **37** 401
Bhakuni D S and Mangla V K 1981 *Indian J. Chem.* **B20** 531
Bhakuni D S, Mangla V K, Singh A N and Kapil R S 1978b *J. Chem. Soc. Perkin I* 4459
Bhakuni D S, Satish S and Dhar M M 1970b *Phytochem.* **9** 2573
Bhakuni D S, Satish S and Dhar M M 1972a *Tetrahedron* **28** 4579
Bhakuni D S, Satish S, Uprety H and Kapil R S 1974b *Phytochem.* **13** 2769
Bhakuni D S, Silva M, Stephen A M and Sammes P G 1976a *Phytochem.* **15** 574

Bhakuni D S and Singh A N 1978a *J. Chem. Soc. Perkin I* 618
Bhakuni D S and Singh A N 1978b *Tetrahedron* **34** 1409
Bhakuni D S, Singh A N and Jain S 1978c *J. Chem. Soc. Perkin I* 1318
Bhakuni D S, Singh A N and Jain S 1980b *Tetrahedron* **36** 2149
Bhakuni D S, Singh A N and Jain S 1981 *Tetrahedron* **37** 2651
Bhakuni D S, Singh A N, Jain S and Kapil R S 1977a *Chem. Commun.* 211
Bhakuni D S, Singh A N, Jain S and Kapil R S 1978d *J. C. S. Chem. Comm.* 266
Bhakuni D S and Singh A N, Tewari S and Kapil R S 1977b *J. Chem. Soc.* (C) 1662
Bhakuni D S and Singh R S 1982 *J. Nat. Prod.* **45** 252
Bhakuni D S, Tewari S and Dhar M M 1972b *Phytochemistry* **11** 1819
Bhakuni D S, Tewari S and Kapil R S 1977c *J. Chem. Soc.* (C) 709
Bhakuni D S, Uprety H, and Widdowson D A 1976b *Phytochem.* **15** 736
Brochman-Hanssen E, Fu C.-C. and Zanati C 1971 *J. Pharm. Sci.* **64** 831
Cava M P, Nomura K, Schlessinger and Buck K T 1964a *Chem. Ind.* 282
Cava M P, Nomura K, Schlessinger R H, Buck K T, Dougles B, Raffauf R F and Weisbach J A 1964b *Chem. Ind.* 282
Chatterjee R, Guha M P and Chatterjee A 1952 *J. Ind. Chem. Soc.* **29** 371
Chatterjee A, Majumdar P L, Mukerjee R, Saha S K and Talpatra S K 1965 *Tetrahedron Lett.* 1539
Constantine Jr. G H, Vitek M R, Sheth K, Catalfomo P and Sciuchetti L A 1966 *J. Pharm. Sci.* **55** 982
Dhar M L, Dhar M M, Dhawan B N, Mehrotra B N and Ray C 1968 *J. Exp. Biol.* **6** 232
Dhar M L, Dhawan B N, Prasad C R, Rastogi R P, Singh K K and Tandon J S 1974 *Indian J. Exp. Biol.* **12** 512
Dhawan B N, Dubey M P, Mehrotra B N, Rastogi R P and Tandon J S 1980 *Indian J. Exp. Biol.* **18** 594
Dubey M P, Srimal R C and Dhawan B N 1969 *Indian. J. Pharm.* **1** 73
Gear J R and Spenser I D 1963 *Can. J. Chem.* **41** 783
Gopinath K W, Govindachari T R, Rajappa S and Ramadas C V 1959 *J. Sci. Ind. Res.* **B18** 444
Govindachari T R, Pai B R and Nagarajan K 1954 *J. Chem. Soc.* 2801
Gupta N C, Bhakuni D S and Dhar M M 1970 *Experientia* **26** 12
Gupta R N and Spenser I D 1965 *Can. J. Chem.* **43** 133
Haynes L J, Stuart, K L, Barton D H R, Bhakuni D S and Kirby G W 1965 *Chem. Commun.* 141
Herbert R B and Jackson F B 1977 *J. Chem. Soc.* (*Chem. Commun.*) 955
Jain S and Bhakuni D S 1977 *Indian J. Chem.* **B15** 389
Joshi P P, Bhakuni D S and Dhar M M 1974 *Indian J. Chem.* **12** 517
Joshi P P, Bhakuni D S and Dhar M M 1974 *Indian J. Chem.* **12** 649
Kar K, Mukherjee K C and Dhawan B N 1976 *Indian J. Exp. Biol.* **15** 547
Kupchan S M, Yang T H, Vasilikiotis G S, Barnes M H and King M L 1967 *J. Am. Chem. Soc.* **89** 3075
Leete E, Bodem G B and Manuel M F 1971 *Phytochemistry* **10** 2687
Leete E and Chedekel M R 1972 *Phytochemistry* **11** 2751
Mangla V K and Bhakuni D S 1980a *Indian J. Chem.* **B19** 748
Mangla V K and Bhakuni D S 1980b *Tetrahedron* **36** 2489
Mehra K, Garg H S, Bhakuni D S and Khanna N M 1978 *Indian J. Chem.* **B14** 58
Mehra K, Garg H S, Bhakuni D S and Khanna N M 1976 *Indian J. Chem.* **B14** 216, 844
Mulchandani N B, Iyer S S and Badheka L P 1969, 1971 *Phytochem.* **8** 1931; **10** 1047
Pande H and Bhakuni D S 1976 *J. Chem. Soc. Perkin I* **21** 97
Pande H, Saxena N K and Bhakuni D S 1976 *Indian J. Chem.* **B14** 366
Patnaik G K, 1983 Personal Communication
Prakash O, Bhakuni D S and Kapil R S 1978 *J. Chem. Soc. Perkin I* 628
Prakash O P, Bhakuni D S and Kapil R S 1979 *J. Chem. Soc. Perkin I* 1515 Robinson R 1955 *The structural relationship of natural products* (Oxford: Clarendon Press) p. 78
Rueppel M L and Rapoport H 1971 *J. Am. Chem. Soc.* **93** 7021
Shamma M 1972 *The isoquinoline alkaloids* (Academic Press) p. 115
Shamma M and Moniot J L 1976 *Heterocycles* **4** 1817
Shamma M and Rothenberg A S 1978 *Lloydia* **41** 169
Shamma M, Shine R J and Dock B S Du 1967 *Tetrahedron* **23** 2887
Sharma V, Jain, S, Bhakuni D S and Kapil R S 1982 *J. Chem. Soc. Perkin I* 1153
Singh A N and Bhakuni D S 1977 *Indian J. Chem.* **15B** 388
Singh A N, Pande H and Bhakuni D S 1977 *Experientia* **32** 1368
Singh A N, Pande H and Bhakuni D S 1977 *Lloydia* **40** 322

Singh R S, Jain S and Bhakuni D S 1978 *Nat. Acad. Sci. Lett.* **1** 93
Singh R S, Kumar P and Bhakuni D S 1981 *J. Nat. Prod.* **44** 664
Sivakumaran M and Gopinath K W 1976 *Indian J. Chem.* **B14** 150
Skerl A R and Gross E G 1971 *Phytochem.* **43** 133
Snatzke G and Wollenberg G 1966 *J. Chem. Soc.* (C) 1981
Srivastava O P 1982 Personal Communication
Stuart K L and Cava M P 1968 *Chem. Rev.* **68** 321
Stuart K L, Haynes L J Barrett M and Husbands G E M 1968 *Tetrahedron Lett.* 4473
Tewari S, Bhakuni D S and Dhar M M 1972 *Phytochem.* **11** 1149
Tewari S, Bhakuni D S and Kapil R S 1975 *J. C. S. Chem. Comm.* 554
Tewari S, Bhakuni D S, and Kapil R S 1974 *J. C. S. Chem. Comm.* 940
Uprety H, Bhakuni D S, and Dhar M M 1972 *Phytochem.* **11** 3057
Uprety H, Bhakuni D S and Kapil R S 1975 *Phytochem.* **14** 1535
Vashistha S K and Siddiqui S 1941 *J. Indian Chem. Soc.* **18** 641