

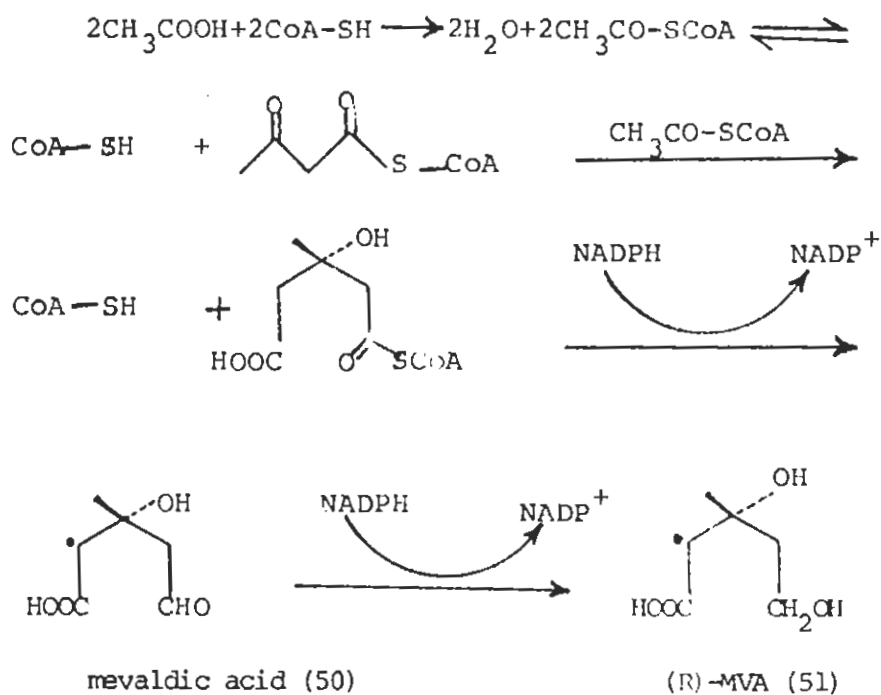
## 1.2 BIOSYNTHESIS OF TRITERPENES

Triterpenoids belong to a group of natural products which are widely distributed throughout the plant kingdom. They usually contain thirty carbon atoms formed by the combination of six isoprene units. Many however contain more or less carbon atoms and some which do not strictly follow the isoprene rule have also been isolated and characterized during recent years.

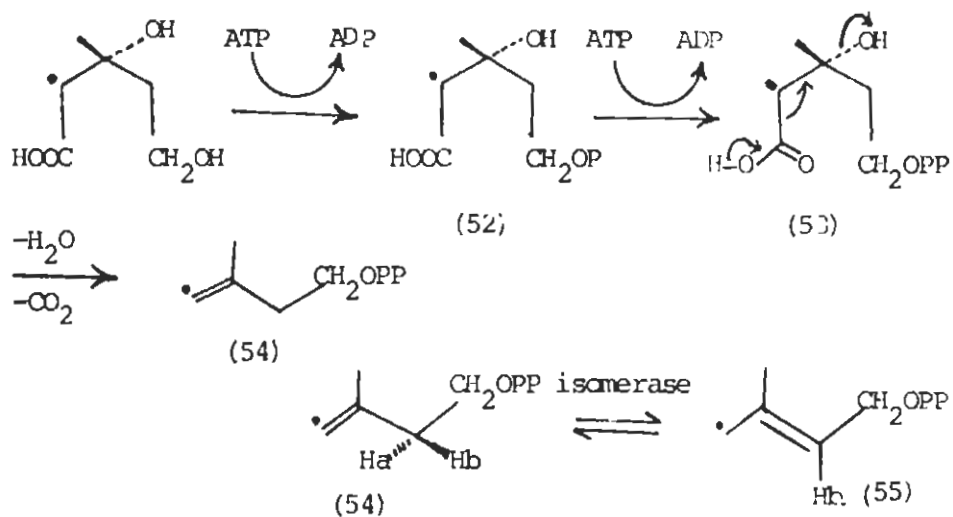
The biosynthesis of terpenoids can be subdivided into three steps.

1. The formation of isopentene unit from acetate.
2. The condensation of this unit to form acyclic terpenoids.
3. The conversion of acyclic terpenoids into cyclic terpenoids.

The conversion of acetate to mevalonic acid (51) proceeds according to Scheme (20). The isopentene unit is in fact formed from mevalonic acid (51) by the loss of one carbon. Labelling experiments have shown that it is the carbonyl group which is lost during the formation of the isopentene unit (Scheme 21).



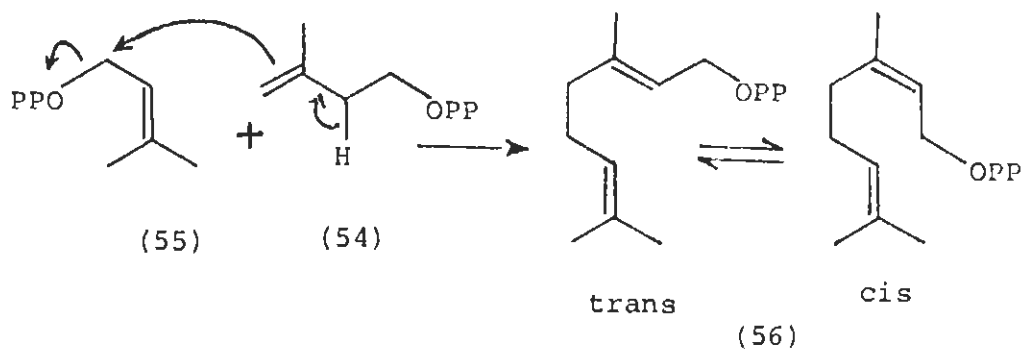
Scheme-20



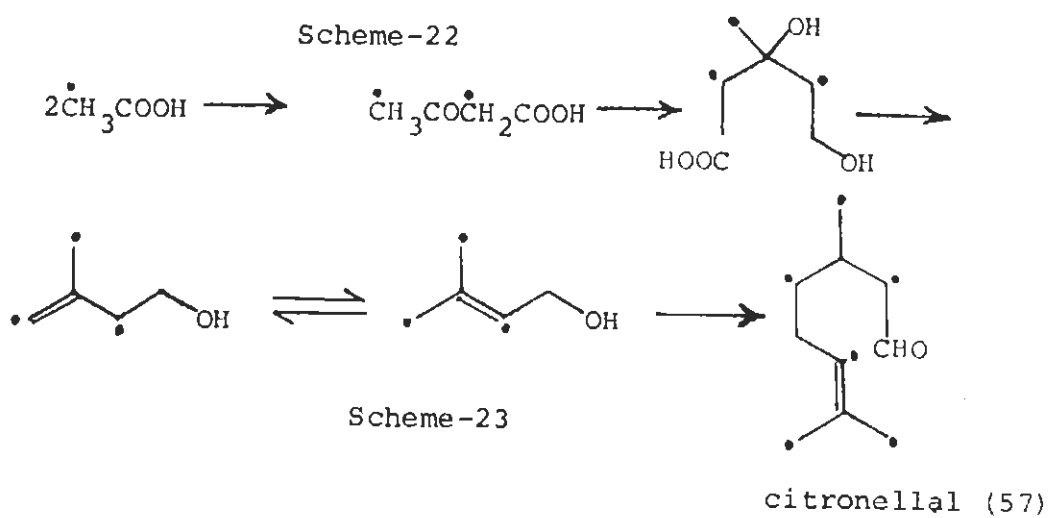
Scheme-21

Phosphorylation of mevalonic acid (51) first produces mevalonic acid-5-phosphate (52,  $P=PO_3H_2$ ) and this is followed by a second phosphorylation to give mevalonic acid-5-pyrophosphate (53,  $PP=P_2O_6H_3$ ). Subsequent loss of a molecule of water affords 3-methyl but-3-enyl pyrophosphate (54). The isoprene unit is in the form of 3-methyl but-3-enyl pyrophosphate but in the presence of an appropriate enzyme isomerises to 3-methyl but-2-enyl ( $\beta,\beta$ -dimethylallyl) pyrophosphate (55). The isomerisation of unit (54) and unit (55) is stereospecific,  $H_a$  being the proton that is eliminated. Moreover, the newly formed methyl group is trans to the  $CH_2OPP$  group in (55) Scheme (21).

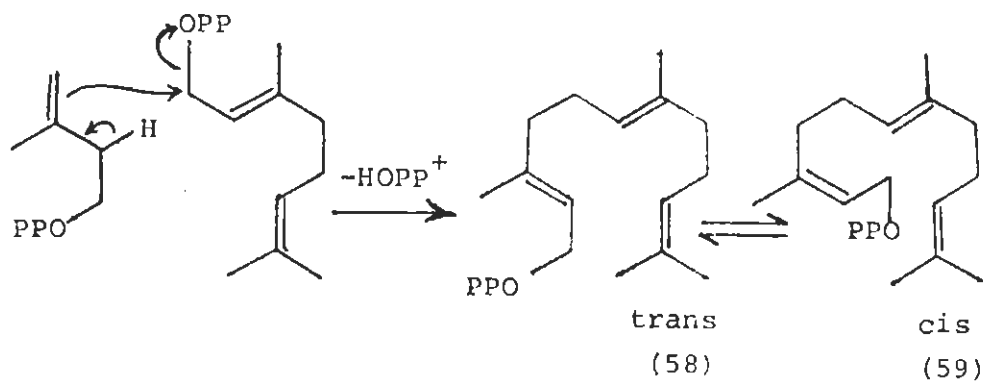
Geranyl pyrophosphate (56) arises by the combination of (54) and (55); 3-methyl but-3-enyl pyrophosphate acts as a nucleophile and 3-methyl but-2-enyl pyrophosphate as the electrophile to afford head-to-tail union as indicated in Scheme(22). This route is supported by biosynthetic experiments with labelled acetate leading to citronellal (57) as present in Scheme(23). Geranyl pyrophosphate reacts with 3-methyl but-3-enyl pyrophosphate to give farnesyl pyrophosphate (59) (Scheme 24).



Scheme-22

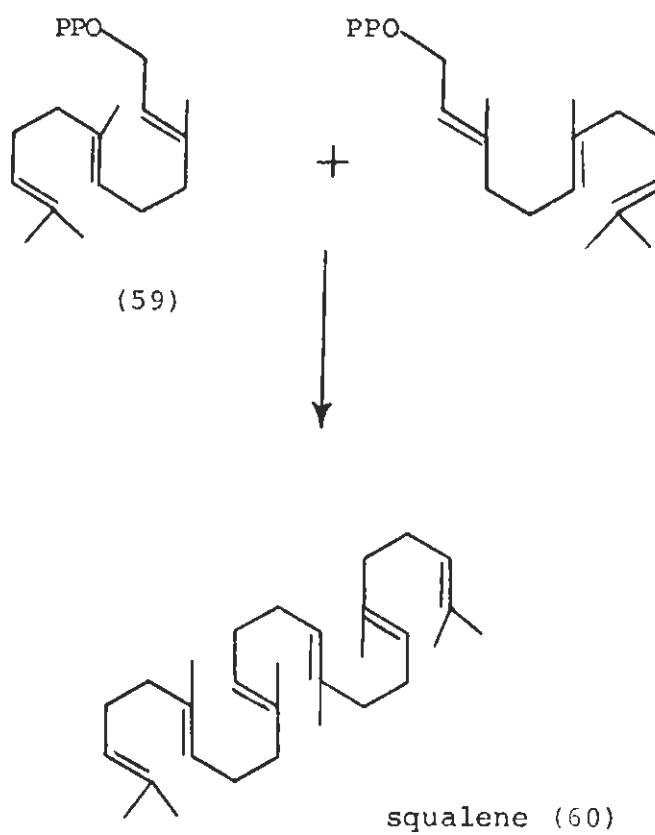


Scheme-23

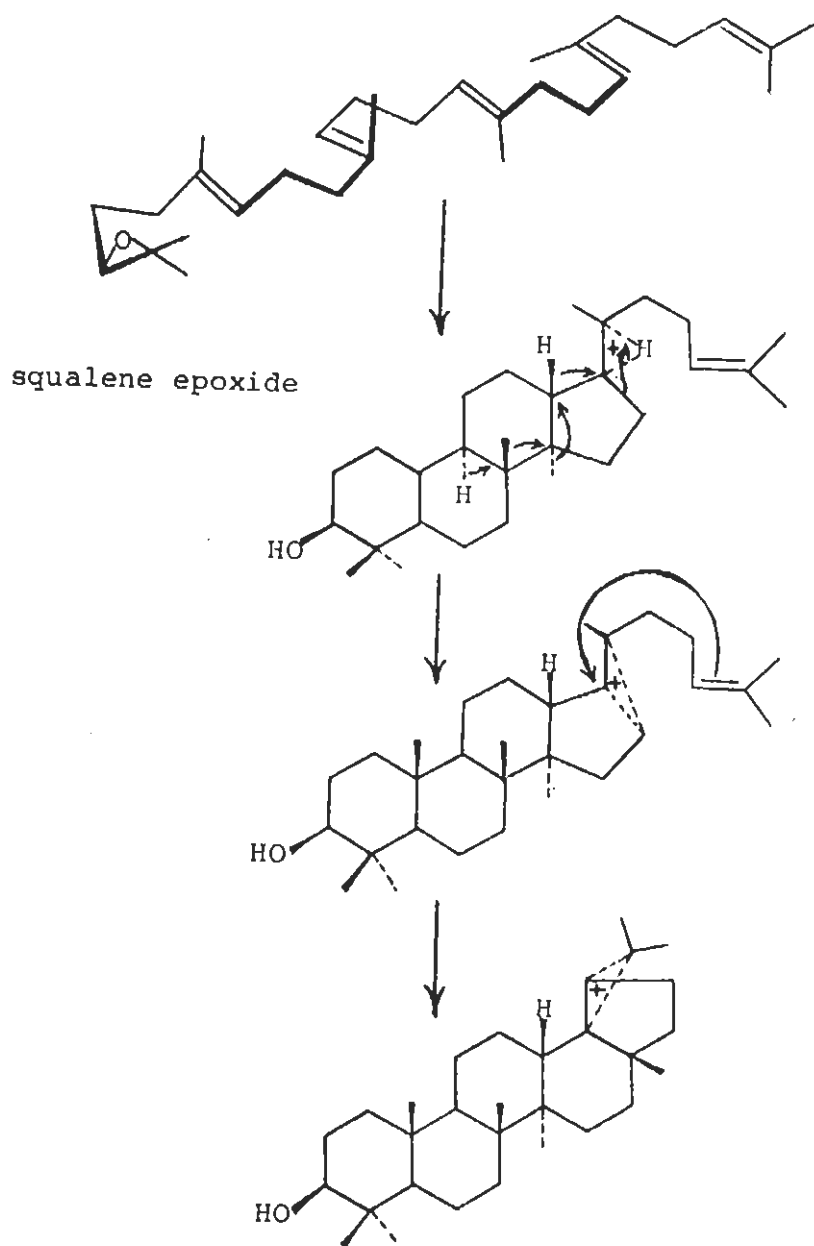


Scheme-24

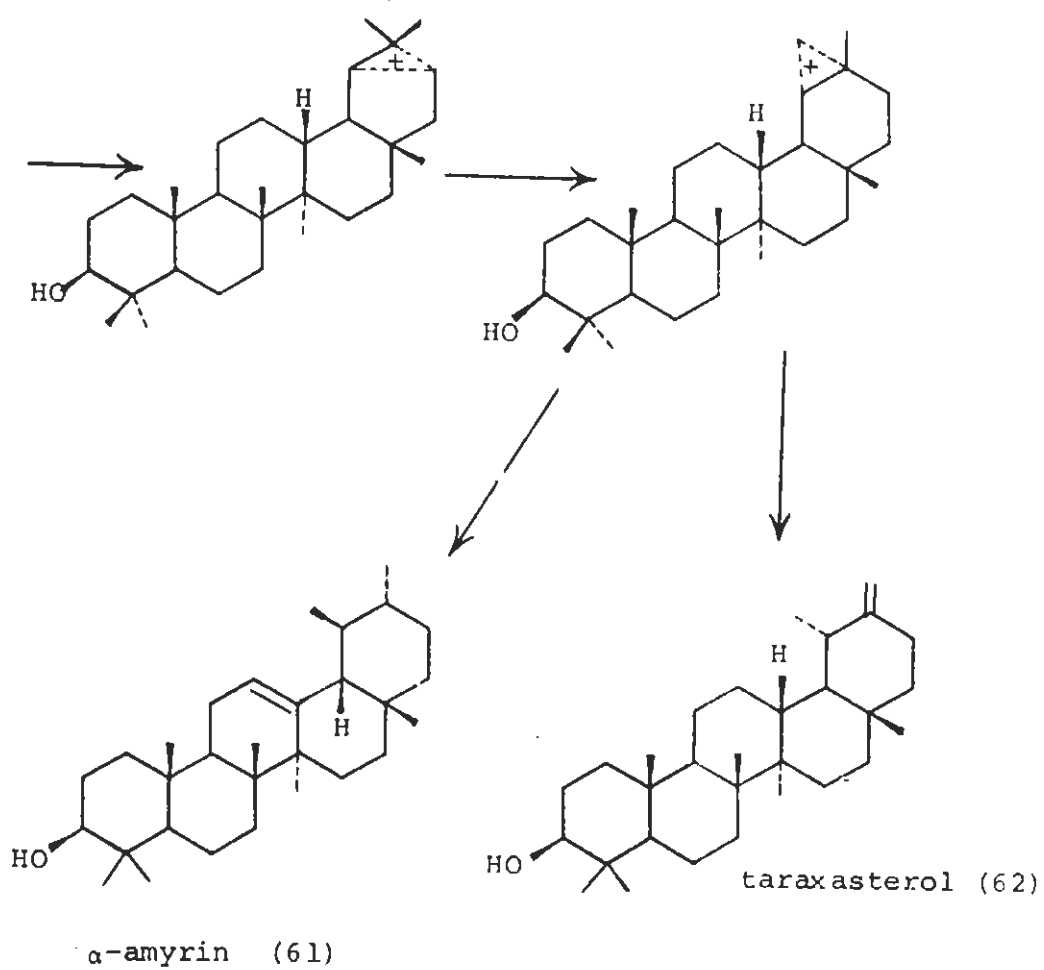
Squalene (60) is formed by the linkage<sup>22,23</sup> of two molecules of farnesyl pyrophosphate (59) joined tail-to-tail as shown in (Scheme 25). Squalene (60) is the biological precursor of all triterpenoids and its stereochemistry was established by an x-ray investigation of its urea adduct.<sup>24</sup> Different types of tetra- and pentacyclic triterpenoids are formed according to the conformation that squalene epoxide adopts.  $\alpha$ -Amyrin (61)<sup>25</sup> and taraxasterol (62)<sup>26</sup> are formed as outlined in Scheme (26) and  $\beta$ -amyrin (63)<sup>27,28</sup> arises according to Scheme (27).



Scheme-25

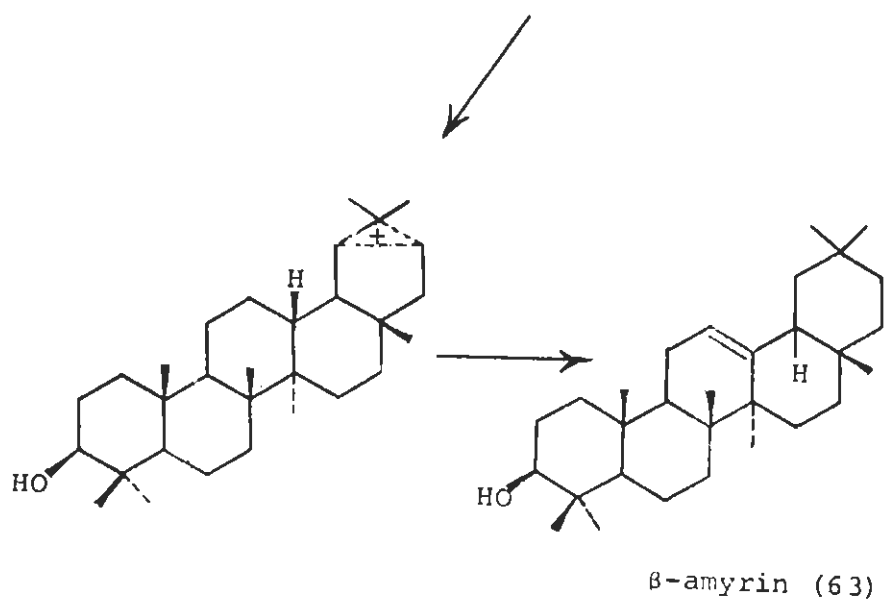


Scheme-26



Scheme-26

Squalene epoxide



Scheme-27

## I N T R O D U C T I O N

*Berberis*, family Berberidaceae is a large genus with nearly 400 species found mostly in Asia, America and the Mediterranean region. It is represented in Pakistan by 20 species distributed largely in the mountainous areas.<sup>29</sup> The plants belonging to the genus *Berberis* are reputed for their curative properties and hence have played a vital role in folkloric medicine for the last few centuries, particularly in the Indo-Pakistan sub-continent.<sup>30</sup> The *Berberis* genus is a rich source of different types of isoquinoline alkaloids. These are highly complex organic molecules and include many physiologically active compounds such as codeine, narcotine and emetine.

Codeine was first isolated from opium by Robiquet in 1832 and is used clinically for the relief of cough, chronic cough as in tuberculosis and as a mild analgesic, codeine resembles morphine but is less toxic.

Narcotine has been found to possess antitussive activity. The alkaloid has only a mild narcotic action and is a much weaker analgesic than morphine or codeine. Ethyl narceinate is used as a narcotic, analgesic and

antitussive agent. Emetine is used clinically against amoebic infections<sup>31</sup> but because of its toxicity and cumulative effects its use is not recommended beyond a 9-10 day period.

Simple tetrahydroisoquinoline alkaloids such as anhalonidine, anhalidine and pelletine have little activity as anticonvulsants, tranquilizers or muscle relaxants and possess no significant hallucinogenic action.<sup>32</sup>

Papaverine hydrochloride has been used for the relaxation of smooth muscles.<sup>33</sup> It also has coronary vasodilator properties, but it is not as effective as nitroglycerine in the treatment of angina pectoris. Papaverine hydrochloride has been used in vasospasm accompanying peripheral arterial embolism, pulmonary embolism and cerebrovascular thrombosis.<sup>34</sup> The drug exhibits a distinct local anaesthetic activity. Papaverine hydrochloride is not a narcotic and is not addictive but its side effects are drowsiness, constipation, increased reflex excitability and gastric distress.

A phenethylisoquinoline alkaloid, methopholine, is a safe analgesic. The effectiveness of methopholine is similar

to that of codeine but unlike codeine methopholine does not lead to physical addiction, sedation, or constipation.<sup>35</sup>

A large number of tetrahydroprotoberberines substituted in ring A and D have shown promising tranquilizing properties in initial tests.<sup>36</sup> Berberine, which has some antibacterial and antiprotozoal activity, has been shown to form a complex with DNA. The alkaloid is probably intercalated into supercoiled mitochondrial DNA to produce configurational changes in the DNA.<sup>37</sup> It is also active in vitro against *Mycobacterium smegmaris* ATcc 607 and is thus of potential importance as an antitubercular drug.<sup>38</sup>

The bisbenzylisoquinoline alkaloid (+)-tubocurarine chloride is used in very small doses in abdominal surgery as a complement to anesthetics since it causes paralysis of the abdominal muscles without stopping the natural movement of the intestines.<sup>39</sup> The alkaloids cis-sampareine,<sup>40</sup> thalidasine<sup>41</sup> and tetrandrine<sup>42</sup> have shown promising tumour-inhibiting properties in initial tests. The oxyacanthine-type alkaloid (+)-cepharanthine is claimed to be highly effective against human tuberculosis and leprosy.<sup>43</sup>

A list of the different types of isoquinoline alkaloids isolated from various species of *Berberis* is given in Table 1.

T A B L E - 1

PLANTS	ALKALOIDS	REF. NO.
<i>Berberis acanthifolium</i>	Berberine, palmatine	44
<i>B. amurensis</i> Ruper	Berberine, berbaminine, berbamine, palmatine, oxyberberine, (berlambine), jatrorrhizine, magnoflorine, base (m.p. 190-191), isoterandrine.	45,46,47
<i>B. aquifolium</i> Pursch.	Berbamine, oxyacanthine, berberine, carypalmine, canadine.	48,49 50
<i>B. aristata</i> ( <i>B. floribunda</i> )	Oxyacanthine, berbamine, jatrorrhizine, berberine, epiberberine, palmatine, dehydrocarboline, columbamine.	51,116,127
<i>B. asiatica</i> Roxb. enDe.	Berbamine, berberine, palmatine, jatrorrhizine, berbamine.	52
<i>B. baluchistanica</i>	Pakistanine, pakistanamine	53,54
	Baluchistanamine	55
	Baluchistine	56
	Dihydrosecoquettamine, secoquettamine, secoquettamine, quettamine chloride, (+)-armepavine methochloride, oblongine chloride.	57
<i>B. buxifolia</i>	Catafacine	58
	Calafatimine	59

Contd..

TABLE-(1) Contd.

<i>B. calliobotrys</i>	Khyberine, pakistanamine, 1-0-methyl pakistanine, pakistanine, chitraline, kalashine.	60
<i>B. coriaria</i>	Berberine	61
<i>B. chilensis</i>	(+)-Reticuline	62
	O-Methylisothalicberine, isothalicberine, 7-0-desmethylisothalicberine	63
<i>B. darwini</i>	Protopine	63
	Berberine	69
<i>B. empetrifolia</i>	Berberine, paquistanine, isotetrandrine, protopine, berlambine.	64
	1,4-dioxo-2-hydroxy-7,8, methylenedioxy-12, 13-dimethoxyaporphoeadane.	64
	Pakistanine	65
<i>B. fortunei</i> Lindl	Berbamine, oxyacanthine	66
	Berberine, jatrorrhizine, palmatine, berbamine, magnoflorine.	67
<i>B. gracilis</i>	Canadine, berberine, cryptopine, protopine.	68
<i>B. hakeoides</i>	Pronuciferine	62
<i>B. heteropoda</i> Schrenk.	Berbamine	70
	Oxyacanthine	71
	Berberine, columbamine, palmatine, berbamine, isotetandrine, oxyacanthine, bervuleine, vulracine.	72

Contd...

TABLE-(H) Contd.

	Jatrorrhizine, columbamine, berberubine.	73
	$C_{19}H_{22}ON_2$	74
<i>B. himalaica</i> Ahrendt.	Himanthine	75
<i>B. integerrima</i>	Berberine	76
	Berbamunie	76,77
	Oxyacanthine, magnoflorine	76
	Isoboldine, thalicmidine N-oxide, reticuline, isocorydine, N-oxide.	78
	Isocorydine, thalicmidine, glaucine, oxyacanthine.	79
<i>B. japonica</i> R.Br.	Berbamine	66
	Isotertrandine	66,80
	Isoterine, berbamine, jatrorrhizine, berberine, palmatine.	81
<i>B. juliana</i> Schneid.	Berbamine, oxyacanthine	82
	Magnoflorine	82,86
	Pakistanamine	83,84,85
	Berberine, jatrorrhizine	85,86
	Glaucine, palmatine	87
<i>B. kawakami</i> Hayata.	Berbamine, isotetrandrine	88
	Berberine, jatrorrhizine, palmatine, berbamine, isotetrandrine, magnoflorine.	89

Contd....

TABLE [1] Contd.

<i>B. koreana</i>	Berberine, palmatine, magnoflurine.	90
<i>B. lamberti</i> R.N. Parker.	Berbamine, oxyacanthine	91,92
	Berberine, columbamine, dihydroberberine, jatrorrhizine, oxyberberine (berlambine).	92
<i>B. laurina</i> (Thumb) Billb.	Belarine	93
	Espinidine, espinine	94
	Lauberine	95
	O-methyl isothalicberine, obaberine.	93,95
	Berberine, hydrastine	96
	Umbellatine, neprotine	97
	Belarine	98,99
<i>B. lycium</i> Royle	Berbamine	100,101
	Umbellatine	101
	Berberine	101,102,103
	Palmatine, oxyberberine	102
	Berbericine	103
<i>B. mingetsensis</i> Hayata.	Berbamine, Isotetrandrine	104
<i>B. morrisonensis</i> Hayata.	Berbamine	105
	Isotetrandrine, berbaminine	106

Contd.....

TABLE (1) Contd.

<i>B. oblonga</i>	2'-n-Methylberbamine	107
	Oblongamine, oxyacanthine	106
	Berberine, berbamine, magnoflorine, palmatine.	106,108
	Columbamine, yatorrhizine, berbamine.	109
	Oblongine	110
	6-hydroxy-7-methoxy-N-methylisoquinolinium, 6-hydroxy-7-methoxy-N-methyl 3-4-dihydroisoquinolinium.	111
<i>B. orthobotrys</i>	Kalashine	112,113
	1-0-methylpakistanine, chitraline, pakistanamine, pakistanine, berbamine, oxyacanthine, aromoline.	113
<i>B. petiolaris</i>	Berbamine	114
<i>B. swaseyi</i> Buckey.	Berbamine	115
	Berberine, berbamine	115,117
	Jatrorrhizine	115,118
<i>B. thunbergii</i> DC	Berbamine, isotetrandrine, oxyacanthine.	119
	Jatrorrhizine, columbamine, palmatine, shobakunine, berberine.	120,121
<i>B. tinctoria</i>	Berbamine	75

Contd.....

TABLE (1) Contd.

<i>B. tschonoskyana</i> Regel.	Obaberine, obamegine, oxyacanthine.	122
	Berberine, jatrorrhizine, oxyberberine, (berbambine), palmatine, magnoflorine, obamegine.	123,124
<i>B. tschonoskyana</i> Regel.	Base (m.p. 89-92)	123,124
<i>B. vulgaris</i> Linn	Berbamine	125
	Oxyacanthine, berberine, columbamine, palmatine, berbamine, isotetandrine, oxyacanthine, bervuleine.	126
	Vulracine	72
	Jatrorrhizine	128
	Berberrubine	73
	$C_{19}H_{22}ON_2$ m.p. 256	74
<i>B. zebiliana</i>	Berbamine, oxoberberine	129
	Berberine	130

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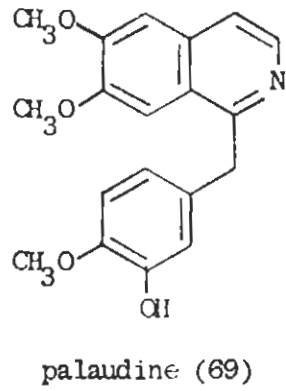
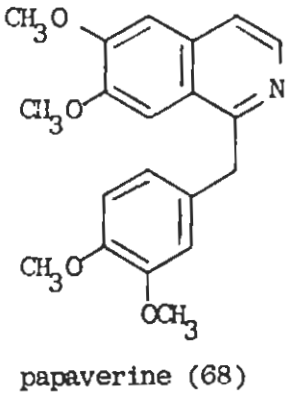
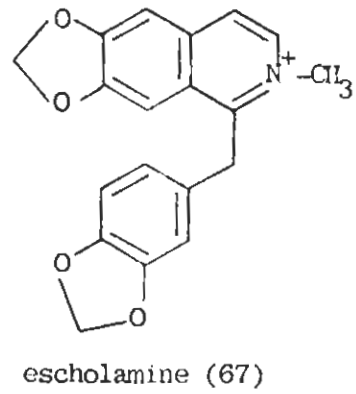
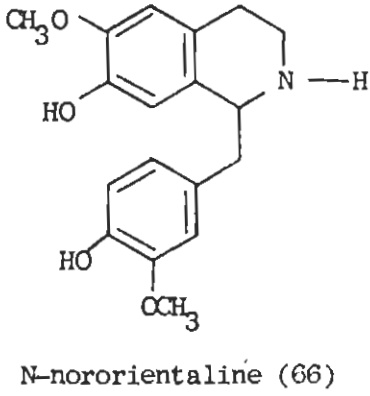
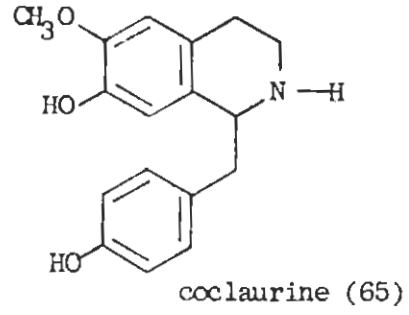
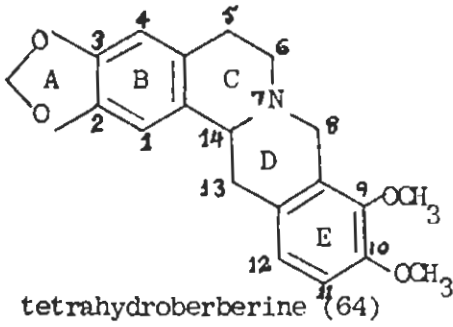
*Berberis aristata*<sup>131</sup> is an erect spinous shrub 6-13 ft. high, often forming gregarious patches. It has a pale yellowish-brown bark, which is deeply furrowed. The flowers are golden yellow. It occurs on the Himalayas from 6,000-10,500ft and also in Nilgiri Hills, and in Ceylon.

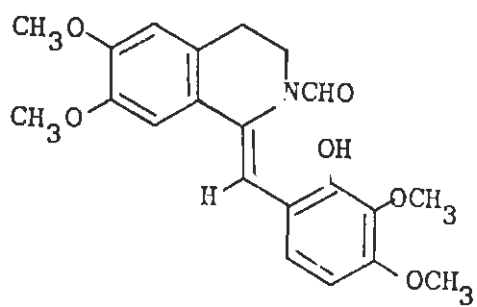
The roots of *Berberis aristata* are used as a bitter tonic, alternative, astringent, diaphoretic and as a curative of piles. "Rasaut" (root bark extract) is highly esteemed in the indigenous medicine. Mixed with butter and alum or with opium and lime juice it is applied over the eyelids as a useful household remedy for the treatment of acute conjunctivitis and in chronic ophthalmia. Washing of unhealthy ulcers and sores with "rasaut" decoction is an ancient practice both in India and in Pakistan.<sup>132</sup> Rasaut is also used in the treatment of skin diseases, menorrhagia, jaundice, and malarial fever.<sup>131</sup>

The protoberberine and bisbenzylisoquinoline types of alkaloids have been isolated from *Berberis aristata* DC. More than fifty different types of protoberberine alkaloids are known in nature. They have the general structure shown in structure (64). Substituents are usually present in them at C-2 and C-3 and either at C-9 and C-10 or at C-10 and

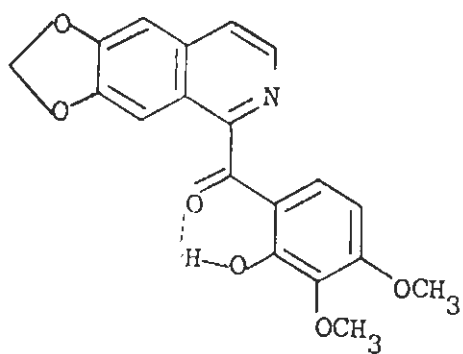
C-11. In some instances a hydroxyl or methoxyl substituent may be present at C-1. A methyl group is sometimes found at C-13 while in a few cases a hydroxyl is located at C-13 or at C-5.

The benzyloisoquinolines occupy a paramount position in alkaloid chemistry because they act as precursors to many of the naturally occurring isoquinoline alkaloids. The benzyloisoquinoline alkaloids are either of the 1,2,3,4-tetrahydro-type, such as coclaurine (65) and N-nororientaline (66) or of the fully aromatised type as in the cases of papaverine (68), palaudine (69) and escholamine (67). Pseudobenzyloisoquinoline alkaloids are benzyloisoquinolines which incorporate three oxygenated substituents in the bottom aromatic ring. Three different types of pseudobenzyloisoquinoline alkaloids are known. The group includes polycarpine (70),<sup>133,134,135</sup> rugosinone (71)<sup>136</sup> and (-)-ledecorine (72).<sup>137</sup> The bisbenzyloisoquinolines constitute the largest group of the isoquinoline alkaloids. They are dimeric bases which for purposes of classification, can be subdivided into 28 subgroups.<sup>138</sup> The aromatic substituents may be hydroxyl, methoxyl or methylenedioxy. Two asymmetric centers are present in the bisbenzyloisoquinoline alkaloids except when one or both of the nitrogen atoms are in the form of an imine.

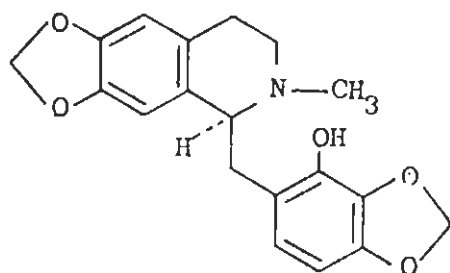




polycarpine (70)



rugosinone (71)



ledecorine (72)

Investigation of the alkaloids in the roots of *Berberis aristata* DC (Berberidaceae) has been carried out by a number of groups. R.P.Chatterjee and co-workers<sup>23</sup> have reported the isolation of eight alkaloids from its roots. These are oxyacanthine, berbamine, berberine, epiberberine, palmatine, dehydrocarboline, jatrorrhizine and columbamine. Investigations on *Berberis aristata* have also been carried out by Ray and Roy<sup>88</sup> who reported the presence of the major alkaloid berberine in 2.23% yield. Later a systematic study of the alkaloidal constituents of this species was carried out by K.K.Chakraverti<sup>99</sup> who isolated two major alkaloids in the form of their hydrochloride salts, one of which was the previously reported alkaloid berberine while the other was palmatine.

## 2.2 RESULTS AND DISCUSSION

*Berberis aristata* has been reputed as a medicinal plant in scientific and folkloric literature but only a limited chemical investigation with particular reference to its tertiary alkaloidal contents has been carried out. In this section of the thesis, the isolation and structure elucidation of the tertiary alkaloids isolated from the root bark extract of *B. aristata* is presented. As a result of present work six different alkaloids have been isolated. These alkaloids belong to three different classes namely protoberberine type, bisbenzylisoquinoline type and pseudobenzylisoquinoline type.

### ISOLATION AND STRUCTURAL STUDIES ON TERTIARY ALKALOIDS OF BERBERIS ARISTATA

"Rasaut" (root bark extract of *B. aristata*) was extracted with hot ethanol. The extract was evaporated to a brown residue and acidified with 20% acetic acid. The acidic aqueous solution was then extracted exhaustively with chloroform. The acidic aqueous extract was then basified with 20% ammonium hydroxide solution to pH-9 and extracted with chloroform to obtain the tertiary bases. The chloroform layer was separated, dried (anhydrous  $\text{Na}_2\text{SO}_4$ ) and evaporated to a brown gum which consisted of the alkaloidal fraction ( $F_1$ ).

i) Isolation and Structure of "Karachine" (73)

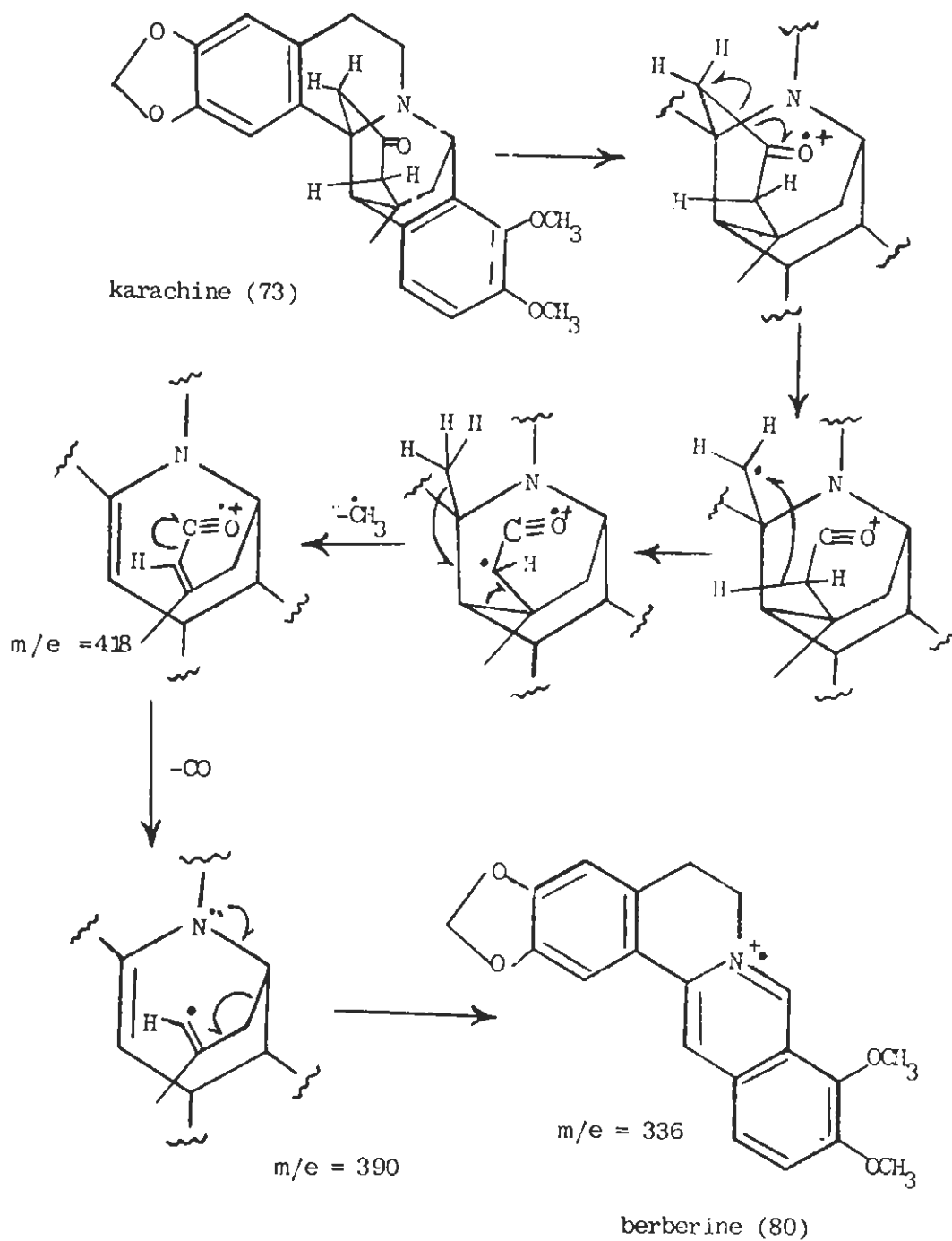
The alkaloidal fraction ( $F_1$ ) was chromatographed on a neutral aluminium oxide column. The column was packed in petroleum-ether and elution was carried out with petroleum-ether, petroleum ether-chloroform, chloroform-methanol and finally with methanol. The fractions eluted with petroleum ether-chloroform ( $FT_1$ ) afforded an oily mass. The oily mass from the petroleum ether-chloroform was rechromatographed on a silica gel column which was packed in petroleum-ether followed by increasingly polar mixtures of petroleum ether-chloroform. A small quantity (8mg) of a colourless and optically inactive alkaloid named "Karachine" (73) m.p. 146-148°C was obtained which afforded its  $M^+$  at  $m/e = 433.1881$  in agreement with the formula  $C_{26}H_{27}O_5N$ . The ultraviolet spectrum of "Karachine" (73) showed absorption maxima at 285 n.m., and 226 (sh) and minima at 255 n.m. The positions of absorption showed the presence of a tetrahydroprotoberberine nucleus. The i.r. spectrum ( $CHCl_3$ ) gave an absorption band at  $1710\text{ cm}^{-1}$  indicating the presence of a nonconjugated carbonyl group. The nuclear magnetic resonance spectrum (360 MHz, FT) in  $CDCl_3$  (recorded by Prof. M. Shamma at Pennsylvania State University) showed a one-proton quartet centered at  $\delta 1.11$  ( $J_{ax} = 1.8\text{ Hz}$ ,  $J_{ab} = 12.6\text{ Hz}$ ) due to

H<sub>a</sub>, another one-proton quartet at  $\delta$  2.08 ( $J_{bx}=4.0\text{Hz}$ ,  $J_{ba}=12.6\text{Hz}$ ) due to H<sub>b</sub>. The protons of C- $\gamma$  and C- $\epsilon$  gave two sets of two proton doublet of doublets at  $\delta$  2.46 and  $\delta$  2.48 ( $J_{gem}=14.0\text{Hz}$ ) and at  $\delta$  2.70 and  $\delta$  2.72 ( $J_{gem}=14.3\text{Hz}$ ) respectively. The one proton quartet at  $\delta$  4.12 ( $J_{xa}=1.8\text{Hz}$ ,  $J_{xb}=4.0\text{Hz}$ ) is assigned to H-8. A singlet at  $\delta$  0.82 is due to the bridgehead methyl group attached to C- $\beta$ . A nuclear overhauser enhancement study was carried out by Prof.M.Shamma and co-workers in order to find out the substitution pattern in the aromatic rings A and D of "karachine" (73). Irradiation of the C-10 methoxy singlet at  $\delta$  3.77 resulted in an overall 11.6% increase in the area of the  $\delta$  6.52 and  $\delta$  6.55 ring D aromatic doublet of doublets. Irradiation of H-1 singlet at  $\delta$  6.73 gave a 2.8% increase of the  $\delta$  2.70 and  $\delta$  2.72 doublet of doublets assigned to the C- $\epsilon$  protons as well as to a  $\delta$  5.6% increase of the signal at  $\delta$  3.07 due to H-13. Significantly irradiation of either the H-1 or H-4 singlets at  $\delta$  6.73 and  $\delta$  6.17 respectively led to no observable NOE for the methoxyl absorptions.

The mass spectrum of "karachine" (73) shows a molecular ion at  $m/e = 433$  and a base peak at  $m/e = 336$ . The base peak exactly fits the molecular ion of berberine

$m/e = 336 (M^+)$ . The loss of 97 mass units from the molecular ion occurs via cleavage  $\alpha$  to the nitrogen atom (C-14 to C- $\epsilon$  bond) followed by a retro-Diels Alder process (Scheme 28). The  $m/e = 97$  fragment corresponds to  $C_6H_9O$  or more specifically to two moles of acetone minus the elements of water, other fragments occur at  $m/e = 418$  (19%), 390 (23%), 375 (4%), 336 (100%), 321 (27%), 292 (16%), 278 (21%), 263 (3%), 189 (21%) and 174 (3%).

In order to gain further insight into the structure for "karachino" (73), it was reduced by Prof. Maurice Shamma and co-workers with sodium borohydride. Reduction was carried out in methanol, to afford amorphous dihydrokarachino (74). It is assumed that reducing agent has approached from the less hindered side of carbonyl. The nuclear magnetic resonance spectrum (360 MHz, FT) in  $CDCl_3$  shows a one-proton quartet at  $\delta$  0.96 ( $J_{ax} = 1.8\text{Hz}$ ,  $J_{ab} = 12.6\text{Hz}$ ) due to  $H_a$  on C- $\alpha$ . There is a complex multiplet in the region  $\delta$  1.60 -  $\delta$  2.30 consisting of two ABX systems due to the C- $\gamma$  and C- $\epsilon$  protons coupled to the C- $\delta$  proton. Irradiation of the C- $\delta$  proton at  $\delta$  4.16 gave the much simpler spin-decoupled pattern of two overlapping AB systems at  $\delta$  1.67 and  $\delta$  2.29 ( $J_{gem} = 14.0\text{ Hz}$ ) and  $\delta$  1.88

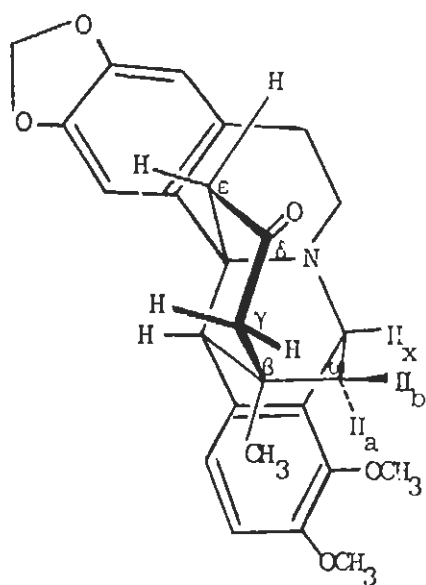


Scheme-28

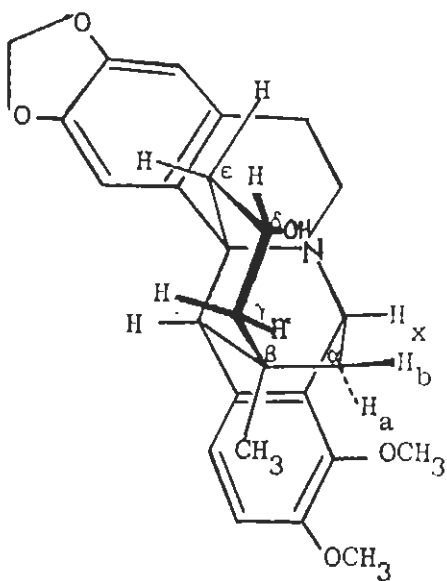
and  $\delta$  2.22 ( $J_{\text{gem}}=14.0\text{Hz}$ ) due to the methylene protons at C- $\gamma$  and C- $\epsilon$ . A three-proton upfield singlet at  $\delta$  0.73 is due to the bridgehead methyl group attached to C- $\beta$ .

The mass spectrum of dihydrokarachine (74) shows a molecular ion at  $m/e = 435$ , the base peak at  $m/e = 336$  and other fragments are at 420 (6%), 390 (4%), 336 (100%), 321 (15%), 292 (8%), 278 (9%), 263 (1%), 189 (16%) and 174 (2%). On the basis of these data structures (73) and (74) are assigned to "karachine" and "dihydrokarachine" respectively.

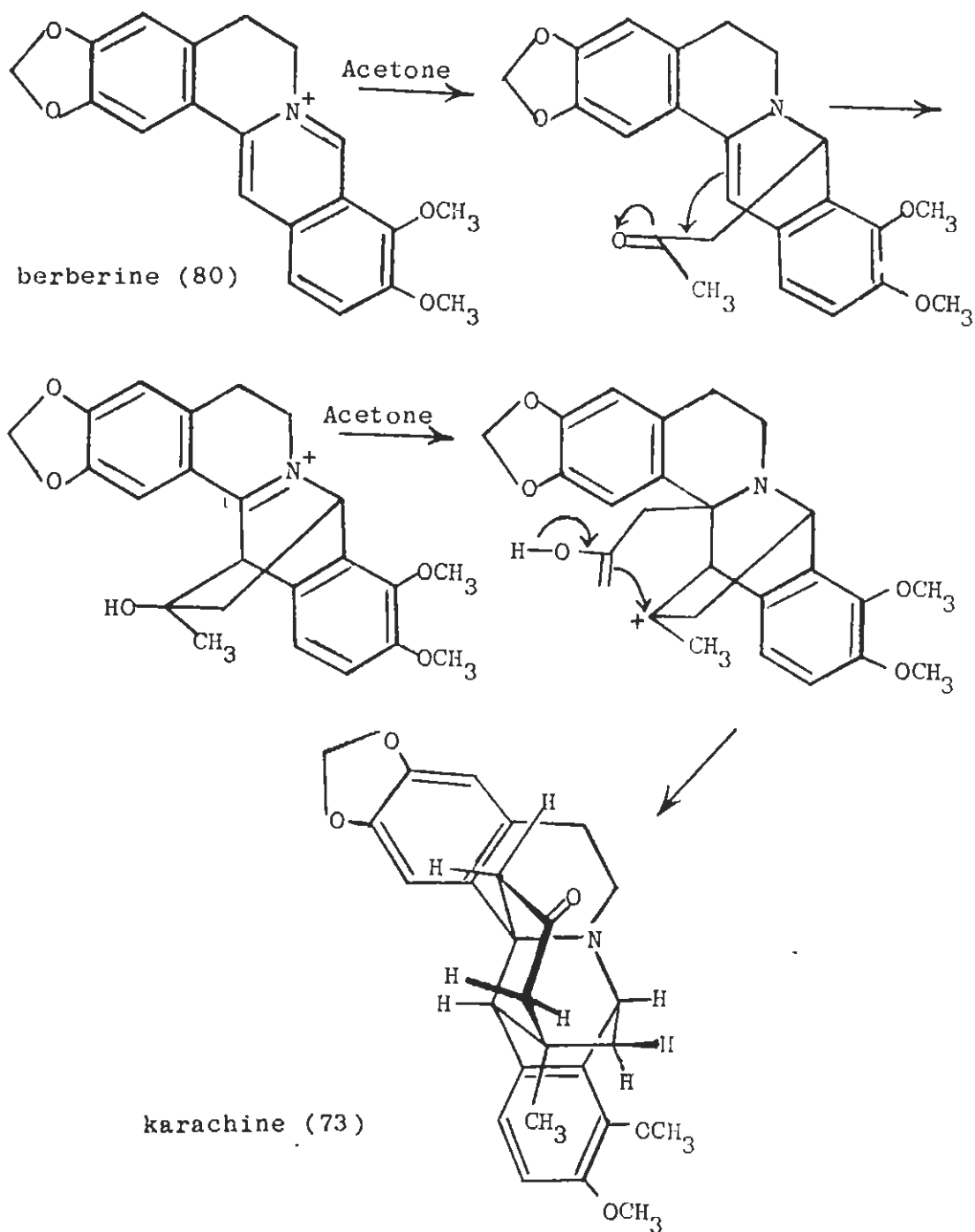
It appears that "karachine" (73) is formed in the plant by the condensation of berberine (80) with 2 mol. of acetone and loss of water as showed in Scheme 29. To make sure that "karachine" (73) is a true alkaloid not an artifact of isolation, berberine was condensed with acetone at varying pH but "karachine" (73) was not observed to be formed. The isolation procedure was repeated with purified acetone-free solvents which led to the reisolation of "karachine" (73). Racemic "karachine" (73) is the first naturally occurring berbinoid incorporating acetone units. The alkaloid "karachine" (73) is the most complex of more than fifty protoberberine alkaloids presently known.



karachine (73)



dihydrokarachine (74)



Scheme-29

ii) Isolation and Identification of Oxyberberine (75)

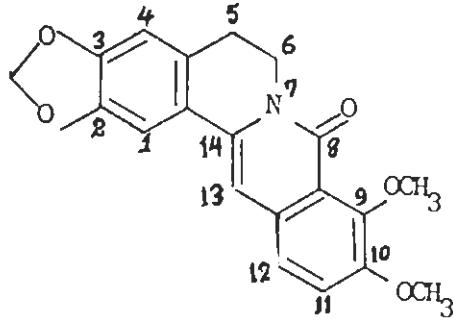
Another protoberberine alkaloid has been isolated from *B. aristata*. "Rasaut" was soaked overnight in 20% acetic acid filtered, and the extract was basified with 20%  $\text{NH}_4\text{OH}$  solution to pH-10. It was extracted with chloroform and the chloroform layer was evaporated to afford the tertiary alkaloidal fraction ( $\text{F}_2$ ) as a brownish gum. This fraction was chromatographed on a neutral aluminium oxide column. Elution was carried out with petroleum-ether, petroleum ether-ethylacetate, ethylacetate, ethylacetate-methanol and finally with methanol. The petroleum ether-ethylacetate fraction (FP) afforded two alkaloids "C" and "D". Alkaloid "C" was purified by repeated preparative thin layer chromatography using silica gel (G 60) plates in ethylacetate: petroleum-ether (80:20). Final purification of alkaloid (C) was carried out by preparative thin layer chromatography on silica gel (G 60) plates using  $\text{CHCl}_3$ : MeOH (100:5). The ultraviolet spectrum of alkaloid "C" showed absorption maxima at 233, 290 and 340 n.m. and minima at 285 and 292.5 n.m. The absorptions were characteristic for a tetrahydroprotoberberine system. An accurate mass measurement of the molecular ion afforded  $\text{M}^+ = 351.1110$  ( $\text{C}_{20}\text{H}_{17}\text{O}_5\text{N}$  requires 351.1106). The nuclear magnetic resonance

spectrum of alkaloid "C" in  $\text{CDCl}_3$  showed two singlets at  $\delta$  3.96 and  $\delta$  4.02 indicating the presence of two  $\text{OCH}_3$  groups. A peak at  $\delta$  6.2 is assigned to the methylenedioxy protons. The protons of C-11 and C-12 gave two doublets at  $\delta$  7.35 and  $\delta$  7.32 respectively ( $J = 7.3\text{Hz}$ ). A one-proton singlet at  $\delta$  7.23 is assigned to C-13. The two-protons adjacent to the nitrogen resonated as a triplet at  $\delta$  4.30 (t, H-6,  $J=6.0\text{Hz}$ ), while another triplet at  $\delta$  2.90 is assigned to H-5, ( $J=6.0\text{Hz}$ ). The mass spectrum showed the molecular ion at  $m/e = 351$  ( $M^+$ , 55%) and other major peaks were at 336 (62%), 322 (61%), 292 (30%) and 168 (13%). Alkaloid "C" was identified as oxyberberine (75) on the basis of the above spectroscopy data. The structure was confirmed by direct chromatographic and spectroscopic comparisons with an authentic sample.

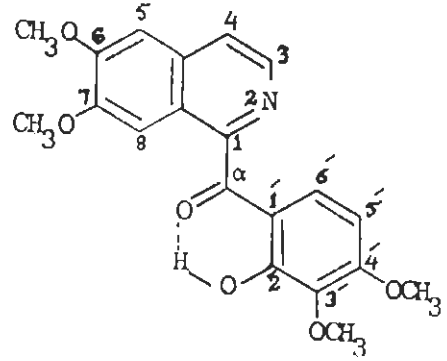
iii) Isolation and Structure of Taxilamine (76)

In continuation of our work on the isolation and structure elucidation of minor alkaloids of *B. aristata*, a new pseudobenzylisoquinoline alkaloid named as "taxilamine" (76) has been isolated.

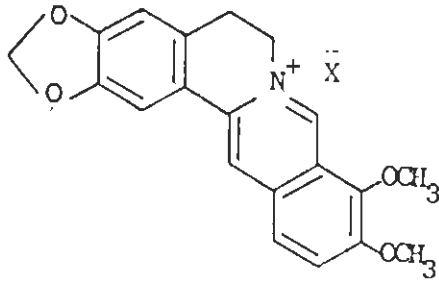
Taxilamine (alkaloid "D") obtained as described above from the petroleum ether - ethylacetate fraction (FP) was further purified by repeated preparative thin layer chromatography. The substance afforded maxima at 238 n.m. and 299 n.m. with a shoulder at 330 n.m., and a minimum at 255 n.m. in its ultraviolet spectrum. On addition of alkali (aqueous sodium hydroxide) a bathochromic shift occurred and bands were observed at 237, 292, 328 and 380 n.m. The nuclear magnetic resonance (360 MHz, FT) in  $\text{CDCl}_3$  (recorded by Prof. Maurice Shamma at Pennsylvania State University) showed the H-3 and H-4 protons in (76) as a doublet of doublets at  $\delta$  8.46 and  $\delta$  7.66 ( $J_{\text{vic}}=5.5\text{Hz}$ ); H-5' and H-6' as another doublet of doublets at  $\delta$  6.44 and  $\delta$  7.28 ( $J_{\text{vic}}=9.1\text{Hz}$ ); two singlets of H-5 and H-8 were found to be present at  $\delta$  7.15 and  $\delta$  7.40 respectively. Four singlets at  $\delta$  3.92,  $\delta$  3.96,  $\delta$  3.97 and  $\delta$  4.06 confirmed the presence of four methoxyl groups in the molecule. The nuclear magnetic resonance spectrum resembled the spectrum of rugosinone (71). Accurate mass measurement on  $\text{M}^+$  of taxilamine afforded the molecular formula  $\text{C}_{20}\text{H}_{19}\text{O}_6\text{N}$ , showing molecular ion  $m/e = 369.1213$  while other fragments were present at  $m/e = 354$  (14%), 352 (15%), 326 (14%), 310 (68%), 296 (33%), 188 (57%), 181 (13%), 149 (75%), 86 (50%), 84 (74%) and 49 (100%).



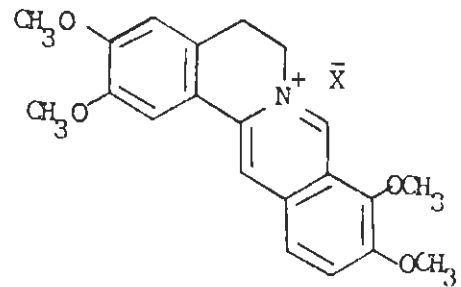
oxyberberine (75)



taxilamine (76)



berberine (80)



palmatine (46)

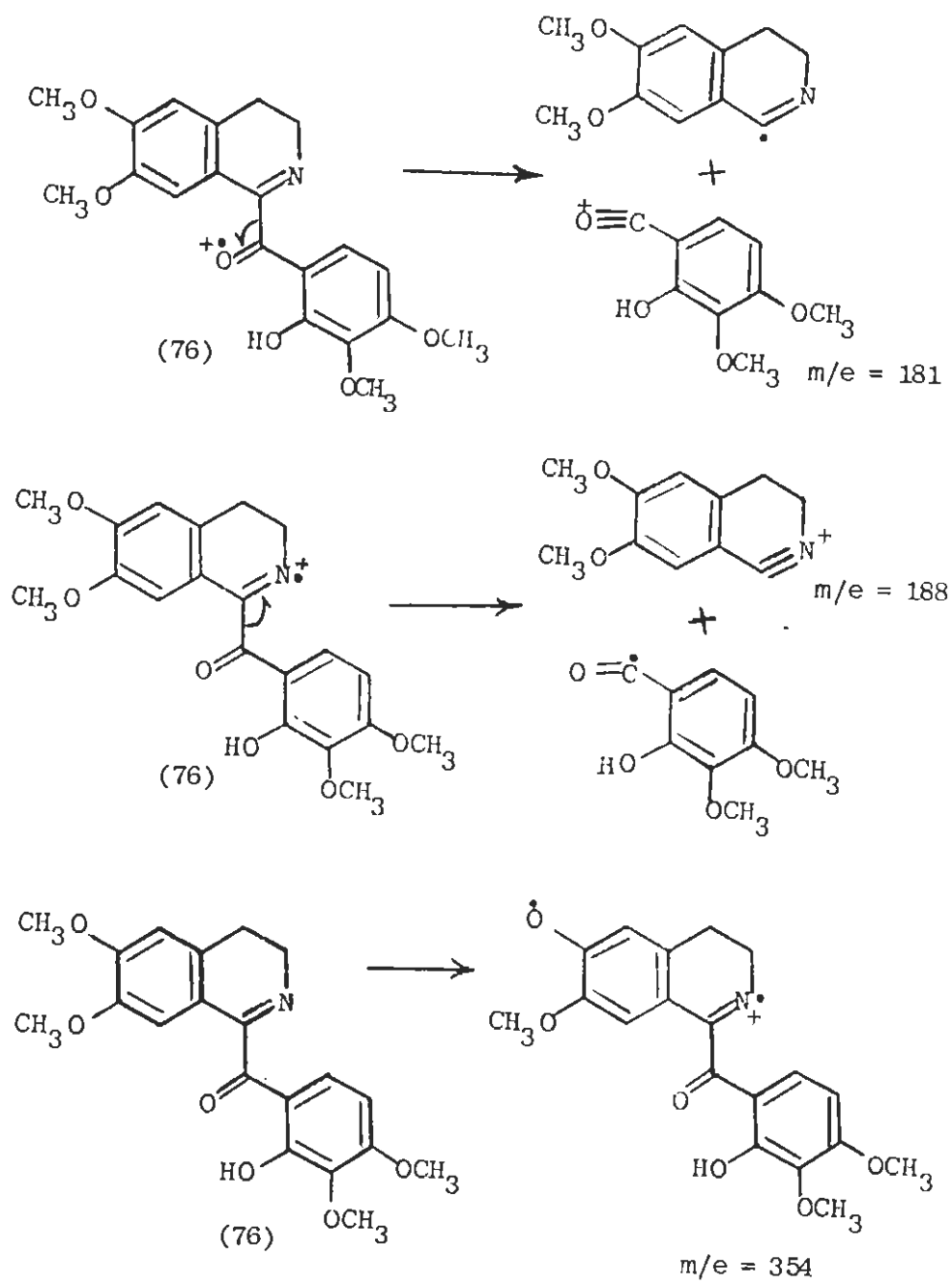
The  $m/e = 181$  and  $188$  fragments may arise by cleavage of C-1 to C- $\alpha$  bond (Scheme 30). On the basis of these spectral data structure (76) is assigned to taxilamine.

"Taxilamine"(76) is probably formed in nature through oxidative rearrangement of palmatine (46) to polycarpine (70). Hydrolytic N-deformylation followed by further oxidation would then afford "taxilamine" (76) (Scheme 31).

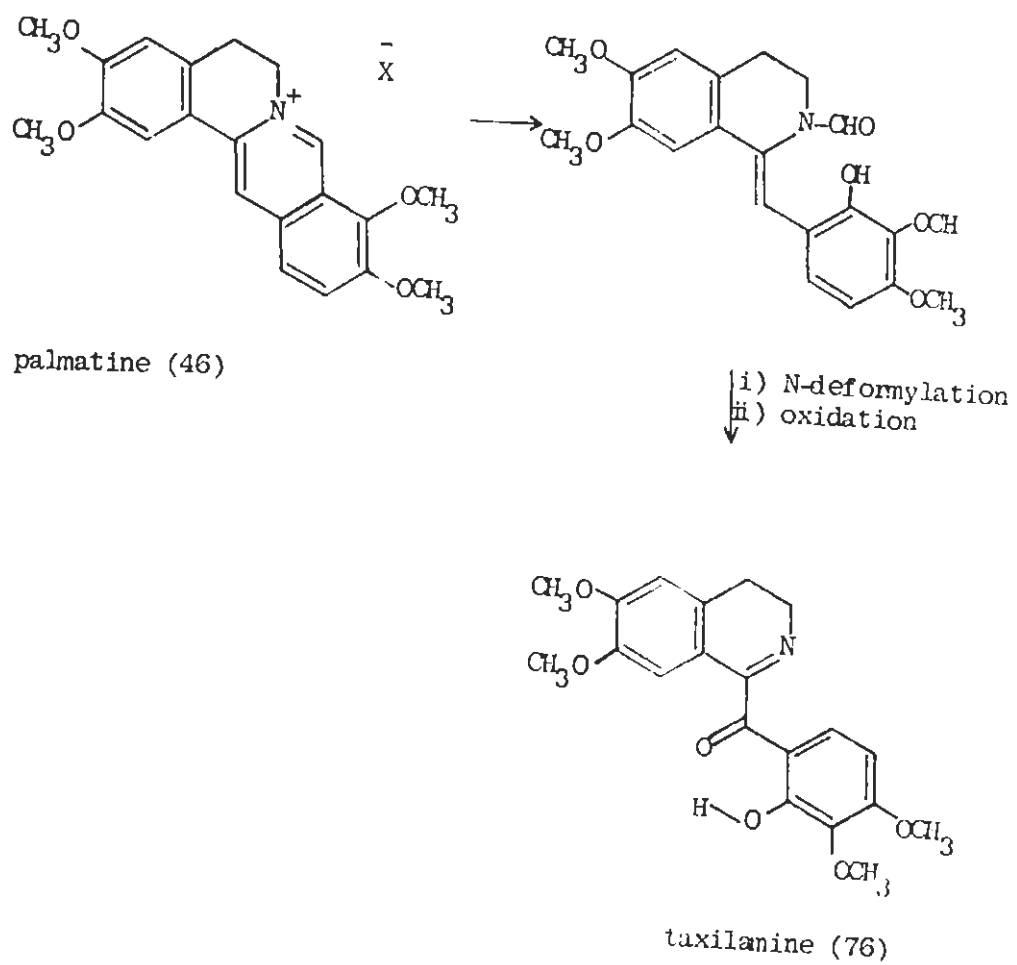
iv) Isolation and Structure of Bisbenzylisoquinoline Alkaloids

A part from the protoberberine and pseudobenzylisoquinoline alkaloids described above, dimeric bisbenzylisoquinoline alkaloids have also been isolated from rasaut namely oxyacathine, berbamine and aromoline. These belong to a group containing two diphenyl ether linkages.

The tertiary alkaloidal fraction ( $F_1$ ) was chromatographed on a neutral aluminium oxide column. Elution was carried out with petroleum-ether followed by increasingly polar mixtures of petroleum-ether and ethylacetate, ethylacetate, ethylacetate-methanol and finally with methanol. The ethylacetate fraction ( $FB_1$ ) afforded a mixture of alkaloids (E) and (F). The ethylacetate and methanol fraction ( $FB_2$ ) afforded yet another alkaloid (G).



Scheme-30



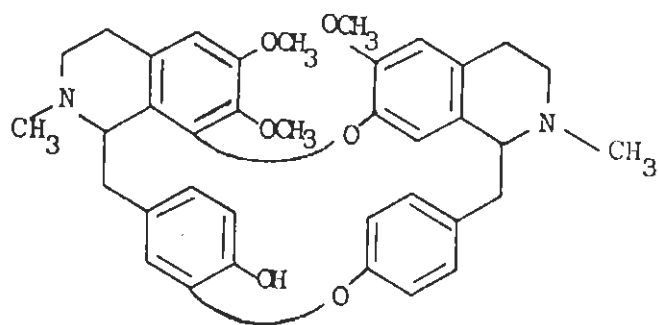
Scheme-31

The alkaloid (E) was further purified by separation on precoated t.l.c. sheets of neutral aluminium oxide (type E) in acetone:methanol (85:15). Alkaloid (E) melted at 212°C. The ultraviolet spectrum showed the absorption maxima at 206 and 282 n.m. with a shoulder at 238 n.m. and minima at 265 n.m. The positions of absorptions coincide with those observed for bisbenzylisoquinoline alkaloids. An accurate mass measurement of the molecular ion afforded  $M^+ = 608.2874$  in agreement with the formula  $C_{37}H_{40}O_6N_2$ . The proton magnetic resonance spectrum (60 MHz) of alkaloid (E) in  $CDCl_3$  gave two sharp singlets at  $\delta$  2.55 and  $\delta$  2.60 integrating for three protons each of which were assigned to the two N-methyl groups. Three sharp singlets at  $\delta$  3.15,  $\delta$  3.65 and  $\delta$  3.75 were present which were assigned to the three  $OCH_3$  groups. The mass spectrum gave the molecular ion peak at  $m/e = 608$  ( $M^+$ , 50%) while other peaks were observed at 607 (30%), 501 (20%), 417 (17%), 416 (10%), 396 (60%), 393 (20%), 381 (30%), 335 (20%), 198 (++, 100%) & 192 (40%). On the basis of these spectral data, the alkaloid (E) was identified as oxyacanthine (79).

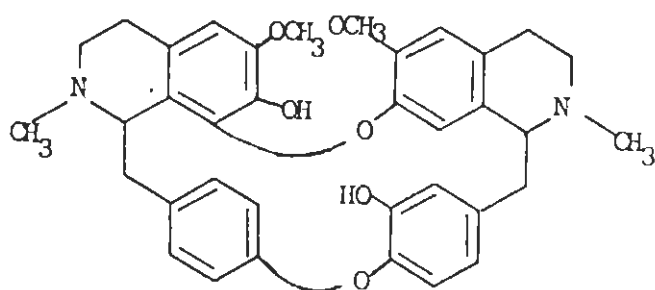
Alkaloid (F) was purified from fraction (FB<sub>1</sub>) by separation on precoated t.l.c. sheets of neutral aluminium oxide type (E) in acetone:methanol (85:15). It melted at 154°C. The ultraviolet spectrum of the alkaloid (F) showed absorption maxima at 284 n.m. and minima at 262 n.m., characteristic of bisbenzylisoquinoline alkaloids. An accurate mass measurement on the molecular ion afforded M<sup>+</sup> = 608.2877 in agreement with the formula C<sub>37</sub>H<sub>40</sub>O<sub>6</sub>N<sub>2</sub>. The proton magnetic resonance spectrum (60 MHz) of alkaloid (F) in CDCl<sub>3</sub> exhibited two singlets at δ 2.24 and δ 2.58 indicating the presence of two N-methyl groups. Three singlets at δ 3.20, δ 3.60 and δ 3.73 indicated the presence of three OCH<sub>3</sub> groups. The mass spectrum of the substance gave the molecular ion peak at m/e = 608 (M<sup>+</sup>, 79%) while other peaks were observed at 607 (50%), 485 (2%), 417 (7%), 395 (68%), 381 (34%) & 198 (++, 100%). On the basis of these spectral data the substance was identified as berbamine (77).

The fraction (FB<sub>2</sub>) was further purified on a basic aluminium oxide column. The column was eluted with ethylacetate and ethylacetate-methanol. The ethylacetate-methanol eluates gave the pure alkaloid (G) on evaporation, m.p. 174°C. The ultraviolet spectrum showed maxima at

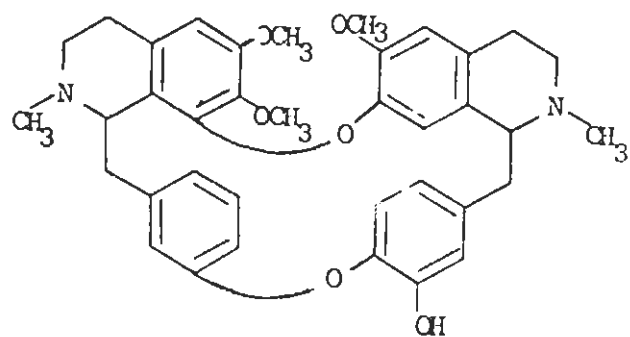
208 & 285 n.m., shoulder at 228 n.m., and minimum at 263 n.m. These values were similar to those observed for other bis-benzylisoquinoline alkaloids. An accurate mass measurement on the molecular ion afforded  $M^+ = 594.2738$  in agreement with formula  $C_{36}H_{38}O_6N_2$ . The proton magnetic resonance spectrum (60 MHz) of alkaloid (G) in  $CDCl_3$  gave two singlets at  $\delta$  2.54 and  $\delta$  2.56 showing the presence of two N-methyl groups. Two singlets at  $\delta$  3.56 and  $\delta$  3.76 indicated the presence of two  $OCH_3$  groups. The mass spectrum of alkaloid (G) gave the molecular ion peak at  $m/e = 594$  ( $M^+$ , 100%) and other peaks were observed at 593 (53%), 382 (46%), 381 (85%), 368 (8%), 367 (40%), 364 (8%), 297 (3%), 192 (17%), 191 (75%), 174 (17%), 168 (14%). On the basis of these spectral data, the alkaloid (G) was identified to be arnomoline (78).



berbamine (77)



aramoline (78)



oxyacanthine (79)

## EXPERIMENTAL GENERAL NOTES

Mass spectra were recorded on VG Micro mass-MM12 mass spectrometer and Varian MAT 112-S mass spectrometer coupled to the spectroscopy 188 computer. High resolution mass spectra were recorded on Varian MAT 312 mass spectrometer. Infrared spectra were recorded on Pye Unicam SP-200G or a Jasco IRA-I spectrophotometer. Nuclear magnetic resonance spectra were recorded on Jeol JNM-PMX 60, Jeol FT100 MHz, Bruker FT360 MHz or FT400 MHz NMR spectrometers.

GLC was performed on a glass column (190x0.150 m) packed with 3% of OV-225 on gas chrom Q (100-120 mesh) GC/MS was performed at an ionization potential of 70 ev. Column chromatography was generally carried out using silica gel-60 F<sub>254</sub> (70-230 or 120-230 mesh) and aluminium oxide (90, neutral).

2.3

E X P E R I M E N T A L

i) Extraction of Root Bark Extract of *Berberis aristata* ("Rasaut")

Lumps of "rasaut" (concentrated root bark extract of *Berberis aristata*, 3 Kg) were crushed and soaked in hot ethyl alcohol (10 litre). The extract was drawn after every fifteen days. After three extractions, the extracts were concentrated in a cyclone evaporator under reduced pressure, the temperature being maintained between 50-55°C. A dark brown coloured semisolid was obtained which was acidified with 20% acetic acid (1 litre) to pH-2. The solution was then extracted exhaustively with chloroform (5 litre). The acidic aqueous extract was basified with 20% ammonium hydroxide solution to pH-9 and extracted with chloroform (7 litre) to obtain the tertiary bases. The chloroform layer was separated, dried (anhydrous  $\text{Na}_2\text{SO}_4$ , 40g) and evaporated to a brown residue. The alkaloidal fraction ( $F_1$ , 12g) were chromatographed on a neutral aluminium oxide column (410g) to isolate the tertiary bases

ii) Isolation of "Karachine"(73)

The alkaloidal fraction ( $F_1$ , 12g) was chromatographed on a neutral aluminium oxide column (410g). The

column was packed in petroleum-ether. Elution was carried out with petroleum-ether (2 litre) followed by increasingly polar mixtures of petroleum ether-chloroform (3 litre), chloroform (1 litre), chloroform-methanol (2 litre), and finally with pure methanol (1 litre). The fractions eluted with petroleum ether-chloroform afforded an oily mass which was further purified on a silica gel column (25.0g). Elution was carried out with petroleum-ether (2 litre) followed by increasingly polar mixtures of petroleum ether-chloroform (500 ml) and ultimately with pure chloroform. A new alkaloid, named "karachine" (8 mg) was isolated from the petroleum ether-chloroform eluates. The alkaloid was further purified by repeated crystallisation in ethylacetate m.p. 146-148°C, optically inactive. Exact mass of the molecular ion was recorded at  $M^+ = 433.1881$  in agreement with the molecular formula  $C_{26}H_{27}O_5N$  (requires 433.1889).

U.V.Spectrum (EtOH):  $\lambda_{\max}$  285 and 226 (sb) n.m. ( $\log \epsilon$  3.62 and 3.90).

$\lambda_{\min}$  255 n.m. ( $\log \epsilon$  2.12)

I.R.Spectrum ( $CHCl_3$ ):  $\nu_{\max}$  1710  $cm^{-1}$  (C=O stretching).

$^1\text{H-N.M.R.}$  ( $\text{CDCl}_3$ , 360 MHz, F.T.): (Recorded by Prof. Maurice Shamma, Pennsylvania State University):

- $\delta$  0.82 (3H, s,  $\text{C}_3\text{-CH}_3$ ),
- $\delta$  1.11 (q,  $\text{H}_a$ ,  $J_{ax}=1.8\text{Hz}$ ,  $J_{ab}=12.6\text{Hz}$ ),
- $\delta$  2.08 (q,  $\text{H}_b$ ,  $J_{bx}=4.0\text{Hz}$ ,  $J_{ba}=12.6\text{Hz}$ ),
- $\delta$  2.46 (d, 2H,  $J_{gem}=14.0\text{Hz}$ , C $\gamma$ )
- $\delta$  2.48 (d, 2H,  $J_{gem}=14.0\text{Hz}$ , C $\gamma$ )
- $\delta$  2.70 (d, 2H,  $J_{gem}=14.3\text{ Hz}$ , C $\epsilon$ )
- $\delta$  2.72 (d, 2H,  $J_{gem}=14.3\text{Hz}$ , C $\epsilon$ )
- $\delta$  4.12 (q,  $\text{H}_x$   $J_{xa}=1.8\text{Hz}$ ,  $J_{xb}=4.0\text{Hz}$ ).

Mass Spectrum:  $m/e = 433$  ( $\text{M}^+$ , 78%), 418 (19%), 390 (23%), 375 (4%), 336 (100%), 321 (27%), 292 (16%), 278 (21%), 263 (3%), 189 (21%) and 174 (3%).

Dihydrokarachine (74): Dihydrokarachine (74) was prepared by Prof. Maurice Shamma's group by reduction of karachine in methanol with sodium borohydride.

$^1\text{H-N.M.R.}$  ( $\text{CDCl}_3$ , 360 MHz, FT):

- $\delta$  0.73 (3H, s,  $\text{C}_5\text{-CH}_3$ ),
- $\delta$  0.96 (q,  $\text{H}_a$ ,  $J_{ax}=1.8\text{Hz}$ ,  $J_{a,b}=12.6\text{Hz}$ ),
- $\delta$  1.60-  $\delta$  2.30 (complex multiplets C- $\gamma$  and C- $\epsilon$  protons coupled to the C- $\delta$  proton).

Mass Spectrum:  $m/e = 435 (M^+, 21\%), 420 (6\%), 390 (4\%), 336 (100\%), 321 (15\%), 292 (8\%), 278 (9\%), 263 (1\%), 189 (16\%)$  and  $174 (2\%)$ .

iii) Extraction of Minor Alkaloids from Root Bark Extract of *B. aristata* ("Rasaut")

Rasaut (2 Kg) was percolated with 10% acetic acid at room temperature for ten hours. The resulting extract was filtered and basified with 20% ammonium hydroxide to pH-10. It was extracted with chloroform (20 litre) to obtain the tertiary bases. The chloroform layer was separated, dried (anhydrous  $NO_2SO_4$ , 20g) and concentrated, in a cyclone evaporator under reduced pressure, the temperature being maintained between  $50-55^{\circ}C$ . A dark brown coloured semisolid tertiary alkaloidal residue was obtained. The alkaloidal fraction ( $F_2$ , 8g) was chromatographed on a neutral aluminium oxide column to isolate the minor tertiary bases.

iv) Isolation of Oxyberberine and Taxilamine

The tertiary alkaloidal fraction ( $F_2$ , 8g) was chromatographed on a neutral aluminium oxide column (280 g) which was packed in petroleum-ether. Elution was carried out with petroleum-ether (2 litre) followed by

petroleum ether-ethylacetate (2 litre), ethylacetate (2 litre), ethylacetate-methanol (2 litre) and ultimately with pure methanol (1 litre). The fraction (FP) eluted with petroleum ether-ethylacetate gave on evaporation a light yellow coloured mixture of two alkaloids (C) and (D). The alkaloids (C) and (D) were further purified by repeated thin layer chromatography on precoated thin layer chromatography sheets of silica gel (G 60) in ethylacetate and petroleum ether (80:20).

The alkaloid (C) was identified as a oxyberberine. The alkaloid (D) was identified as a new alkaloid and named as "taxilamine". Oxyberberine was purified by preparative t.l.c. in  $\text{CHCl}_3$  : MeOH (100:5) system.

Oxyberberine (75): Exact mass of the molecular ion was recorded at  $M^+ = 351.1110$   $\text{C}_{20}\text{H}_{17}\text{O}_5\text{N}$  (requires 351.1106).

U.V.Spectrum (EtOH):  $\lambda_{\text{max}}$  233, 290 and 340 n.m. ( $\log \epsilon$  4.71, 4.01, and 4.46)

$\lambda_{\text{min}}$  285 and 292.5 ( $\log \epsilon$  3.90 and 3.94).

$^1\text{H-N.M.R.}$  ( $\text{CDCl}_3$ , 360 MHz F.T.):

- $\delta$  2.90 (t, H-5,  $J=6.0\text{Hz}$ ),
- $\delta$  4.30 (t, H-6,  $J=6.0\text{Hz}$ ),
- $\delta$  3.96 (3H, s,  $\text{OCH}_3$ ),
- $\delta$  4.02 (3H, s,  $\text{OCH}_3$ ),
- $\delta$  6.73 (s, H-4),
- $\delta$  6.72 (s, H-1),
- $\delta$  7.32 (d, H-12,  $J=7.3\text{Hz}$ ),
- $\delta$  7.35 (d, H-11,  $J=7.3\text{ Hz}$ ),
- $\delta$  7.23 (s, H-13).

Mass Spectrum:  $m/e = 351(\text{M}^+, 55\%), 336(62\%), 322(61\%), 292(30\%), 168(13\%)$ .

"Taxilamine"(76): Exact mass of the molecular ion was recorded at  $\text{M}^+=369.1213$  in agreement with the molecular formula  $\text{C}_{20}\text{H}_{19}\text{O}_6\text{N}$  (requires 364.1212).

U.V.Spectrum (MeOH):  $\lambda_{\text{max}} 238, 299$  and  $330$  (sh) n.m.  
( $\log \epsilon 4.48, 4.02$  and  $3.85$ ).

$\lambda_{\text{min}} 255$  n.m.  
MeOH  $\text{OH}^-$   $\lambda_{\text{max}} 237, 292, 328$  (sh) and  $380$  n.m.  
( $\log \epsilon 4.53, 3.97, 3.80$  and  $3.77$ ).

$^1\text{H-N.M.R.}$  ( $\text{CDCl}_3$ , 360 MHz F.T.): (Recorded by Prof.M. Shamma, Pennsylvania State University)

$\delta$  7.15 (s, H-5),  
 $\delta$  7.40 (s, H-8),  
 $\delta$  8.46 (d, H-3,  $J_{\text{vic}}=5.5\text{Hz}$ ),  
 $\delta$  7.66 (d, H-4,  $J=5.5\text{Hz}$ ),  
 $\delta$  6.44 (d, H-5,  $J_{\text{vic}}=9.1\text{Hz}$ ),  
 $\delta$  7.28 (d, H-6,  $J_{\text{vic}}=9.1\text{Hz}$ ),  
 $\delta$  3.92 (3H, s,  $\text{OCH}_3$ ),  
 $\delta$  3.96 (3H, s,  $\text{OCH}_3$ ),  
 $\delta$  3.97 (3H, s,  $\text{OCH}_3$ ),  
 $\delta$  4.06 (3H, s,  $\text{OCH}_3$ ).

Mass Spectrum:  $m/e = 369$  ( $\text{M}^+$ , 31%), 354 (14%), 352 (15%), 326 (14%), 310 (68%), 296 (33%), 188 (57%), 181 (13%), 149 (75%), 86 (50%), 84 (74%) and 49 (100%).

v) Isolation of Bisbenzylisoquinolines Alkaloids

The alkaloidal fraction ( $\text{F}_1$ , 12g) was chromatographed on a neutral aluminium oxide column (410g). The column was packed in petroleum-ether. Elution was carried out with petroleum-ether (2 litre) followed by increasingly polar mixtures of petroleum ether-ethylacetate (3 litre),

ethylacetate (2 litre), ethylacetate-methanol (2 litre) and ultimately with pure methanol (1 litre). The fractions eluted with ethylacetate was designated as (FB<sub>1</sub>) and that eluted with ethylacetate-methanol was designated as (FB<sub>2</sub>). The fraction (FB<sub>1</sub>) on evaporation gave a light yellow coloured mixture of two alkaloids (E) and (F) which were separated on precoated thin layer chromatography sheets of neutral aluminium oxide (type E) in acetone and methanol(85:15). Alkaloid (E) was identified as oxyacanthine and alkaloid (F) was identified as berbamine.

Oxyacanthine (79): m.p. 211-212°C. Exact mass of the molecular ion was recorded at  $M^+ = 608.2874$  in agreement with the molecular formula  $C_{37}H_{40}O_6N_2$  (requires 608.2880).

U.V.Spectrum (MeOH):  $\lambda_{\max}$  238 (sh) 282 n.m. (log  $\epsilon$  5.45, 3.92)  
 $\lambda_{\min}$  265 n.m (log  $\epsilon$  2.38)

I.R.Spectrum (CHCl<sub>3</sub>):  $\lambda_{\max}$  3435 cm<sup>-1</sup> (OH, stretching)  
2801 cm<sup>-1</sup> (N-CH<sub>3</sub>, stretching)

<sup>1</sup>H-N.M.R. (CDCl<sub>3</sub>, 60MHz):

$\delta$  2.55 (3H, s, NCH<sub>3</sub>),

$\delta$  2.60 (3H, s, NCH<sub>3</sub>),

$\delta$  3.15 (3H, s, OCH<sub>3</sub>),

$\delta$  3.65 (3H, s, OCH<sub>3</sub>),

$\delta$  3.75 (3H, s, OCH<sub>3</sub>).

Mass Spectrum:  $m/e = 608 (M^+, 50\%), 607 (30\%), 501 (20\%), 417 (17\%), 416 (10\%), 396 (60\%), 393 (20\%), 381 (30\%), 335 (20\%), 198 (++, 100\%), 192 (40\%).$

Berbamine (77): m.p. 154-155°C. Exact mass of the molecular ion was recorded at  $M^+ = 608.2877$  in agreement with the molecular formula  $C_{37}H_{40}O_6N_2$  (requires 608.2880).

U.V. Spectrum (MeOH):  $\lambda_{\max} 284 \text{ n.m. } (\log \epsilon 3.79).$   
 $\lambda_{\min} 262 \text{ n.m. } (\log \epsilon 2.3)$

I.R. Spectrum ( $CHCl_3$ ):  $\nu_{\max} 3440 \text{ cm}^{-1}$  (OH, stretching),  
 $2808 \text{ cm}^{-1}$  (N- $CH_3$ , stretching),

$^1H$ -N.M.R. ( $CDCl_3$ , 60MHz):

$\delta 2.24$  (3H, s, N- $CH_3$ ),  
 $\delta 2.58$  (3H, s, N- $CH_3$ ),  
 $\delta 3.20$  (3H, s, O $\underline{C}H_3$ ),  
 $\delta 3.60$  (3H, s, O $\underline{C}H_3$ ),  
 $\delta 3.73$  (3H, s, O $\underline{C}H_3$ ).

Mass Spectrum  $m/e = 608 (M^+, 79\%), 607 (50\%), 485 (2\%), 417 (7\%), 395 (68\%), 381 (34\%), 198 (++, 100\%).$

Aromoline (78): m.p. 174°C. The fraction (FB<sub>2</sub>, 1.5g) afforded impure aromoline. Aromoline was further purified on a basic aluminium oxide column (60g). The column was eluted with ethylacetate and ethylacetate-methanol. The fraction eluted with ethylacetate-methanol gave aromoline on evaporation. Exact mass of the molecular ion was recorded at M<sup>+</sup>=594.2738 in agreement with molecular formula C<sub>36</sub>H<sub>38</sub>O<sub>6</sub>N<sub>2</sub> (requires 594.2742).

U.V.Spectrum (MeOH):  $\lambda_{\max}$  208, 228 (sh), 285 n.m (log  $\epsilon$  4.94, 4.69, 3.95).  
 $\lambda_{\min}$  263 n.m. (log  $\epsilon$  2.1)

I.R.Spectrum (CHCl<sub>3</sub>):  $\nu_{\max}$  3440 cm<sup>-1</sup> (OH, stretching)  
2808 cm<sup>-1</sup> (N-CH<sub>3</sub>, stretching)

<sup>1</sup>H-N.M.R. (CDCl<sub>3</sub>, 60 MHz):

$\delta$  2.54 (3H, s, NCH<sub>3</sub>),  
 $\delta$  2.56 (3H, s, NCH<sub>3</sub>),  
 $\delta$  3.56 (3H, s, OCH<sub>3</sub>),  
 $\delta$  3.76 (3H, s, OCH<sub>3</sub>).

T A B L E - V I I

<sup>13</sup>C-N.M.R. CHEMICAL SHIFT VALUES OF PROSAPOGENIN "PR"

=====			
Carbon	Chemical Shift(s) (ppm)	Carbon	Chemical Shift(s) (ppm)
=====			
C(1)	38.5	C(16)	23.1
C(2)	26.0	C(17)	47.2
C(3)	82.0	C(18)	41.5
C(4)	42.4	C(19)	46.1
C(5)	48.4	C(20)	30.5
C(6)	18.5	C(21)	33.7
C(7)	32.4	C(22)	32.5
C(8)	39.9	C(23)	69.1
C(9)	47.8	C(24)	13.5
C(10)	57.1	C(25)	15.9
C(11)	23.5	C(26)	17.2
C(12)	122.2	C(27)	26.0
C(13)	143.5	C(28)	176.0
C(14)	41.9	C(29)	33.0
C(15)	28.1	C(30)	23.8
Glc	C(1)	105.0	
	C(2)	75.3	
	C(3)	78.4	
	C(4)	71.6	
	C(5)	75.6	
	C(6)	61.9	
=====			