BIOPROSPECTING STUDIES ON *SARCOCOCCA CORIACEA* (HOOK. F.) OF NEPALESE ORIGIN

THESIS SUBMITTED FOR THE FULFILLMENT
OF THE DOCTOR OF PHILOSOPHY

By

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Achyut Adhikari
Karachi, Pakistan
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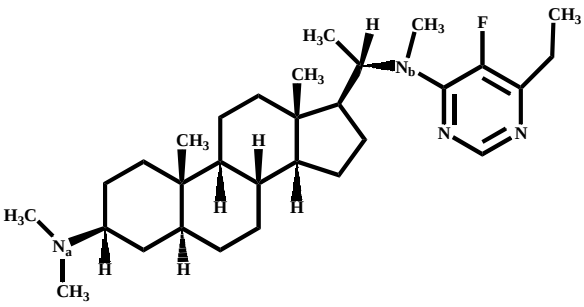
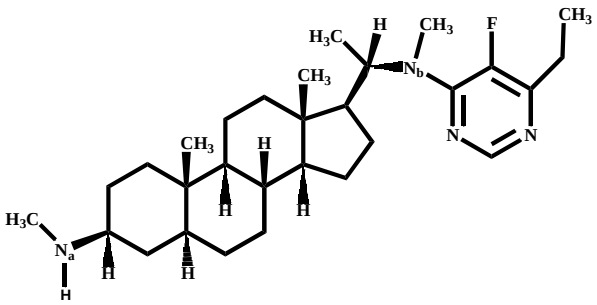
SUMMARY

Although fluorine is the most abundant halogen in the Earth's crust, fluorine containing natural products are extremely rare in nature. This is due to the special properties of fluorine such as: a) it is found in highly insoluble form, therefore it has less bioavailability, b) it has very high heat of hydration due to which it is a poor nucleophile in aqueous solution, and also c) redox potential which is required for the oxidation of fluoride is higher than that generated by the reduction of hydrogen peroxide, thus fluorine cannot be incorporated into organic compounds *via* the haloperoxidase reaction. Due to these reasons, the mechanism by which C-F bond formation can take place has attracted a considerable scientific interest. Only 18 organofluorine containing natural products have been reported so far. All of them are primary metabolites and no fluorine containing secondary metabolite has been reported from any natural source.

This Ph. D. study has led to an exciting discovery of a novel class of fluoropyrimidine substituted alkaloids from a medicinal plant, *Sarcococca coriacea* of Nepalese origin. Seven fluoropyrimidine containing steroidal alkaloids **1-7** and one novel fluoropyrimidine substituted ionone **8** have been obtained. This is the first time that fluorine containing secondary metabolites have been reported from any natural source, which has opened up a new avenue for further research in the chemistry of fluorine- containing secondary metabolites.



Adhikarimine A (**1**)



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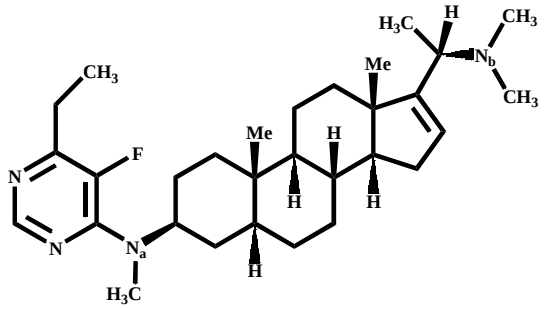
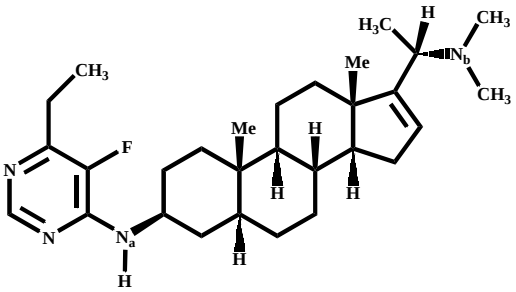
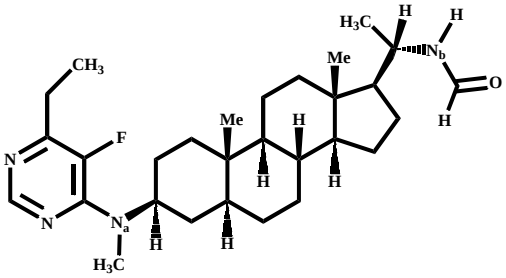


Adhikarimine C (**3**)



Adhikarimine D (**4**)



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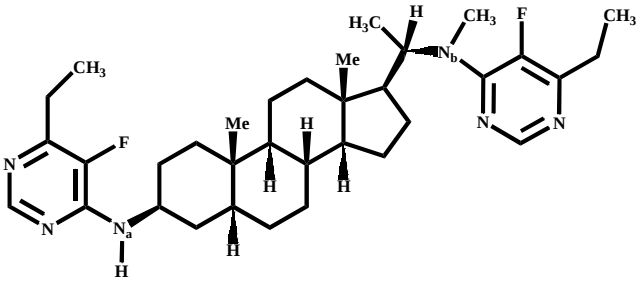
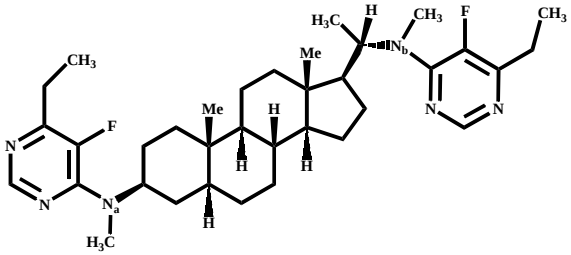
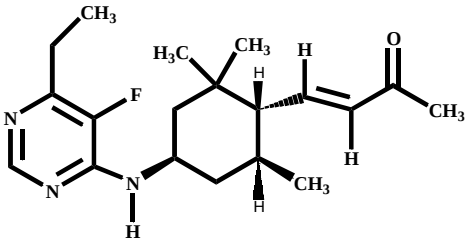


Adhikarimine F (**6**)



Adhikarimine G (**7**)



Adhikarione (**8**)

Additionally, nine known pregnane-type steroidal alkaloids **9-17** have also been isolated for the first time from *S. coriacea*, which include alkaloid-C (**9**), *N*a-methylepipachysamine D (**10**), sarcovagenine C (**11**), sarcovagine D (**12**), *N*-methylpachysamine A (**13**)**,** dictyophlebine (**14**)**,** 5,6-dihydrosarconidine (**15**)**,** terminaline (**16**)**,** and *iso*-*N*-formylchonemorphine (**17**)**.**

1.0 General Introduction

Throughout the ages, human race have fulfilled its basic needs from nature, such as foodstuffs, shelters, clothing, fertilizers, flavors and fragrances. Progressively they started to use parts of plants (leaves, stems, roots, rhizomes, etc.), and some animal parts, along with minerals, for the treatment of diseases. History of medicine thus started with the genesis of human civilization. The modern medicine or allopathy is a result of continuous scientific and observational development of traditional medicines and practices.

Traditional medicine systems which have been in use for years are mainly depending on plants. The first record, which was written at about 2,600 BC on clay tablets in cuneiform, is from Mesopotamia. Oils of *Cedrus* species (cedar) and *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrth), and *Papaver somniferum* (poppy juice) are some examples amongst approximately 1,000 plant-derived substances which people have used in ancient times and are still in use for the treatment of diseases such as cough and cold, parasitic infections and inflammation.[1]

Nearly all ancient cultures have used plants as a source of medicine. Egyptian pharmaceutical record *Ebers Papyrus* at 1,500 BC; includes 700 drugs, mostly plants, though animal organs and some minerals were also used.[1]

Ayurveda is an ancient system of medicine which is still widely practiced in South Asia and has a sound philosophical and experimental basis. *Atharvaveda* (around 1,200 BC), *Charak Samhita* and *Sushrut Samhita* (1,000 - 500 BC) are the classic texts that give detailed descriptions of over 700 herbs used in Ayurveda.[2]

The Chinese *Materia Medica*, which was first compiled in 1,100 BC (Wu Shi Er Bing Fang, containing 52 prescriptions), has been extensively revised over the centuries. This was followed by recorded history such as the Shennong Herbal (~100 BC; 365 drugs) and the Tang Herbal (659 AD; 850 drugs).[1]

The Greeks have contributed significantly in the development of the herbal drugs in prehistoric western world. Theophrastus (A Philosopher and Natural Scientist) reported the medicinal properties of many plants and changes in their characteristics through cultivation, in his book '*History of Plants*,' (300 BC). Dioscorides, a Greek Physician (100 AD), reported the method of collection, storage, and use of many medicinal herbs. Galen (130-200 AD), who was a teacher of pharmacy and medicine in Rome, published nearly 30 books on prescription and formulae of herbal drugs.[1]

In many developing countries, people are still depending on traditional medicines for their healthcare. In developed countries too, people are now turning to herbal remedies for multiple reasons. Many modern medicines still contain substances of plant origin.

The World Health Organization (WHO) estimates that about 80% inhabitants of developing countries rely almost entirely on traditional medicines for their primary healthcare needs. Over 3 billion people in the developing countries utilize plant-based medicines on a regular basis. Natural pharmaceuticals (Naturaceuticals), nutraceuticals and cosmeceuticals have tremendous

significance in global healthcare regime. Plants possessed an immense chemical diversity which can be used in different types of drug discovery, such as antimicrobial, cardiovascular, immunosuppressive and anticancer. According to the WHO survey, the international market of herbal products or botanicals is estimated to be US$ 62 billion per annum. It is estimated to grow to US$ 5 trillion by the year 2050.[3] The nutraceutical marketplace in Europe is estimated to be US$ 9 billion, while in US marketplace it is about US$ 10-12 billion. It is expanding at a rate of more than 20% per year.[2, 3]

Natural products chemistry actually began with the isolation of morphine from opium (*Papaver somniferum*) by Serturner. This was followed by the isolation of quinine from *Cinchona* tree in 1860. A German chemist Carl Koler isolated cocaine from *Erythroxylon coca*, the chemical responsible for paralyzing the nerve endings responsible for transmitting pain. Reserpine, an anti-hypertensive alkaloid was obtained from *Rauwolfia serpentine*.[2]

Secondary metabolites which are produced by living organisms (microbes and plants) for the purpose of their own survival have a major importance in drug discovery. Secondary metabolites with therapeutic indications include cyclosporine (immunosuppression), mevinolin (hypercholesterolaemia), avermectin (parasitic disease), artemisinin (malaria), vinblastine, vincristine and taxol (cancer).

Today, natural product-based drug discovery competes with the combinatorial chemistry, which has given a significant complementary way for drug development. It produced thousands of compounds of almost similar structures in a single experiment, which are immediately available for bio-assay screening. In contrast, plants and microorganisms have the potential to synthesize novel chemicals in unpredictable ways. The genes and metabolic pathways by which plants and

microbes produce novel structures are now understood in many cases and this knowledge has been used in drug discovery. Plants and microbes used same technique as in combinatorial chemistry to biosynthesized secondary metabolites. Natural product biosynthesis is mostly catalyzed by enzymes, which result from an enormous gene pool that has evolved over billions of years.[4]

Recent improvements in instrumentation, robotics, and bioassay technology have increased the speed of bioassay-guided isolation and structure elucidation of natural products significantly. These improvements have allowed natural product research to be more competitive and cost effective, as compared to synthetic medicinal chemistry.

Since 2005, thirteen natural product (NP)-derived drugs have been lunched in the market (**Table-1.1**), in addition to the 37 late stage clinical development NP-based candidates (6 in registration and 31 in phase III). The traditional strengths of NPs in oncological and transmittable diseases is still explored with 19 (51%) compounds being evaluated for the treatment of cancer and 10 (27%) for the treatment of bacterial infections. The remaining compounds are for the treatment of metabolic diseases (4), pain (2) and multiple sclerosis (2). A large number of NP-derived compounds are in various stages of clinical development which indicates that natural products are still the major feasible source of new drug candidates. In absence of NP-derived compounds, it is predicted that there would be a considerable therapeutic shortage in a number of important areas, such as neurodegenerative and cardiovascular diseases, most solid tumors and immune-inflammatory diseases.[5]

During the current study, bioprospecting of an extremely rare fluorine containing novel class of alkaloids from a medicinal plant *Sarcococca coriacea* was carried out. The medicinal plant *Sarcococca coriacea* is a member of family Buxaceae.

Table. 1.1: NP-derived drugs launched since 2005.[5]

Year	Trade name	Lead compound	Classification	Disease area
2005	Dronabinol/Cannabidol (Sativex ®)	Dronabinol/ Cannabidol	NPs	pain
2005	Fumagilin (Flisint®)	fumagillin	NP	antiparasitic
2005	Doripenem (Finibax ®/ Doribax™)	thienamycin	NP-derived	antibacterial
2005	Tigecycline (Tygacil®)	tetracycline	Semi-synthetic NP	antibacterial
2005	Ziconotide (Prialt®)	ziconotide	NP	pain
2005	Zotarolimus (Endeavor ™ stent)	sirolimus	Semi-synthetic NP	Cardiovascular surgery
2006	Anidulafungin (Eraxis ™ / Ecalta ™)	Echinocandin B	Semi-synthetic NP	antifungal
2006	Exenatide (Byetta ™)	Exenatide-4	NP	diabetes
2007	Lisdexamfetamine (Vyuanse ™)	amphetamine	NP-derived	ADHD
2007	Retapamulin (Altabax ™/Altargo ™)	pleuromutilin	Semi-synthetic NP	antibacterial
2007	Temsirolimus (Torisel ™)	sirolimus	Semi-synthetic NP	oncology
2007	Trabectedin (Yondelis ™)	trabectedin	NP	oncology
2007	Ixabepilone (Ixempra ™)	Epothilone B	Semi-synthetic NP	oncology

2.0 Plant *Sarcococca coriacea*

2.1 Introduction

2.1.1 The Family Buxaceae

Plants of the family Buxaceae are generally evergreen trees, shrubs and rarely perennial herbs, monoecious or dioecious. It comprises four genera, *Buxus*, *Sarcococca*, *Pachysandra* and *Simondsia*, with over one hundred species. It is usually distributed in tropical and temperate regions,[6] mainly in Himalayan regions ranging from West Afghanistan to East Myanmar, including the Philippines. A large number of steroidal alkaloids have been reported from different plants of this family during various phytochemical investigations in last three decades.[7, 8]

2.1.2 The Genus *Sarcococca*

The genus *Sarcococca* is an evergreen shrub, which is free from many diseases and resistant to insects attack. It has very attractive dark green glossy leaves and intoxicating fragrance when blooming, followed by small red or black berries. It grows best in moist and well-drained soil. It is usually distributed in South-East Asia, Europe and Africa. In South-East Asia, it is mainly distributed from Afghanistan through Pakistan, India, Nepal, Central China, Sri Lanka, Thailand, Java and Sumatra (Indonesia), Taiwan, and the Philippines.[9]

The genus *Sarcococca* comprises of several species.[10] Some important species are *S. saligna*, *S. coriacea*, *S. hookeriana*, *S. wallichii*, *S. rusciofolia*, *S. humilis*, *S. vegans*, *S. brevifolia* and *S. zeylanica*. In Nepal, the genus *Sarcococca* comprises of four species named *S. coriacea*, *S. hookeriana*, *S. wallichii* and *S. saligna*.[11] Phytochemical and biological investigations on different species of this genus have been carried out in last two decades, in which the major contributions have been made by the research group of Prof. Dr. Atta-ur-Rahman and Prof. Dr. M. Iqbal Choudhary.[7, 8]

2.1.2.1 Phytochemistry of Genus *Sarcococca*

Since the first phytochemical investigation on the genus *Sarcococca* in 1963,[12] extensive studies on this genus have been carried out which led to the isolation of a large number of bioactive steroidal alkaloids. The survey of compounds on this genus till date is presented below:

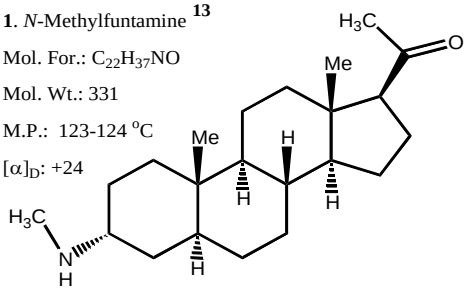
1. *N*-Methylfuntamine [13]

Mol. For.: $C_{22}H_{37}NO$

Mol. Wt.: 331

M.P.: 123-124 ^oC

$[\alpha]_D$: +24

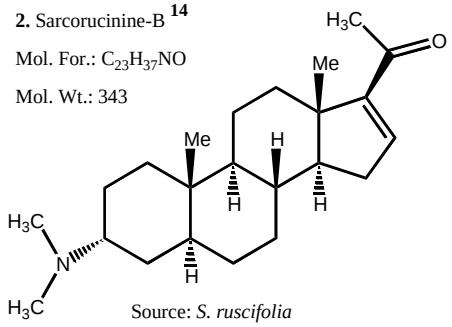


Source: *S. coriacea*
Activity: AChE/BChE inhibitior

2. Sarcorucinine-B [14]

Mol. For.: $C_{23}H_{37}NO$

Mol. Wt.: 343



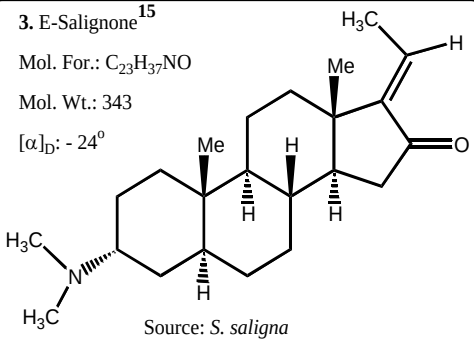
Source: *S. ruscifolia*
Activity: not reported

3. E-Salignone[15]

Mol. For.: $C_{23}H_{37}NO$

Mol. Wt.: 343

$[\alpha]_D$: - 24^o



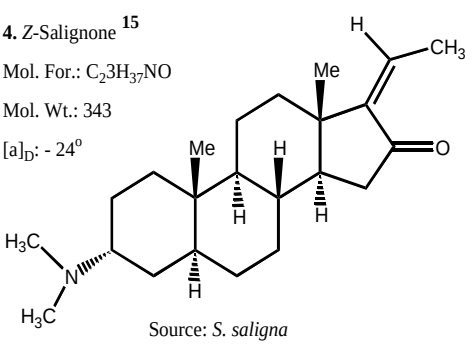
Source: *S. saligna*
Activity: not reported

4. Z-Salignone [15]

Mol. For.: $C_2 3H_{37}NO$

Mol. Wt.: 343

[a]$_D$: - 24^o



Source: *S. saligna*
Activity: not reported

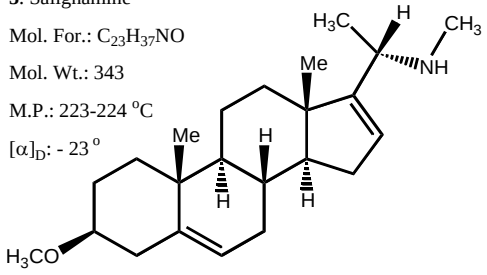
5. Salignamine [16]

Mol. For.: $C_{23}H_{37}NO$

Mol. Wt.: 343

M.P.: 223-224 ^oC

$[\alpha]_D$: - 23 ^{o}



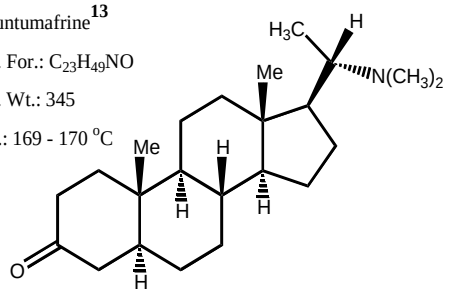
Source: *S. saligna*
Activity: AChE/ BChE inhibitior

6. Funtumafrine[13]

Mol. For.: $C_{23}H_{49}NO$

Mol. Wt.: 345

M.P.: 169 - 170 ^oC



Source: *S. coriacea*
Activity: AChE / BChE inhibitior

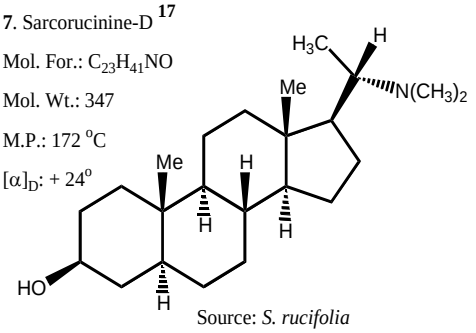
7. Sarcorucinine-D [17]

Mol. For.: $C_{23}H_{41}NO$

Mol. Wt.: 347

M.P.: 172 ^{o}C

$[\alpha]_D$: + 24^{o}



Source: *S. rucifolia*
Activity: not reported

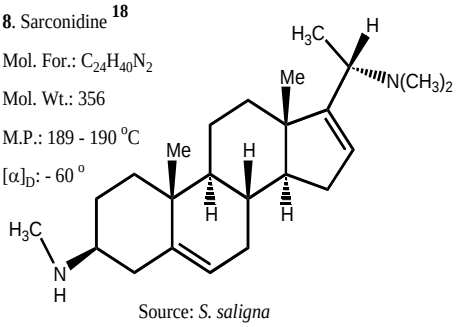
8. Sarconidine [18]

Mol. For.: $C_{24}H_{40}N_2$

Mol. Wt.: 356

M.P.: 189 - 190 ^{o}C

$[\alpha]_D$: - 60 ^{o}

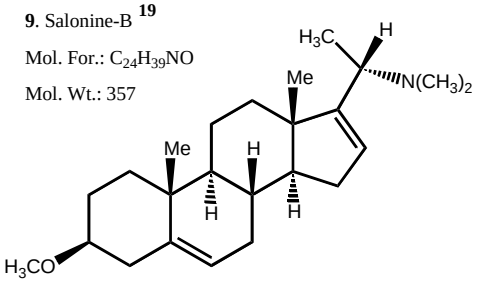


Source: *S. saligna*
Activity: not reported

9. Salonine-B [19]

Mol. For.: $C_{24}H_{39}NO$

Mol. Wt.: 357



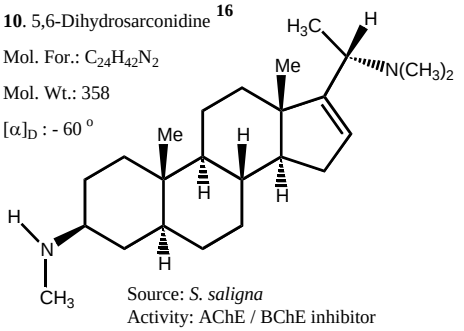
Source: *S. saligna*
Activity: AChE / BChE inhibitor

10. 5,6-Dihydrosarconidine [16]

Mol. For.: $C_{24}H_{42}N_2$

Mol. Wt.: 358

$[\alpha]_D$: - 60 ^{o}



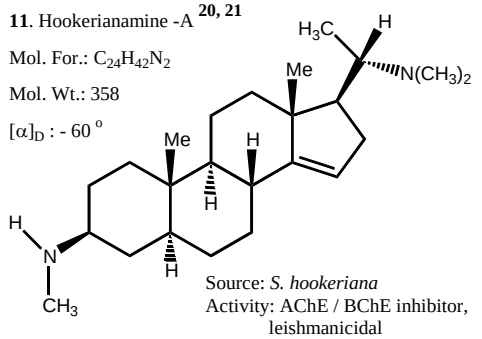
Source: *S. saligna*
Activity: AChE / BChE inhibitor

11. Hookerianamine -A [20, 21]

Mol. For.: $C_{24}H_{42}N_2$

Mol. Wt.: 358

$[\alpha]_D$: - 60 ^{o}



Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, leishmanicidal

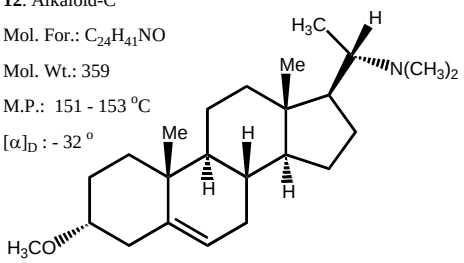
12. Alkaloid-C [22]

Mol. For.: $C_{24}H_{41}NO$

Mol. Wt.: 359

M.P.: 151 - 153 ^{o}C

$[\alpha]_D$: - 32 ^{o}



Source: *S. purniformis / S. saligna*
Activity: AChE / BChE inhibitor

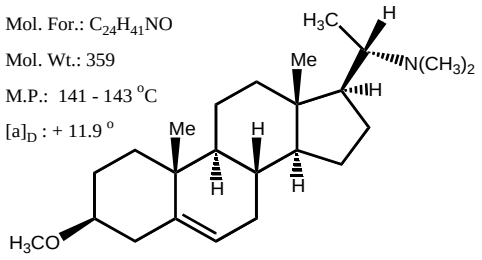
13. Pachyaximine-A [23]

Mol. For.: $C_{24}H_{41}NO$

Mol. Wt.: 359

M.P.: 141 - 143 ^{o}C

$[a]_D$: + 11.9 ^{o}



Source: *S. saligna*
Activity: not reported

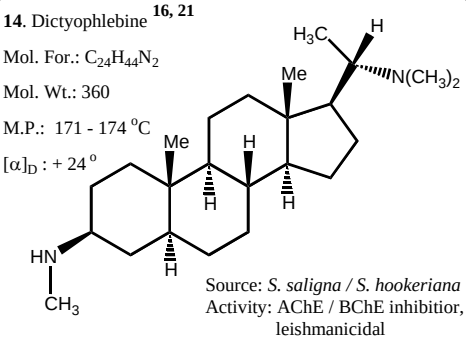
14. Dictyophlebine [16, 21]

Mol. For.: $C_{24}H_{44}N_2$

Mol. Wt.: 360

M.P.: 171 - 174 ^{o}C

$[\alpha]_D$: + 24 ^{o}



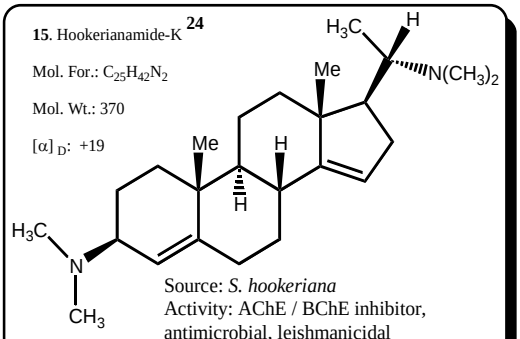
Source: *S. saligna / S. hookeriana*
Activity: AChE / BChE inhibitior, leishmanicidal

15. Hookerianamide-K [24]

Mol. For.: $C_{25}H_{42}N_2$

Mol. Wt.: 370

$[\alpha]_D$: +19

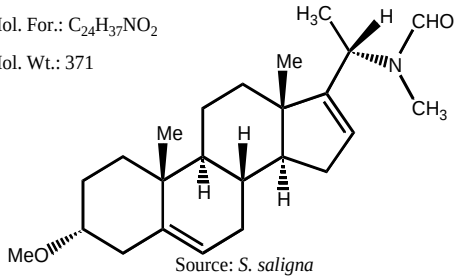


Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, antimicrobial, leishmanicidal

16. *N*-[formyl(methyl)amino] salonine B [16]

Mol. For.: $C_{24}H_{37}NO_2$

Mol. Wt.: 371

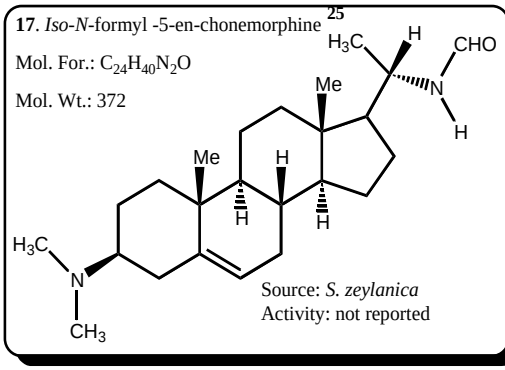


Source: *S. saligna*
Activity: AChE / BChE inhibitor

17. *Iso-N*-formyl -5-en-chonemorphine [25]

Mol. For.: $C_{24}H_{40}N_2O$

Mol. Wt.: 372



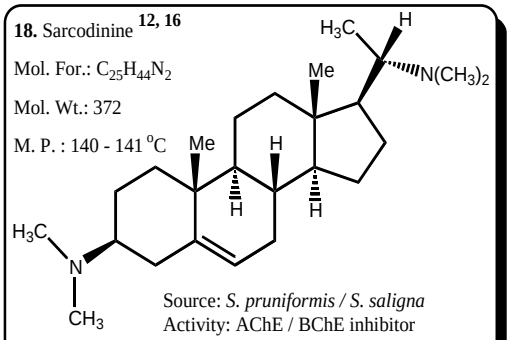
Source: *S. zeylanica*
Activity: not reported

18. Sarcodinine [12, 16]

Mol. For.: $C_{25}H_{44}N_2$

Mol. Wt.: 372

M. P. : 140 - 141 °C



Source: *S. pruniformis / S. saligna*
Activity: AChE / BChE inhibitor

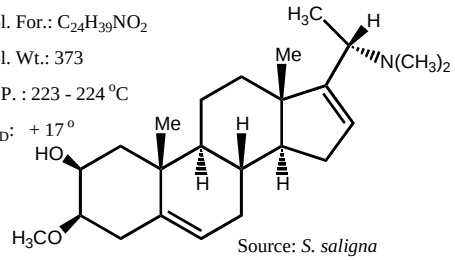
19. 2-Hydroxysalignamine [16]

Mol. For.: $C_{24}H_{39}NO_2$

Mol. Wt.: 373

M. P. : 223 - 224 °C

$[\alpha]_D$: + 17 °



Source: *S. saligna*
Activity: not reported

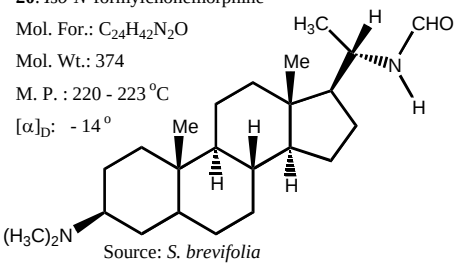
20. *Iso-N*-formylchonemorphine [26, 16]

Mol. For.: $C_{24}H_{42}N_2O$

Mol. Wt.: 374

M. P. : 220 - 223 °C

$[\alpha]_D$: - 14 °



Source: *S. brevifolia*
Activity: AChE / BChE inhibitor, antibacterial

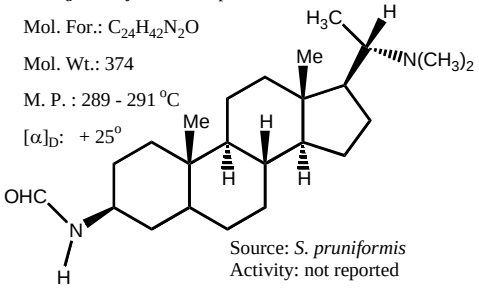
21. N_a -Formylchonemorphine [27, 28]

Mol. For.: $C_{24}H_{42}N_2O$

Mol. Wt.: 374

M. P. : 289 - 291 °C

$[\alpha]_D$: + 25°

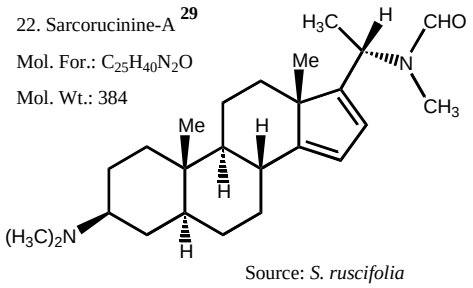


Source: *S. pruniformis*
Activity: not reported

22. Sarcorucinine-A [29]

Mol. For.: $C_{25}H_{40}N_2O$

Mol. Wt.: 384

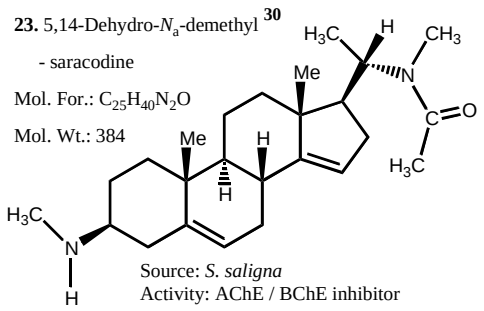


Source: *S. ruscifolia*
Activity: not reported

23. 5,14-Dehydro-N_a-demethyl [30] - saracodine

Mol. For.: $C_{25}H_{40}N_2O$

Mol. Wt.: 384

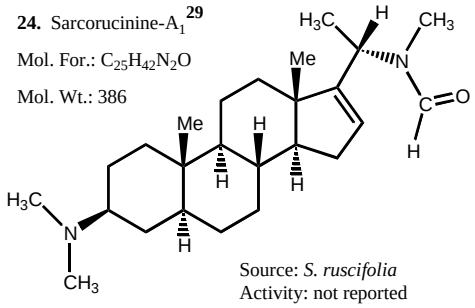


Source: *S. saligna*
Activity: AChE / BChE inhibitor

24. Sarcorucinine-A_1 [29]

Mol. For.: $C_{25}H_{42}N_2O$

Mol. Wt.: 386

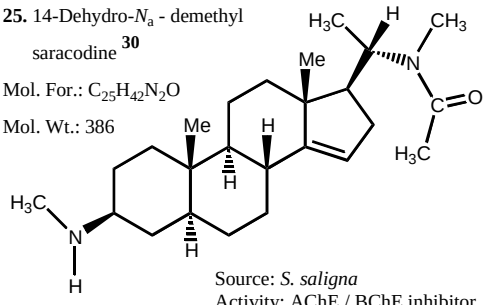


Source: *S. ruscifolia*
Activity: not reported

25. 14-Dehydro-N_a - demethyl saracodine [30]

Mol. For.: $C_{25}H_{42}N_2O$

Mol. Wt.: 386

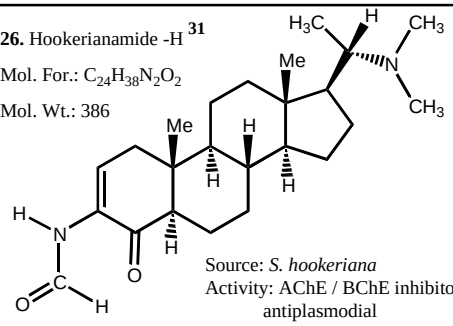


Source: *S. saligna*
Activity: AChE / BChE inhibitor

26. Hookerianamide -H [31]

Mol. For.: $C_{24}H_{38}N_2O_2$

Mol. Wt.: 386

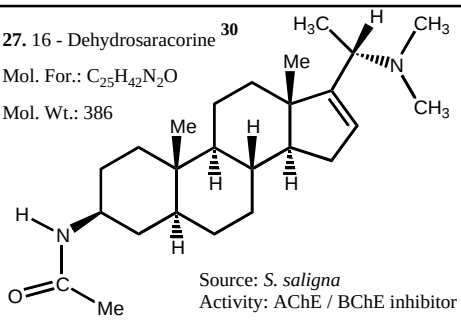


Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, antiplasmodial

27. 16 - Dehydrosaracorine [30]

Mol. For.: $C_{25}H_{42}N_2O$

Mol. Wt.: 386



Source: *S. saligna*
Activity: AChE / BChE inhibitor

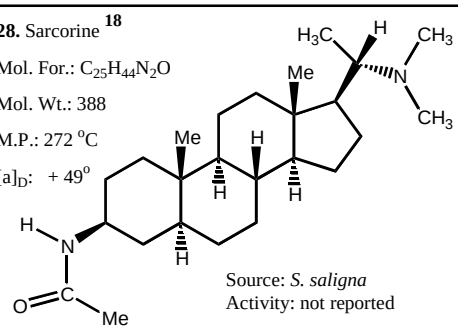
28. Sarcorine [18]

Mol. For.: $C_{25}H_{44}N_2O$

Mol. Wt.: 388

M.P.: 272 ^oC

$[a]_D$: + 49^o



Source: *S. saligna*
Activity: not reported

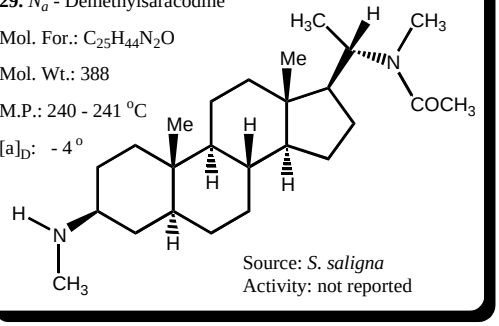
29. N_a - Demethylsaracodine [32]

Mol. For.: $C_{25}H_{44}N_2O$

Mol. Wt.: 388

M.P.: 240 - 241 ^oC

$[a]_D$: - 4 ^{o}



Source: *S. saligna*
Activity: not reported

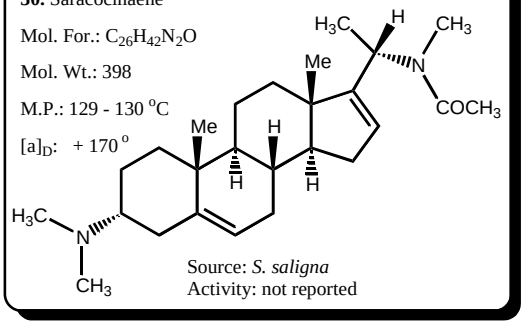
30. Saracocinaene [23]

Mol. For.: $C_{26}H_{42}N_2O$

Mol. Wt.: 398

M.P.: 129 - 130 ^oC

$[a]_D$: + 170 ^{o}



Source: *S. saligna*
Activity: not reported

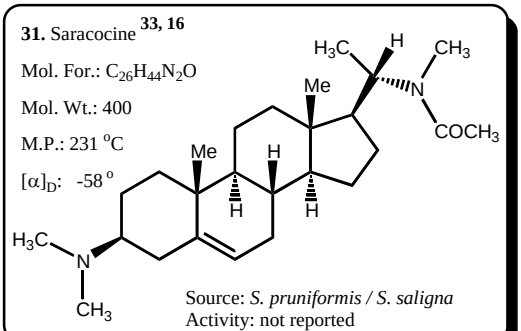
31. Saracocine [33, 16]

Mol. For.: $C_{26}H_{44}N_2O$

Mol. Wt.: 400

M.P.: 231 °C

$[\alpha]_D$: -58 °



Source: *S. pruniformis / S. saligna*
Activity: not reported

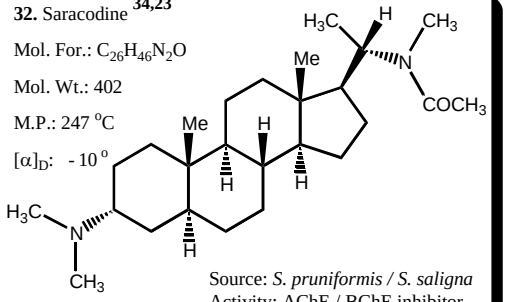
32. Saracodine [34,23]

Mol. For.: $C_{26}H_{46}N_2O$

Mol. Wt.: 402

M.P.: 247 °C

$[\alpha]_D$: - 10 °



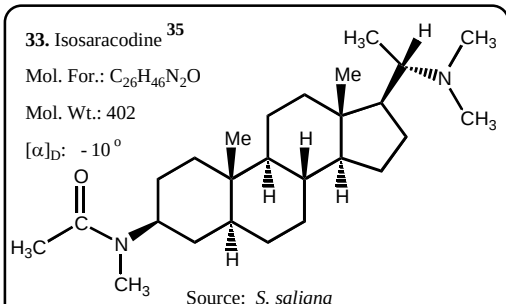
Source: *S. pruniformis / S. saligna*
Activity: AChE / BChE inhibitor

33. Isosaracodine [35]

Mol. For.: $C_{26}H_{46}N_2O$

Mol. Wt.: 402

$[\alpha]_D$: - 10 °

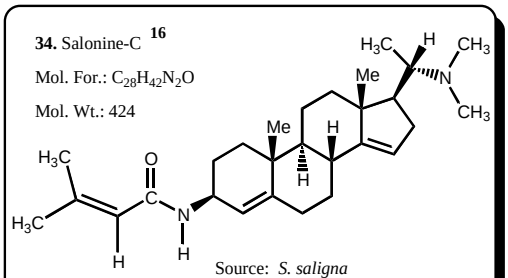


Source: *S. saligna*
Activity: AChE / BChE inhibitor

34. Salonine-C [16]

Mol. For.: $C_{28}H_{42}N_2O$

Mol. Wt.: 424



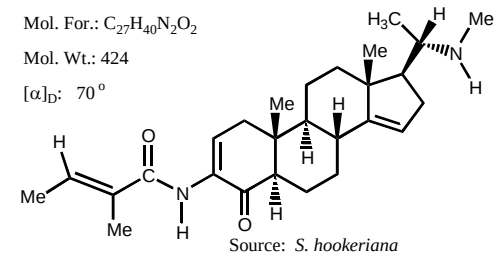
Source: *S. saligna*
Activity: AChE / BChE inhibitor

35. Hookerianamide-F [36, 21]

Mol. For.: $C_{27}H_{40}N_2O_2$

Mol. Wt.: 424

$[\alpha]_D$: 70 °



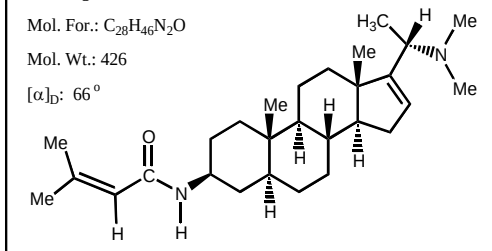
Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, leishmanicidal

36. Salignarine -D [37]

Mol. For.: $C_{28}H_{46}N_2O$

Mol. Wt.: 426

$[\alpha]_D$: 66 °



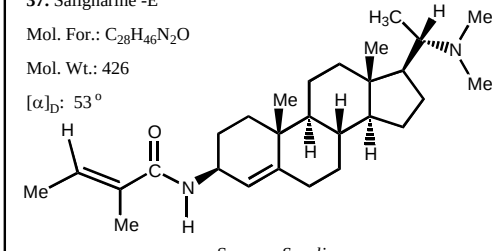
Source: *S. saligna*
Activity: not reported

37. Salignarine -E [37]

Mol. For.: $C_{28}H_{46}N_2O$

Mol. Wt.: 426

$[\alpha]_D$: 53 °



Source: *S. saligna*
Activity: not reported

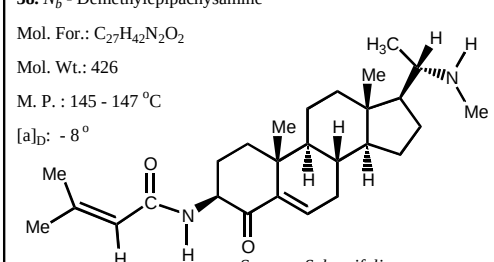
38. N_b - Demethylepipachysamine [26]

Mol. For.: $C_{27}H_{42}N_2O_2$

Mol. Wt.: 426

M. P. : 145 - 147 °C

[a]$_D$: - 8 °



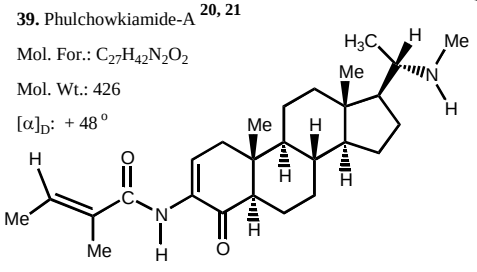
Source: *S. bervifolia*
Activity: Antibacterial

39. Phulchowkiamide-A [20, 21]

Mol. For.: $C_{27}H_{42}N_2O_2$

Mol. Wt.: 426

$[\alpha]_D$: + 48 °



Source: *S. hookeriana*
Activity: AChE / BChE inhibitior, leishmanicidal

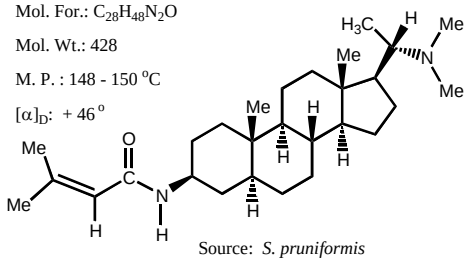
40. Alkaloid-A [38]

Mol. For.: $C_{28}H_{48}N_2O$

Mol. Wt.: 428

M. P. : 148 - 150 °C

$[\alpha]_D$: + 46 °



Source: *S. pruniformis*
Activity: not reported

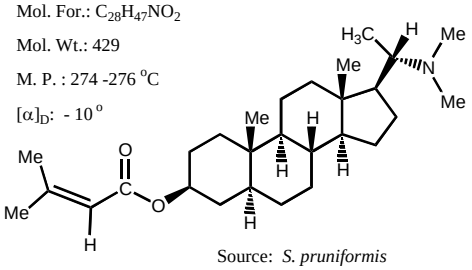
41. Alkaloid-B [38]

Mol. For.: $C_{28}H_{47}NO_2$

Mol. Wt.: 429

M. P. : 274 -276 °C

$[\alpha]_D$: - 10 °



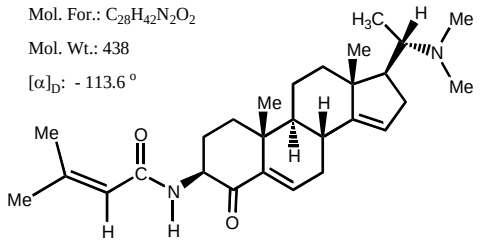
Source: *S. pruniformis*
Activity: not reported

42. Sarsaligenone [28]

Mol. For.: $C_{28}H_{42}N_2O_2$

Mol. Wt.: 438

$[\alpha]_D$: - 113.6 °



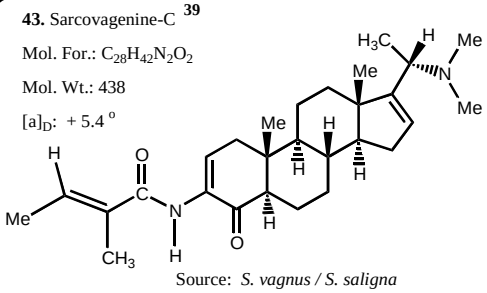
Source: *S. saligna*
Activity: Antibacterial, AChE / BChE inhibitor

43. Sarcovagenine-C [39]

Mol. For.: $C_{28}H_{42}N_2O_2$

Mol. Wt.: 438

$[a]_D$: + 5.4 °



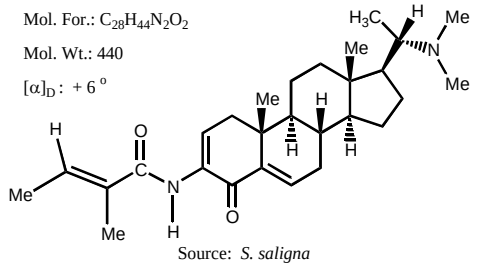
Source: *S. vagnus / S. saligna*
Activity: AChE / BChE inhibitor

44. 2,3-Dehydrosarsalignone [30]

Mol. For.: $C_{28}H_{44}N_2O_2$

Mol. Wt.: 440

$[\alpha]_D$: + 6 °



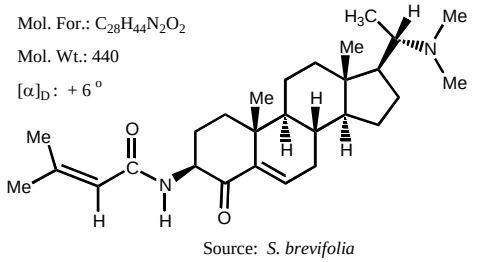
Source: *S. saligna*
Activity: AChE / BChE inhibitor

45. Epipachysamine-E-5-ene-4-one [26]

Mol. For.: $C_{28}H_{44}N_2O_2$

Mol. Wt.: 440

$[\alpha]_D$: + 6 °



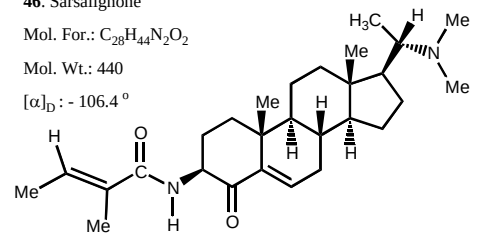
Source: *S. brevifolia*
Activity: Antibacterial

46. Sarsalignone [28]

Mol. For.: $C_{28}H_{44}N_2O_2$

Mol. Wt.: 440

$[\alpha]_D$: - 106.4 °



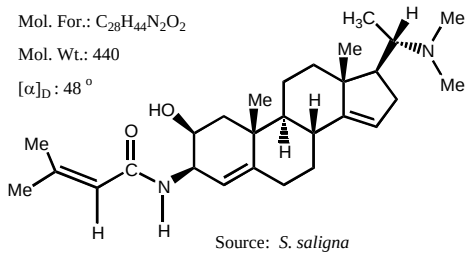
Source: *S. saligna*
Activity: Antibacterial, AChE / BChE inhibitor

47. Salignenamide-B [40]

Mol. For.: $C_{28}H_{44}N_2O_2$

Mol. Wt.: 440

$[\alpha]_D$: 48 °



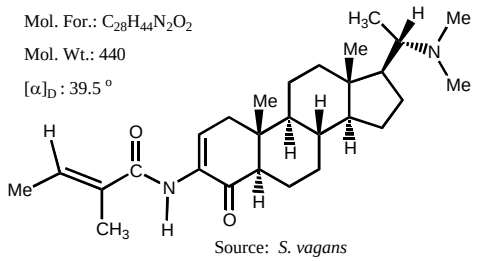
Source: *S. saligna*
Activity: not reported

48. Sarcovagine - D [41, 21]

Mol. For.: $C_{28}H_{44}N_2O_2$

Mol. Wt.: 440

$[\alpha]_D$: 39.5 °



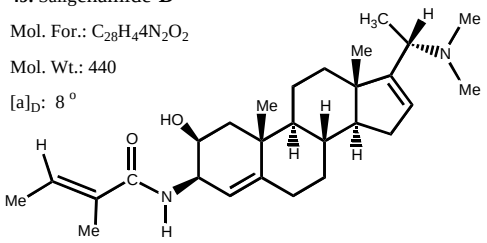
Source: *S. vagans*
Activity: Leishmanicidal

49. Saligenamide-**D** [10]

Mol. For.: $C_{28}H_{4}4N_2O_2$

Mol. Wt.: 440

$[a]_D$: 8 °

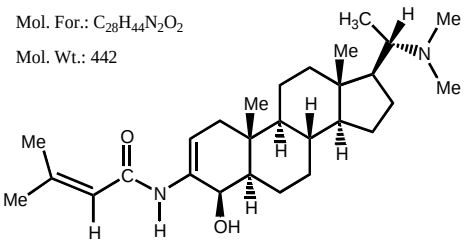


Source: *S. saligna*
Activity: AChE / BChE inhibitor

50. Vaganine-B [42]

Mol. For.: $C_{28}H_{44}N_2O_2$

Mol. Wt.: 442



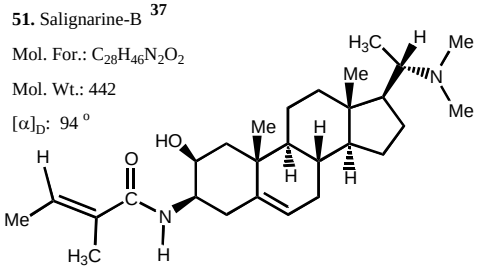
Source: *S. vagans*
Activity: not reported

51. Salignarine-B [37]

Mol. For.: $C_{28}H_{46}N_2O_2$

Mol. Wt.: 442

$[\alpha]_D$: 94 °



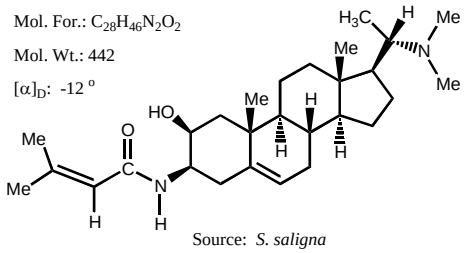
Source: *S. saligna*
Activity: AChE / BChE inhibitor

52. Salignarine-C [37]

Mol. For.: $C_{28}H_{46}N_2O_2$

Mol. Wt.: 442

$[\alpha]_D$: -12 °



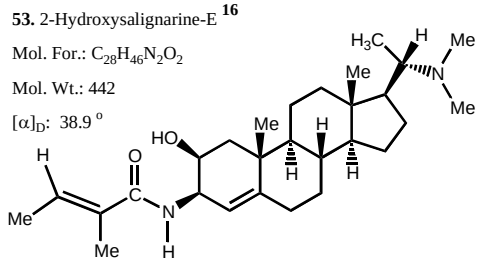
Source: *S. saligna*
Activity: not reported

53. 2-Hydroxysalignarine-E [16]

Mol. For.: $C_{28}H_{46}N_2O_2$

Mol. Wt.: 442

$[\alpha]_D$: 38.9 °

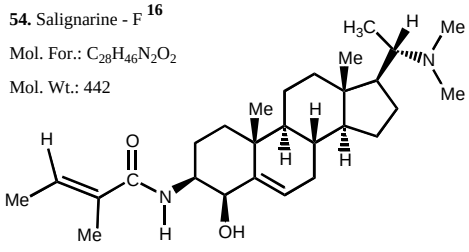


Source: *S. saligna*
Activity: not reported

54. Salignarine - F [16]

Mol. For.: $C_{28}H_{46}N_2O_2$

Mol. Wt.: 442



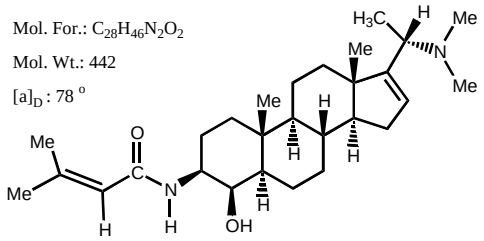
Source: *S. saligna*
Activity: AChE / BChE inhibitor

55. Hookerianamide - J [24]

Mol. For.: $C_{28}H_{46}N_2O_2$

Mol. Wt.: 442

$[a]_D$: 78 °



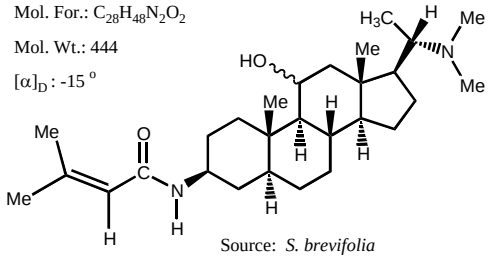
Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, leishmanicidal, antimicrobial

56. 11-Hydroxyepipachysamine-E [43]

Mol. For.: $C_{28}H_{48}N_2O_2$

Mol. Wt.: 444

$[\alpha]_D$: -15 °

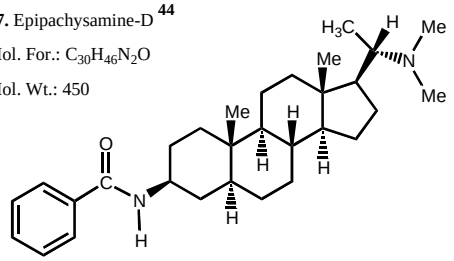


Source: *S. brevifolia*
Activity: not reported

57. Epipachysamine-D [44]

Mol. For.: $C_{30}H_{46}N_2O$

Mol. Wt.: 450



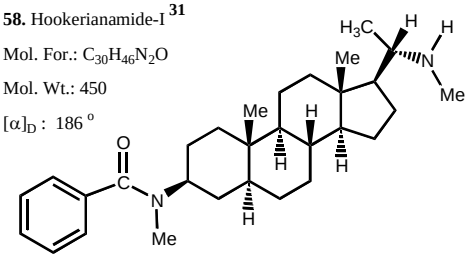
Source: *S. saligna*
Activity: Antibacterial, AChE / BChE inhibitor

58. Hookerianamide-I [31]

Mol. For.: $C_{30}H_{46}N_2O$

Mol. Wt.: 450

$[\alpha]_D$: 186 °



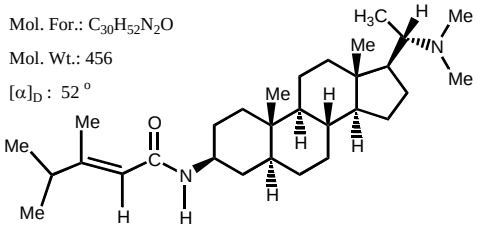
Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, antiplasmodial

59. Salignenamide-A [40]

Mol. For.: $C_{30}H_{52}N_2O$

Mol. Wt.: 456

$[\alpha]_D$: 52 °



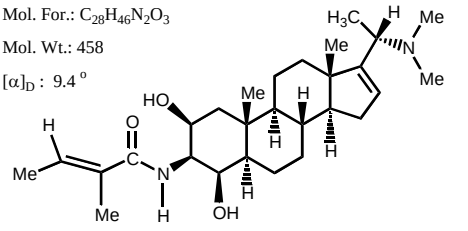
Source: *S. saligna*
Activity: not reported

60. Sarcovagenine-A [39]

Mol. For.: $C_{28}H_{46}N_2O_3$

Mol. Wt.: 458

$[\alpha]_D$: 9.4 °



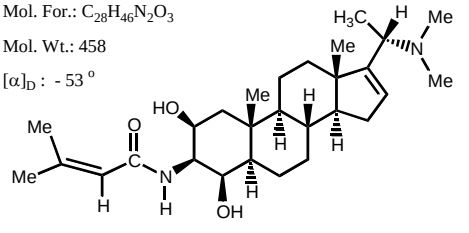
Source: *S. vaganas*
Activity: not reported

61. Hookerianamide - A [20, 21]

Mol. For.: $C_{28}H_{46}N_2O_3$

Mol. Wt.: 458

$[\alpha]_D$: - 53 °

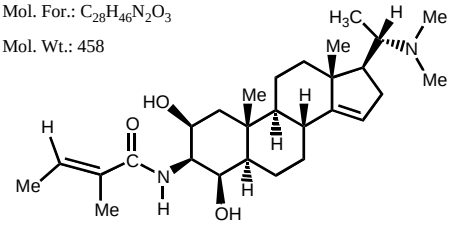


Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, leishmanicidal

62. Salonine - A [19]

Mol. For.: $C_{28}H_{46}N_2O_3$

Mol. Wt.: 458



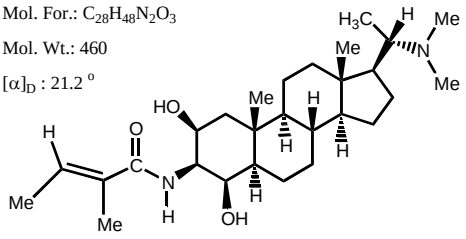
Source: *S. saligna*
Activity: AChE / BChE inhibitor

63. Sarcovagine - A [41]

Mol. For.: $C_{28}H_{48}N_2O_3$

Mol. Wt.: 460

$[\alpha]_D$: 21.2 °



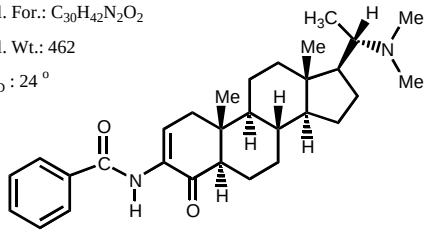
Source: *S. vagans*
Activity: not reported

64. Axillaridine A [10]

Mol. For.: $C_{30}H_{42}N_2O_2$

Mol. Wt.: 462

$[\alpha]_D$: 24 °



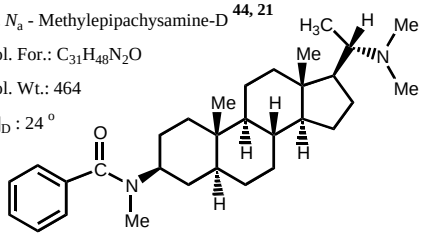
Source: *S. saligna*
Activity: AChE / BChE inhibitor

65. N_a - Methylepipachysamine-D [44, 21]

Mol. For.: $C_{31}H_{48}N_2O$

Mol. Wt.: 464

$[\alpha]_D$: 24 °



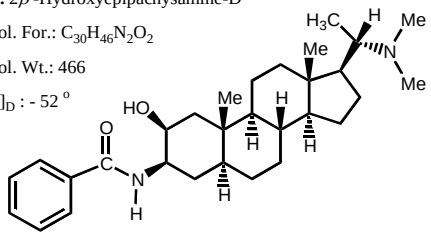
Source: *S. saligna / S. hookeriana*
Activity: Antibacterial, leishmanicidal

66. 2β-Hydroxyepipachysamine-D [10]

Mol. For.: $C_{30}H_{46}N_2O_2$

Mol. Wt.: 466

$[\alpha]_D$: - 52 °

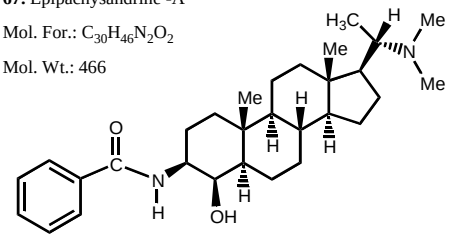


Source: *S. saligna*
Activity: AChE / BChE inhibitor

67. Epipachysandrine -A [18]

Mol. For.: $C_{30}H_{46}N_2O_2$

Mol. Wt.: 466



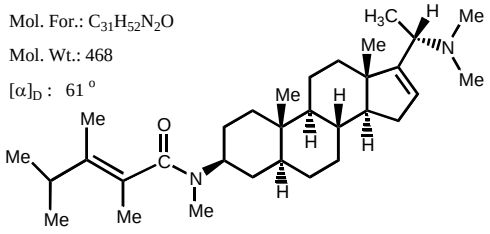
Source: *S. saligna*
Activity: Antitumor

68. Salignenamide-E [10]

Mol. For.: $C_{31}H_{52}N_2O$

Mol. Wt.: 468

$[\alpha]_D$: 61 °



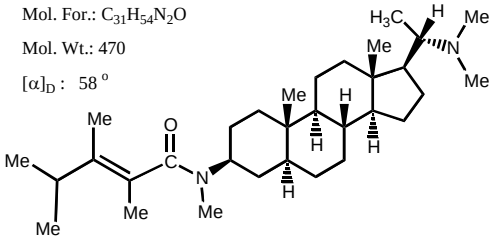
Source: *S. saligna*
Activity: AChE / BChE inhibitior

69. Salignenamide-F [10]

Mol. For.: $C_{31}H_{54}N_2O$

Mol. Wt.: 470

$[\alpha]_D$: 58 °

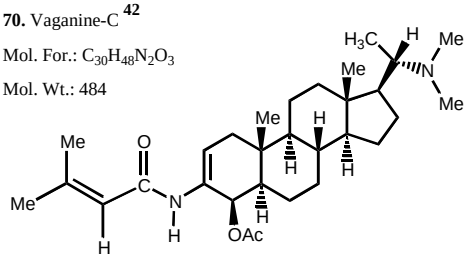


Source: *S. saligna*
Activity: AChE / BChE inhibitior

70. Vaganine-C [42]

Mol. For.: $C_{30}H_{48}N_2O_3$

Mol. Wt.: 484



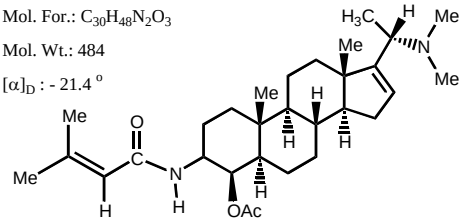
Source: *S. vaganas*
Activity: not reported

71. Vaganine-D [45]

Mol. For.: $C_{30}H_{48}N_2O_3$

Mol. Wt.: 484

$[\alpha]_D$: - 21.4 °



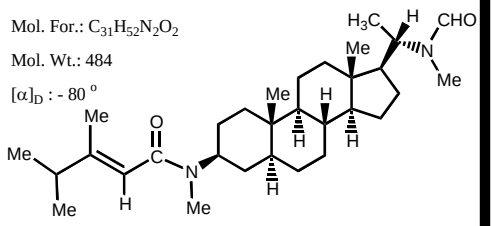
Source: *S. coriacea*
Activity: AChE / BChE inhibitor

72. Hookerianamide-D [36, 21]

Mol. For.: $C_{31}H_{52}N_2O_2$

Mol. Wt.: 484

$[\alpha]_D$: - 80 °



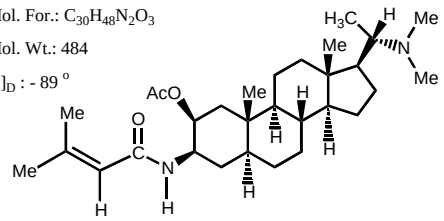
Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, leishmanicidal

73. Hookerianamide-E [36, 21]

Mol. For.: $C_{30}H_{48}N_2O_3$

Mol. Wt.: 484

[a]$_D$: - 89 °



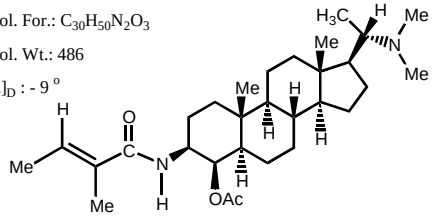
Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, leishmanicidal

74. Sarcovagine-C [41,8]

Mol. For.: $C_{30}H_{50}N_2O_3$

Mol. Wt.: 486

$[\alpha]_D$: - 9 °



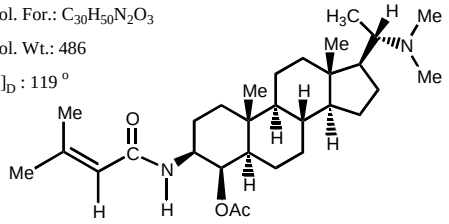
Source: *S.vagans*
Activity: not reported

75. Vaganine-A [42]

Mol. For.: $C_{30}H_{50}N_2O_3$

Mol. Wt.: 486

$[\alpha]_D$: 119 °



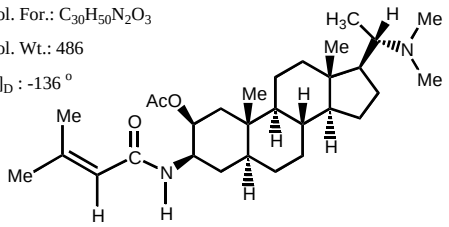
Source: *S.vagans*
Activity: not reported

76. Hookerianamide-C [20, 21]

Mol. For.: $C_{30}H_{50}N_2O_3$

Mol. Wt.: 486

$[\alpha]_D$: -136 °



Source: *S. hookeriana*
Activity: AChE / BChE inhibitor, leishmanicidal

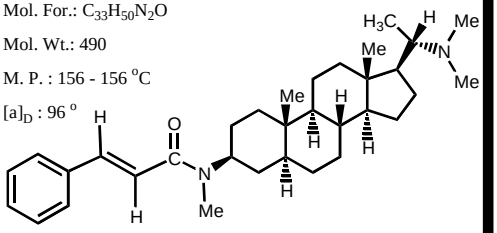
77. Saligcinnamide [44]

Mol. For.: $C_{33}H_{50}N_2O$

Mol. Wt.: 490

M. P. : 156 - 156 °C

[a]$_D$: 96 °



Source: *S. saligna*
Activity: Antibacterial, AChE / BChE inhibitor

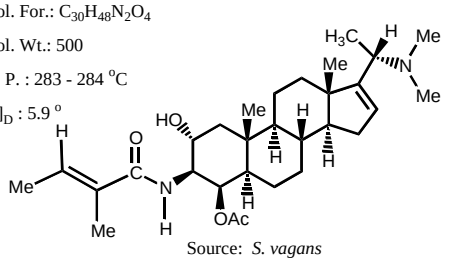
78. Sarcovagenine-B [39]

Mol. For.: $C_{30}H_{48}N_2O_4$

Mol. Wt.: 500

M. P. : 283 - 284 °C

$[\alpha]_D$: 5.9 °

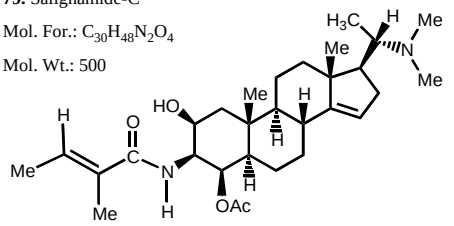


Source: *S. vagans*
Activity: not reported

79. Salignamide-C [10]

Mol. For.: $C_{30}H_{48}N_2O_4$

Mol. Wt.: 500



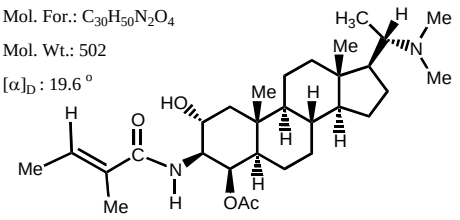
Source: *S. saligna*
Activity: AChE / BChE inhibitor

80. Salignamide-B [41]

Mol. For.: $C_{30}H_{50}N_2O_4$

Mol. Wt.: 502

$[\alpha]_D$: 19.6 °



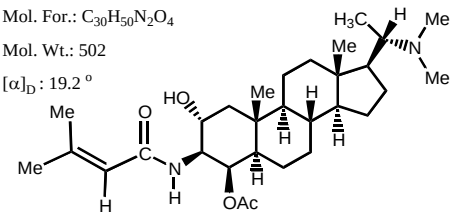
Source: *S. vagans*
Activity: not reported

81. Hookerianamide-B [20, 21]

Mol. For.: $C_{30}H_{50}N_2O_4$

Mol. Wt.: 502

$[\alpha]_D$: 19.2 °

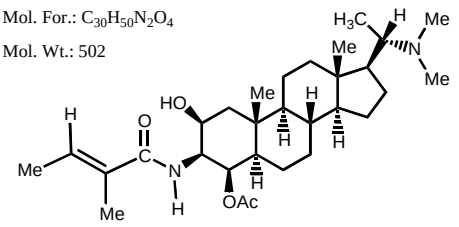


Source: *S. hookeriana*
Activity: AChE / BChE inhibitior, leishmanicidal

82. Axillarine -F [10]

Mol. For.: $C_{30}H_{50}N_2O_4$

Mol. Wt.: 502



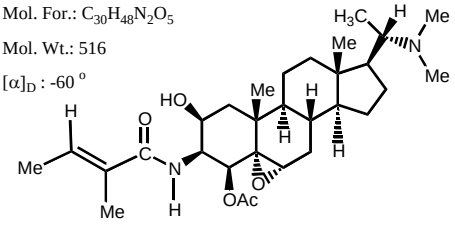
Source: *S. saligna*
Activity: AChE / BChE inhibitior

83. Salignarine -A [37]

Mol. For.: $C_{30}H_{48}N_2O_5$

Mol. Wt.: 516

$[\alpha]_D$: -60 °



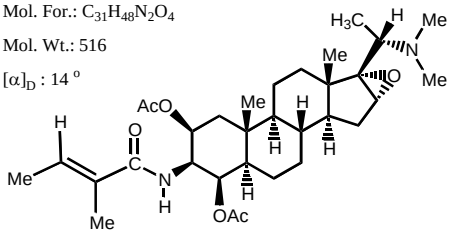
Source: *S. saligna*
Activity: not reported

84. Epoxynepakistamine-A [13]

Mol. For.: $C_{31}H_{48}N_2O_4$

Mol. Wt.: 516

$[\alpha]_D$: 14 °



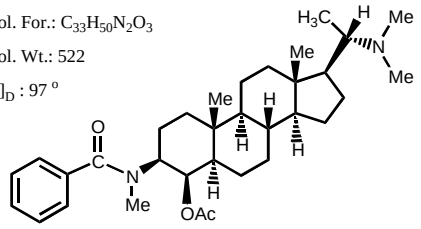
Source: *S. coriacea*
Activity: AChE / BChE inhibitior

85. Hookerianamide -G [36]

Mol. For.: $C_{33}H_{50}N_2O_3$

Mol. Wt.: 522

$[\alpha]_D$: 97 °



Source: *S. hookeriana*
Activity: AChE / BChE inhibitior

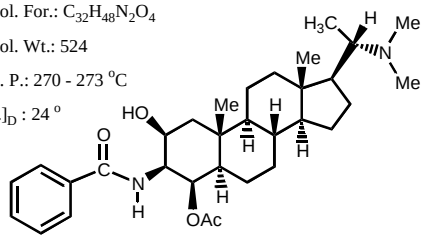
86. Axillarine- C [10]

Mol. For.: $C_{32}H_{48}N_2O_4$

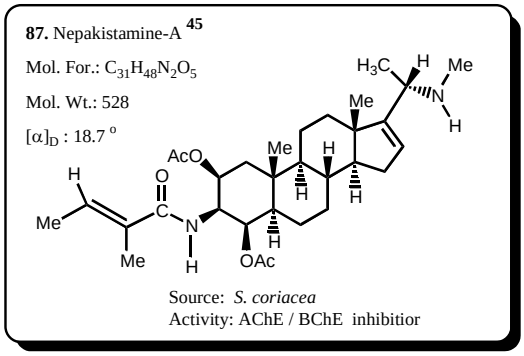
Mol. Wt.: 524

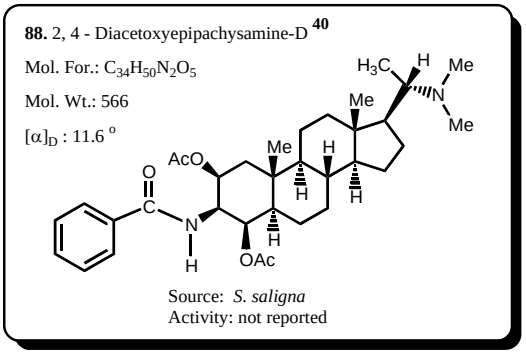
M. P.: 270 - 273 °C

$[\alpha]_D$: 24 °



Source: *S. saligna*
Activity: AChE / BChE inhibitior





2.1.2.2 Pharmacology of Steroidal Alkaloids of Genus *Sarcococca*

Many of the steroidal alkaloids have shown a variety of biological activities, which led to an increasing research interest during last four decades. Some important activities of steroidal alkaloids of *Sarcococca* plants are as follows:

a) Acetyl- and butyrylcholinesterases inhibition:

The bioassay-guided phytochemical investigation on *Sarcococca* species revealed that the extracts, fractions and compounds of this genus are potent AChE and BChE inhibitors.[8, 20]

b) Antibacterial activities:

Some compounds isolated from *S. saligna* have shown a significant antibacterial activity.[46]

c) Antileishmanial activities:

Seventeen steroidal alkaloids from *S. hookeriana*, along with five synthetic derivatives, have shown a potent to mild antileishmanial property.[21]

d) Antiplasmodial activities:

Some steroidal alkaloids, isolated from *Sarcococca hookeriana*, showed a moderate *in vitro* antiplasmodial activity against W2 strain of *P. falciparum*.[8]

e) Spasmolytic activities:

Extracts and compounds of *S. saligna* showed a dose dependent spasmolytic activity and found to relax high K^+ (80 mM)-induced contraction in the rabbit jejunum intestinal preparations. This was indicative of a calcium channel-blocking mechanism.[35, 47]

f) Cytotoxicity:

The dichloromethane extract of *S. coriacea* was found to be cytotoxic, while the methanol extract showed a polygalacturonase inhibition.[48]

2.1.2.3 *Sarcococca coriacea*

The genus *Sarcococca* has several species, one of them *S. coriacea* (Buxaceae) is an evergreen shrub which grow up to a height of two meters. It is widely distributed in Bhutan, North Assam, South Tibet, and in Central Nepal at altitudes of 600-1600 m.[11] Flowers are white and creamy, blossom in February and fruiting takes place in August. Local people call this plant Fitifiya or Pipiree in Nepal. This plant is used by the common people as a drug for the treatment various diseases like malaria, and skin infection in folk system of medicine.[49]

The literature survey shows that four new and two known cholinesterase inhibiting steroidal alkaloids were previously reported from the aerial parts of this plant.[13, 45] During the course of present study, we have worked on the roots of *Sarcococca coriacea* and a class of novel fluorine-containing steroidal alkaloids, along with some known compounds, were obtained.

3.0 Steroidal Alkaloids

3.1 Introduction

Steroidal alkaloids are nitrogen- containing compounds with a typical steroidal skeleton (cyclopentenophenanthrene), with nitrogen in the ring or in a side chain.[50] These can be defined as

steroidal amines, instead of pure alkaloids, due to their biosynthesis as they are derived from mevalonic acid, and not from amino acid.

3.2 Classification

Steroidal alkaloids are mainly present in plants of Apocyanaceae, Solanaceae, Liliaceae, and Buxaceae families, as well as in amphibians and marine invertebrates. On this basis, steroidal alkaloids can be divided into three major categories.

A) Steroidal Alkaloids from Plants

Higher plants belonging to the families of angiosperms are main source of steroidal alkaloids whereas plants belong to the families of gymnosperms rarely contains steroidal alkaloids. According to the origin, steroidal alkaloids are mainly classified into following four major groups:

I) Steroidal Alkaloids of Apocynaceae

II) Steroidal Alkaloids of Buxaceae

III) Steroidal Alkaloids of Liliaceae

IV) Steroidal Alkaloids of Solanaceae

I) Steroidal Alkaloids of Apocynaceae

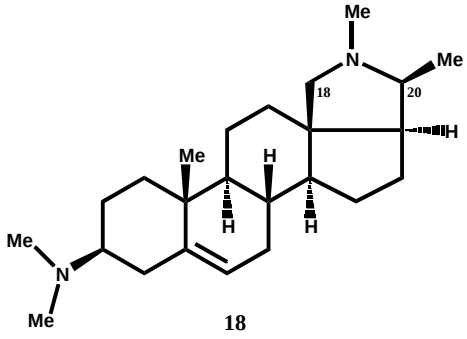
Apocynaceae family is the one of the richest sources of steroidal alkaloids. More than 150 new steroidal alkaloids have been isolated from the different genera of this family, such as *Holarrhena*, *Paravallaris*, *Funtumia*, *Kibatalia* and *Malovetia*.[51] Most of the earlier work in this area was performed by Goutarel and Co-workers in France in the 1960s and early 1970s.[52] According to the structures, Apocynaceae alkaloids can be further classified in two types:

a) The Conanine- type Alkaloids

Distinguished feature of this type of alkaloids is a five-membered heterocyclic ring, adjacent to ring D of the common steroidal skeleton (the heterocyclic ring can be either pyrrolidine or pyrroline) and either oxygen or an amine functionality is present at C-3 position of ring A. Conessine (**18**) was the first and the most common member of conanine-type alkaloids, isolated from many plants of the genera *Holarrhena*, *Malovetia*, and *Funtumia.* Due to its C-18 substituted steroidal nature, it has attracted a major scientific interest, because it can lead to the synthesis of important hormones through quite simple chemical conversions.[53] Other examples of this sub-class are holonamine (**19**) [54] and reghoholarrhenine-C (**20**).[55]

b) The Pregnane-type Alkaloids

Distinguished feature of this type of alkaloids is a simple pregnane skeleton bearing amino substituents on either C-3 or on C-20 or on both positions. Funtuphyllamines A (**21**), B (**22**) and C (**23**) are common examples of this class, isolated from *Funtumia africana*.[12]



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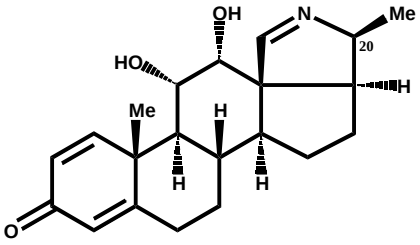

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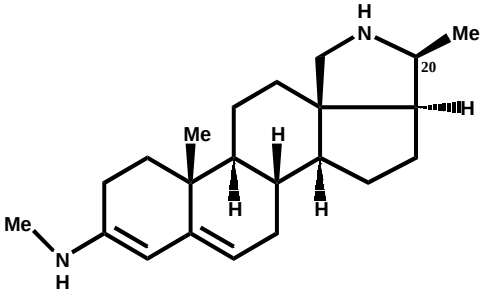

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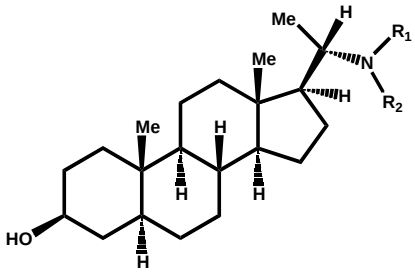
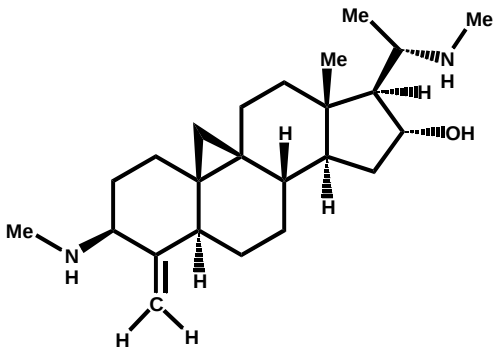


21 $R_1 = R_2 = H$
22 $R_1 = H$, $R_2 = CH_3$
23 $R_1 = R_2 = CH_3$

II) Steroidal Alkaloids of Buxaceae

A number of genera of the family Buxaceae, such as *Buxus*, *Sarcococca* and *Pachysandra*, were found to be rich sources of steroidal alkaloids. The genus *Sarcococca* has already yielded more than 90 new steroidal alkaloids,[8] whereas the genus *Buxus* has so far yielded nearly 250 new alkaloids.[8]

A prototype of a new class of steroidal alkaloid cyclobuxine-D (**24**), which contains a cyclopropane ring and a substitution pattern at C-4 and C-14, was isolated from *B. microphylla* in 1964. This skeleton was predicted to be biogenetic intermediate between the lanosterol- and cholesterol-type steroids.[56]



24

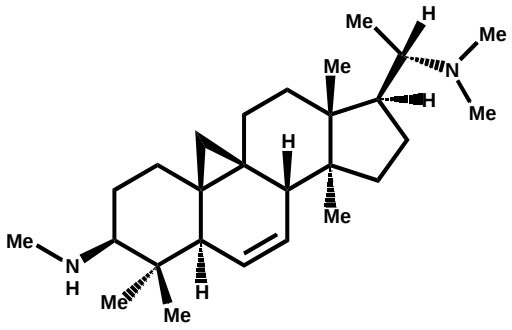
Buxus alkaloids generally divided into following groups:

a) Derivatives of 9*β*, 19-cyclo-4, 4, 14*α*-trimethyl-5*α*-pregnane

Distinguished feature of this type of alkaloids are a cyclopentanophenanthrene ring and substitution pattern at C-4 and C-14. The specific examples include cyclobuxapaline-C (**25**).[57]

b) Derivative of *Abeo* -9(10 ⟶ 19)-4, 4, 14 *α*-trimethyl-5*α*-pregnane

Breaking of C-9/C-10 bond and formation of cycloartane skeleton in ring B are the characteristic features of this type of alkaloids. Papilamine (**26**) is an example of this class of steroidal alkaloids.[58]



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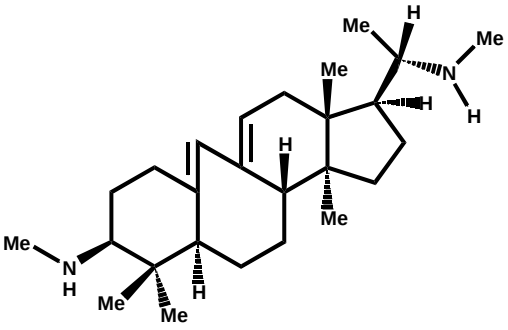

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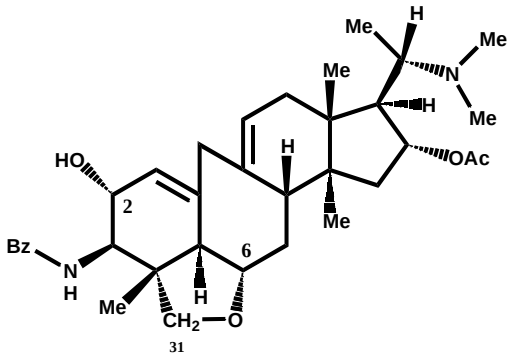
O [6]-Buxafurnamine (**27**) and *O*[10]-buxafurnamine (**28**) with a tetrahydrofuran ring, were isolated from *B. hildebrandtii* and *B. papillosa*.[59] An interesting structural varaiation in this type is the presence of a tetrahydrooxazine ring in a number of steroidal alkaloids, isolated from *B. papillosa* and *B. sempervirens.* Representive examples are harappamine (**29**) and moenjodaramine (**30**).[60]



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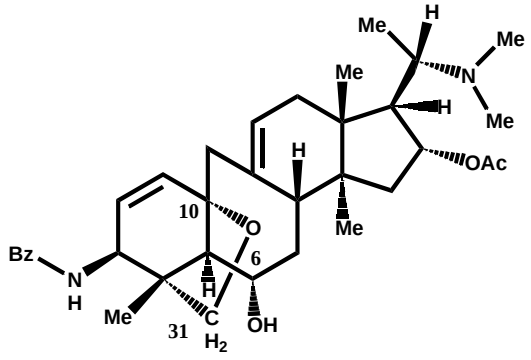

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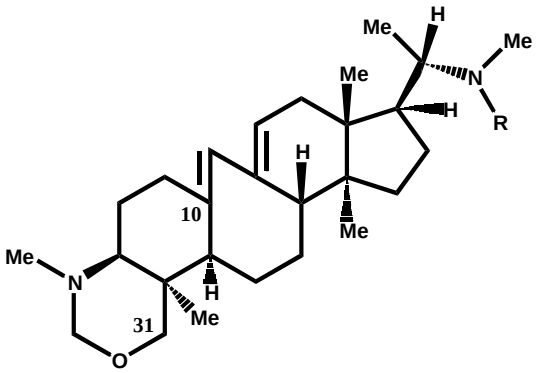
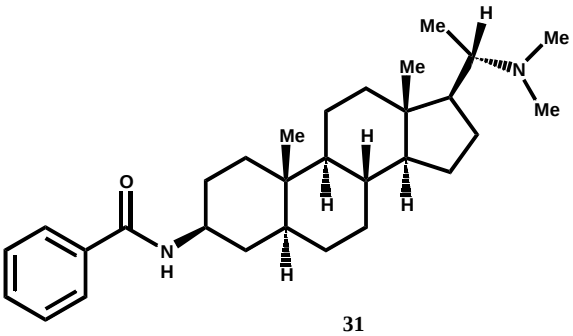


29 Harappamine, R =H
30 Moenjodaramine, R = CH_3

Plants of the genus *Pachysandra* and *Sarcococca* are also a major source of simple pregnane-type steroidal alkaloids with nitrogen atoms at C-3 and C-20 positions. Epipachysamine-D (**31**), isolated from *P. procumbens*,[61] and saracocine (**32**) obtained from *Sarcococca* saligna,[16] are the representative examples of this type of alkaloids.



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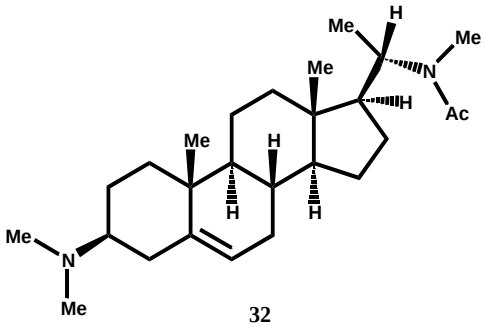

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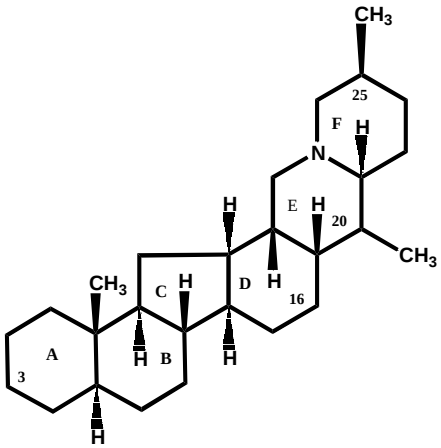
C) Steroidal Alkaloids of Liliaceae

Distinguished feature of the steroidal alkaloids, isolated from the family Liliaceae, is a C_{27} cholestane skeleton with five or six carbocyclic or heterocyclic rings. On the basis of the carbon skeleton, they can be classified into two groups:

A) Isosteroidal Alkaloids (**33**)

B) Steroidal Alkaloids (**34**) [62]



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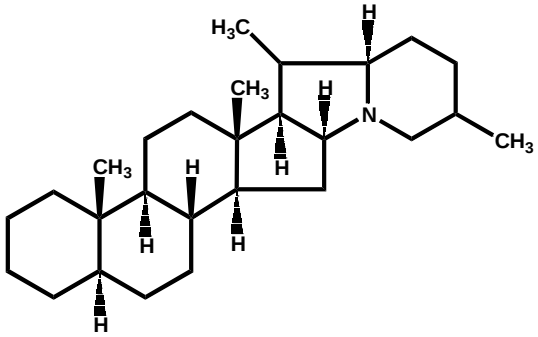

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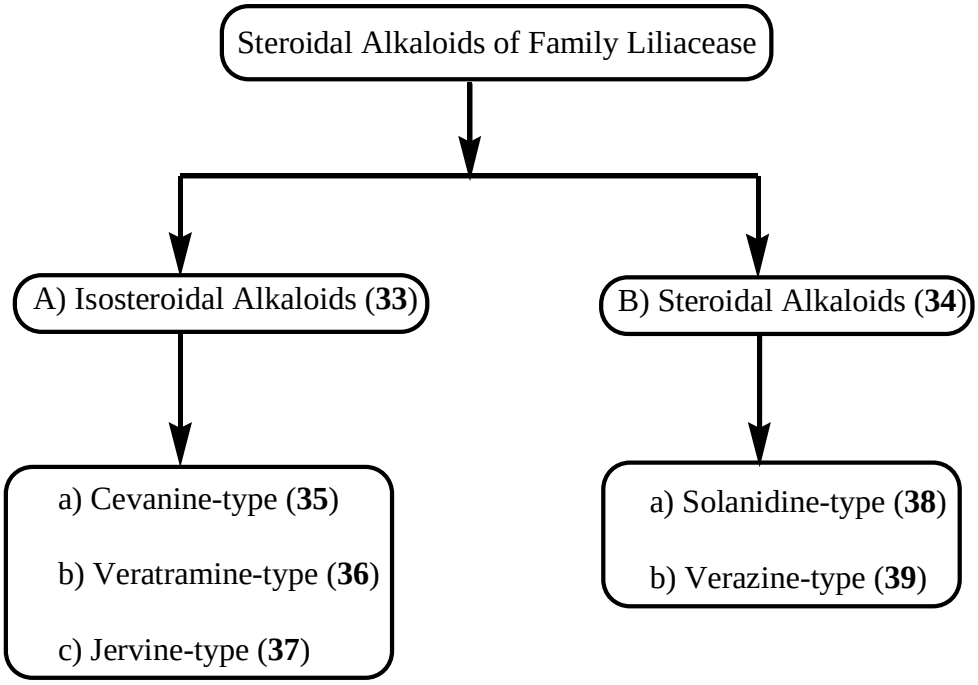


Scheme- 3.2.1: Classification of steroidal alkaloids of family Liliaceae.

The isosteroidal alkaloids, which have basic skeleton like **33,** are characterized by a C-nor-D-homo-[14(13→12)-*abeo*] ring system. They can be further divided on the basis of the linkages between rings E and F into:-

a) Cevanine-type

b) Veratramine-type

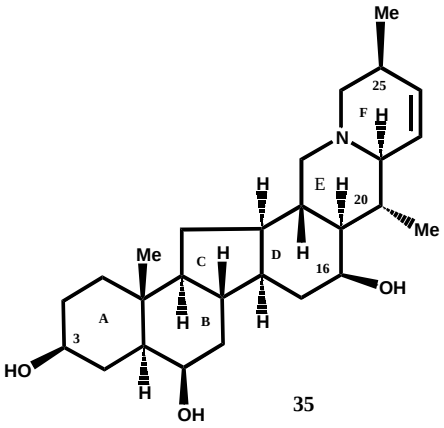
c) Jervine-type

a) Cevanine- type Alkaloids

Distinguished feature of the cevanine-type alkaloids is a hexacyclic benzo [7, 8], fluoreno [2, 1-b] quinolizine nucleus. It is the largest group of steroidal alkaloids with over 60 alkaloids isolated from *Veratrum* and *Fritillaria* species. Impericine (**35**) is an example of this type of alkaloids.[63]

b) Veratramine-type alkaloids

The characteristic feature of veratramine-type of alkaloids is the absence of ring E and the presence of an aromatic ring D. However many analogous with an unaromatized ring D are also placed in this group. Over 10 veratramine-type alkaloids have been isolated from *Veratrum* and *Fritillaria* species. 20-Isoveratramine (**36**) is an example of this type of alkaloids.[64]



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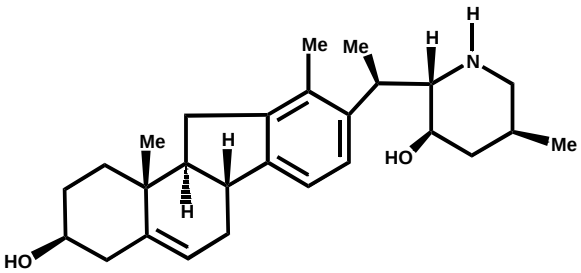

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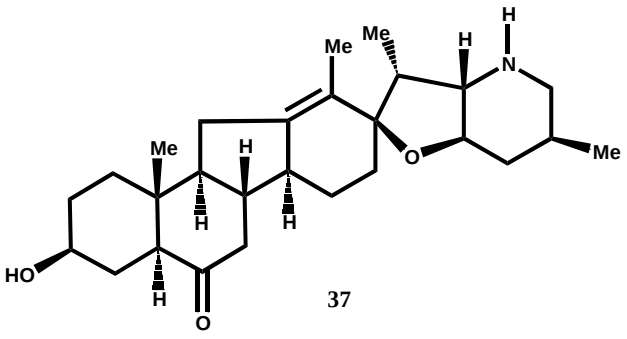
c) Jervine -type Alkaloids

Distinguished features of jervine-type alkaloids include a C-*Nor*-D-homosteroidal alkaloid with hexacyclic rings and a furan ring E, fused with a piperidine ring system, forming an ether bridge between C-17 and C-23. Over 12 jervine-type alkaloids have been reported so far. Peimisine (**37**) is a typical example of this group of alkaloid.[65]



37

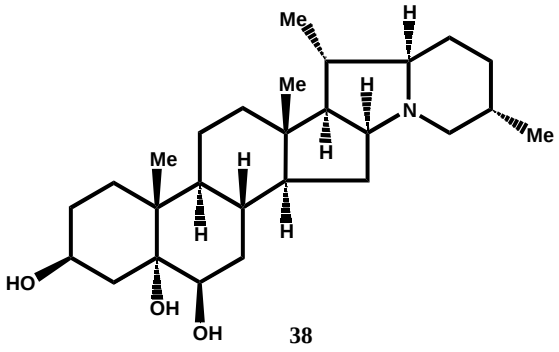
The steroidal alkaloids which have a basic skeleton **34,** contains a six membered C-ring and five membered D- ring, can be further divided into following types;

a) Solanidine-type alkaloids

The solanidine-type alkaloids have a basic skeleton **34**. They arise in nature from epiminocholestanes in which the amino group is incorporated into an indolizine ring to form a hexacyclic skeleton. *Veratrum* and *Fritillaria* species are the main sources of this type of alkaloids. Alkaloid **38** is an example of this type.[66]

b) Verazine-type Alkaloids

Distinguished feature of verazine-type alkaloids is the 22/23, 26-epiminocholestane heterocyclic skeleton. Most of the members of this group of alkaloids have been reported from *Veratrum* and *Fritillaria* species. Verazine (**39**) is an example of this group of alkaloids.[67]



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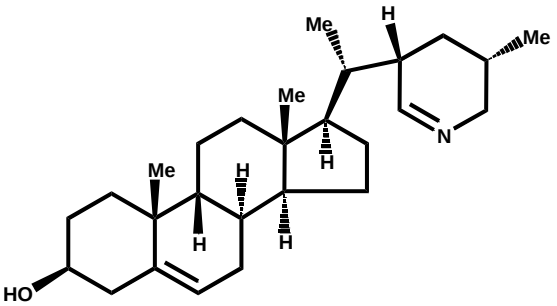


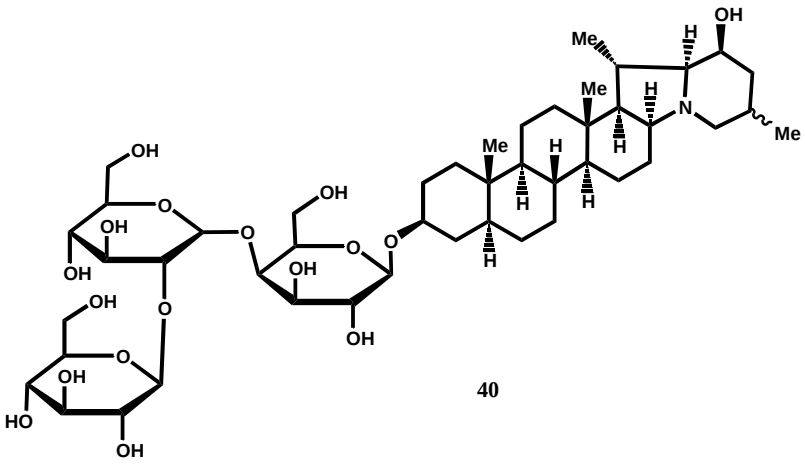
39

V) Steroidal Alkaloids of the Solanaceae

Over 200 alkaloids have been isolated from the different genera of family Solanaceae, including *Solanum* and *Lycopersium* (*Lycopersicum*). All of these alkaloids possess a C_{27} cholestane skeleton and can be divided into following structural types;[56]

a) Solanidine-type

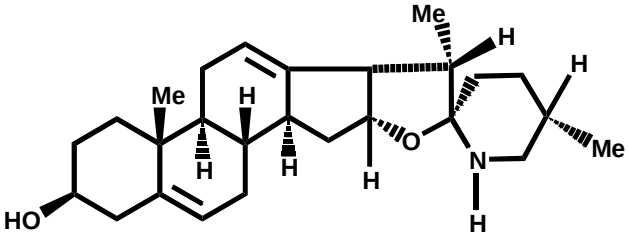
These types of alkaloids are common in the family Solanaceae and Liliaceae. 3-*O*-*β*–Lycotriaoside (**40**), isolated from *S. lyratrum*, is an example of this type of compounds.[67]



40

b) Spirosolanes-type

These alkaloids have a methyl piperidine ring (ring F), with the α-position joined to C-22 of the steroidal framework to form an oxazaspirane unit. Veramine (**41**) is the typical example of this type of compounds.[68]



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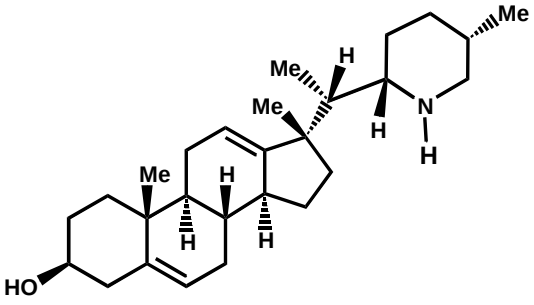
c) Solacongestidine-type

Metabolites of this type show a structural character in which the side chain (C-20-C-27) of the cholestane skeleton is rearranged to an *N*-containing six-membered ring without being conjugated to C-16. Thus forms a 5-methylpiperidine ring, attached to C-20 in the steroidal framework *via* the

2-position of the heterocycle. The alkaloids veralkamine (**42**) from *Vertanum album* and etioline (**43**) from *Solanum* and *Veratrum* species are typical examples of this type of alkaloids.[69, 56]



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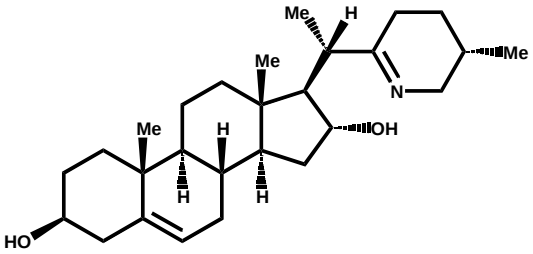

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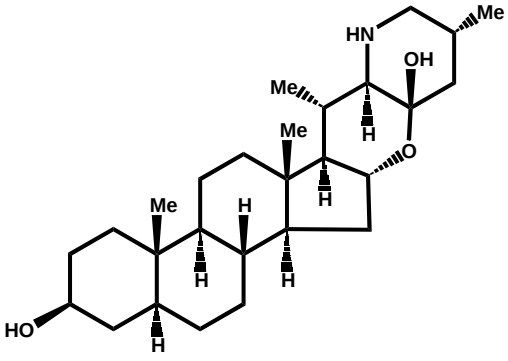
d) Solanocapsine -type

This type of alkaloids contains an unusual epiminocyclohemiketal moiety in their structure. They are mostly reported from *Solanum* species. Solanocapsine (**44**), isolated from *S. capsicastrum*, is a typical example of this class.[70]

e) Jurubidine-type

Distinguished characteristics of this type of alkaloids are presence of amino group at C-3 and spirostane skeleton (rings E and F). Jurubidine (**45**) is an example of this type of alkaloids.[71]



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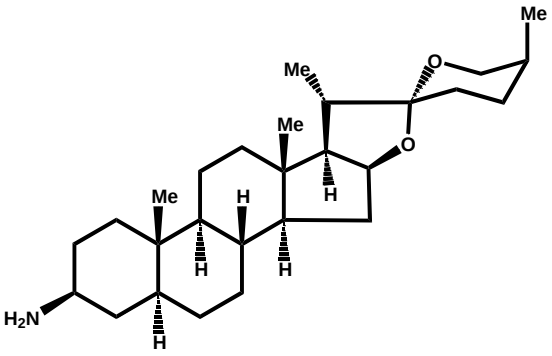
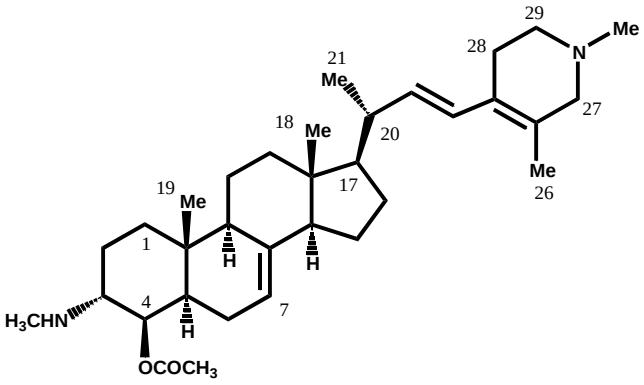


45

B) Steroidal Alkaloids from Marine Organisms

A number of steroidal alkaloids have been isolated from marine animals, mostly from marine invertebrates. A stigmastane-type steroidal alkaloid, 4-acetoxy-plakaminamine B (**46**), was isolated from a Thai marine sponge *Corticium* sp.[72] A new series of cytotoxic steroidal alkaloids were isolated from a Philippine Sponge *Corticium niger*. Plakinaminamine I (**47**) is an example of this group of alkaloids.[73]



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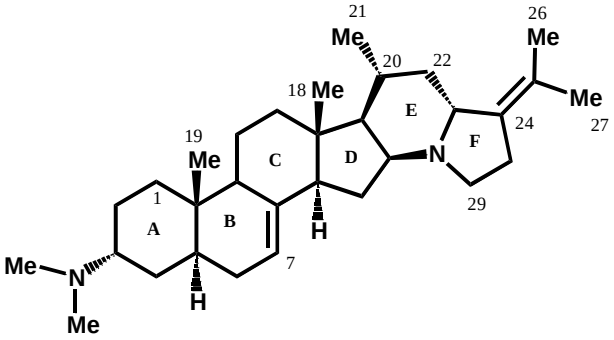
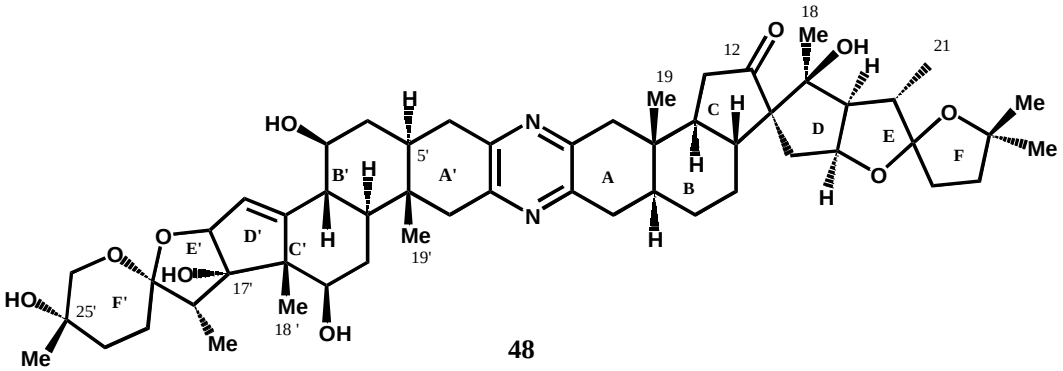


47

Nearly 50 cytotoxic, dimeric alkaloids were isolated from the different species of *Ritterella* and *Cephalodiscus.* The ritterazines and cephalostatins share many structural features in which two highly oxygenated C_{27} steroidal units are fused *via* a pyrazine ring at C-2 and C-3 and both chains of the steroidal units usually form either 5/5 or 5/6 spiroketals. Ritterazine-A (**48**) is a typical example of this series.[74]



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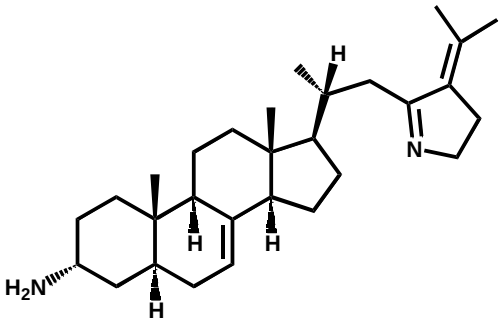
A marine sponge of the genus *Plakina* has yielded many antimicrobial steroidal alkaloids, for example plakinamine A (**49**).[75] Recently 9 (10-19)- *abeo*-andorstane-type steroidal alkaloids, cortistatins A- D, have been reported from marine sponge *Corticium simplex*. Cortistatin A (**50**) is a representative example of this series.[76]



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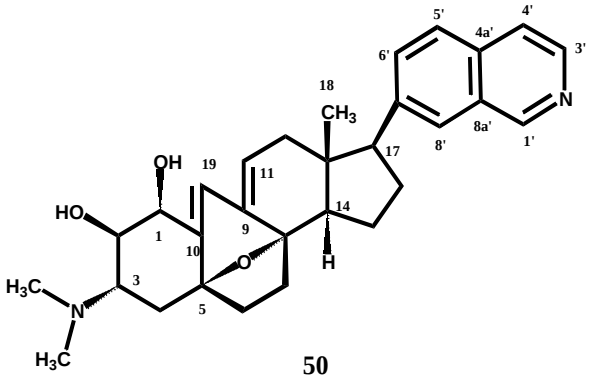

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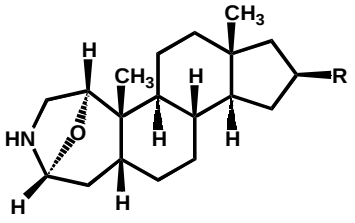
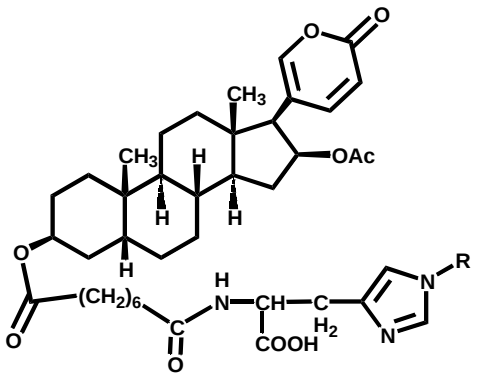
C) Steroidal Alkaloids from Terrestrial Animals

More than thirty new steroidal alkaloids have been obtained from various species of *Salamandra*, *Phyllobates* and *Bufo*.[56] These alkaloids are generally found in secretions from the skin glands of these amphibians and appear to protect the skin against fungal and bacterial infections. Most of these have a basic skeleton **51**. The interesting features of these alkaloids include a *cis* junction between rings A and B, and the presence of an expanded ring A with the formation of an isoxazoline system. Some representative examples are samandarin (**52**), bufotalin A (**53**) and bufotalin B (**54**).[77, 68, 78]

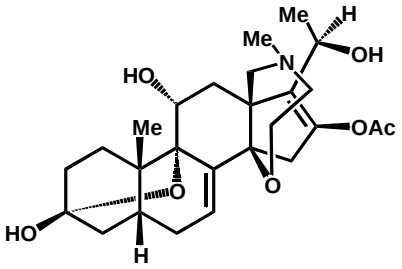


51 R = H, 52 R = OH



53 R = H, 54 R = Me

Highly toxic alkaloids, batrachotoxins (-) batrachotoxinin A (**55**) and homobatrachotoxin (**56**) were isolated from different species of poison-dart frog of genus *Phyllobates*.[79]



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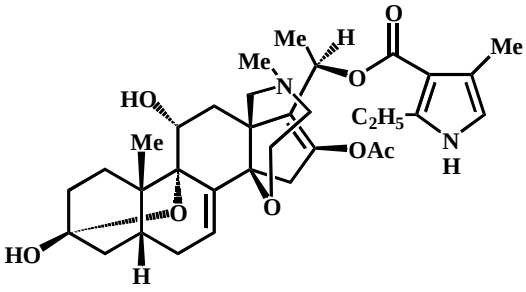


56

3.3 Spectral Properties of *Sarcococca* Alkaloids

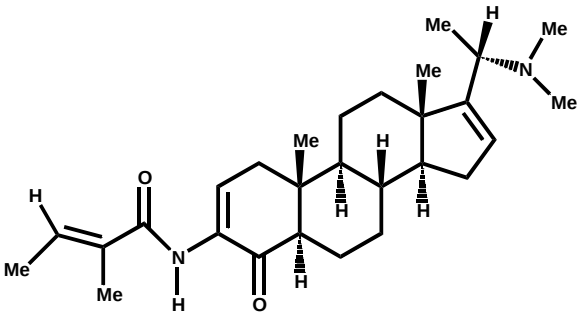
Steroidal alkaloids, isolated from genus *Sarcococca*, can be distinguished from other types of steroidal alkaloids on the basis of characteristic spectral behavior. Their structures are generally deduced by using UV, IR, mass, 1D- and 2D-NMR spectral data.

A) UV Spectrophotometry

Most of the compounds of genus *Sarcococca* show characteristic UV absorptions, representing functionalities present, whereas some alkaloids show only the terminal absorption. For example, sarcovagenine-C (**57**) shows UV absorption at 254 nm for α, β- unsaturated carbonyl functionality.[14]

B) IR Spectrophotometry

To detect the functionalities, such as amine (3300-3500 cm^{-1}), hydroxyl (3200-3600 cm^{-1}), amides (1630-1680 cm^{-1}) and carbonyl group (1700-1750 cm^{-1}), and the presence of olefinic bonds (1600-1680 cm^{-1}), which are frequently present in steroidal alkaloids, IR spectrophotometry is very useful. For example, sarcovagenine A (**58**) shows IR absorptions at 3602 (NH), 3349 (OH), 1641 (amide C=O) and 1616 (C=C) cm^{-1}.[15]



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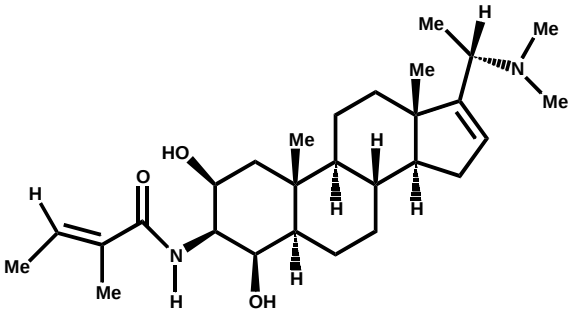


58

C) Mass Spectrometry

Mass spectrometry plays a key role in the structural elucidation of *Sarcococca* alkaloids. Some of the distinguishing features of mass spectra of *Sarcococca* alkaloids are as follows:

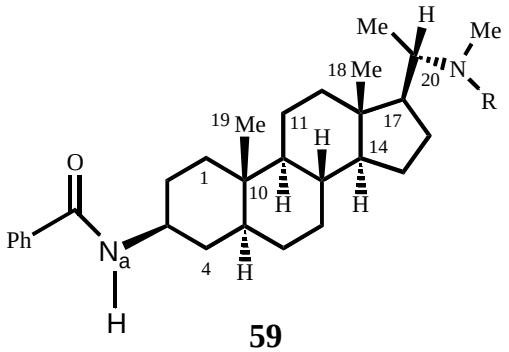
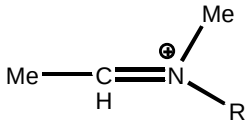
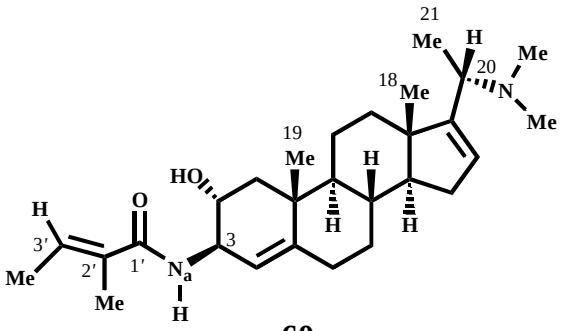
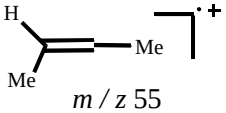
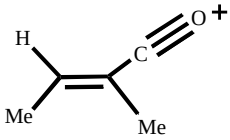
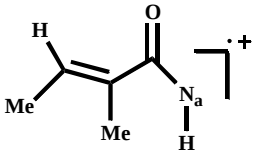
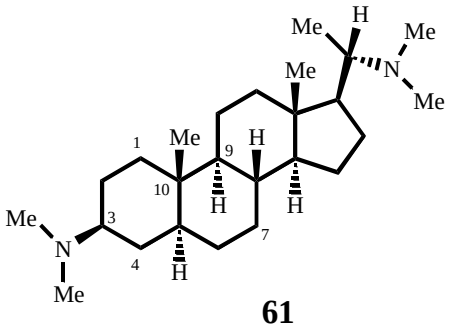
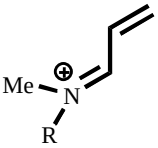
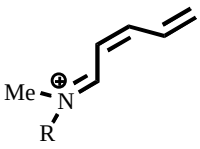
1) It is well known that the fragmentation in nitrogen- bearing substances is preferentially initiated by the cleavage of the bond between the carbons α and β to the nitrogen atom. The fragment ions resulting from the cleavage of nitrogen containing side chain on the ring-D are more abundant and mostly form the base peaks at *m/z* 58 and 72, representing *N*-methyl-*N*-ethyliminium and *N, N*-dimethyl-ethyliminium, cleaved from C-17 of the site, as shown in structure **59** in Table- 3.3.1.

2) Steroidal alkaloids with a double bond between C-16/C-17, as in saligenamide D (**60**), characteristically showed the M^+ -15 as a base peak due to the loss of C-21 secondary methyl group.[18]

3) The compounds having the tigloyl or senecoyl moiety at C-3 position gives characteristic peaks at *m/z* 55, 83, and 98 as in **60**.[14]

4) The compounds with a benzoyl moiety at C-3 exhibit a characteristic peak at *m/z* 105, as in compound **59** (Table-3.3.1).

Table- 3.3.1: Key EI MS Fragmentation of Steroidal Alkaloids of *Sarcococca*.

Compound	Breaking bonds	Fragments
 **59**	C-17/C-20	 R = Me, *m/z* 72 R = H, *m / z* 58
	N_a/ COPh	 *m/z* 105
	C-20/C-21	M^+ -15 = base peak
 **60**	C-1'/C-2'	 *m / z* 55
	N_a/C-1'	 *m / z* 83 (C_5H_7O)
	C-3/ N_a	 *m / z* 98 (C_5H_8NO)
 **61**	C-1/C-10 C-3/C-4	 R = Me, *m / z* 84 R = H, *m / z* 70
	C-2/C-3 C-5/C-10 C-7/C-8	 R = Me, *m / z* 110 R = H, *m / z* 96

5) The mass spectra of 3-dimethylaminopregnane-types of alkaloids exhibit characteristic ions at *m/z* 84 and 110, resulting from the cleavage of ring A, as shown in compound **61** of Table-3.3.1 (as in sarconidine).[56]

D) 1D- and 2D- NMR Spectroscopy

The *Sarcococca* alkaloids possess a cyclopentenophenanthrene skeleton with different functionalities, such as hydroxyl, carbonyl, acetoxy, methoxy, and olefins. The distinguishing features of NMR spectra of *Sarcococca* alkaloids are as follows:

1) The methyl, methylene and methine protons of the skeleton resonate in the range of δ 0.6 to 2.6, as overlapping signals. However, 2D NMR spectroscopic techniques, such as COSY, TOCSY, ROESY, HMQC, and HMBC have been efficiently used for the interpretation of NMR data.

2) All the reported *Sarcococca* alkaloids, except a few, have a basic skeleton **62**, with two tertiary methyl groups at C-18 and C-19 which resonate in the range of δ 0.6 to 1.2. In general, protons of C-18 methyl resonate up-field than the protons of C-19 methyl.

3) Most of them contain a secondary methyl at C-21, which appear as a doublet in the range of δ 0.9 to 1.4 ($J_{20,\,21}$ = 6.2 - 6.6 Hz).

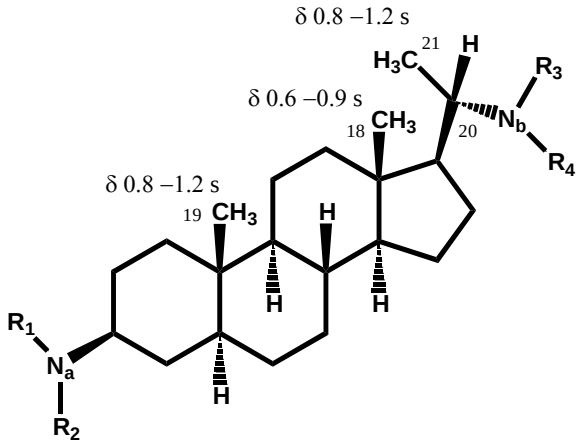
4) Most of these alkaloids have 3H or 6H downfield singlets between δ 2.0 to 2.4 due to N_a or N_b mono- or dimethyl groups at C-3 and / or at C-20.

5) *Sarcococca* alkaloids frequently contain *N*-senecioyl or *N*-tigloyl moieties at C-3. The C-4′ and C-5′ methyl protons of C-3 senecioyl moiety resonate as singlets between δ 1.7 - 2.1, while C-2′ methine proton appears as a singlet at δ 5.4, as observed in salignarine-D (**63**).[15] Similarly, the C-4′ and C-5′ methyls of tigloyl moiety resonates as doublets

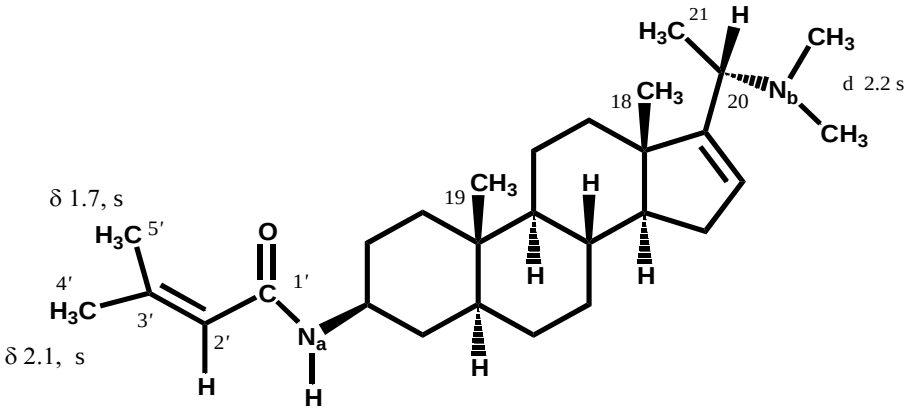
between δ 1.7 - 1.9 ($J_{4',\ 3'}$ = 6.4 - 6.7 Hz), and as a singlet between δ 1.7 - 2.0, respectively. The C-3′ methine proton in this group appeared as a quartet at δ 6.2 - 6.5 ($J_{3',\ 4'}$ = 6.3 - 6.7 Hz), as shown in sarcovagenine B (**64**).[14]

6) Multiplets resonate in the region of δ 2.8 - 5.2 are characteristic of methine protons, geminal to the hydroxyl, acyloxy and amidic functionalities.

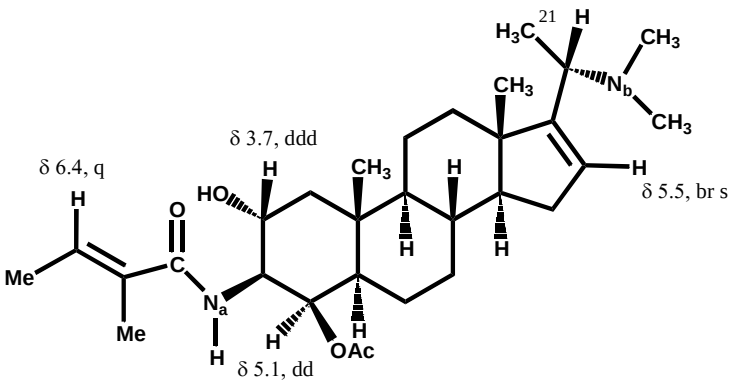
7) In some cases, vinylic hydrogens at C-6 and C-16 resonate between δ 5.2 - 5.8, as in compound **64**.



62



63



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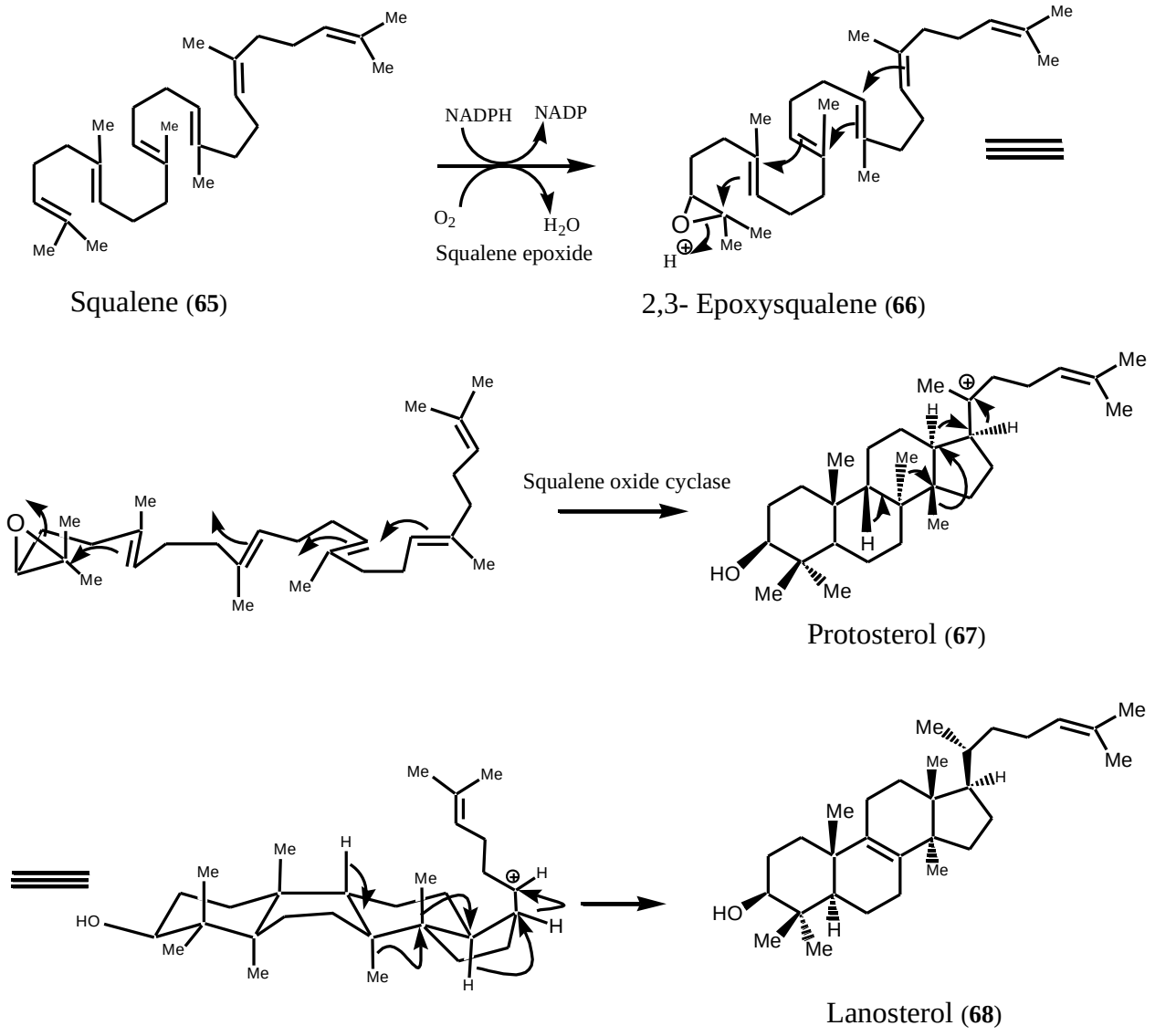
3.4 Biosynthesis of *Sarcococca* Alkaloids

The *Sarcococca* alkaloids are classified as unique pregnane-type steroidal derivatives with a C-20 degraded side chain. These are biogenetically derived from lanosterol which is a precursor of cholesterol. Lanosterol is derived from the polyunsaturated triterpene-squalene (**65**) *via* enzymatic cyclization and rearrangement. The biosynthesis of squalene starts from glucose (the isoprenoid pathway), which is itself produced by reduction of CO_2 in plants and autotropic microorganisms.[80, 81]

A) Biosynthesis of Lanosterol from Squalene (65)

The biosynthesis of lanosterol starts from squalene (**65**), which is converted into 2, 3-epoxysqualene (**66**), catalyzed by an enzyme squalene epoxidase in the presence of NADPH and oxygen[82] (an aerobic phase of steroidogenesis) (Scheme-3.1). Then cyclization of 2, 3-epoxy squalene (**66**) occurred in the presence of an enzyme, squalene oxide cyclase, which forms a protosterol (**67**). The process of cyclization is carbocation mediated and proceed in a step-wise sequence. First proton attacks on the oxide, followed by a concerted electron shift resulting in to ring closure with a formation of carbonium ion at C-20 position of protosterol (**67**)**.** Then the carbonium ion of C-20 is neutralized by a series of 1, 2-*trans* migration of hydride and methyl groups, which result in the elimination of a proton from C-9 to give lanosterol (**68**)**.** In this case,

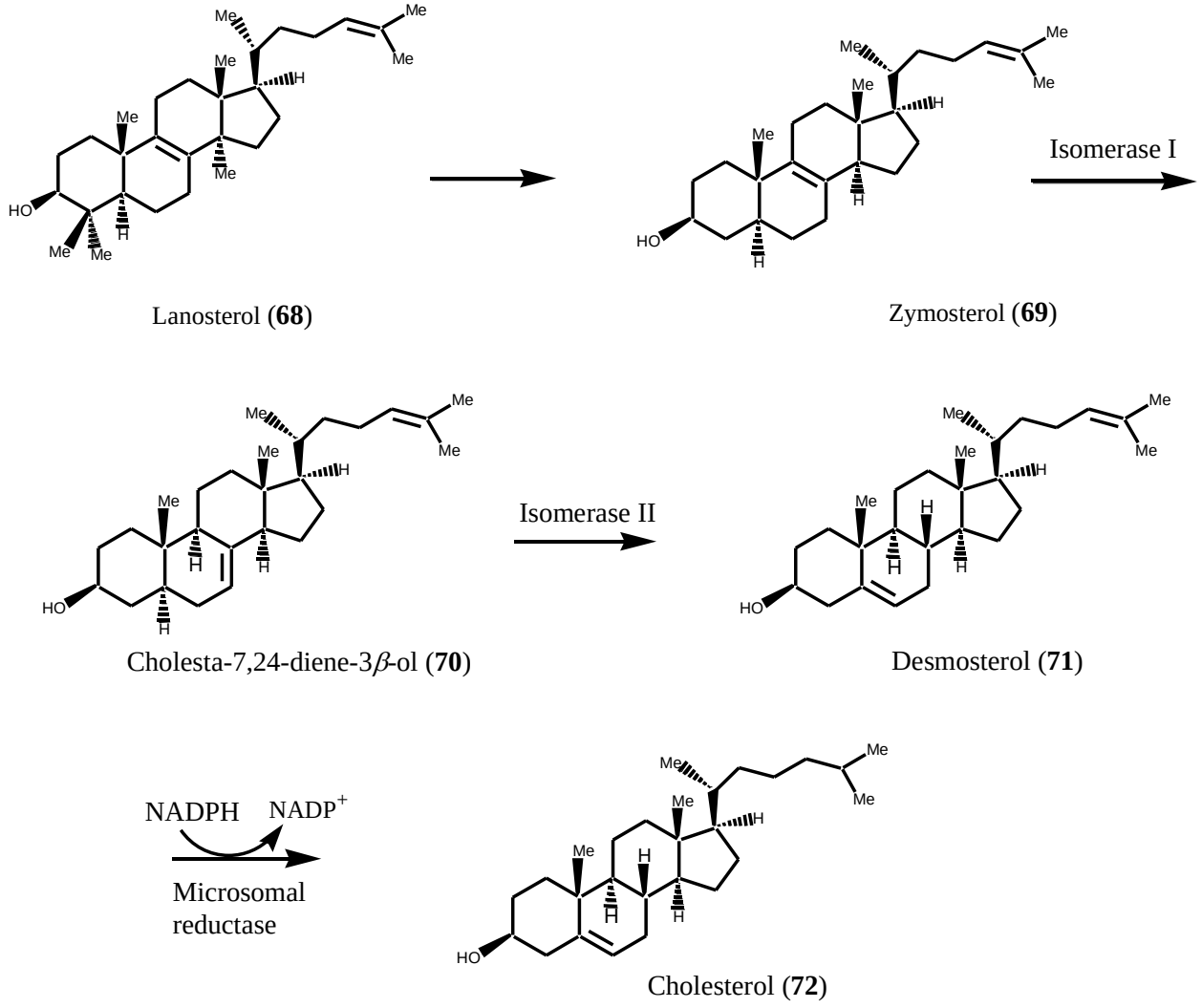
2,3-epoxy squalene (**66**) is folded in a chair-boat-chair-boat form, followed by proton-initiated cyclization to produce the protosterol (**67**) with the *trans-syn-trans-anti-trans-anti* configuration with the appropriate stereochemistry at the stereogenic centers (5*α*, 8*β*, 9*α*, 10*β*, 13*β*, 14*α*, 17*β*, 20 *R*).[83] Finally, a series of 1, 2-*trans* migration of hydride and methyl group leads to the elimination of C-9 proton, giving rise to lanosterol (**68**).



Scheme-3.1: Biosynthesis of lanosterol (**68**) from squalene (**65**).

B) Biosynthesis of Cholesterol from Lanosterol (68)

Conversion of lanosterol into cholesterol (Scheme- 3.2) is one of the key steps in the biosynthesis of steroids. This conversion includes three importants steps, (a) the oxidative elimination of C-4 and C-14 methyl groups to give zymosterol (**69**), (b) migration of Δ^8 double bond to Δ^7 position and (c) then to Δ^5 position to give desmosterol (**71**) *via* intermediate **70**. The reduction of the side chain Δ^{24} double bond by NADPH in the presence of microsomal Δ^{24} reductase gives rise to cholesterol (**72**).[84]



Lanosterol (**68**)

Zymosterol (**69**)

Cholesta-7,24-diene-3β-ol (**70**)

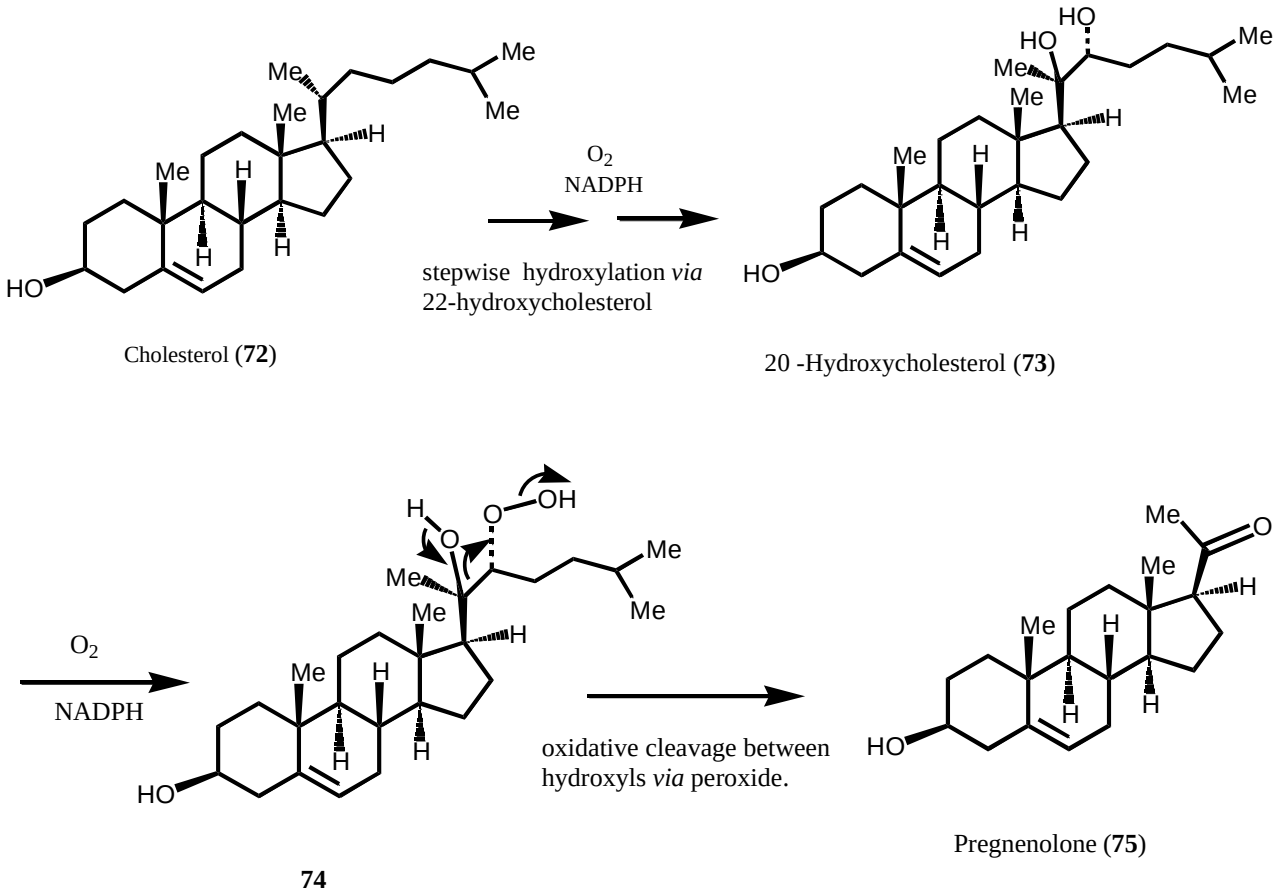
Desmosterol (**71**)

Cholesterol (**72**)

Scheme- 3.2: Biosynthesis of cholesterol (**72**) from lanosterol (**68**).

C) Biosynthesis of Pregnenolone (75) from Cholesterol (72)

The pathway starts with the stepwise hydroxylations at C-22 and C-20 of cholesterol to form **73**. Oxidative cleavage between hydroxyls, perhaps *via* peroxide results in the formation of pregnenolone (**75**).[84]



Cholesterol (**72**)

20 -Hydroxycholesterol (**73**)

74

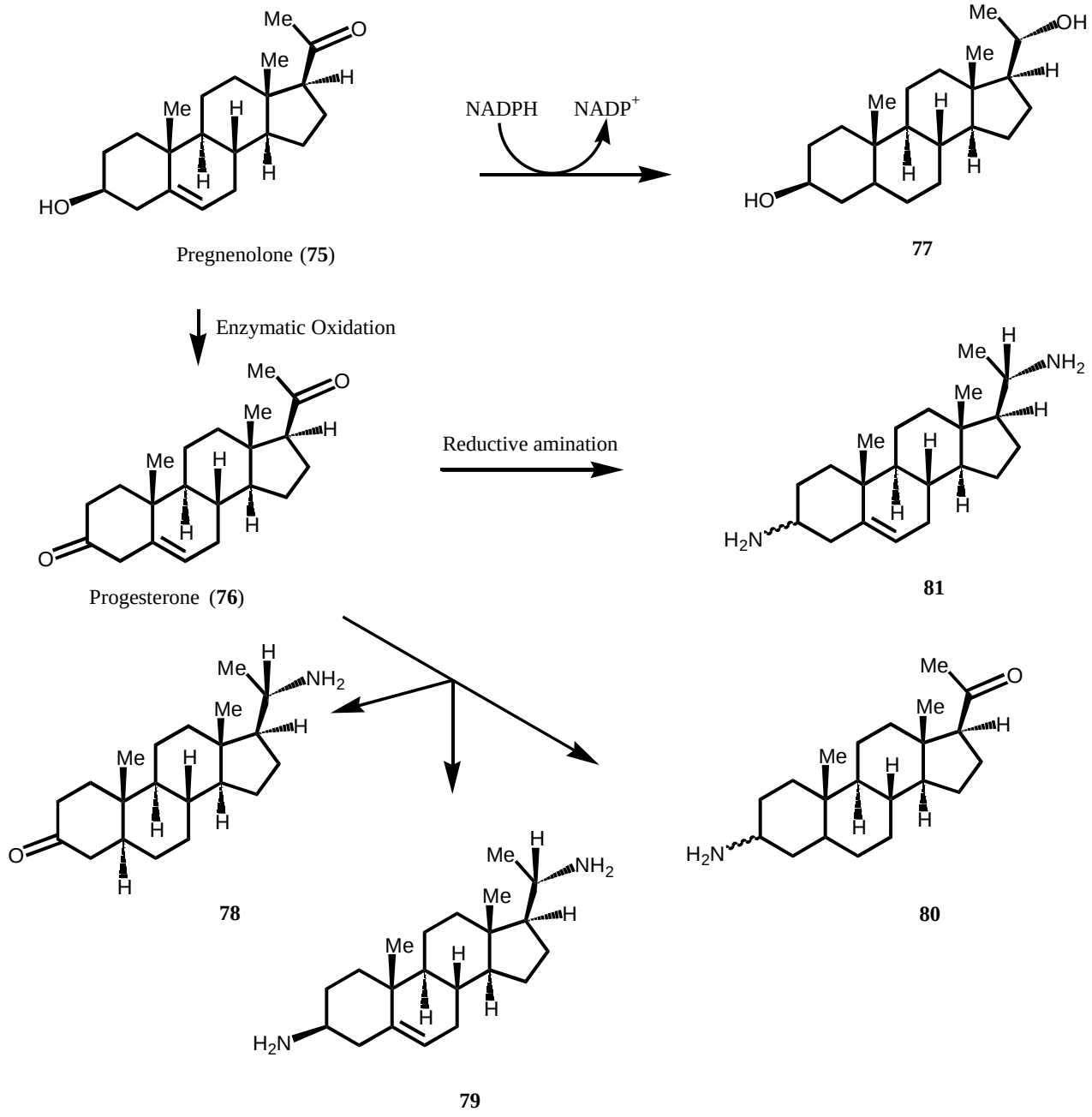
Pregnenolone (**75**)

Scheme-3.3: Biosynthesis of pregnenolone (**75**) from cholesterol (**72**).

D) Biosynthesis of *Sarcococca* Alkaloids

It has been speculated that the nitrogen atom/atoms are incorporated into the steroidal skeleton at the later stages of the biosynthesis.[85] It is proposed that the pregnane-type steroidal alkaloids are derived from a reductive amination of the steroidal ketones, such as pregnenolone (**75**).

Enzymatic oxidation and reduction of pregnenolone (**75**) yield an oxidized product**,** progesterone (**76**) and reduced product **77,** respectively. The amination or reductive amination of compounds **76** and **77** give rise to the compounds **78**, **79**, **80**, and **81,** as shown in Scheme-3.4.



Scheme-3.4: Biosynthesis of *Sarcococca* alkaloids from pregnenolone (**75**).

4.0 Fluorinated Natural Products

4.1 Introduction

Fluorine is the most abundant halogen in the Earth's crust, ranking 13th in all the elements. The fluorine content is 270-740 ppm, higher than that of chlorine (10-180 ppm), in igneous and sedimentary rocks, but much of this fluorine is in an insoluble form which is biologically unavailable. Thus sea water contains only 1.3 ppm of fluorine, in contrast to 19,000 ppm of chloride.[86] Interestingly some of the marine organisms and terrestrial plants contain inorganic fluoride in significant amount, such as the sponge *Halichondria moorei* is reported to accumulate as much as 10% fluorine on a dry weight basis as potassium fluorosilicate.[87] Similarly the genus *Camellia*, which includes the tea plant (*Camellia sinensis*), can selectively concentrate inorganic fluoride from relatively low concentrations in the soil. Thus commercial tea itself can contain 70-80 μg of fluoride/gram of dry weight while and levels of up to 300 μg of fluoride/gram of dry weight have been observed in older leaves of ornamental *Camellia* species, such as *C. japonica.*[86] Interestingly, despite its abundance, fluorine bound with organic compounds appears to be very rare in nature. It has been reported in relatively small number of tropical and sub tropical plants, in only two actinomycetes amongst microorganisms, and only one marine sponge amongst a massive number of marine organisms.[86, 88] Only 18 organofluorine compounds have been reported as natural products, and all of them are primary metabolites. No fluorine containing secondary metabolite has ever been reported up to date. The underlying reasons for this dearth of fluorinated natural products, as compared to the relative abundance of other halogenated metabolites, are as follows:

I. There is a relatively low bioavailability of fluorine in comparison with the other halogens, since it is bound in a largely insoluble form.

II. Fluorine's heat of hydration is very high (117 Kcal mol^{-1}), as compared to Cl, and Br (84 and 78 Kcal mol^{-1}, respectively). Therefore fluoride ion is a poor nucleophile in aqueous solution, thereby limiting its participation in displacement reactions.

III. Fluorine cannot be incorporated into organic compounds *via* the haloperoxidase reaction, since the redox potential, required for oxidation of fluoride, is much greater (-3.06 V) than that generated by the reduction of hydrogen peroxide. Therefore the mechanism by which C-F bonds are formed biologically is of considerable interest.[86, 88]

Fluorine occurs in nature as a single isotope ^{19}F, which possesses a spin quantum number of one half (I = ½) and also a magnetogyric ratio similar to that of the proton. Coupling constants and chemical shifts values of fluorine are larger in magnitude than proton. Fluorine can couple with ^{1}H and ^{13}C nuclei up to long range (^{4}J-^{5}J) with characteristic coupling constants. The NMR spectroscopy can therefore play a key role in identifying the organofluorine compounds.[86]

4.2 Classification of Fluorinated Natural Products

According to the biological sources, fluorinated natural products can be classified as follows:

A) Fluorinated Natural Products from Plants

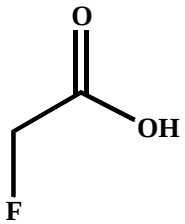
I) Fluoroacetic Acid

Most common naturally occurring organofluorine compound is the fluoroacetic acid (**82**). It was first isolated from the South African plant *Dichapetalum cymosum*, by Marasis in 1943. Fluoroacetic acid was responsible for considerable cattle loss in the Transvaal region of South Africa.[86] The young leaves of the plant can accumulate fluoroacetate up to 2,500 μg g^{-1} dry weight, which are particularly toxic in early spring. Since then it has been found that many other species of the Dichapetalaceae produce fluoroacetate. Highest levels of fluoroaceate (up to 8,000

μg g^{-1} dry weight) have been reported in the young leaves and seeds of *D. braunii* from Southeastern Tanzania.[90] High concentration of fluoroacetate is found in the leaves of *D. toxicarium*, a West African species which accumulates *ω*-fluorinated lipids in the seeds.[91] The leaves of *D. heudelotti* and the leaves and seeds of *D. stuhlmanni* are another source of fluoroacetate.[92] The presence of fluoroacetate in *D. michelsonii*, *D. guineense*, *D. venenatum*, *D. macrocarpum*, *D. ruhlandii*, *D. barterie*, *D. deflexum*, *D. mossambicense*, and *D. tomentosum* is responsible of their toxicity. Fluoroacetate has also been identified in *Spondianthus preusii* from West Africa.[86]

A number of Australian plants are recognized as a source of fluoroacetate. The most widely studied is *Acacia georginae,* found in Queensland. It has reported that leaves and seeds of this species contain fluoroacetate up to 250-400 *μ*g g^{-1} dry weight, respectively.[86, 92] Fluoroacetate is also found in the plants of the *Gastrolobium* and *Oxylobium* genera, which belong to the family Leguminosae. These plants accumulate relatively high quantities of the fluoroacetate and have been recognized as toxic, as evidenced by their local names. The most toxic species, *Oxylobium parviform* (Box poison), can accumulate as much as 2,500 *μ*g g^{-1} of fluoroacetate.[86, 93, 94]

It has been observed that fluoroacetate concentrations can be markedly different between different plant organs within a species and in different seasons and age of the plant.[90, 86]



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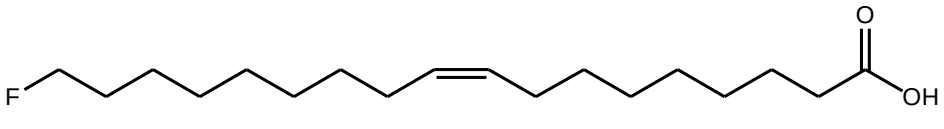
II) *ω*-Fluorofatty Acids

Nine *ω*- fluoro fatty acids have been reported from the seed oil of *Dichapetalum toxicarium*. The *ω*-fluorooleic acid ($C_{18:1}$) (**83**), which is a major fluorinated component, is about 80% of the total organo fluorine present. It is present up to 3% of the seed oil and was first isolated by Peters and co-workers in 1959. The presence of *ω*-fluoropalmitic acid ($C_{16:0}$) (**84**), and small quantities of *ω*-fluorocapric acid ($C_{10:0}$) (**85**) and *ω*-fluoromyristic acid ($C_{14:0}$) (**86**) were deduced by gas chromatographic study.[95, 86] A reexamination of seed oil in 1990, by using gas chromatography/ mass spectrometry (GC-MS), indicated the presence of *ω*-fluoro derivatives of palmitoleic ($C_{16:1}$) (**87**), stearic ($C_{18:0}$) (**88**), linoleic ($C_{18:2}$) (**89**), arachidic ($C_{20:0}$) (**90**) and eicosenoic ($C_{20:1}$) (**91**) acids and threo-18-fluoro-9,10-dihydroxystearic acid (**92**).[96]



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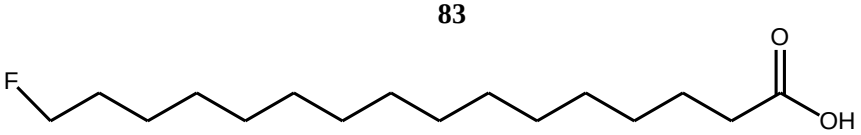

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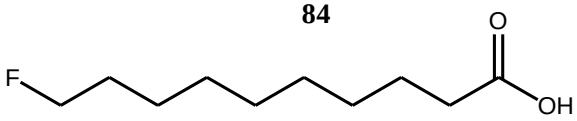

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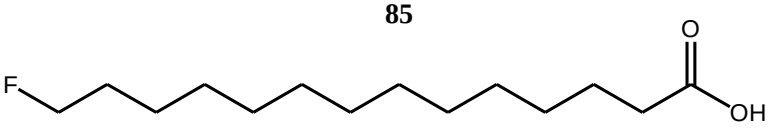

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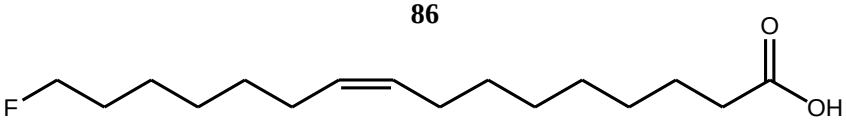

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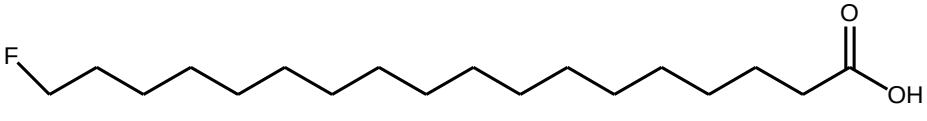

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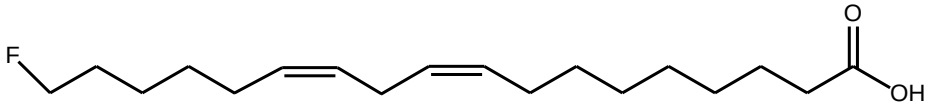

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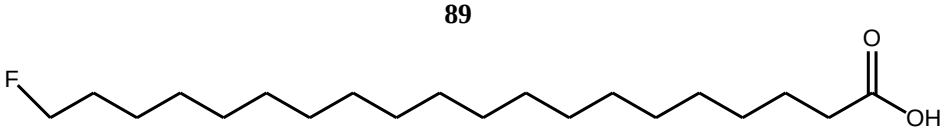

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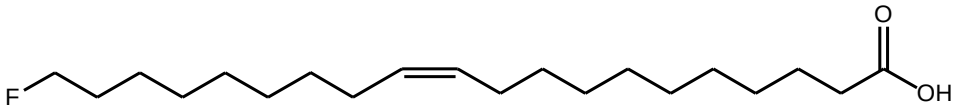

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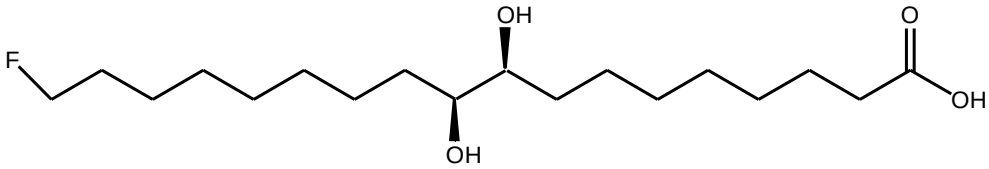


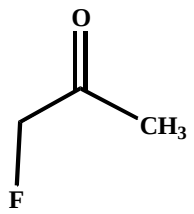
92

III) Fluoroacetone

In 1967, Peters and Shorthouse reported the presence of fluoroacetone (**93**) in the homogenates of *Acacia georginae* and other plants.[97] They transfered the volatile components of *Acacia georginae* homogenates to a solution of 2, 4-dinitrophenylhydrazine, and detected the presence of the 2, 4-dinitrophenylhydrazone derivative of fluoroacetone (**93**). The amount of **93** was correlated with the concentration of fluoride in the homogenate.[98]

IV) (2*R*, 3*R*)-2-Fluorocitrate

(2*R*, 3*R*)-2-Fluorocitrate (**94**) is accumulated, along with fluoroacetate, by the many plants such as soybean, alfalfa, and crested wheatgrass, when they are incubated with fluoride.[99, 86] Cell cultures of the tea plant (*Thea sinesis*) can accumulate 5-10 μg of fluorocitrate g^{-1} of dry weight in the tissues, and commercial tea can contain up to 30 μg g^{-1} dry weights.



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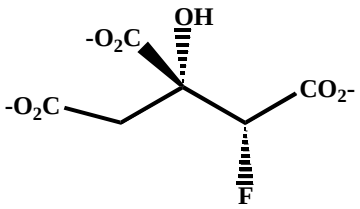


94

B) Fluorinated Natural Products from Microorganisms

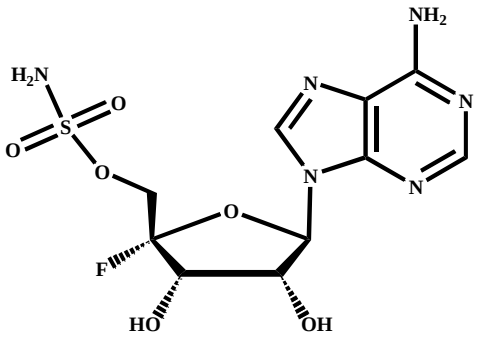
I) Nucleocidin

An adenine-containing antibiotic nucleocidin (**95**) was isolated from the fermentation broths of the microorganism *Streptomyces calvus* in 1957. Initially proposed empirical formula of $C_{11}H_{16}N_6SO_8$ was revised to $C_{10}H_{14}N_6SO_7$ {9-[4-*O*-sulfamoyl pentofuranosyl] adenine} in 1968. Before 1969, it was considered that doubling of certain peaks in the ^{1}H-NMR spectrum was due to the hindered rotation within the molecule. Finally, on the basis of spectroscopic evidences, including ^{1}H- and ^{19}F- NMR analyses, it was attributed to the presence of fluorine. The empirical formula was thus further revised to $C_{10}H_{13}N_6O_6SF$ and a new structure, 4′-fluoro-5′-*O*-sulfamoyladenosine (**95**) was proposed**.** A *β*-D-configuration of the ribose moiety was postulated. Structure of molecule was finally confirmed by the total synthesis in 1976. Later attempts to re-isolate nucleocidin from various strains of *S. calvus*, including original strain, remain unsuccessful and compound was therefore declared "extinct".[100, 101]

II) 4-Fluorothreonine and Fluoroacetic Acid

4-Fluorothreonine (**96**) was isolated during the course of studies on improving the production of the *β*-lactam antibiotic, thienamycin, by the actinomycete *Streptomyces cattleya* in 1986.[102] It was observed under certain conditions, the organism biosynthesizes a metabolite, 4-fluorothreonine (**96**), which showed antimicrobial activity against a range of bacteria. The compound is a stereochemical analoges of L-threonine, and was obtained as a stereoisomer.

It was inferred from the ^{19}F-NMR studies that conditions, conductive to 4-fluorothreonine biosynthesis, fluoro-acetate also accumulates in the culture medium up to a concentration of 2-3 mM.



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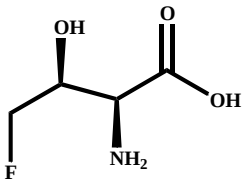
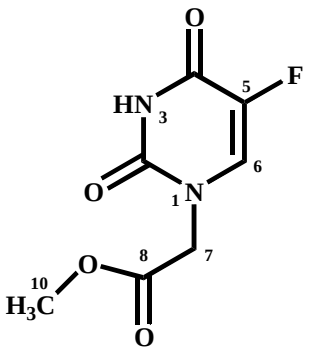


96

C) Fluorinated Natural Products from the Sponge

Five 5-fluorouracil derivatives **97-101** were obtained from the marine sponge *Phakellia fusca*, collected around the Yongxing Island in the South Sea of China. Compounds **99** and **100** were previously reported as synthetic compounds with antitumor activity.[88]



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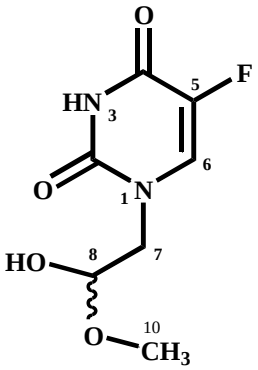

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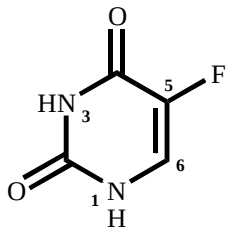

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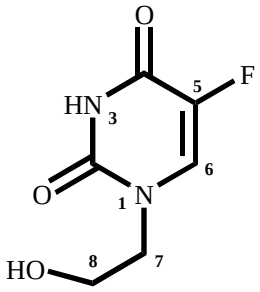

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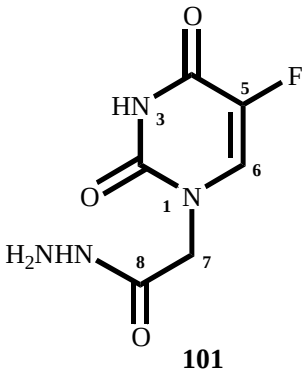
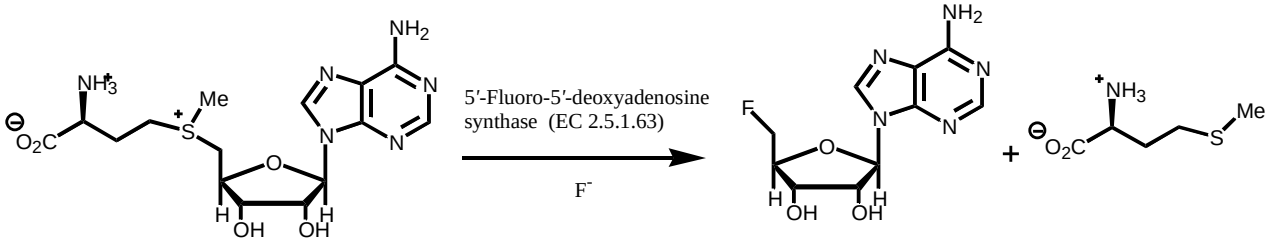


101

4.3 Biosynthesis of Organofluorine Compounds

It was observed that when fluoride was added to the fermentation media of *S. cattleya*, both fluoroacetate and 4-fluorotheronine were accumulated at millimolar level.[102] On the basis of this observation, it was assumed that *S. cattleya* has a capacity to synthesize organofluorine compounds from inorganic fluoride. It was also assumed that an enzyme exists which is capable of forming the C-F bond. Successive biosynthetic investigations have discovered that an enzyme, 5′-fluoro-5′-deoxyadenosine (5′-FDA) synthase (EC 2.5.1.63), catalyze the reaction of *S*-adenosyl-L-methionine (SAM) (**102**) with fluoride ion to yield 5′-FDA (**103**), as shown in Scheme-4.1. This was the first enzyme isolated from *S. cattleya* and trivially termed as 'fluorinase', which is responsible for the biosynthesis of organofluorine compounds in nature.[103, 104]

Identification of the 'fluorinase' was a landmark achievement in the study of the biosynthesis of organofluorine metabolites.[105]



102 **103**

Scheme-4.1: Fluorinase from *Streptomyces cattleya* catalyzes the reaction of fluoride ion and SAM to generate 5′-FDA and L-methionine.[104]

Single-crystal X-ray diffraction technique was used to study the structure of fluorinase. This study revealed that fluorinase is a dimer of a trimer. Three active sites are present in the trimer, where SAM (**102**) can bind. The X-ray based structures of the binding of fluorinase with SAM (**102**), as

well as with the product 5′-FDA (**103**), has shown that ribose ring of the adenosine moiety is unusually planar in structure. Hydrogen bonding between the 2′- and 3′-hydroxyl groups of the ribose and the carboxylate residue of aspartate-16 facilitate the planarity of ribose, as shown in Fig. 4.1.[106]

Assays of the enzyme, both in the forward and reverse directions, have revealed that the fluorinase enzyme has a reversible action and catalyzes the conversion of 5′-FDA (**103**) into SAM (**102**).[107] Interestingly when a 2′-deoxy-substrate, e.g. 2′-deoxy-5′-FDA, is presented to the enzyme, it is also converted, although at a reduced rate.[104]

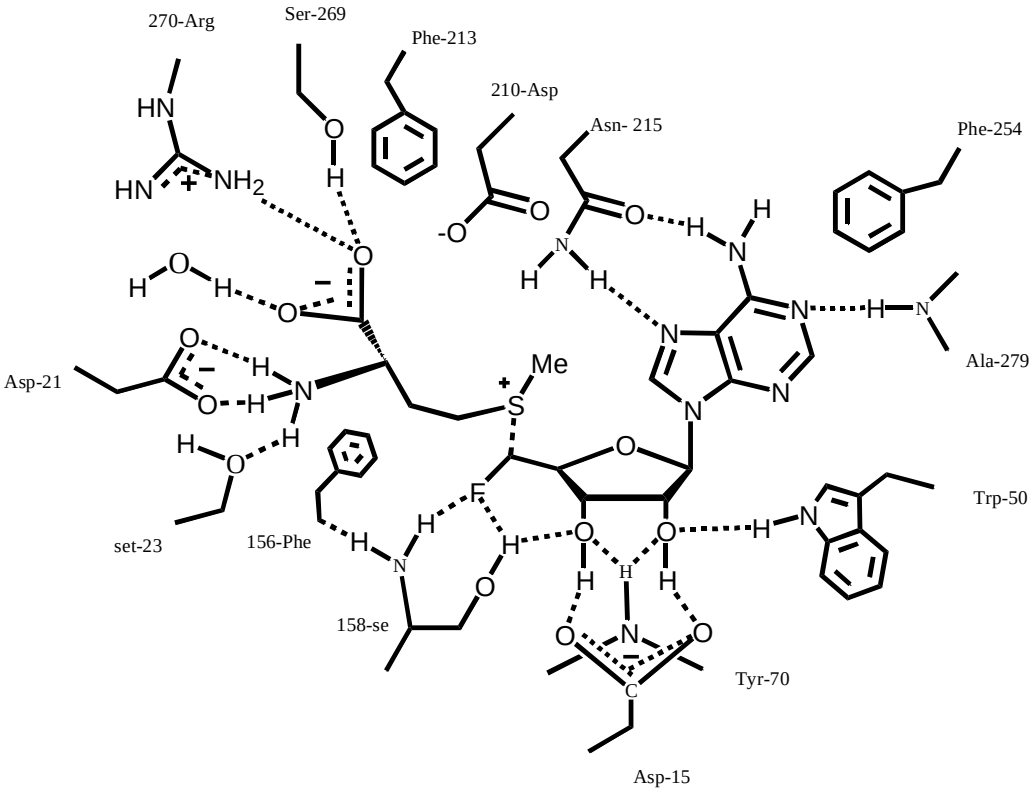
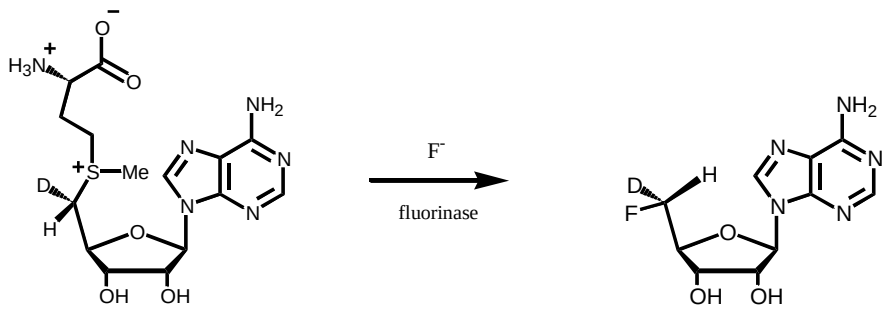


Fig. 4.1: Hydrogen bondings on the surface of the fluorinase between the enzyme 5′-FDA and L-methionine, as deduced by the X-ray crystallography.[104]

Mechanism of action of the fluorinase

Fluorination occurs with an inversion of configuration, as shown in scheme-**4.2**, as revealed with the help of stereo-specifically labeled SAM, carrying deuterium at the 5′-pro-*S* site. This rely on S_N2 substitution reaction which is further supported by a QM/MM study.[108] Quantum mechanical and molecular mechanics studies were required to determine the conformation of reactant and product in the reactive complexes and of the transition state. Both the crystallographic[106] and theoretical studies[108] have shown that all the hydrated water molecules that cover the fluoride ion, are removed. The loss of heat of hydration is remunerated by at least three hydrogen bonds to the



Scheme- 4.2: The fluorination reaction occurs with an inversion of configuration, as revealed by deuterium labeling, consistent with an S_N2 reaction mechanism.[104]

surface of the protein. It has been found that in the transition state, fluoride ion forms hydrogen bonding contacts with both the -NH and -OH hydrogen of Ser-158, and the -OH side chain of Thr-80, as illustrated in Fig. 4.2.[104] Electrostatic interaction between F^- and the positively charged sulphur (R_3S^+) of SAM also plays a key role in stabilizing the fluoride ion in the active site of the fluorinase.[109] The enzyme catalyzes the reaction with the help of hydrogen bonding interactions. Calculated activation energy for this reaction in the presence of enzyme is 53 $KJmol^{-1}$. This has significantly less activation barrier than the barrier found in the solution reaction (92 $KJmol^{-1}$). Due to this the rate of reaction is accelerated by one million fold (10^6), in the presence of enzyme.[108, 104]

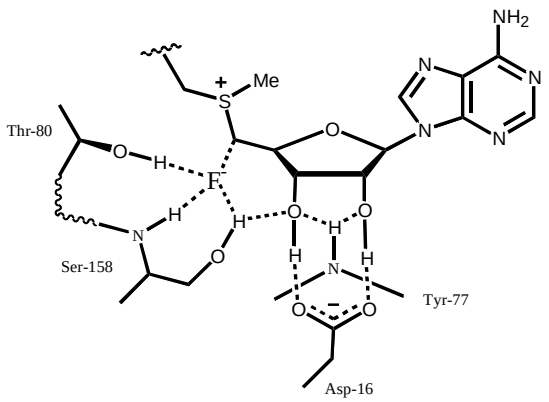
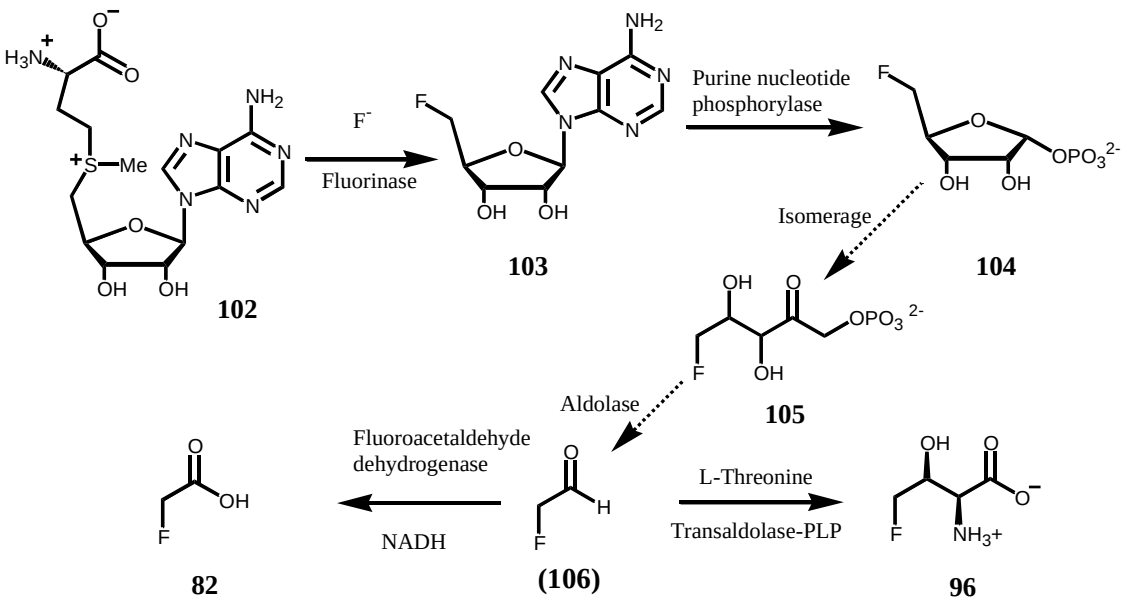


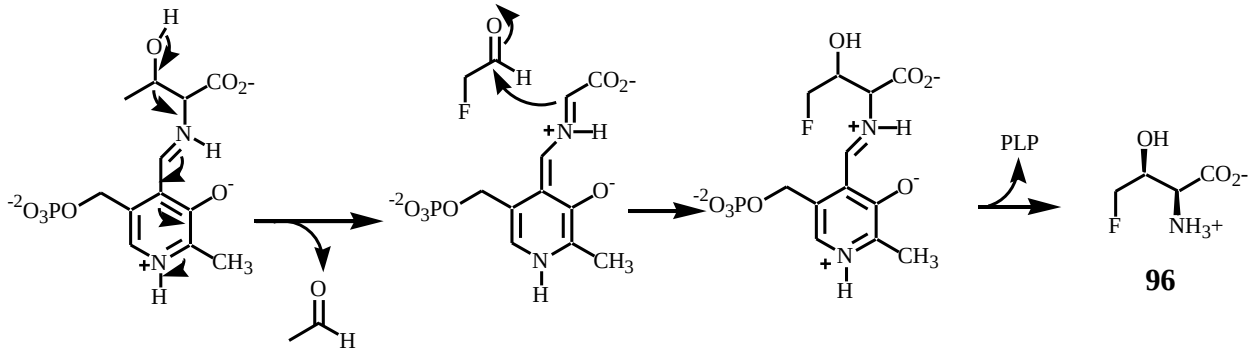
Fig. 4.2: Fluoride ion- based hydrogen bonding, prior to nucleophilic attack at the active site of the fluorinase, as deduced by QM/MM calculations.[104]

Another biosynthetic pathway was proposed for fluoroacetate and 4-fluorothreonine.[110] In this pathway, 5′-FDA (**103**) converts into fluoroacetaldehyde (**106**)[111] *via* 5-fluororibose-1-phosphate (**104**).[112] Isomerase and aldolase enzymes play a major role to convert 5-fluororibose-1-phosphate (**104**) to fluoroacetaldehyde. Fluoroacetaldehyde (**106**) is oxidized to fluoroacetate (**82**) by the action of an NADH dependent aldehyde dehydrogenase (Scheme-4.3).[113]



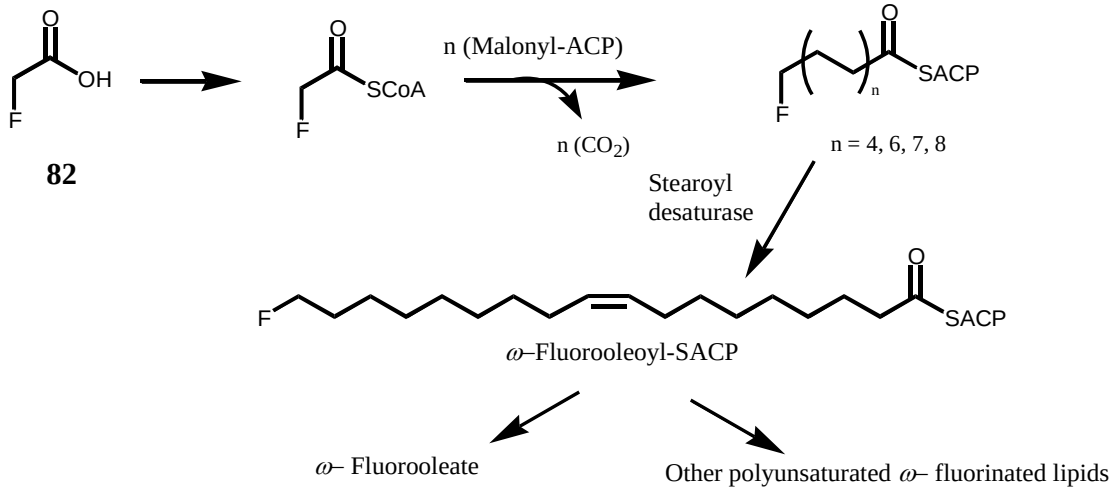
Scheme-4.3: The biosynthetic pathway to the fluorometabolites **82** and **96** in *S. cattleya*. The dotted arrows indicate enzymes, which remains to be purified.[86]

In another reaction, combination of fluoroacetaldehyde with the amino acid, L-threonine, in a pyridoxal phosphate (PLP) takes place. This is a transaldol type reaction which generates an antibiotic, 4-fluorothreonine (**96**) (Scheme-4.4).[114, 86]

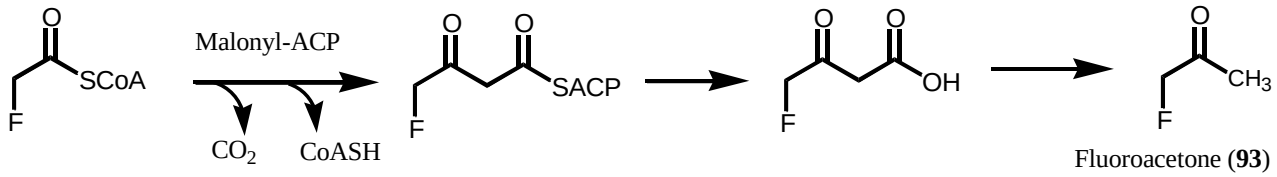


Scheme-4.4: Proposed mechanism of 4-fluorothreoine production catalyzed by threonine transaldolase.[86]

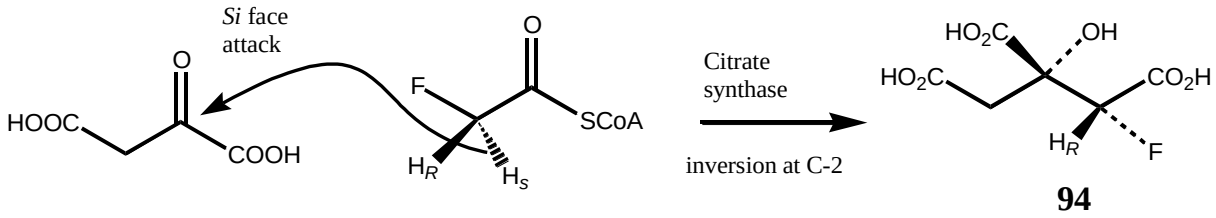
Biosynthesis of ω-fluorofatty acid probably takes place through a normal fatty acid anabolic pathway. At first, acetyl acyl carrier protein (acetyl-ACP) is replaced with fluoroacetyl-ACP then condensation with malonyl-ACP takes place. The absence of fluorine at any other position of fatty acid chain indicates that either fluoroacetyl CoA is not a substrate for acetyl CoA carboxylase, or fluoromalonyl-ACP is not involved in the chain elongation process (Scheme-4.5).[86]



Scheme-4.5: Biosynthesis of ω-fluoro fatty acids from fluoroacetate.[86]



Scheme-4.6: Biosynthesis of fluoroacetone.[86]



Scheme-4.7: Biosynthesis of (2*R*, 3*R*)-2-fluorocitrate.[86]

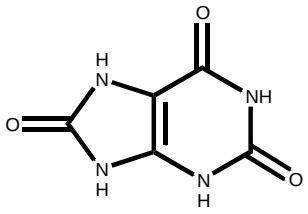
Recent attempts to re-isolate nucleocidin (**95**) from *S. calvus* have proved unsuccessful. From this, it was clear that nucleocidin is not obviously derived from fluoroacetate and its biosynthesis might therefore involve a C-F bond-forming enzyme, unique to *S. calvus.* It is very likely that other fluorinated natural products and the enzymes that are responsible for biosynthesis do exist and are yet to be identified.

Recently Prof. Dr. M. Iqbal Choudhary's research group has also isolated a series of fluoropyrimidine containing secondary metabolites from the plant *Osyris wightiana* (Wallich ex Wight). Plant *Osyris wightiana* was also collected from the close vicinity of the area from where *Sarcococca coriacea* (Hook. f.) was collected. Same fluoropyrimidine ring substituted secondary metabolites from two unrelated medicinal plants from close vicinity and collected during same time has raised an interesting question about the bio-synthesis of fluorine containing secondary metabolites.

5.0 Pyrimidine Ring Containing Secondary Metabolites

5.1 Introduction

As early as 1776, a purine derivative uric acid (**107**) was isolated from urinary calculi (stones) by Scheele.[115] Pyrimidines (‘*m*-diazine’) are known as breakdown products of uric acid since the early history of organic chemistry. The first pyrimidine derivative {alloxan (**108**)} was obtained in 1818 by Brugnateli by oxidizing the uric acid with HNO_3. Systematic study of the ring system began with the work of Pinner, who first used the name pyrimidine (Combination of the words pyridine and amidine) to the unsubstituted parent body.[115]



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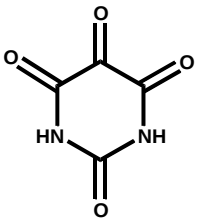

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5.2 Classification

A number of pyrimidine ring containing secondary metabolites have been reported from different sources and found to have excellent medicinal value. Classification of pyrimidine ring containing secondary metabolites on the basis of biological origin is presented below:

A) Pyrimidine containing secondary metabolites from plants

B) Pyrimidine containing secondary metabolites from marine organisms

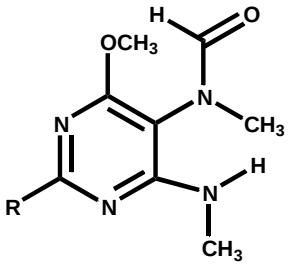
C) Pyrimidine containing secondary metabolites from microorganisms

A) Pyrimidine containing secondary metabolites from plants

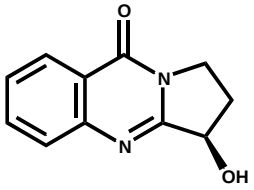
Plants have been the richest source of pyrimidine-containing secondary metabolites. A number of pyrimidine- and purine- ring containing secondary metabolites has been reported from different plants. Some of the representative examples are as follows:

I) Pyrimidine and Quinazoline Alkaloids

Three pyrimidines, heteromines F, G and H (**109a -109c**, resp.), were obtained from the aerial parts of *Heterostemma brownii*. In Taipei folk medicine, this plant is used for the treatment of cancer.[116, 117] The first known quinazoline alkaloid from plant was vasicine (**110**), isolated in 1888 from *Adhatoda vasica*.[118] Seven quinolizine alkaloids were reported from the plants *Glycosmis pentaphylla* used in the Ayurvedic medicine as a febrifuge and antihelmetic. Compound **111** is a representative example of these types of compounds.[119]



(109 a) R = Me_2N
(109 b) R = MeNH
(109 c) R = NH_2



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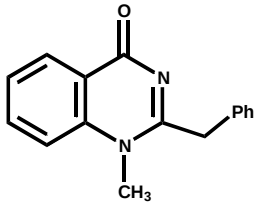
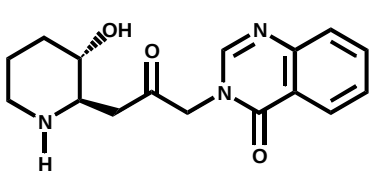


111

Two interconvertible alkaloids, febrifugine (**112a**) and isofebrifugine (**112b**), were reported from *Dichroa febrifuga*,[120] which is an ingredient in traditional Chinese herbal medicine, effective against malaria. Febrifugine (**112a**) is the first alkaloid, other than those of *Cinchona* group, with antimalarial in nature.



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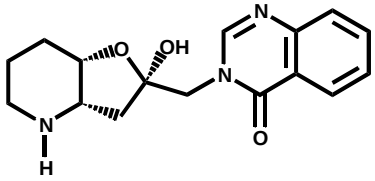
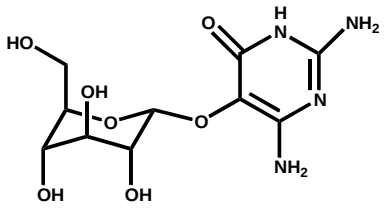


112 b

II) Glycosylated pyrimidines

Glycosylated pyrimidine compound, vicine (**113**), causative agent of favism, a metabolic disorder due to deficiency of glucose-6-phosphate dehydrogenase (G6PD) and glutathione (GSH), has been

reported from the seeds of *Vicia sativa.* Charine (**114**) was reported from *Momordica charantia* L., a climbing vine, which is used in folk medicines for the treatment various ailments.[115]



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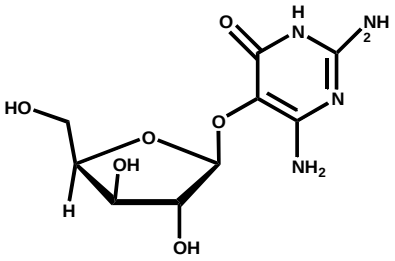

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B) Pyrimidine Containing Secondary Metabolites from Marine Organisms

Marine organisms are rich sources of pyrimidine containing secondary metabolites. A number of compounds have been reported, especially from marine sponges.

I) Pyrimidine Containing Secondary Metabolites from Sponges

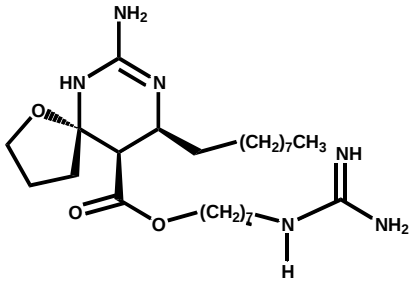
A number of pyrimidine containing metabolites have been reported from sponges. According to structures, these metabolites are divided into following classes:

a) Pyrimidine and Quinazoline Alkaloids

Three cytotoxic compounds, crambescin A-C, with 2-aminopyrimidine moieties, were isolated from a Mediterranean sponge, *Crambe crambe*. Crambescin B (**115**) is a representative example of these types of compounds.[121, 115]

Bromopyrrole- containing derivatives, manzacidin A-C (**116a-116c**, resp), have been reported from an Okinawan sponge *Hymeniacidon* sp. These alkaloids contain ester- linked pyrrole and pyrimidine moieties.[122]

Three variolin- type secondary metabolites have been reported from the sponge *Kirkpatrickia varialosa.* Variolin B (**117**) is a representative example of these types of compounds.[123]



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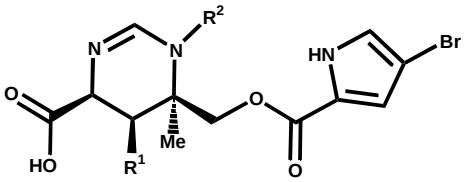
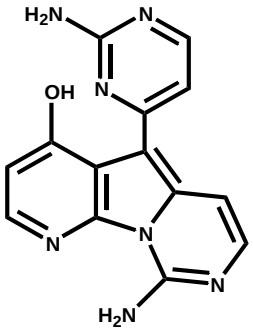


116 a $R^1 = R^2 = H$
116 b $R^1 = OH$, $R^2 = H$
116 c $R^1 = R^2 = H$ **(9 -epi)**



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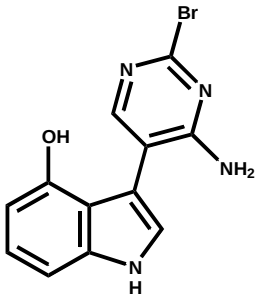
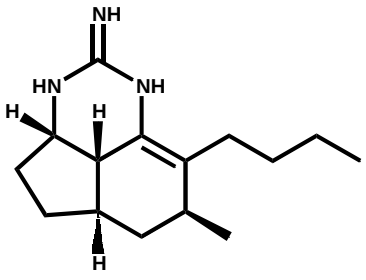


118

Psammopemmins group of alkaloids, with a novel 4′-amino-2′-bromopyrimidine-5-yl moiety, were isolated from an Atlantic sponge, *Psammopemma* species. Psammopemmin A (**118**) is an example of this class.[124]

Toxic cyclic 2-iminopyrimidine derivatives, ptilocaulin (**119**) and isoptilocaulin (**120**), were obtained from the Caribbean sponge *Ptilocaulis spiculifer*.[125]



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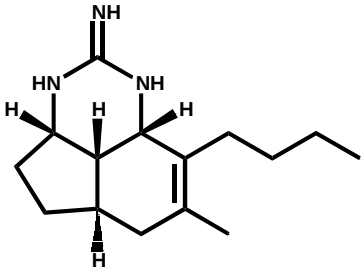


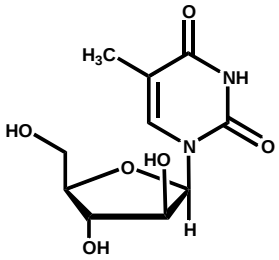
120

b) Glycosylated Pyrimidines

Spongothymine (**121**) and spongouridine (**122**), were obtained from a Caribbean sponge, *Cryptotethia crypta*.[126]

II) Pyrimidine Containing Secondary Metabolites from Puffer Fish

Tetrodotoxin (**123**) [127] is one of the most powerful non-protein neurotoxins isolated from liver and ovaries of the Japanese puffer fishes *Spoerides rubripes* and *S. phyreus*. Structure of this compound is based on a 2-iminooctahydro-1*H*-quinazoline skeleton.



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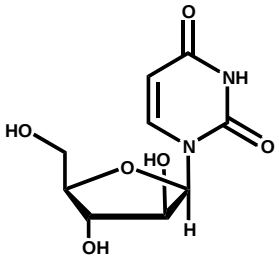

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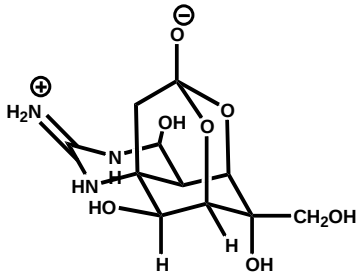


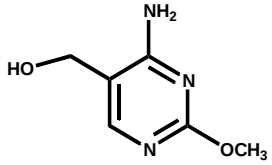
123

C) Pyrimidine Containing Secondary Metabolites from Microorganisms

Microorganisms, such as bacteria and algae, are the richest source of pyrimidine containing secondary metabolites with high medicinal value.

I) Secondary Metabolites from Bacteria

The simplest pyrimidine antibiotic bacimethrin (4-amino-5-(hydroxymethyl)-2-methoxy pyrimidine) (**124**),[128] was reported from *Bacillus megaterium*. Sparsomycin (**125**),[129] is a metabolite of *Streptomyces sparsogens* or *S. cuspidosporus*. A toxic metabolite epicylindrospermopsin (**126**)[130] has been reported from the cyanobacterium, *Aphanizomenon ovalisporum*.



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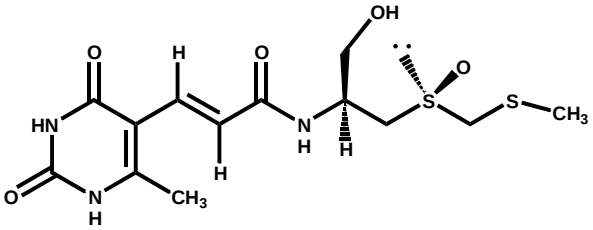
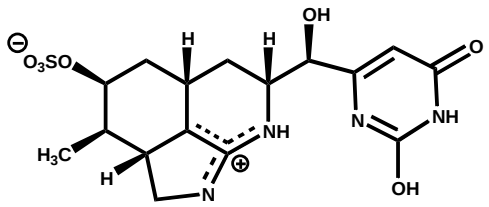


125

Polyoxins and neopolyoxins were reported from culture broth of *Streptomyces cacoi* var. *asoenis* and *S. tendae*, respectively. Polyoxin A (**127**) and nikkomycin A (**128**) are the representative examples of these types of metabolites.[130]



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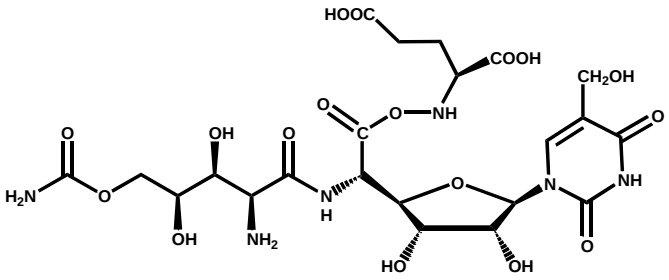

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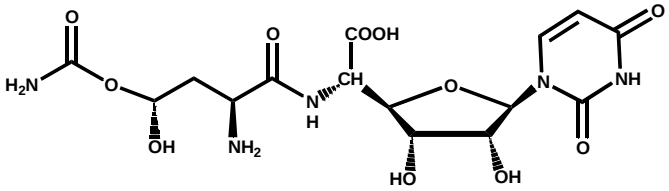
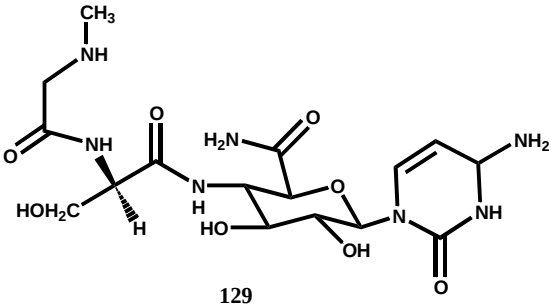


128

Gougerotin (**129**) was reported from *Streptomyces gougerotii*.[115] Blasticidin S (**130**) an antibiotic, was reported from *Streptomyces griseochromogenes.*[115]



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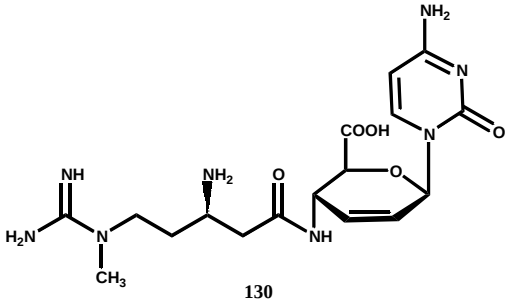
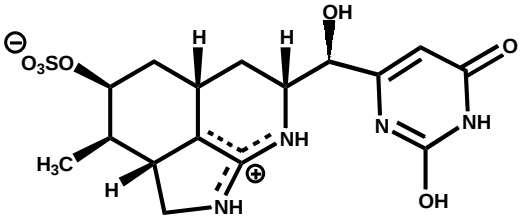


130

II) Pyrimidine Containing Secondary Metabolites from Algae

The hepatotoxin cylindrospermopsin (**131**) was reported from the blue green alga *Cylindrospermopsis raciborskii*. This compound is produced by algal blooms and contaminates drinking water and is responsible of a severe outbreak of hepatoenteritis in tropical Australia.[132] Moreover pyrimidine is the constituent of many vital molecules, including DNA, RNA, amino acids and vitamins.



131

5.3 Pharmacology of Pyrimidine Containing Secondary Metabolites

Over the years, the pyrimidine system turned out to be an important pharmacophore, with a variety of medicinal uses. Bacimethrin (**124**) and related compounds are good antibiotics. Bacimethrin is active against several yeasts and bacteria *in vitro*, as well staphylococcal infections *in vivo*.[128] Sparsomycin (**125**) is one of the few antibiotics which can inhibit the protein synthesis in bacteria. Moreover, sparsomycin and some of its derivatives selectively act on several human tumors.[129]

Heteromines F, G, and H (**109a-109c**) are known to be cytotoxic against several cancer cell lines.[115, 116] Crambescin B (**115**) has a IC_{50} value of 1 μg/mL against L1210 murine leukemia cells.[121] Variolin B (**117**) and related compounds have shown activity against P388 murine leukemia cells.[123] Benzopyrimidines febrifugine (**112a**) and isofebrifugine (**112b**) have shown a good antimalarial property.[120]

Polyoxins (**127**) and nikkomycins (**128**) are the most effective chitin synthase, with marked antifungal activities.[131] They represents potentially useful model for the development of effective

agents for the treatments of opportunistic fungal infections. Gougerotin (**129**) inhibits the protein synthesis by preventing the transfer of amino acids from amino acyl tRNA to polypeptide, and acts as an effective antibiotic.[115] Blasticidin S (**130**) has been extensively used as an excellent fungicide against *Pyricularia oryzae.*[115] Spongothymine (**121**) and spongouridine (**122**) possess a good antiviral activity.[126]

5.4 Biosynthesis of Pyrimidine Ribonucleotides

Isotopic labeling experiments have shown that N-1, C-4, C-5 and C-6 of the pyrimidine ring are derived from aspartic acid. C-2 arose from HCO_3^- and N-3 is contributed by the glutamine (**Fig. 5.1**). In animals, the amino group is donated by glutamine, whereas in bacteria it is supplied directly by ammonia.

The first reaction during pyrimidine biosynthesis is the synthesis of carbamyl phosphate from HCO_3^- and amide nitrogen of glutamine which is catalyzed by the cytosolic enzyme carbamoyl phosphate synthase Π (**Scheme-5.1**). This reaction consumes two molecules of ATP, one provides phosphate and other energizes the reaction.

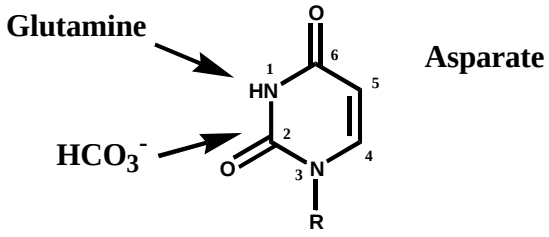
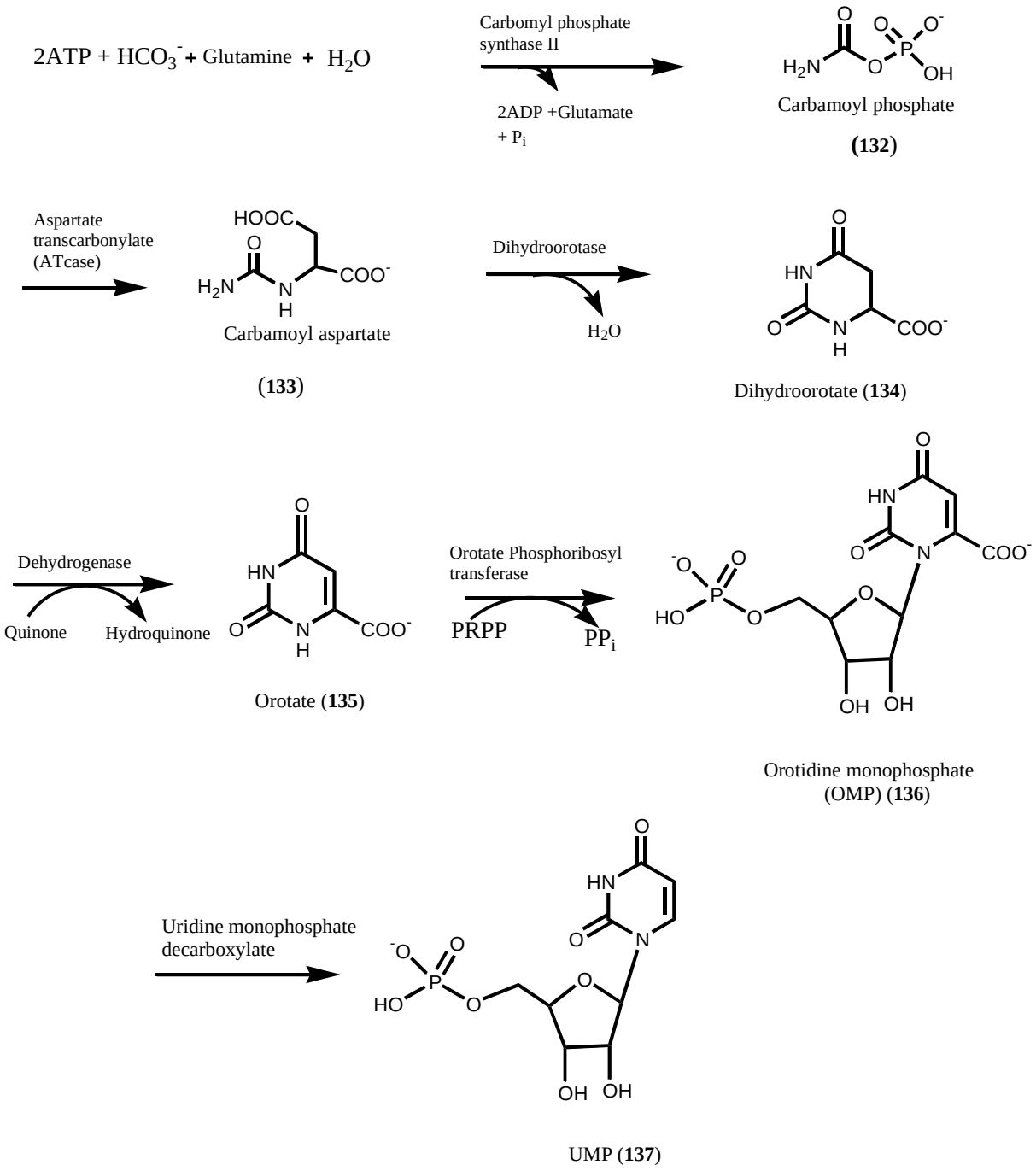


Fig. 5.1: Biosynthetic origin of pyrimidine ring atoms.

The second step in the biosynthesis is the condensation of carbamoyl phosphate (**132**) with aspartate to form carbamoyl aspartate (**133**), which is catalyzed by an enzyme aspartate transcarbamoylase.



Scheme-5.1: Biosynthesis of UMP (**137**).

The third reaction of the pathway is ring closure to form dihydroorotate (**134**), which is further oxidized to orotate (**135**) in the presence of an enzyme, dihydroorotate dehydrogenase. Orotate reacts further with PRPP (5′-phosphoribosyl-1′-pyrophosphate) in the presence of enzyme orotate

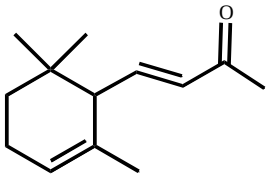
phosphoribosyl transferase to give orotidine-5′-monophosphate (OMP) (**136**). The energy for this reaction is obtained from the hydrolysis of eliminated PP. The OMP formed has *β*-configuration at the anomeric position. The final reaction in the biosynthesis of pyrimidine is the decarboxylation of OMP by OMP decarboxylate to form UMP (**137**).

The synthesis of UTP from UMP occurs in the presence of enzymes nucleoside monophosphate kinase (MK) and nucleoside diphosphate kinase (DK).[133]

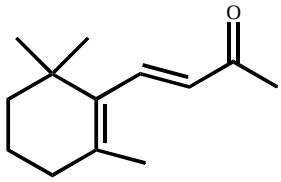
6.0 Ionones

6.1 Introduction

The thirteen carbon containing ketones, *α*- and *β*-ionones, are cyclic terpenoids that occur in many essential oils. Ionones are aromatic constituents and contributor of the aroma of roses, despite their relatively low concentration. They are important fragrances used in perfumery. *β*-ionone is also a raw material for the production of retinol. Biochemically, the ionones are derived from the degradation of carotenoids. Ionones are also called as apocarotenoids.



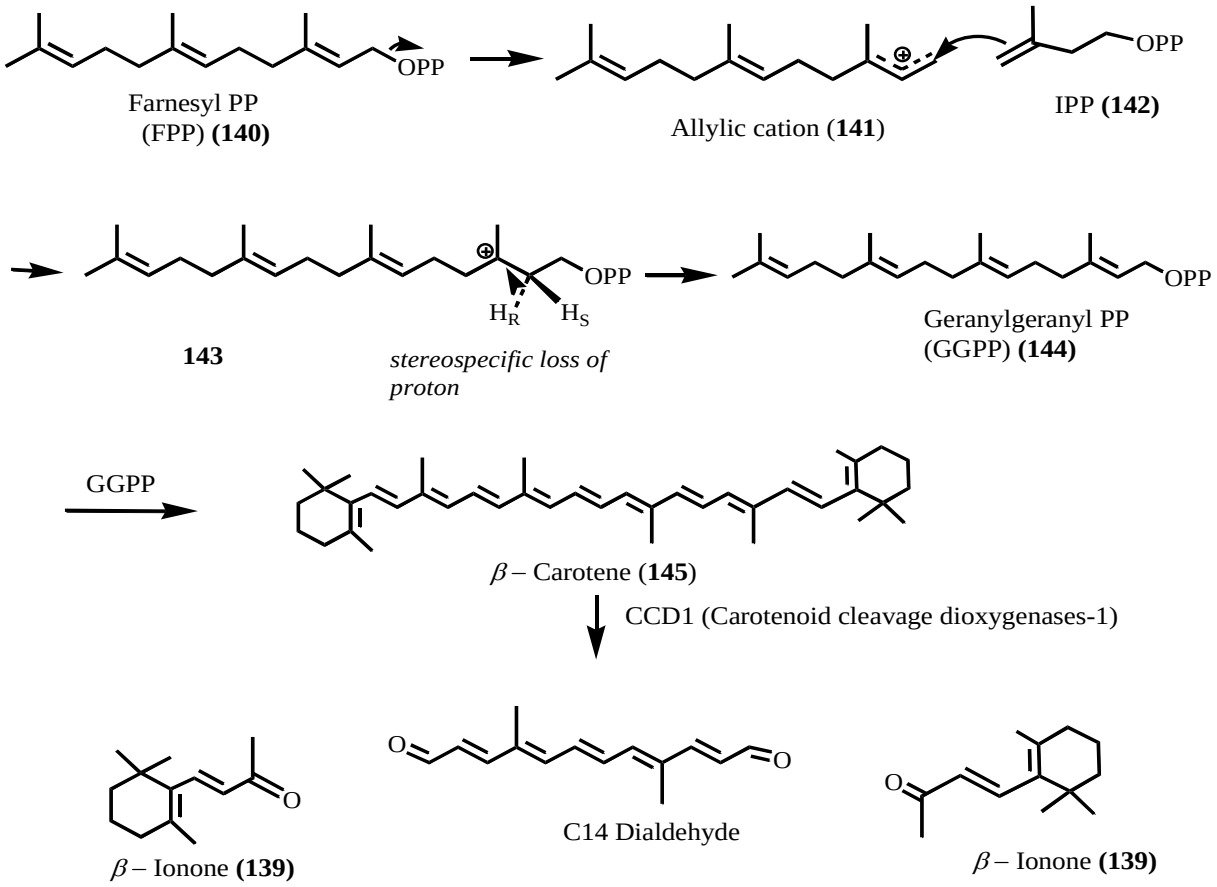
α – Ionone (**138**)



β – Ionone (**139**)

6.2 Biosynthesis of Ionones

Biochemically ionones are derived from oxidative cleavage of carotenoids, as shown in Scheme-6.1. The enzymes named carotenoid cleavage dioxygenases (CCDs) is responsible for the cleavage of carotenoids at specific double bonds.[134]



Scheme-6.1: Biosynthesis of Ionone.

7.0 OBJECTIVE OF THE CURRENT STUDY

The nature is the main source of novel chemical compounds. Plants have a complex chemical defense system, which is based on the production of a large number of chemically diverse compounds. Natural products are capable of providing the complex molecules that would not be accessible by other way. Around 5-15% of the total higher plants have been investigated and a large number of plants and marine species remain uninvestigated. In addition, only less than 1% of bacterial and 5% of fungal species are currently known.[135] Microorganisms have a great potential to yield novel bio-active compounds, because they survive in extreme and competitive environments.

One of the interesting fields of natural product chemistry which has not well explored till now is the isolation of fluorinated secondary metabolites. Only 18 primary metabolites of organofluorine compounds have been reported in literature. Fluorine containing compounds have attracted a major scientific interest as a large number of synthetic organofluorine compounds are used as drugs. About 20-25% of drugs in pharmaceutical pipeline contain at least one fluorine atom.[136] When fluorine is bound to the carbon, it forms the strongest bonds in organic compounds, while high electronegativity of fluorine makes the C-F bond more polarizable. This electronic effect assists the reaction at adjacent carbon centers. The presence of fluorine in molecule has less steric requirement but more electronic effect. This influences directly in substrate- enzyme binding. Fluorine can change the acidity of neighboring functional groups which affect in enzyme-substrate binding. Synthesis of organofluorine compounds is not so easy and as a result, natural product can be the major source for novel fluorinated secondary metabolites.

Isolation and structural elucidation of novel secondary metabolites, such as fluorinated secondary metabolites from plant and the study of the biological activities of these metabolites is the major objective of the current study.

8.0 Results and Discussion

The roots of *Sarcococca coriacea* (Hook, F.) were collected from Champadevi, Kathmandu, Nepal, during July 2005. Air-dried roots (16.0 kg) of *S. coriacea* were extracted with 80% methanol in water (60 L) (Experimental -9.3, Scheme-9.1, Page-131). The concentrated aqueous methanolic extract (1.5 Kg) was dissolved in cold distilled water and defatted with petroleum ether (30 L), (90.0 g, **Sc-A**). The aqueous layer then extracted with CH_2Cl_2 (30 L) to obtain "neutral fraction" (45.0 g, **Sc-B**). The aqueous layer was then made alkaline by adding ammonia solution (pH 9-10) and extracted with CH_2Cl_2 (30 L) to obtain "alkaline fraction" (30.0 g, **Sc-C**). The aqueous "alkaline fraction" was then acidified by acetic acid to pH 3-4 and extracted with CH_2Cl_2 (30 L) to obtain so called "acidic fraction" (6.0 g, **Sc-D**).

Based on the TLC results, the "alkaline" fraction **Sc-D** was initially subjected to the silica gel column chromatography and eluted with gradients of petroleum ether and acetone mixtures (Experimental-9.3, Scheme-9.2, Page-132), which afforded a number of fractions **A-K**. Elution with 20% acetone in petroleum ether afforded a fraction **E** (1.5 g), which was further chromatographed on neutral alumina column to afford a number of sub- fractions $\mathbf{E_1}$-$\mathbf{E_5}$. Compounds **1-2,** and **13-15** were obtained from the fraction **E**. Similarly, fraction **D** (2.5 g), through the same isolation procedure (Experimental-9.3, Scheme-9.3, Page- 133), afforded six sub-fractions $\mathbf{D_1}$-$\mathbf{D_6}$, Compounds **4**, **6**, and **8** were obtained from the fraction **D**, while fraction **A** also afforded four sub-fractions $\mathbf{A_1}$-$\mathbf{A_4}$ (Experimental-9.3, Scheme-9.2, Page-132) and yielded the compounds **3** and **7.** Fraction I (800 mg) was subjected to the neutral alumina column chromatography to obtain sub-fractions $\mathbf{I_1}$-$\mathbf{I_4}$ (Experimental-9.3, Scheme-9.4, Page-133) and yielded the compounds **5**, **16** and **17**.

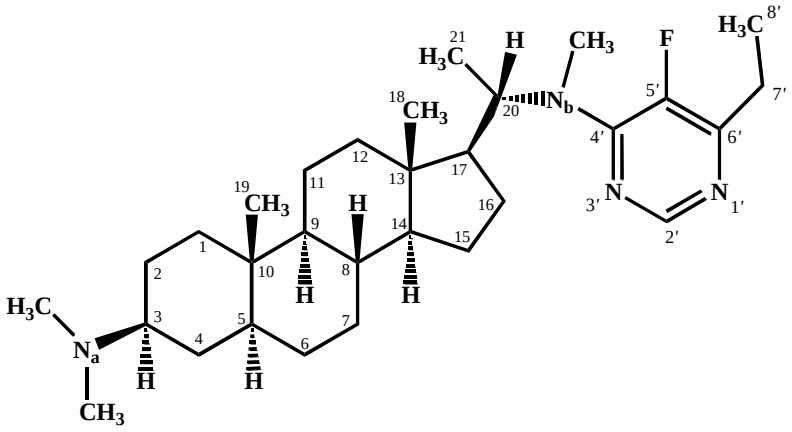
Different types of column chromatographic techniques were used for the purification of the compounds. A variety of spectroscopic and X-ray diffraction techniques were employed for structure elucidation of these compounds.

8.1 Novel Fluorinated Alkaloids

Seven fluoropyrimidine substituted novel steroidal alkaloids **1-7**, and one fluoropyrimidine substituted ionone **8** were isolated from the alkaline CH_2Cl_2 fraction (pH 9-10), obtained from the 80 % MeOH/H_2O extract of air-dried roots of *Sarcococca coriacea.*

8.1.1 Adhikarimine A (1)

Adhikarimine A (**1**), which showed a positive alkaloidal test with Dragendorff's reagent, was isolated as white crystals from the alkaline fraction (pH 9-10) of the root extract of *S. coriacea* (Experimental Section, Scheme-9.2, Page-132). It was isolated from the neutral alumina column chromatography of sub-fraction E_1, by using pet. ether/EtOAc (9:1) as eluent, in semi pure form, which was further passed over Sephadex LH-20 column (elution with MeOH), yielding needle-type crystals.



Adhikarimine A (**1**)

The optical rotation of compound **1** $[\alpha]_D^{26}$ = + 26.3º (*c* = 0.022, MeOH) indicated the presence of chiral centers in the molecule. The UV spectrum of compound **1** displayed absorptions at λ_{max} 255 and 287 and λ_{min} 366, 276, 222 nm, characteristic of a hetero-aromatic ring.[137] The IR spectrum showed absorptions at 2933 (CH) 1591 and 1495 (C = C aromatic) and 1381 (C-F) cm^{-1}.

Molecular formula and mass spectrometry:

The EI MS exhibited an M^+ at *m/z* 484, which was further supported by the FAB^{+ve} MS (*m/z* 485). The HREI MS of compound **1** showed the M^+ at *m/z* 484.7481, corresponding to the formula $C_{30}H_{49}N_4F$ (calcd 484.7487), with eight degrees of unsaturation. Alkaloids with four or more degrees of unsaturation, reported from this genus, are usually of pregnane-type.[44] The presence of two diagnostic fragments at *m/z* 84 (100%) and 110 (97.7%), suggested a 3-(dimethylamino) pregnane skeleton.[138] The EI MS also supported the presence of a 6-ethyl-5-fluoropyrimidine substituent by an ion at *m/z* 182 $[C_9H_{13}N_3F]^+$, resulted from the cleavage of C-17/C-20 bond.

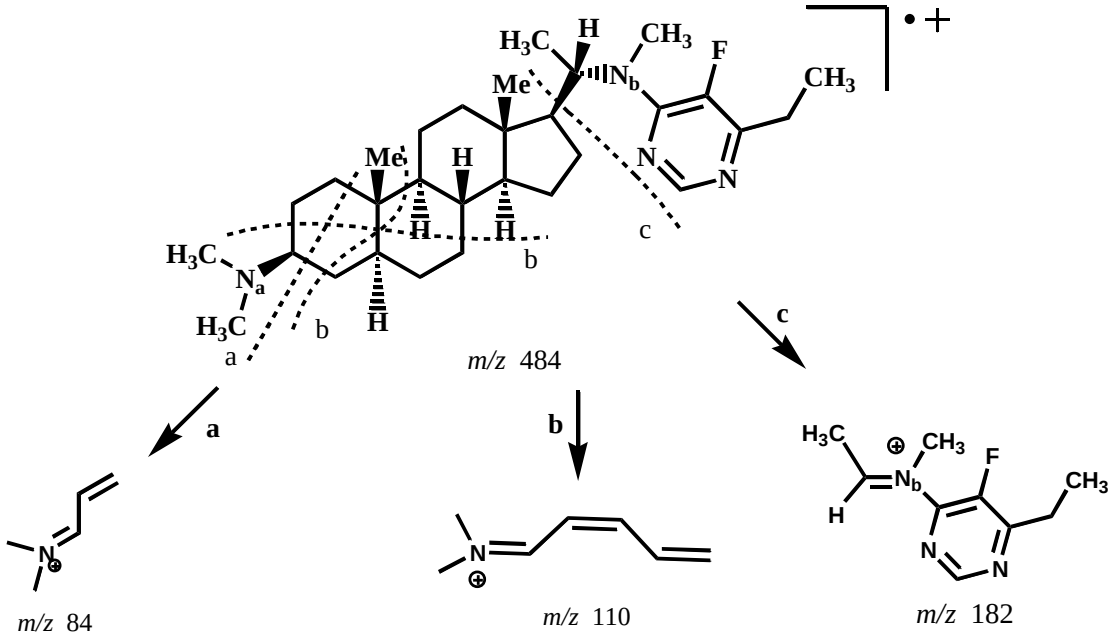


Fig. 8.1.1: Key mass fragmentation pattern of compound **1.**

One-dimensional NMR spectroscopy:

The ^{1}H-NMR (Table-8.1.1) of **1** exhibited two up-field 3H sharp singlets at δ 0.73 and 0.75, which were ascribed to C-18 and C-19 angular methyls, respectively. A 3H doublet, resonating at δ 1.17 ($J_{20,\ 21}$ = 6.4 Hz) was assigned to C-21 secondary methyl protons. While a 1H multiplet at δ 1.60 was assigned to C-20 methine proton. Relatively up-field shift of C-20 methine proton signal was due to the shielding effect of the pyrimidine ring, substituted at the amino group. A 1H broad multiplet at δ 2.11 ($W_{1/2}$ = 13.4 Hz) was attributed to C-3*ax* proton, suggesting an *equatorial* (*β*) orientation of the geminal amino group.[139] A downfield 6H singlet at δ 2.24 was due to the $(CH_3)_2N_a$ protons, A 3H doublet at δ 2.95 was ascribed to CH_3-N_b protons. Appearance of CH_3-N_b protons as a doublet, instead of simple singlet, was due to the long-range coupling with fluorine atom present at C-5′ position of pyrimidine ring.[140]

A 3H triplet at δ 1.23 ($J_{8',\ 7'}$ = 7.5 Hz) was assigned to C-8′ methyl protons, substituted on the pyrimidine ring, while a 2H quartet of doublet at δ 2.67 ($J_{H\text{-}H}$ = 6.5 Hz, $J_{H\text{-}F}$ =2.4 Hz) was due to H-7′. Splitting of H-7′ as quartet of a doublet, instead of simple quartet, was again due to the long-range coupling with fluorine atom present at C-5′ position.[140] A downfield doublet at δ 8.22 ($J_{H\text{-}F}$ = 2.4 Hz) was attributed to H-2′ of the pyrimidine ring. Splitting of H-2′ as a doublet, instead of singlet, was again due to the same reason.

The ^{13}C-NMR spectrum (broad-band decoupled) of **1** displayed 30 signals (Table.8.1.1). DEPT spectrum[141] indicated seven methyl, ten methylene, eight methine and five quaternary carbons. The downfield signals at δ 152.0 ($^{4}J_{C\text{-}F}$ = 4.6, C-2′), 152.5 ($^{2}J_{C\text{-}F}$ = 9.2, C-4′), 145.1 ($^{1}J_{C\text{-}F}$ = 254 Hz, C-5′) and 155.5 ($^{2}J_{C\text{-}F}$ = 16 Hz, C-6′), methylene carbon signal at δ 23.9 ($J_{C\text{-}F}$ = 5.3 Hz, C-7′) and methyl signal at δ 12.7, indicated the presence of a 6-ethyl-5-fluoropyrimidine ring. All the

Table- 8.1.1: ^{1}H- and ^{13}C- NMR chemical shift values of compound **1** ($CDCl_3$, ppm, 300 and 100 MHz, respectively).

C. No	^{1}H-NMR δ (*J* and $W_{½}$ in Hz)	^{13}C-NMR δ ($J_{C\text{-}F}$ in Hz)	Multiplicity
1	0.90, 1.71 (m)	37.8	CH_2
2	1.30, 1.70 (m)	24.6	CH_2
3	2.11 m ($W_{1/2}$ = 13.4)	64.0	CH
4	1.41, 1.20 (m)	31.0	CH_2
5	1.15 (m)	45.7	CH
6	1.16, 1.53 (m)	23.8	CH_2
7	1.01, 1.51 (m)	32.1	CH_2
8	1.05 (m)	35.4	CH
9	0.70 (m)	54.4	CH
10	-	35.7	C
11	1.25, 1.52 (m)	21.1	CH_2
12	1.21, 1.91 (m)	28.9	CH_2
13	-	41.9	C
14	1.59 (m)	54.3	CH
15	1.21, 1.51 (m)	26.5	CH_2
16	1.15, 1.81 (m)	39.6	CH_2
17	0.59 (m)	56.9	CH
18	0.73 (s)	12.2	CH_3
19	0.75 (s)	12.3	CH_3
20	1.60 m	54.3/54.4	CH
21	1.17 d (6.4)	18.2	CH_3
N_b-Me	2.95 d ($J_{H\text{-}F}$ = 4)	30	CH_3
N_a-Me_2	2.24 s	41.7,41.9	CH_3
2′	8.22 d ($J_{H\text{-}F}$ = 2.4)	151.9/152.0 (4.6)	CH
4′	-	152.4/152.5 (9.2)	C
5′	-	142.6/145.1 (254)	C
6′	-	155.5/155.6 (16)	C
7′	2.67 q d (6.5, 2.4)	23.8/23.9 (5.3)	CH_2
8′	1.23 t (7.5)	12.7	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques.

carbons of pyrimidine ring (C-2′, C-4′, C-5′ and C-6′), as well as C-20 and C-7′, were resonated as doublets, instead of singlets due to the coupling with the fluorine substituent.[140, 88] However $^{2}J_{C\text{-}F}$ values of C-4′ and C-6′ showed some variations from the reported values in literature, due to the substituent effects of the amino and ethyl groups.[140]

These observations suggested the following structural features in compound **1**:

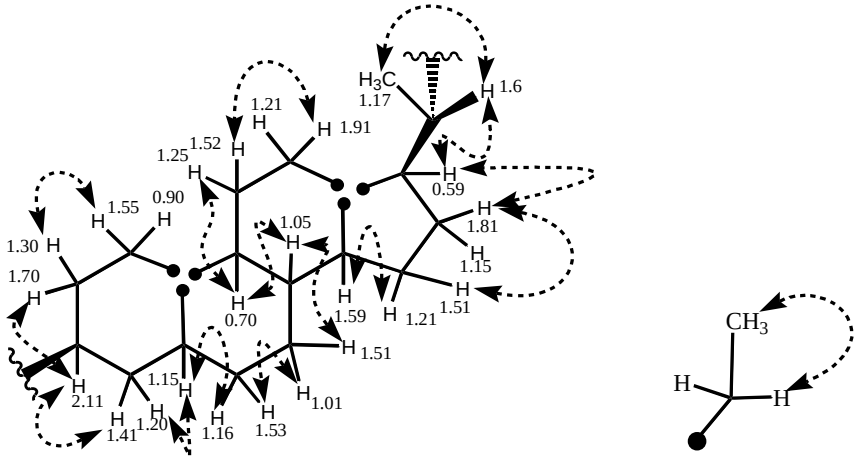
A) 3-(dimethylamino) pregnane skeleton

B) 6-ethyl-5-fluoropyrimidine ring.

Additionally, two-dimensional NMR spectroscopic techniques (^{1}H-^{1}H COSY 45º, HMQC, and HMBC) were used to deduce the final structure of the molecule.

Two-dimensional NMR Spectroscopy:

The deduction of main structural fragments and their joining to obtain the final structure of compound **1** was carried out with the help of ^{1}H-^{1}H COSY 45º, HMQC [142] and HMBC [143] NMR experiments. The presence of two spin systems **'I - II'**, was deduced by the ^{1}H-^{1}H COSY and TOCSY [144] spectra of compound **1** (Scheme-8.1.1).



Fragment 'I' Fragment 'II'

Scheme-8.1.1: Key COSY 45º interactions in fragments 'I-II'.

The major spin system '**I**' of the steroidal skeleton was deduced from the vicinal couplings of C-3 methine proton (δ 2.11) with C-2 methylene (δ 1.30 and 1.70), and C-4 methylene protons (δ 1.41 and 1.20). While at the end of the fragment 'I', the C-20 methine proton (δ 1.60) correlates with C-21 methyl protons (δ 1.17) and C-17 methine proton (δ 0.59). The homonuclear coupling in the small spin system 'II' was also been worked out. In this fragment, coupling between C-7′ methylene protons (δ 2.67) and C-8′ methyl protons (δ 1.23) was also observed.

Long-range HMBC interactions between the protons and carbons were used to join the spin systems together, as well as to find out the position of the quaternary carbons. The N_b-Me protons, resonating at δ 2.95, displayed HMBC correlations with C-4′ and C-20, which supported that C-4′ of 6-ethyl-5-fluoropyrimidine ring was connected to the C-20 amino group. H-2′ resonating at δ 8.22, showed a direct correlation with a carbon resonating at δ 152.0 in the HMQC spectrum, and also displayed HMBC correlations with C-4′, C-6′ and C-5′. H-7′ displayed HMBC correlations with C-6′ and C-5′, while H-8′ displayed correlations with C-7′ and C-6′. The key HMBC interactions are presented in Fig. 8.1.2.

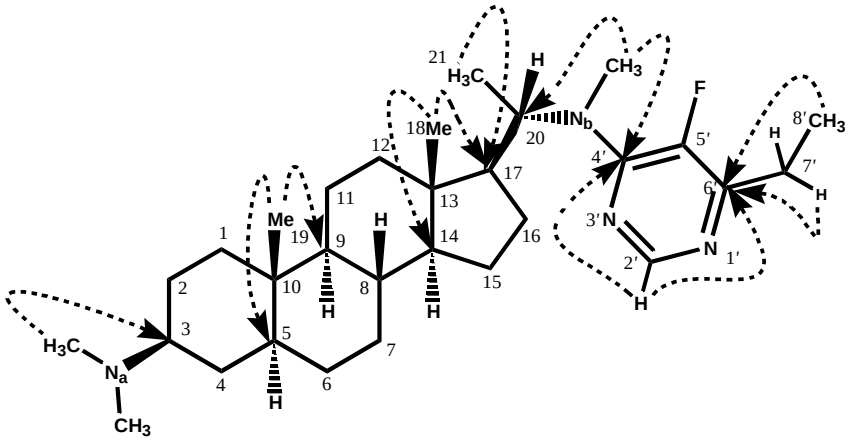


Fig. 8.1.2: Key HMBC interactions in compound **1.**

^{19}F-NMR spectrum displayed resonance at δ -147.0 which further confirmed the presence of fluorine in compound **1**.

Positions of the fluorine and ethyl substituents on the pyrimidine ring were deduced with the help of C-F coupling constants and HMBC correlations, and finally unambiguously determined by the single-crystal X-ray diffraction techniques.

Initially the stereochemistry and configuration of compound **1** was assigned on the basis of spectroscopic evidences and biogenetic grounds, since all the known steroidal alkaloids are biosynthesized from cholesterol *via* pregnenolone (Scheme-3.4, Page-41).[145] The assigned configuration was further supported by NOESY spectrum (Fig. 8.1.3) and finally confirmed by the single-crystal X-ray diffraction studies (Fig. 8.1.4).

The *axial* orientation of C-3 methine proton (δ 2.11 br.m), geminal to amino group, was assigned on the basis of its $W_{1/2}$ = 13.6 Hz, representing the *equatorial* orientation of geminal C-3 amino group.[139]

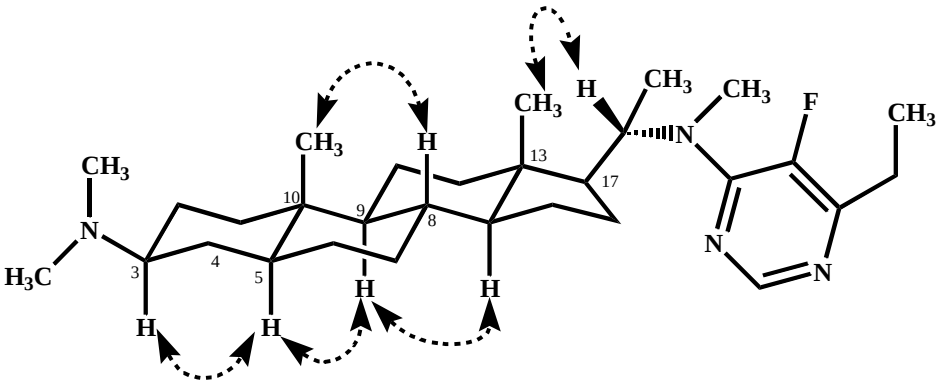


Fig. 8.1.3: Key NOESY interactions in Compound **1.**

A colorless crystal of dimensions 0.50 × 0.35 × 0.32 mm^3 was selected for single-crystal X-ray diffraction studies. Molecular formula $C_{30}H_{49}FN_4$, molecular mass 484.73 amu, crystal system-monoclinic, space group $P2_1$, unit cell dimensions **a** = 7.279 (4) Å, **b** = 10.405 (5) Å, **c** = 18.908 (8) Å, volume = 1431.2 (12) $Å^3$, D_{calcd} = 1.125 mg/m^3, Z = 2. Unit cell dimensions were determined by the Full-matrix least square fit of 7,727 reflections in the range $2.16 < \theta < 25.5^o$ by

using Bruker Smart Apex CCD area detector diffractometer with graphite-monochromator MoKα radiations (λ = 0.71073 Å). A total of 7,727 reflections were collected by using the ω-scan technique at 298(2) ºK. All nonhydrogen atoms were refined anisotropically in a full matrix least square refinement process with the SHELXL-97 software package. The final residual *R* values were 0.0464 for observed data and 0.0608 on the whole data. Final bond distances and angles were found to be in the normal range.

The molecule contains four fused rings A/B/C/D, all ring junctions were found to be *trans*. The ORTEP diagram of the final X-ray structure of compound **1** showed the bond length between N-1′ – C-2′ was 1.312 Å , N-3′ – C-2′ was 1.322 Å, N-3′ – C-4′ was 1.334 Å, C-4′ – C-5′ was 1.407 Å, C-5′ – C-6′ was 1.358 Å , C-5′ – F was 1.354 Å, N-1′ – C-6′ was 1.350 Å, N_b – C-4′ was 1.364 Å and bond angles between C-6′ – N-1′ – C-2′ was 114.9º, C-2′ – N-2′-C-4′ was 117.1º, N-2′ – C-4′ – C5′ was 117.5º, C-4′-C-5′ – C-6′ was 120.9º , N-1′ – C-6′-C-5′ was 120.3º indicated the presence of an fluoropyrimidine ring, substituted to the nitrogen of C-20 side chain.

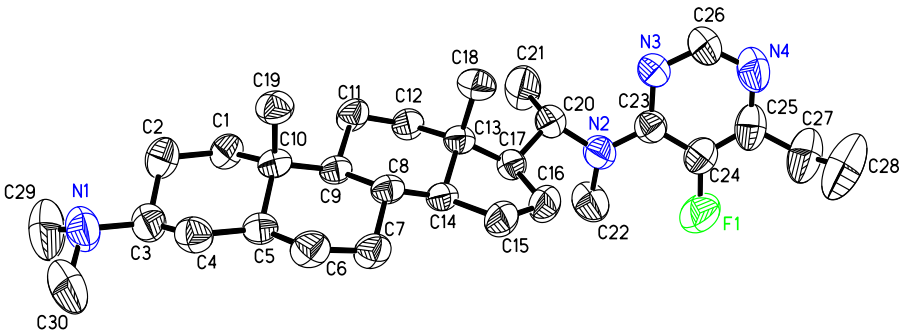
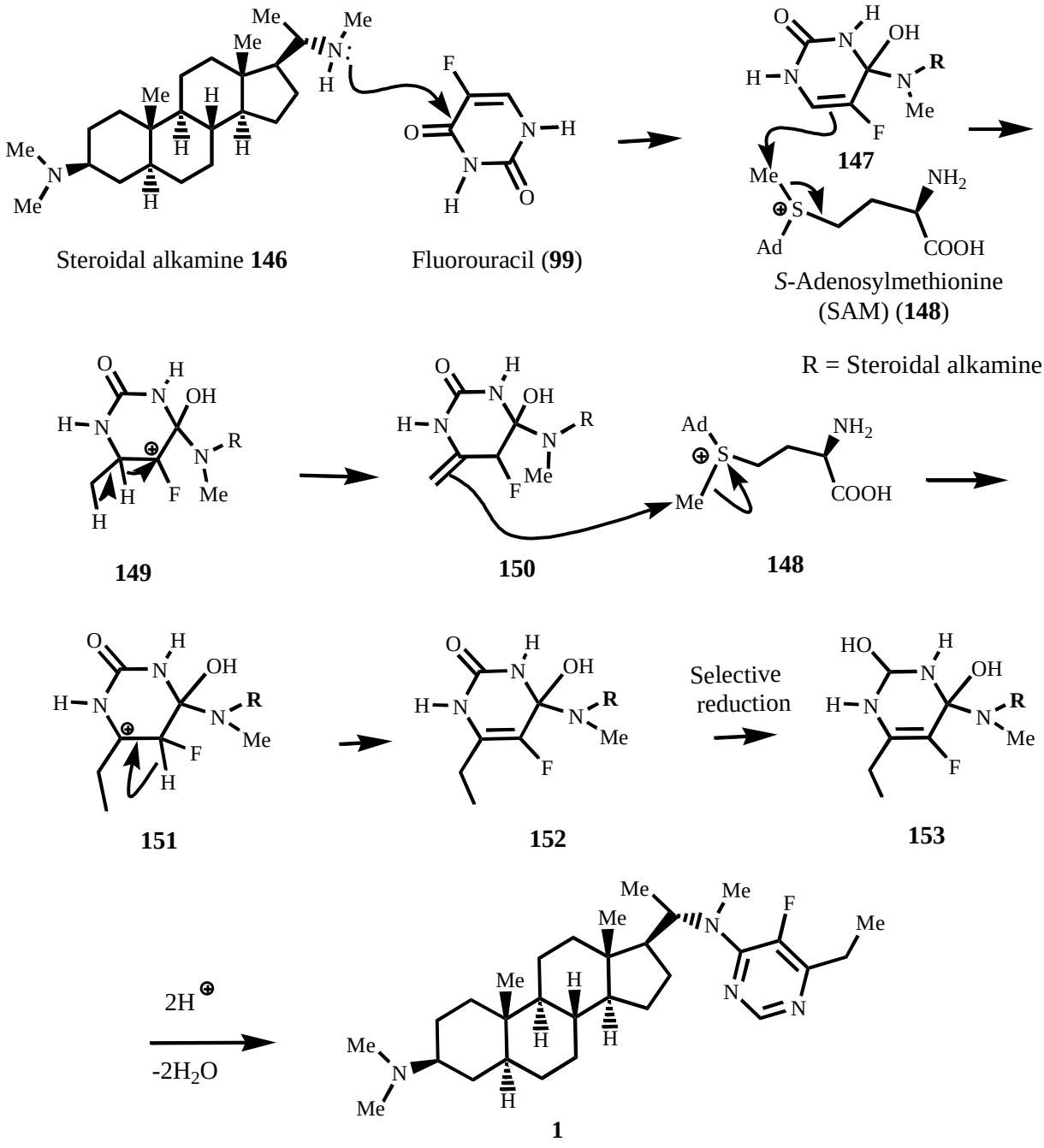


Fig. 8.1.4: Computer-generated ORTEP diagram of the single-crystal X-ray structure of compound **1.**

All these facts led to the structure {(3*β*, 20*S*)-20-[6-ethyl-5-fluoro-4-pyrimidinyl) (methyl) amino]-3-(dimethylamino)-5α-pregnane} for compound **1**, which was trivially named as adhikarimine-A.

***Proposed Biogenesis of Adhikarimine A* (1)**

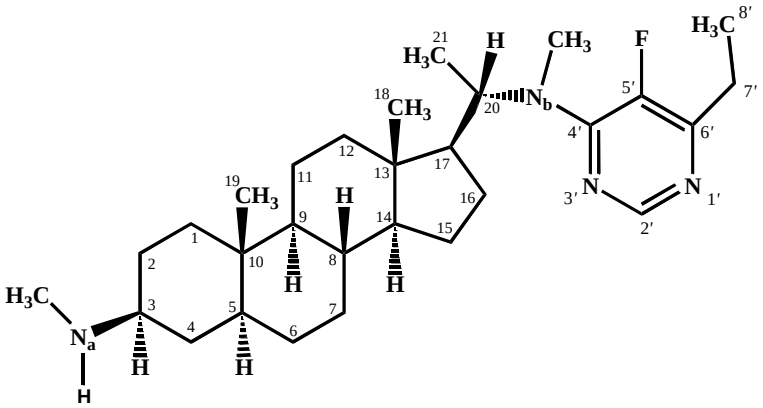
5-Fluorouracil (**99**) has been reported from natural source. It is proposed that electrons of nitrogen of steroidal alkamine **146** attack to the C-6 of 5-fluorouracil (**99**). SAM (**148**) acts as a source of methyl group. Migration of hydride is then taking place, followed by selective reduction and dehydration. Detail of plausible biogenesis of compound **1** is shown in Scheme-8.1.2.



Scheme-8.1.2: Proposed biogenesis of adhikarimine A (**1**).

8.1.2 Adhikarimine B (2)

Adhikarimine B (**2**) was isolated as a white amorphous solid by the column chromatography of the sub-fraction E_2 (Experimental Section, Scheme-9.2, Page-132) on neutral alumina by using pet. ether/EtOAc (9:1) as eluent. Compound **2** was appeared as an orange red spot on the silica gel TLC plate when sprayed with the Dragendorff's spray reagent, indicating its alkaloidal nature.



Adhikarimine B (**2**)

The optical rotation of compound **2** $[\alpha]_D^{25}$ = +19.2º (*c* = 0.05, MeOH) indicated its chiral nature. The UV spectrum of compound **2** exhibited absorptions at λ_{max} 286 and 253 and λ_{min} 376, 274.6, and 223 nm, respectively, representing a hetero-aromatic ring,[137] whereas the IR spectrum exhibited characteristic absorptions at 2929 (CH), 1591 and 1495 (C = C aromatic) and 1377 (C-F) cm^{-1}.

Molecular formula and mass spectrometry:

The EI and FAB $^{+ve}$ MS of compound **2** supported the M^+ *m/z* 470. The HREI MS showed the M^+ at *m/z* 470.7221, in agreement the formula $C_{29}H_{47}N_4F$ (calcd 470.7217) with eight degrees of unsaturation. Two prominent ions at *m/z* 70 and 96 were resulted from the cleavage of ring A. This suggested the presence of a 3-(methylamino) pregnane skeleton.[56] The EI MS also indicated the

presence of 6-ethyl-5-fluoropyrimidine substituent at C-20 amino group, by the presence of the base peak at *m/z* 182 $[C_9H_{13}N_3F]^+$, resulted from the cleavage of C-17/C-20. The key mass fragmentation pattern of compound **2** is presented in Fig. 8.1.5.

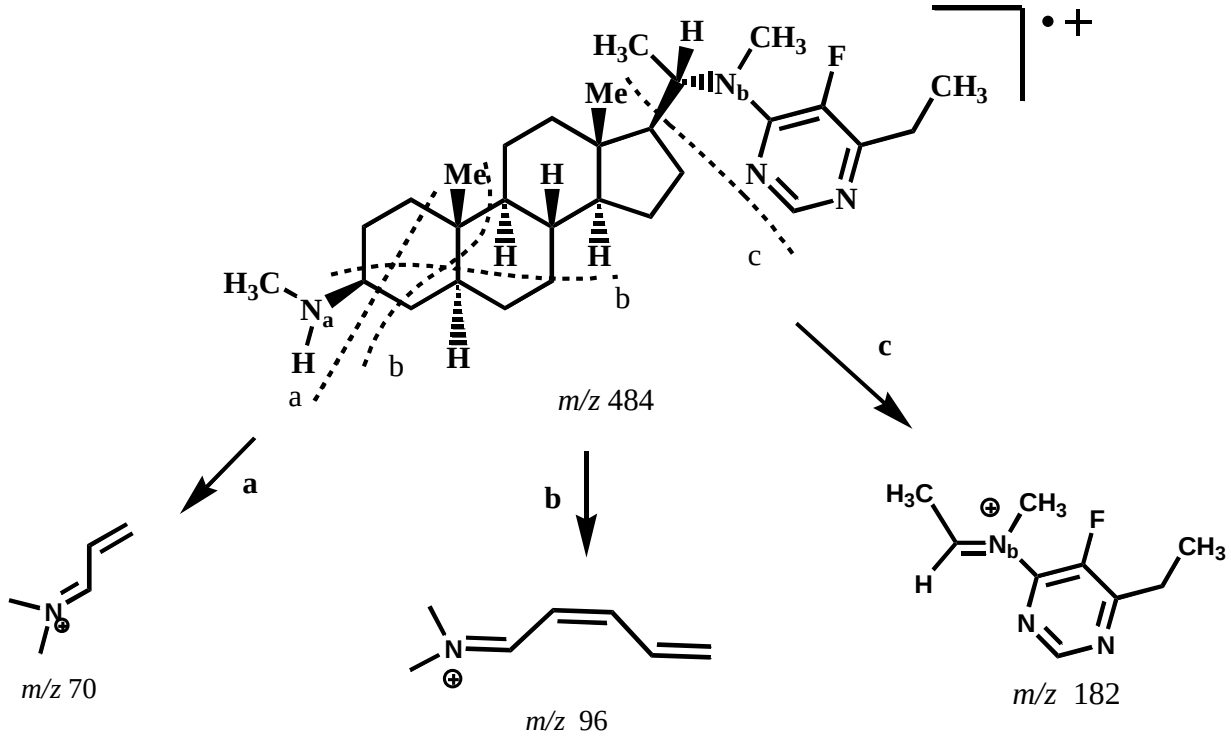


Fig. 8.1.5: Key mass fragmentation in compound **2.**

One-dimensional NMR spectroscopy:

The ^{1}H-NMR spectrum of compound **2** was found to be closely similar to that of compound **1,** with the only difference being the absence of one methyl group at C-3 side chain. The ^{1}H-NMR (Table-8.1.2) of **2** exhibited two up-field sharp 3H singlets at δ 0.72 and 0.78, which were ascribed to C-18 and C-19 angular methyls, respectively. A 3H doublet at δ 1.21 ($J_{20,21}$ = 6.4 Hz) was due to C-21 secondary methyl protons. While a 1H multiplet at δ 1.60 was assigned to the H-20. The up-field shift of C-20 proton was due to the shielding effect of the pyrimidine ring, substituted at the C-20 amino group. A 1H broad singlet at δ 2.74 was attributed to H-3. A downfield 3H singlet

Table. 8.1.2: ^{1}H- and ^{13}C- NMR chemical shift values of compound **2** ($CDCl_3$, ppm, 500 and 100 MHz, respectively).

C. No	^{1}H-NMR δ (*J* and $W_{½}$ in Hz)	^{13}C-NMR δ ($J_{C\text{-}F}$ in Hz)	Multiplicity
1	1.11, 1.86 (m)	39.6	CH_2
2	1.01, 1.50 (m)	25.4	CH_2
3	2.74 br. s ($W_{1/2}$ = 13.3)	54.7	CH
4	1.31, 1.49 (m)	31.9	CH_2
5	1.84 (m)	39.5	CH
6	1.21, 1.50 (m)	23.8	CH_2
7	1.32, 1.39 (m)	32.6	CH_2
8	1.31 (m)	35.4	CH
9	0.70 (m)	54.2*	CH
10	-	36.2	C
11	1.29, 1.41 (m)	20.7	CH_2
12	1.12, 1.18 (m)	28.7	CH_2
13	-	42	C
14	1.59 (m)	54.0*	CH
15	1.04, 1.51 (m)	26.5	CH_2
16	1.13, 1.60 (m)	31.9	CH_2
17	1.05 (m)	56.7	CH
18	0.72 (s)	11.6	CH_3
19	0.78 (s)	12.2	CH_3
20	1.60 m	54.1*	CH
21	1.21 d (6.4)	18.3	CH_3
N_b-Me	2.95 d ($J_{H\text{-}F}$ = 3.9)	30.0	CH_3
N_a-Me	2.39 s	34.0	CH_3
2′	8.23 d ($J_{H\text{-}F}$ = 2.3)	152.5/152.4 (4.6)	CH
4′	-	152.1/152.0 (9.2)	C
5′	-	145.2/142.7 (253.5)	C
6′	-	155.7/155.5 (15.6)	C
7′	2.67 q d (6.5, 2.4)	23.8/23.9 (5.3)	CH_2
8′	1.23 t (7.5)	12.7	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques. * Assignments are interchangeable.

at δ 2.39 was due to the (CH_3)-N_a protons. A 3H doublet at δ 2.95 was ascribed to CH_3-N_b proton. Splitting of CH_3-N_b protons signal as a doublet, instead of a singlet, was due to its long-range coupling with fluorine at C-5′ position.[140]

A 3H triplet at δ 1.23 ($J_{8', 7'}$ = 7.5 Hz) was assigned to C-8′ methyl protons, substituted on the pyrimidine ring, while a 2H quartet of a doublet at δ 2.67 (J_{H-H} = 6.5 Hz, J_{H-F} =2.4 Hz) was assigned to H-7′. Splitting of H-7′ signal as a quartet of doublet, instead of a simple quartet, was again due to its long-range coupling with fluorine at C-5′ position.[140] A downfield 1H doublet at δ 8.23 (J_{H-F} = 2.3 Hz) was attributed to H-2′ of the pyrimidine ring.

The ^{13}C-NMR spectrum (broad-band decoupled and DEPT) of **2** displayed 29 signals (Table-8.1.2), indicating six methyl, ten methylene, eight methine and five quaternary carbons. The downfield signals at δ 152.5 ($^4J_{C-F}$ = 4.4 Hz, C-2′), 152.0 ($^2J_{C-F}$ = 9.2 Hz, C-4′), 145.2 ($^1J_{C-F}$ = 253.5 Hz, C-5′), and 155.5 ($^2J_{C-F}$ = 15.6 Hz, C-6′), methylene signal at δ 23.9 (J_{C-F} = 5.3 Hz, C-7′) and methyl signal at δ 12.7 indicated the presence of a 6-ethyl-5-fluoropyrimidine ring. All pyrimidine ring carbons, were resonated as doublets, due to the coupling with the fluorine, as observed in compound **1.**[140]

Following structural features for compound **2** were deduced from the above cited spectral data:

A) A C-3 methyl amino pregnane-type steroidal skeleton.

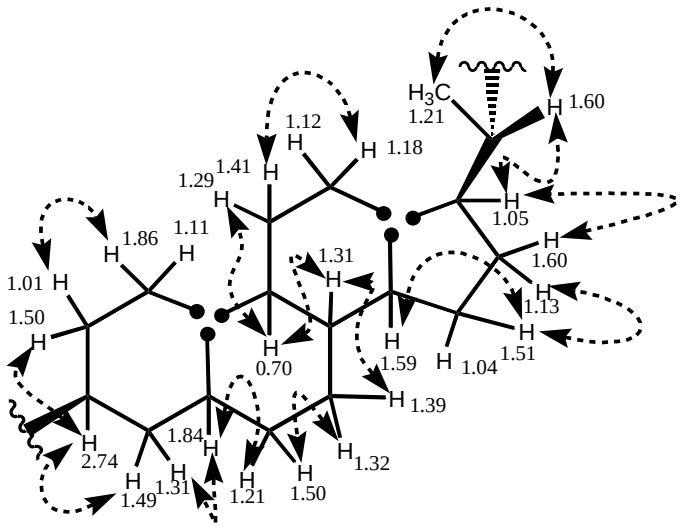
B) A 6-ethyl-5-fluoropyrimidine ring substituted at C-20 amino group.

Finally, two-dimensional NMR experiments, such as ^{1}H-^{1}H COSY 45°, HMQC and HMBC, were recorded to finally deduce the structure of the compound **2**.

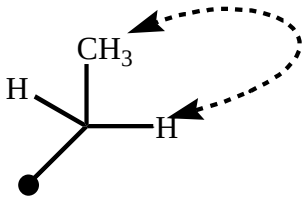
***Two-dimensional NMR Spectroscopy*:**

The one-bond ^{1}H-^{13}C correlations were deduced with the help of HMQC spectrum, while the long-range ^{1}H-^{13}C connectivities were established through HMBC technique. The ^{1}H-^{1}H COSY and TOCSY spectra of compound **2** indicated the presence of a main spin system fragment '**I**', and a smaller spin system, fragment 'II' (Scheme-8.1.3).

The main spin system 'I' was deduced from the vicinal coupling of C-3 methine proton (δ 2.74) with C-2 methylene (δ 1.01 and 1.50) and C-4 methylene protons (δ 1.31 and 1.49). This spin system extended up to C-20 methine proton (δ 1.60), coupled with C-21 methyl protons (δ 1.21). The HMBC correlations were used as a key tool for structural confirmation (Fig. 9.1.6). The H-2′ (δ 8.23) displayed HMBC correlations with C-6′ (δ 155.5) and C-4′ (δ 152.0), while H-7′ (δ 2.67) showed HMBC correlation with C-6′ (δ 155.5). Protons of N_b (Me) group displayed HMBC correlations with C-20 (δ 54.1) and C-4′ (δ 152.0) of pyrimidine. This further supported the early inference that C-4′ of 6-ethyl 5-fluoropyrimidine was connected to the C-20 amino group.



Fragment 'I'



Fragment 'II'

Scheme-8.1.3: Key COSY 45° and TOCSY interactions in fragments 'I-II'.

The stereochemistry in compound **2** was assigned on the basis of biosynthesis of pregnane skeleton,[145] as well as ROESY experiment (Fig. 9.1.7). The C-3 methine proton (δ 2.74, br. s), geminal to amino group, was assigned to be *axial* on the basis of its $W_{1/2}$ = 13.3 Hz, indicated that the C-3 amino group is equatorially orientated.[139]

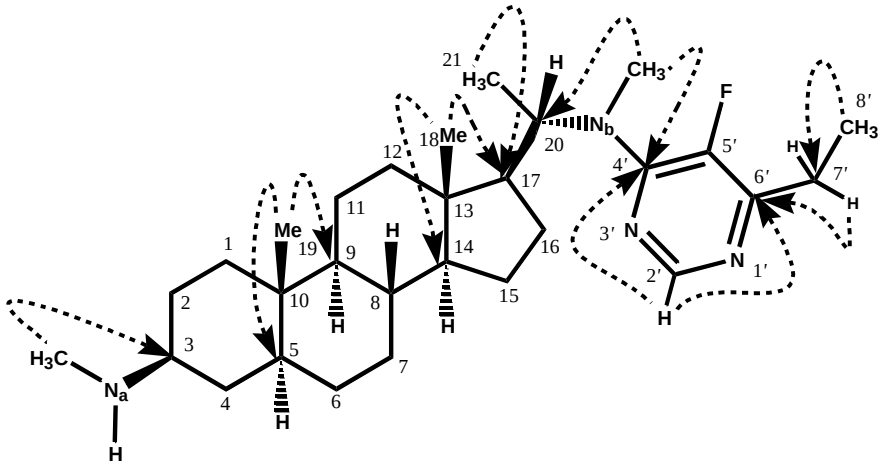


Fig. 8.1.6: Key HMBC interactions in compound **2**.

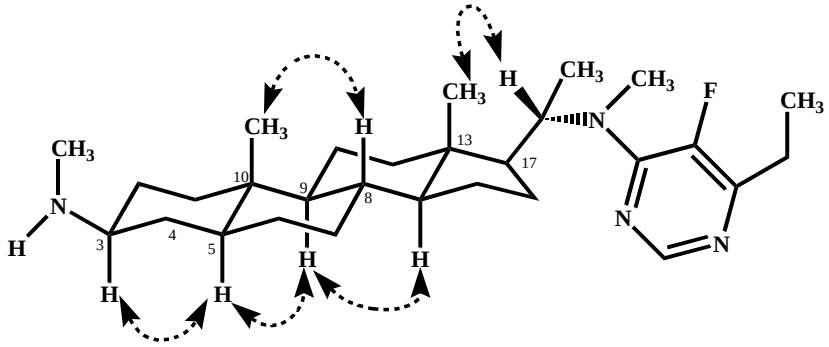


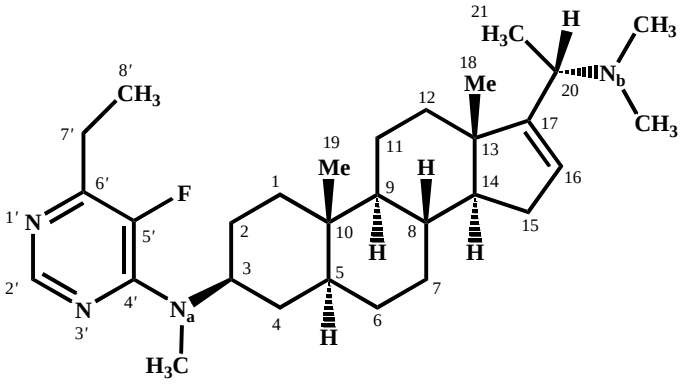
Fig. 8.1.7: Key ROESY interactions in compound **2**.

With the help of the above spectroscopic studies, the structure for compound **2** was deduced as {(3*β*, 20*S*)-20-[6-ethyl-5-fluoro-4-pyrimidinyl) (methyl) amino]-3-(methylamino)-5α-pregnane}, and was trivially named as adhikarimine B.

8.1.3 Adhikarimine C (3)

Compound **3** was obtained from a sub fraction A_2 of dichloromethane extract (pH 9.0) (Experimental Section, Scheme-9.2, Page-132). Sub-fraction A_2 was subjected to neutral alumina column chromatography by using EtOAc/pet. ether as eluent, which resulted in the isolation of a pure compound **3.** Compound **3** was alkaloidal in nature as infered from a positive color test on silica gel TLC plates with the Dragendorff's reagent.

The optical rotation of compound **3** $[\alpha]_D^{25}$ = + 9.6º (*c* = 0.062, MeOH), indicated the presence of asymmetric centers in the molecule. The UV spectrum showed absorptions at λ_{max} 287 and 255, and λ_{min} 367, 264.8 and 225 nm, characteristics of a hetero-aromatic ring.[137] The IR spectrum displayed absorptions at 2930 (C-H), 1591 and 1489 (C=C aromatic), and 1260 (C-F) cm^{-1}.



Adhikarimine C **(3)**

Molecular formula and mass Spectrometry:

The EI MS showed the M^+ at *m/z* 482, which was further confirmed by FAB $^{+ve}$ MS (*m/z* 483). The HREI MS of compound **3** showed the M^+ at *m/z* 482.7330, corresponding to the formula $C_{30}H_{47}N_4F$ (calcd 482.7328), with nine degrees of unsaturation. The base peak at *m/z* 467 in the EI MS was attributed to the loss of a methyl group from the M^+. The peak at *m/z* 72 was due to Me_2N^+ =C(H)Me ion, resulting from cleavage of C-17/C-20 bond.[40] However this ion had a lower

abundance, in comparison to the corresponding signal in common steroidal alkaloids with a 20-$N(Me)_2$ group, where it appears as the base peak. The lower abundance of this ion may be due to the presence of a double bond between C-16/C-17.[20] This ion also supported that the 6-ethyl-5-fluoropyrimidine ring was attached to C-3 amino group, and not at C-20.

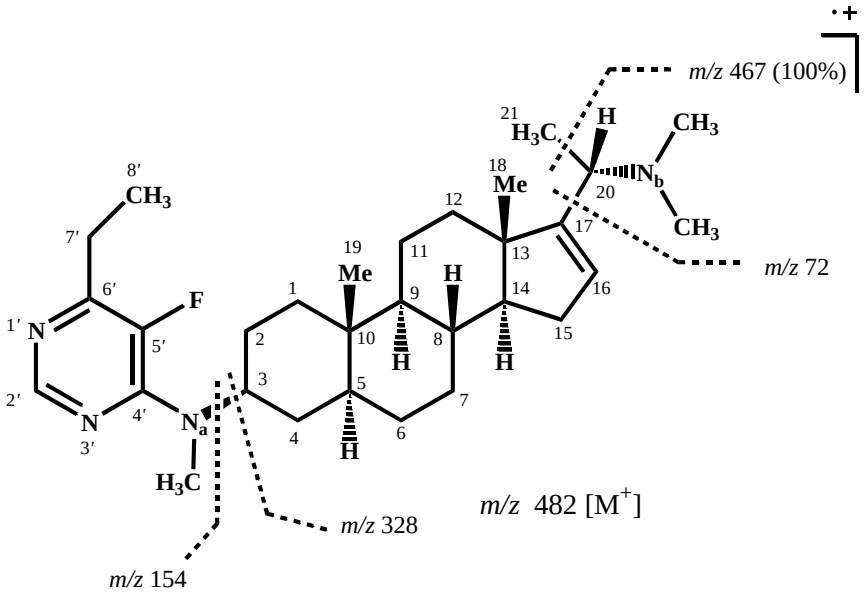


Fig. 8.1.8: Key mass fragments in compound **3.**

***One-dimensional NMR spectroscopy*:**

The ^{1}H-NMR (Table- 8.1.3) of compound **3** exhibited two 3H singlets at δ 0.82 and 0.85 for Me (18) and Me (19), respectively. A 3H doublet at δ 1.08 ($J_{21,\ 20}$ = 6.4 Hz) was assigned to the C-21 methyl protons. A 6H singlet at δ 2.20 was ascribed to the N_b-Me_2 protons. A 1H quartet at δ 2.80 was assigned to H-20, which further supported the presence of a double bond between C-16 and C-17, while a 3H doublet at δ 3.18 ($J_{H\text{-}F}$ = 3.0 Hz) was attributed to N_a-Me protons. Splitting of N_a-Me protons as a doublet, instead of simple singlet, was due to the long-range coupling with fluorine atom present at C-5' position of pyrimidine ring.[140] A 1H multiplet at δ 4.52 ($W_{1/2}$ = 15.0 Hz) was assigned to H-3.[139] A 1H broad singlet at δ 5.51 was due to H-16.

Table 8.1.3: ^{1}H- and ^{13}C- NMR chemical shift values of compound **3** ($CDCl_3$, ppm, 500 and 100 MHz)

C. No	^{1}H-NMR δ (*J* and $W_{½}$ in Hz)	^{13}C-NMR δ ($J_{C\text{-}F}$ in Hz)	Multiplicity
1	1.61 , 1.75	36.8	CH_2
2	1.63 , 1.82	24.6	CH_2
3	4.52 m ($W_{1/2}$ = 15.0)	52.3/52.4 (5.8)	CH
4	1.59 , 1.78	32	CH_2
5	1.50	42.8	CH
6	1.25 , 1.35	29.8	CH_2
7	1.59 , 1.78	33.4	CH_2
8	1.32	35.5	CH
9	0.85	57.3	CH
10	-	38.7	C
11	1.40 , 1.70	21.8	CH_2
12	1.59 , 1.78	36	CH_2
13	-	48.2	C
14	1.32	58.6	CH
15	1.90, 2.10	33	CH_2
16	5.51 (br, s)	124.9	CH
17	-	157.7	C
18	0.82 s	12.8	CH_3
19	0.85 s	16.4	CH_3
20	2.80 q (6.4)	61	CH
21	1.08 d (6.4)	19.1	CH_3
N_b-Me_2	2.20 s	43.4	CH_3
N_a-Me	3.18 d ($J_{H\text{-}F}$ = 3)	34.5/34.6 (8)	CH_3
2′	8.27 d ($J_{H\text{-}F}$= 2.4)	153.1/153.2 (8.8)	CH
4′	-	154.1/154.3 (15)	C
5′	-	144.6/146.7 (254)	C
6′	-	156.6/156.7 (17)	C
7′	2.67 q d (6.5, 2.4)	24.6	CH_2
8′	1.23 t (7.5)	13.5	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques. * Assignments are interchangeable.

A 3H triplet, resonating at δ 1.23 ($J_{8', 7'}$ = 7.5 Hz), was assigned to the C-8′ methyl protons, substituted on the pyrimidine ring. A 2H quartet of doublets at δ 2.67 ($J_{H\text{-}H}$ = 6.5 Hz, $J_{H\text{-}F}$ = 2.4 Hz) was assigned to H-7′. Splitting of H-7′ as quartet of a doublet instead of simple quartet, was again due to the long-range coupling with fluorine atom present at C-5′ position of pyrimidine ring.[140] While a 1H doublet at δ 8.27 ($^5J_{H\text{-}F}$ = 2.4 Hz) was assigned to H-2′ of the pyrimidine substituent.

The ^{13}C- NMR spectrum (broad-band decoupled, DEPT) of **3** displayed 30 signals (Table- 8.1.3), corresponding to seven methyl, nine methylene, eight methine and six quaternary carbons. As in compound **1**, the carbons of the pyrimidine ring, N_a-Me carbon and C-3 were all resonated as doublets due to their couplings with the fluorine substituent.[140, 88]

Above spectral data suggested the following structural features in compound **3**:

A) a basic pregnane-type steroidal skeleton

B) 6-ethyl-5-fluoropyrimidine substituent at C-3 amino moiety

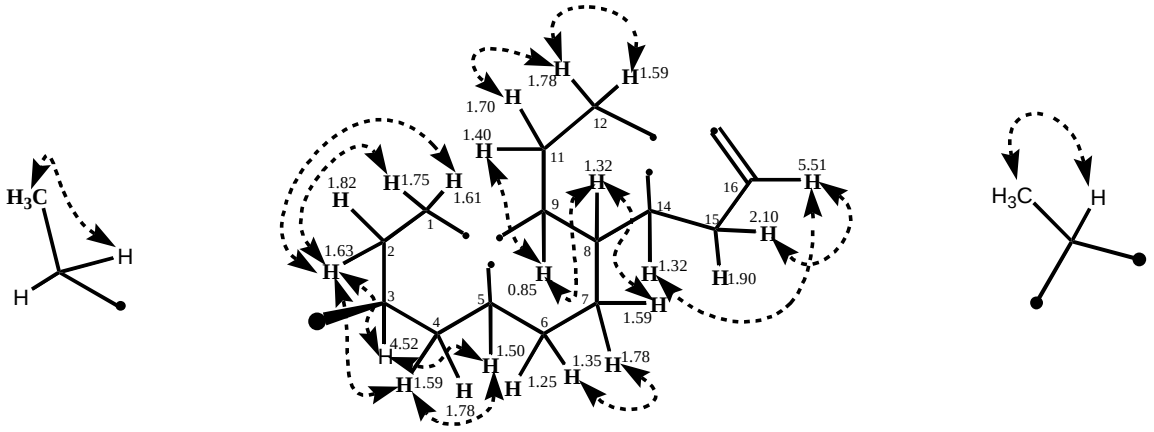
C) a *N*,*N*-dimethylamino moiety at C-20, and

D) a double bond between C-16 and C-17.

Two-dimensional NMR spectroscopy:

The study of COSY 45° and TOCSY spectra of compound **3** indicated the presence of three spin systems (Scheme-8.1.4). Spin system 'I' comprise of an ethyl group, substituted on the pyrimidine ring. The major spin system 'II' of compound **3** comprising on the entire steroidal skeleton, and traced down from the vicinal coupling of C-3 methine proton (δ 4.52) with C-2 (δ 1.63 and 1.82) and C-4 methylene protons (δ 1.59

and 1.78), as well as with C-5 methine proton (δ 1.50). At the other end of the spin system 'II' , the C-16 proton at δ 5.51 showed cross peaks with C-15 methylene (δ1.90, 2.10) and C-14 methine (δ 1.32) protons.



Scheme-8.1.4: Key COSY 45° and TOCSY interactions in fragments 'I-III'.

The correlations between various protons of fragments 'I', 'II' and 'III', as well as possible positions of various functionalities and quaternary carbons were deduced from the HMBC interactions (Fig. 8.1.9). The N_a-Me protons (δ 3.18) showed HMBC correlations with C-3 and C-4′ of pyrimidine. This indicated that the 6-ethyl-5-fluoropyrimidine was connected to the C-3 amino group through C-4′. The N_b-Me_2 (δ 2.20) and H-21 (δ 1.08) displayed HMBC correlations with C-20, while H-20, H-21 and H-18 displayed HMBC correlations with C-17 (δ 157.7). H-16 (δ 5.51) showed HMBC correlations with C-17 and C-15. H-2′ of pyrimidine ring (δ 8.27) displayed HMBC correlations with C-4′, C-6′ and C-5′. In addition, H-7′ displayed HMBC correlations with C-6′ and C-5′.

The assignment of stereochemistry in compound **3** was mainly based on biogenetic consideration, and ROESY cross peaks (Fig. 8.1.10). Where as chemical shifts and coupling constants values

also helped in these assignments. The C-3 methine proton (δ 4.52), geminal to amino group, was assigned to be *axial*, with *equatorial* orientation of the geminal amino group, on the basis of its $W_{1/2}$ = 15.0 Hz.[139]

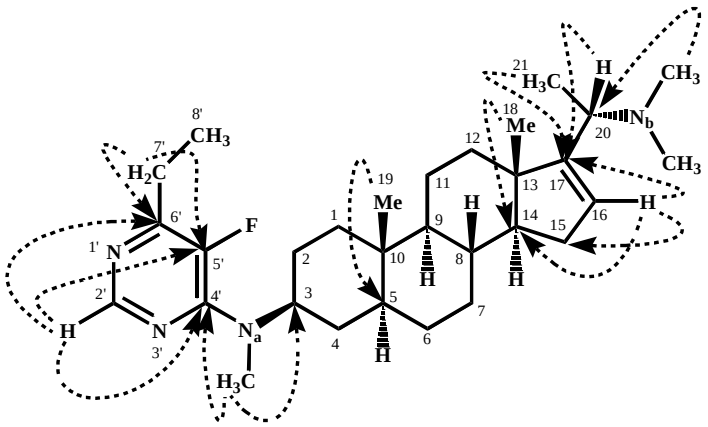


Fig. 8.1.9: Key HMBC interactions in compound **3.**

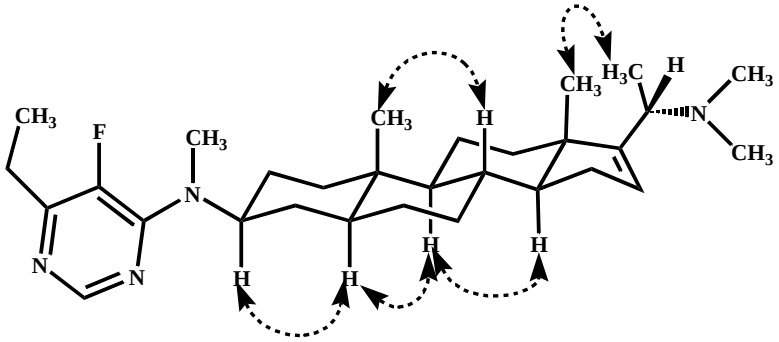
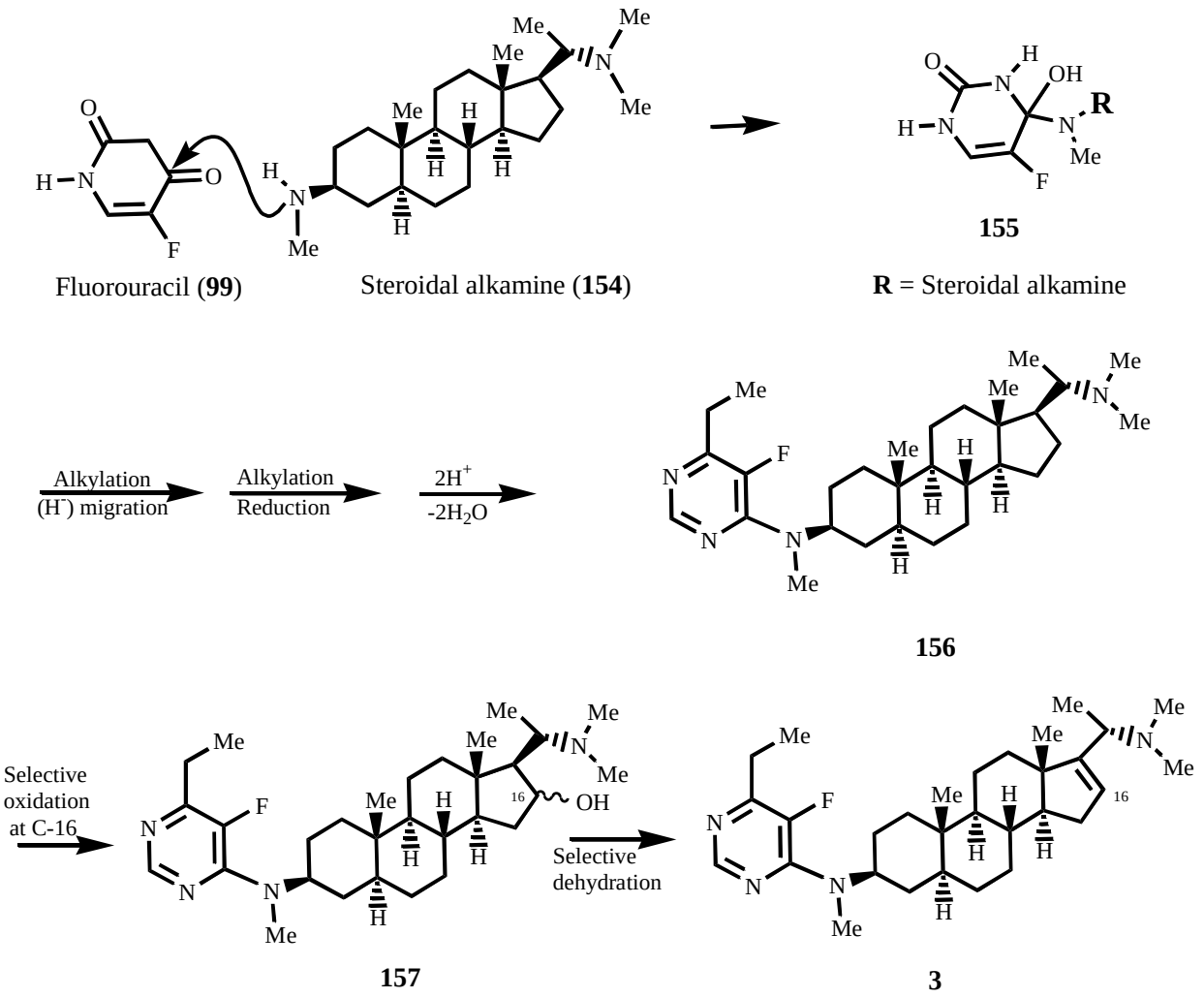


Fig. 9.1.10: Key ROESY interactions in compound **3**.

These assignments led to the structure {(3*β*, 20*S*)-20-(dimethylamino)-3-[(6-ethyl-5-fluoro-4-pyrimidinyl) (methyl) (amino)]-5α-pregn-16-ene} for compound **3,** which was trivially named as adhikarimine C.

Biogenesis of adhikarimine C (3):

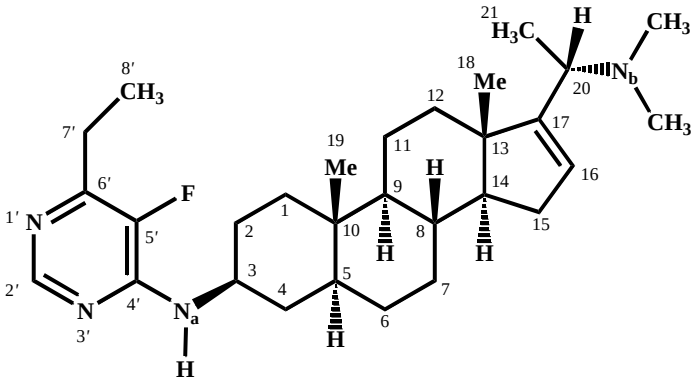
A proposed biogenesis of compound **3** is presented in Scheme-8.1.5.



Scheme-8.1.5: Proposed biogenesis of adhikarimine C (**3**).

8.1.4 Adhikarimine D (4)

Adhikarimine D (**4**) was obtained as a white amorphous solid from the alkaline dichloromethane fraction of the extract of *S. coriacea* (Experimental Section, Scheme-9.3, Page-133). It was isolated from sub-fraction D_1 on a neutral alumina column with EtOAc/pet. ether (5:95) as a semi pure solid. It was further subjected to thin-layer chromatography on pre-coated silica gel plates by using acetone-petroleum ether- diethylamine (1:8.5:0.5) to afford compound **4** in a pure form. Its alkaloidal characteristic was identified by using Dragendorff's spray reagent.



Adhikarimine D (**4**)

The optical rotation of compound **4** was found to be $[\alpha]_D^{25}$ = +19^o (*c* = 0.02, MeOH), which indicated the presence of chiral centers in the molecule. The UV spectrum displayed absorptions at λ_{max} 275 and 243.8 nm and λ_{min} 328 and 217.6 nm, characteristic of the hetero-aromatic ring,[137] while the IR spectrum showed absorptions at 3421 (N-H), 2932 (C-H) , 1617 and 1511 (C=C hetero-aromatic ring) and 1326 (C-F) cm^{-1}.[88]

***Molecular formula and mass spectrometry*:**

The EI MS of compound **4** was characteristic of a pregnane-type steroidal skeleton. It showed the M^+ at *m/z* 468.3, which was further supported by the FAB $^{+ve}$ MS *m/z* 469. The HREI MS showed the M^+ at *m/z* 468.7061, corresponding to the formula $C_{29}H_{45}N_4F$ (calcd 468.7058) with nine degrees of unsaturation. The base peak at *m/z* 453 in the EI MS was attributed to the loss of a methyl group from the M^+. The peak at *m/z* 72 was due to Me_2N^+ = C(H)Me ion, resulting from cleavage of C-17/C-20 bond.[40] The lower abundance of this ion may be due to the presence of a double bond between C-16 and C-17.[20] This ion also supported that the 6-ethyl-5-fluoro pyrimidine ring was substituted at C-3 amino group, and not at C-20 as discussed in case of compound **2**. The ion at *m/z* 142, instead of *m/z* 154, in compound **3** indicated that secondary

amino group is present instead of a tertiary amino group. Key mass fragmentation pattern of compound **4** is shown in Fig. 8.1.11.

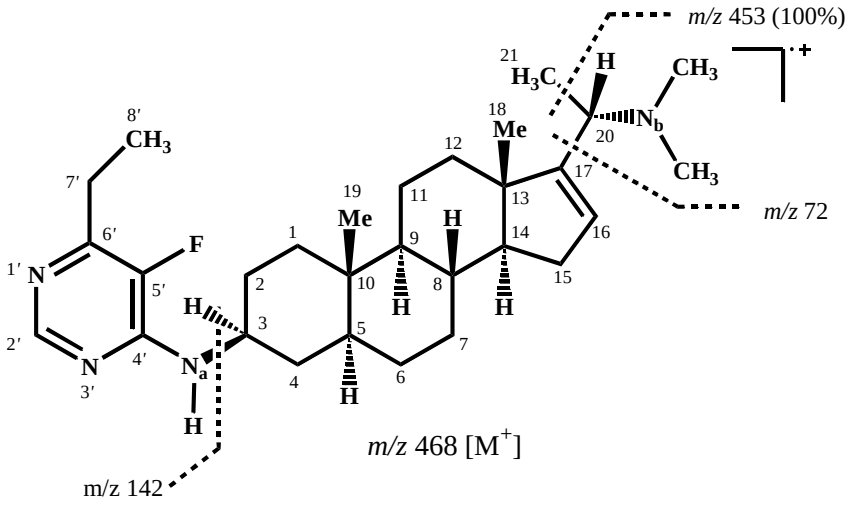


Fig. 8.1.11: Key mass fragmentation in compound **4.**

One-dimensional NMR Spectroscopy:

The one-dimensional NMR spectra of compound **4** was found to be distinctly similar to compound **3**, with the only difference being the absence of a methyl at C-3 amino group. The ^{1}H-NMR spectrum (Table-8.1.4) displayed two 3H singlets in the up-field region at δ 0.82 and 0.85, which were assigned to the C-18 and C-19 methyl protons, respectively. A 3H doublet at δ 1.08 ($J_{21,\,20}$ = 6.5 Hz) was assigned to the C-21 methyl protons. A 6H singlet at δ 2.22 was ascribed to the N_b-Me_2 protons. A 1H quartet at δ 2.81 ($J_{20,\,21}$ = 6.4 Hz) was assigned to H-20, which further supported the presence of a double bond between C -16 and C -17, while a 1H multiplet at δ 3.94 ($W_{1/2}$ = 21.8 Hz) was assigned to H-3.[139] A 1H broad doublet at δ 4.75 (J = 6.4 Hz) was assigned to the proton of the amino group substituted at C-3. A 1H broad singlet at δ 5.53 was assigned to H-16.

Table-8.1.4: ^{1}H- and ^{13}C- NMR chemical shift values of compound **4** (400 and 150 MHz in $CDCl_3$, ppm).

C. No	^{1}H-NMR δ (*J* and $W_{½}$ in Hz)	^{13}C-NMR δ ($J_{C\text{-}F}$ in Hz)	Multiplicity
1	1.15 , 1.05 (m)	37.3	CH_2
2	1.35 , 1.15 (m)	29.0	CH_2
3	3.94 m ($W_{1/2}$ = 21.8)	49.9	CH
4	1.70 , 1.24 (m)	35.5	CH_2
5	1.25 (m)	45.0	CH
6	1.24 , 1.33 (m)	28.5	CH_2
7	1.69 , 0.98 (m)	31.1	CH_2
8	1.35 (m)	34.0	CH
9	0.75 (m)	55.0	CH
10	-	35.8	C
11	1.59 , 1.35 (m)	20.9	CH_2
12	1.21 , 1.64 (m)	34.6	CH_2
13	-	46.8	C
14	1.31 (m)	57.4	CH
15	2.05, 1.85 (m)	31.8	CH_2
16	5.53 (br, s)	124.0	CH
17	-	156.0	C
18	0.82 s	12.0	CH_3
19	0.85 s	12.3	CH_3
20	2.81 q (6.4)	59.2	CH
21	1.08 d (6.4)	16.0	CH_3
N_b-Me_2	2.22 s	42.4	CH_3
N_a-H	4.75 d (*J* = 6.4)	-	-
2′	8.25 d ($J_{H\text{-}F}$ = 2.4)	153.3/153.2 (8.8)	CH
4′	-	151.6/151.5 (15.0)	C
5′	-	144.2/142.5 (252.8)	C
6′	-	152.1/152.0 (15)	C
7′	2.66 qd (6.5, 2.4)	23.6	CH_2
8′	1.22 t (7.5)	16.3	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques.

A 3H triplet at δ 1.22 ($J_{8', 7'}$ = 7.5 Hz) was assigned to the C-8′ methyl protons, substituted on the pyrimidine ring. A 2H quartet of a doublet at δ 2.66 ($J_{H\text{-}H}$ = 6.5 Hz, $J_{H\text{-}F}$ = 2.4 Hz) was assigned to H-7′, while a 1H doublet at δ 8.25 ($^5J_{H\text{-}F}$ = 2.4 Hz) was due to H-2′ of the pyrimidine substituent.

The ^{13}C- NMR spectrum (broad-band decoupled, DEPT) of **4** displayed 29 signals (Table-8.1.4) corresponding to six methyl, nine methylene, eight methine and six quaternary carbons. As in compound **3**, the carbons of the pyrimidine ring were resonated as doublets due to their couplings with the fluorine substituent.[140, 88]

The above observations suggested the following structural features in compound **4:**

A) a basic pregnane- type steroidal skeleton.

B) C-20 dimethyl amino group.

C) 6-ethyl-5-fluoropyrimidine ring substituted to C-3 amino group, and

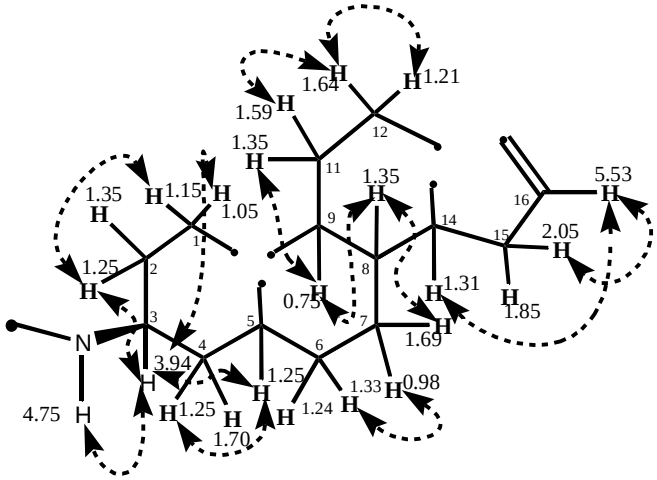
D) a double bond between C-16 and C-17.

Finally, two-dimensional NMR spectroscopic techniques (^{1}H-^{1}H COSY 45º, TOCSY, HMQC, and HMBC) were employed to deduce the structural framework and positions of various functionalities.

Two-dimensional NMR Spectroscopy:

The ^{1}H-^{1}H COSY 45º and TOCSY spectra of **4** clearly indicated the presence of a major spin system 'I' of the steroidal skeleton (Scheme- 8.1.6). This spin system was deduced from the vicinal coupling of C-3 methine proton (δ 3.94) with the C-2 methylene protons (δ 1.15 and 1.35) and C-3 amino proton (δ 4.75), as well as with C-4 methylene protons (δ 1.24 and 1.70). Furthermore, the C-3 methine proton also showed *W* coupling with C-5 methine protons (δ 1.25)

and C-1 methylene proton (δ 1.05). Similarly the C-16 olefinic proton displayed cross peaks with C-15 methylene protons (δ 1.85 and 2.05) and C-14 methine proton (δ 1.31).



Scheme-8.1.6: Key COSY 45º and TOCSY interactions in compound **4.**

Long-range HMBC correlations between protons and carbons were used to join various functionalities together (Fig. 8.1.12). The C-3 methine proton displayed HMBC correlations with C-4′ (δ 151.6) of pyrimidine. Similarly H-N_a showed HMBC correlations with both C-3 (δ 49.9) and C-4′ (δ 151.6) of pyrimidine. These correlations strongly supported that the 6-ethyl-5-fluoropyrimidine ring was substituted on C-3 amino group through C-4′. $N_b(Me)_2$ protons displayed HMBC correlations with C-20 (δ 59.2), while C-21 methyl protons (δ 1.08) showed HMBC correlations with C-20 (δ 59.2) and C-17 (δ 156.0).

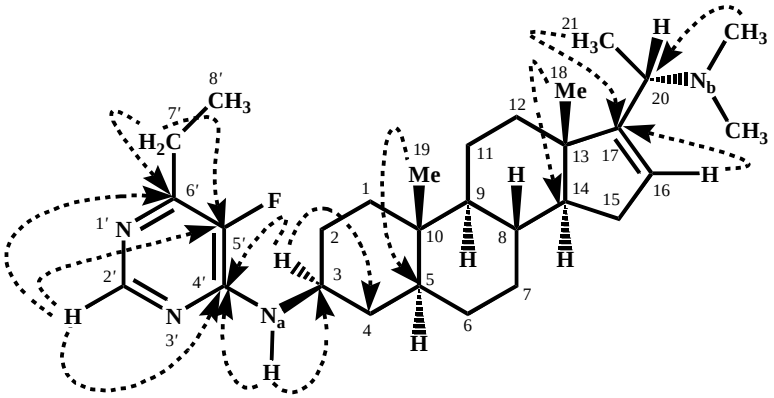


Fig. 8.1.12: Key HMBC interactions in compound **4.**

The stereochemistry and configuration of compound **4** was deduced on the basis of biogenetic considerations, cross peaks in ROESY spectrum (Fig. 8.1.13), and comparison of chemical shift, and coupling constants with the reported values. The C-3 methine proton, resonated at δ 3.94 ($W_{1/2}$ = 21.8 Hz), was found to be *axially* oriented, indicating an *equatorial* orientation of the geminal side chain. [139]

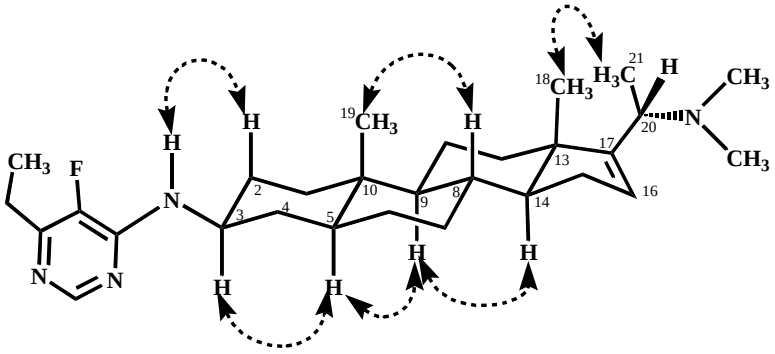


Fig. 8.1.13: Key ROESY interactions in compound **4.**

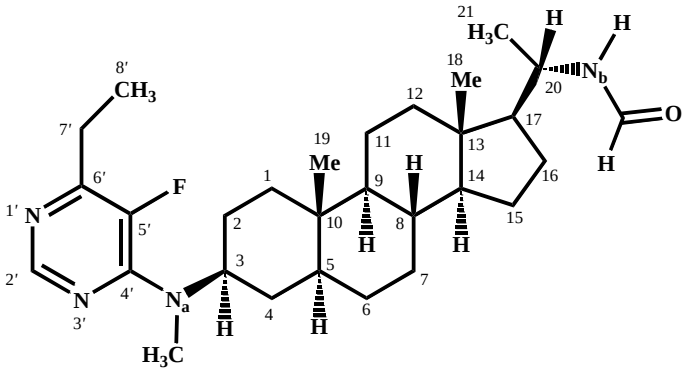
From the above discussions, the structure of compound **4** was deduced as {(3*β*, 20*S*)-20-(dimethylamino)-3-[(6-ethyl-5-fluoro-4-pyrimidinyl) (amino)]-5α-pregn-16-ene}, which was trivially named as adhikarimine D.

8.1.5 Adhikarimine E (5)

Compound **5** was isolated from the column chromatography of sub-fraction I_3 on neutral alumina by using EtOAc/pet. ether as eluting solvent (Experimental Section, Scheme-9.4, Page-133). The alkaloidal nature of compound **5** was inferred from the orange color test on silica gel TLC plate with Dragendorff's reagent spray.

Optical rotation of compound **5** was found to be $[\alpha]_D^{25}$ = + 10.8º (*c* = 0.026, MeOH), which indicated the presence of chiral centers in molecule. The UV spectrum displayed the absorptions at λ_{max} 286.8 and 254.2, and λ_{min} 366 and 200.6 nm, characteristic of hetero-aromatic ring.[137] The

IR absorptions were at 3260 (N-H), 2931 (C-H), 1665 (amide), 1593 and 1496 (C=C aromatic) cm^{-1}.



Adhikarimine E (**5**)

Molecular formula and mass spectroscopy:

The molecular ion peak of **5** was at *m/z* 484 in EI MS, which was further confirmed by FAB^{+ve} MS (*m/z* 485). The HREI MS showed the M^+ at *m/z* 484.7056, corresponding to the formula $C_{29}H_{45}N_4OF$ (calcd 484.7052), with nine degrees of unsaturation. The EI MS fragmentation pattern of compound **5** (Fig. 8.1.14) was largely similar to the previous compounds **1**-**4**. The base

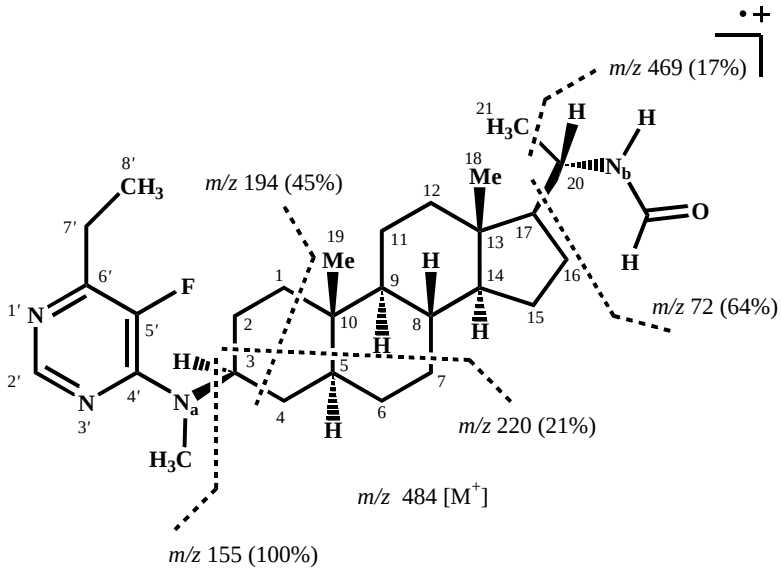


Fig. 8.1.14: Key mass fragmentation in compound **5.**

peak at *m/z* 155 was due to the cleavage of bond between -NCH_3 and C-3, and addition of hydrogen atom to the amino group. The peak at *m/z* 72 was due to cleavage of C-20/C-17 bond.[40] A peak at *m/z* 72 and the base peak at *m/z* 155 clearly indicated that a 6-ethyl-5-fluoropyrimidine ring was substituted at the C-3 side amino group, and not at C-20.

One-dimensional NMR Spectroscopy:

The ^{1}H-NMR spectrum of compound **5** (Table- 8.1.5) was found to be closely similar to the previous compounds, with only difference of an *N*-formyl amino substituent at C-20. A 1H multiplet at δ 4.07 was assigned to H-20. A 1H broad multiplet at δ 4.36 ($W_{1/2}$ = 18.1 Hz) was assigned to H-3α.[139] A 1H doublet at δ 5.31 was assigned to the C-20 amino proton. A downfield splitted signal at δ 8.01/8.06 was attributed to the formamide proton.[36]

The ^{13}C- NMR spectrum (broad-band decoupled, DEPT) of **5** displayed 29 signals (Table- 8.1.5) corresponding to five methyl, ten methylene, nine methine and five quaternary carbons. As in previous compounds, the carbons of the pyrimidine ring along with C-3 and N_a-Me carbons were resonated as doublets due to their couplings with the fluorine substituent.[140, 88]

This suggested the following structural features in compound **5:**

A) a basic pregnane- type steroidal skeleton

B) a *N*-formyl amino moiety

C) 6-ethyl-5-fluoropyrimidine ring.

Finally, two-dimensional NMR spectroscopy (^{1}H-^{1}H COSY 45^o, TOCSY, HMQC, and HMBC) was used to deduce the main skeleton (Scheme -8.1.7)**.**

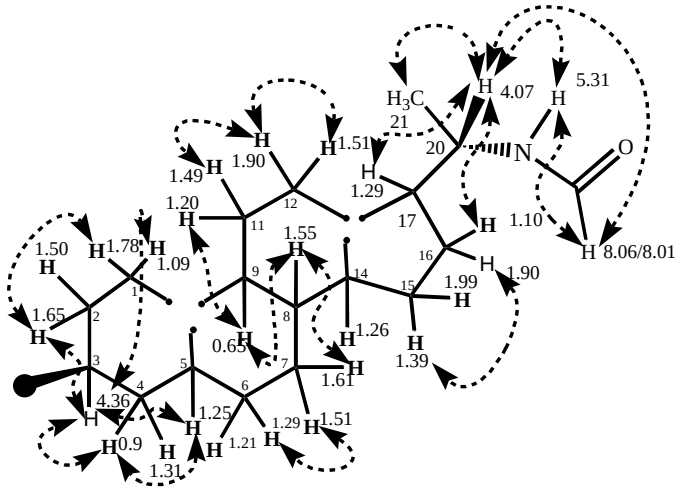
Table-8.1.5: ^{1}H- and ^{13}C- NMR chemical shift values of compound **5** (400 and 125 MHz in $CDCl_3$, ppm).

C. No	^{1}H-NMR δ (*J* and $W_{½}$ in Hz)	^{13}C-NMR δ ($J_{C\text{-}F}$ in Hz)	Multiplicity
1	1.78 , 1.09 (m)	37.6	CH_2
2	1.65 , 1.50 (m)	25.1	CH_2
3	4.36 br m ($W_{1/2}$ =18.1)	56.6/56.5	CH
4	0.9 , 1.31 (m)	31.8	CH_2
5	1.25 (m)	45.8	CH
6	1.21 , 1.29 (m)	28.7	CH_2
7	1.61 , 1.51 (m)	31.9	CH_2
8	1.55 (m)	35.3	CH
9	0.65 (m)	54.2	CH
10	-	35.5	C
11	1.20 , 1.49 (m)	21.0	CH_2
12	1.51 , 1.90 (m)	39.3	CH_2
13	-	42.2	C
14	1.26 (m)	45.7	CH
15	1.39, 1.59 (m)	26.8	CH_2
16	1.10, 1.90 (m)	39.7	CH_2
17	1.29 (m)	57.0	CH
18	0.70 s	12.2	CH_3
19	0.82 s	12.4	CH_3
20	4.07 (m)	46.7	CH
21	1.17 d (6.4)	21.7	CH_3
N_b-C<u>H</u>O	8.01/8.06	159.9/163.2	CH
N_b-<u>H</u>	5.31 d	-	-
N_a-CH_3	3.01	31.1/31.0	CH3
2′	8.25 d ($J_{H\text{-}F}$ = 2.4)	152.5/152.4 (12.5)	CH
4′	-	152.1/152.0 (12.5)	C
5′	-	145.1/143.1 (254.4)	C
6′	-	155.7/155.6 (15)	C
7′	2.66 qd (6.5, 2.4)	23.9 d	CH_2
8′	1.22 t (7.5)	16.3	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques.

Two-dimensional NMR Spectroscopy:

The C-20 methine proton (δ 4.07) displayed cross peaks in COSY-45º and TOCSY spectra with C-21 methyl protons (δ 1.17), N_b-H (δ 5.31), NCHO (δ 8.06), C-17 methine proton (δ 1.29) and C-16 methylene protons (δ 1.10 and 1.90), which clearly indicated the substitution of *N*-formyl amino group at C-20. The key COSY and TOCSY correlations are presented in scheme-8.1.7.



Scheme-8.1.7: Key ^{1}H-^{1}H COSY 45º and TOCSY interactions in compound **5.**

The HMBC correlations were used to join the different functionalities with each other. The N_a-Me protons (δ 3.01) displayed the HMBC correlations with C-3 (δ 56.6) and C-4′ (δ 152.0), which supported that 6-ethyl- 5-fluoropyrimidine ring was connected to C-3 amino group through C-4′. The H-2′ showed connectivities with C-4′ and C-6′ in HMBC spectrum. The C-20 methine proton (δ 4.07) displayed HMBC correlations with C-17 (δ 57.0) and formamide carbonyl carbon (δ 159.9). Key HMBC correlations of compound **5** are shown in Fig. 8.1.15.

The stereochemical assignments in compound **5** are based on biogenetic consideration, many of which were supported by NOESY spectrum. The C-3 methine proton (δ 4.36) was assigned as *axial* on the basis of its $W_{1/2}$ = 18.1 Hz value,[139] representing an *equatorial* orientation of the

geminal C-3 amino group. All the key NOESY interactions of compound **5** are presented in Fig. 8.1.16.

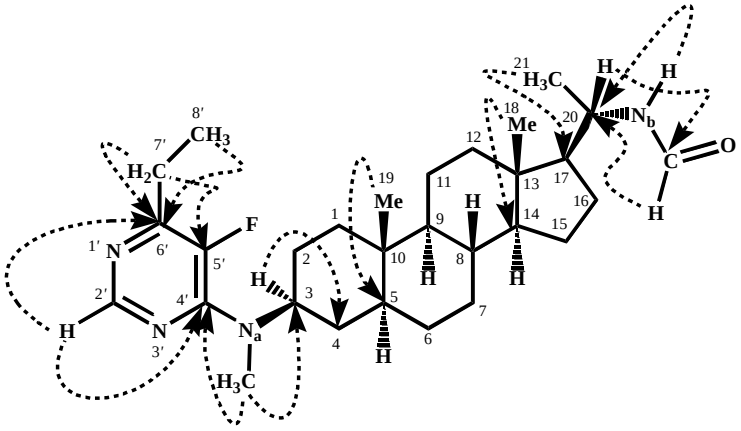


Fig. 8.1.15: Key HMBC interactions in compound **5.**

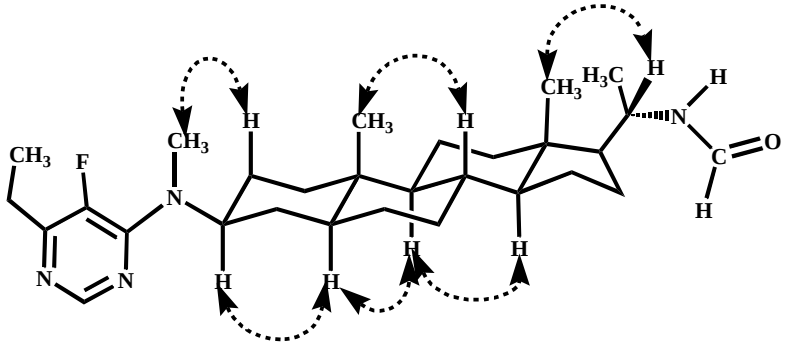
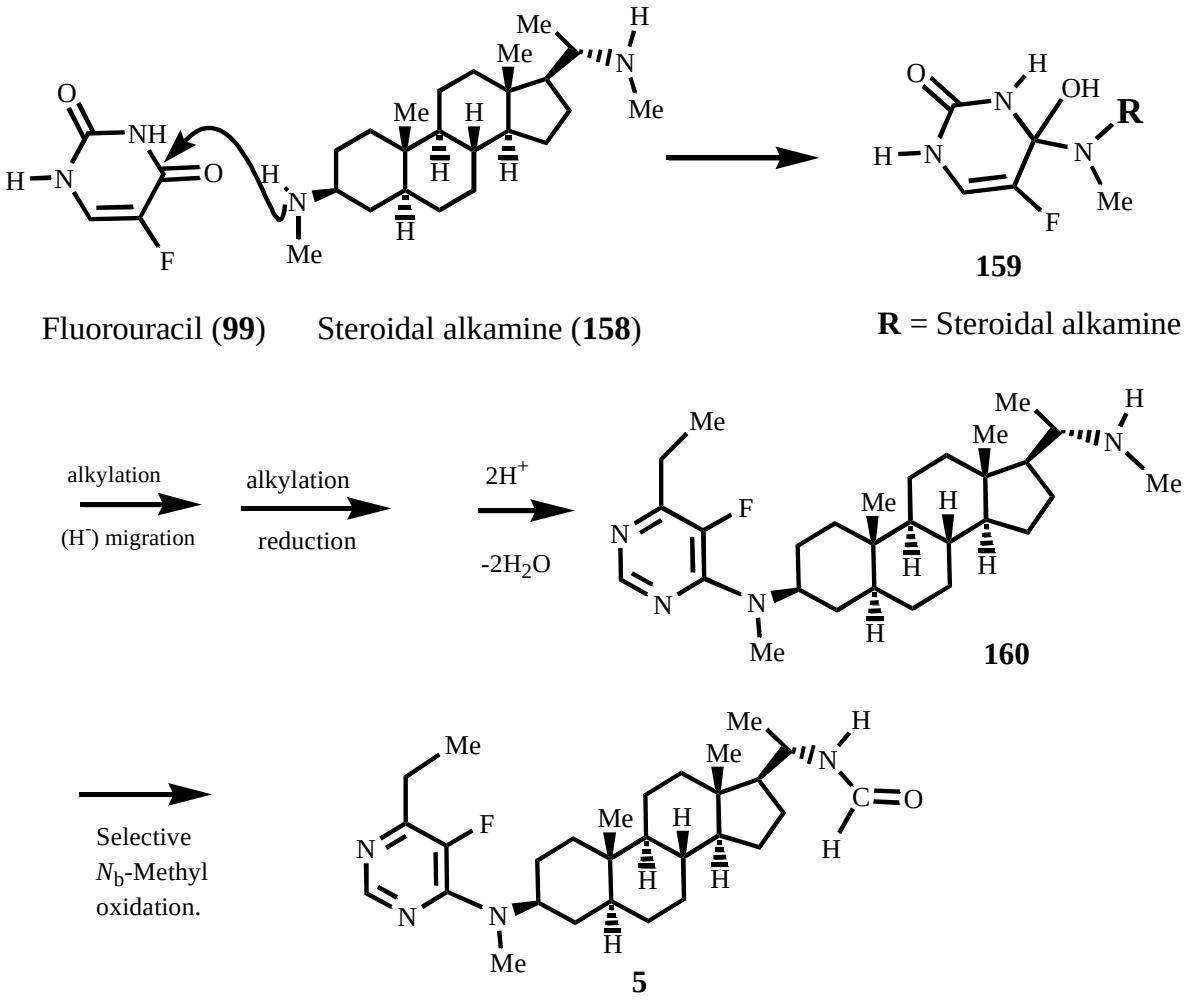


Fig. 8.1.16: Key NOESY interactions in compound **5.**

From the above mentioned spectroscopic studies, the structure of compound **5** was deduced as {(3*β*, 20*S*)-20-(*N*-formyl)-3-[6-ethyl-5-fluoro-4-pyrimidinyl) (methyl) amino]-5α-pregnane}, trivially named as adhikarimine E.

Biogenesis of adhikarimine-E (5):

A proposed biogenesis of compound **5** is presented in Scheme-8.1.8

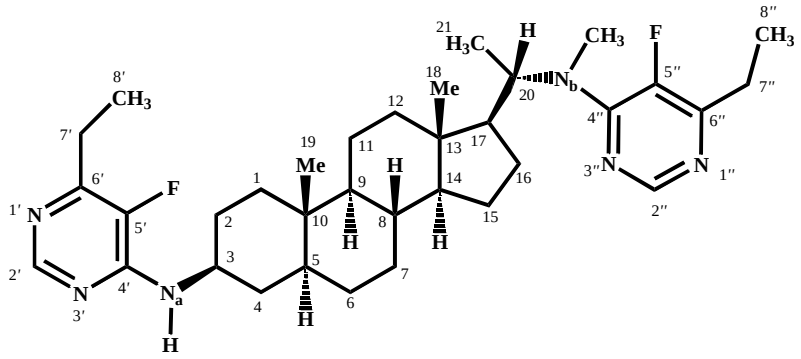


Scheme-8.1.8: Proposed biogenesis of compound **5.**

8.1.6 Adhikarimine F (6)

The dichloromethane fraction of *S. coriacea* extract (at pH 8-9) yielded a white amorphous compound **6** (Experimental Section, Scheme-9.3, Page-133), the optical rotation of which was found to be $[\alpha]_D^{25} = +11^o$ (*c* = 0.02, MeOH), indicated its chiral nature.

The UV spectrum displayed absorptions at λ_{max} 285 and 248.4, and λ_{min} 367 and 219.8 nm, characteristic of a hetero-aromatic ring.[137] The IR spectrum displayed absorptions at 3394 (NH), 2926 (CH), 1734 (NCOH), 1595 and 1502 (C=C aromatic) cm^{-1}.



Adhikarimine F (**6**)

Molecular formula and mass spectrometry:

The EI MS displayed the M^+ peak at *m/z* 580, which was further confirmed by FAB $^{+ve}$ MS (*m/z* 581). The HREI MS of compound **6** showed the exact M^+ at *m/z* 580.8253, corresponding to the formula $C_{34}H_{50}N_6F_2$ (calcd 580.8250), with twelve degrees of unsaturation. The EI MS supported the presence of 6-ethyl-5-fluoropyrimidine moiety at C-20 amino group by the presence of base peak at *m/z* 182 $[C_9H_{13}N_3F]^+$, resulted from the cleavage of C-17/C-20. The EI MS also showed an ion at *m/z* 142 which indicated the presence of an additional 6-ethyl-5-fluoropyrimidine ring, attached to the C-3 amino group. Key mass fragmentation in compound **6** is presented in Fig. 8.1.17.

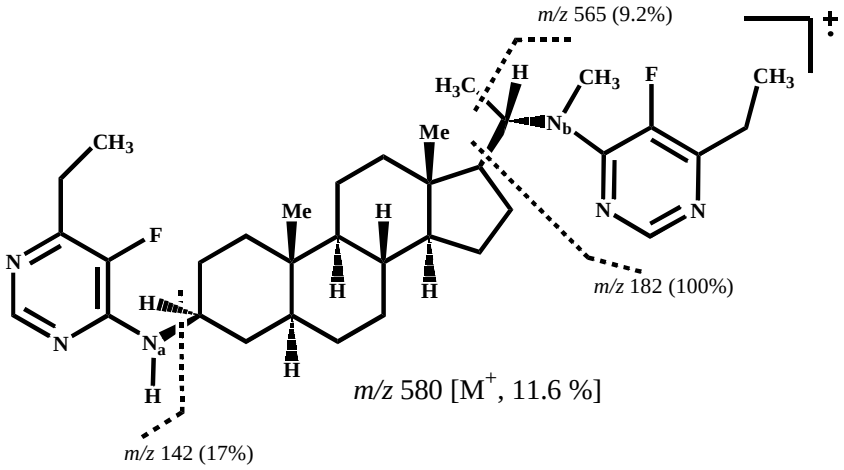


Fig. 8.1.17: Key mass fragmentation in compound **6.**

One –dimensional NMR Spectroscopy:

The ^{1}H- NMR spectrum of compound **6** (Table-8.1.6) was found to be similar to previous compounds, with only difference being the presence of two fluoropyrimidine rings substituted at C-3 and C-20 amino groups. A 1H multiplet at δ 1.60 was attributed to C-20 methine proton. The up-field shift of this signal was due to the shielding effect of the pyrimidine ring, substituted to the amino group. A downfield doublet at δ 2.96 ($J_{H\text{-}F}$ = 4.0 Hz) was attributed to the N_b-Me protons. A 1H broad multiplet at δ 3.95 ($W_{1/2}$ = 21.4 Hz) was assigned to the C-3α proton, representing equatorial orientation of the geminal amino group.[139]

A 6H triplet at δ 1.22 ($J_{8',\,7'}$ = 7.5 Hz) was assigned to the C-8′ and C-8″ methyl protons, substituted on the pyrimidine ring, while a 4H signal at δ 2.66 (q d, $J_{H\text{-}H}$ = 6.5 Hz, $J_{H\text{-}F}$ = 2.4 Hz) was assigned to the C-7′ and C-7″ methylene protons. Two downfield 1H doublets at δ 8.23 and 8.26 ($J_{H\text{-}F}$ = 2.3 Hz) were assigned to the C-2′ and C-2″ protons, respectively.

The broad-band (BB) decoupled ^{13}C-NMR spectrum of compound **6** (Table-8.1.6) displayed resonances for 34 carbons. The DEPT spectrum showed signals for six methyl, eleven methylene, nine methine, and eight quaternary carbons.

Following structural features were thus deduced from the spectroscopic data:

A) A basic pregnane- type steroidal skeleton

B) Two 6-ethyl-5-fluoropyrimidine rings.

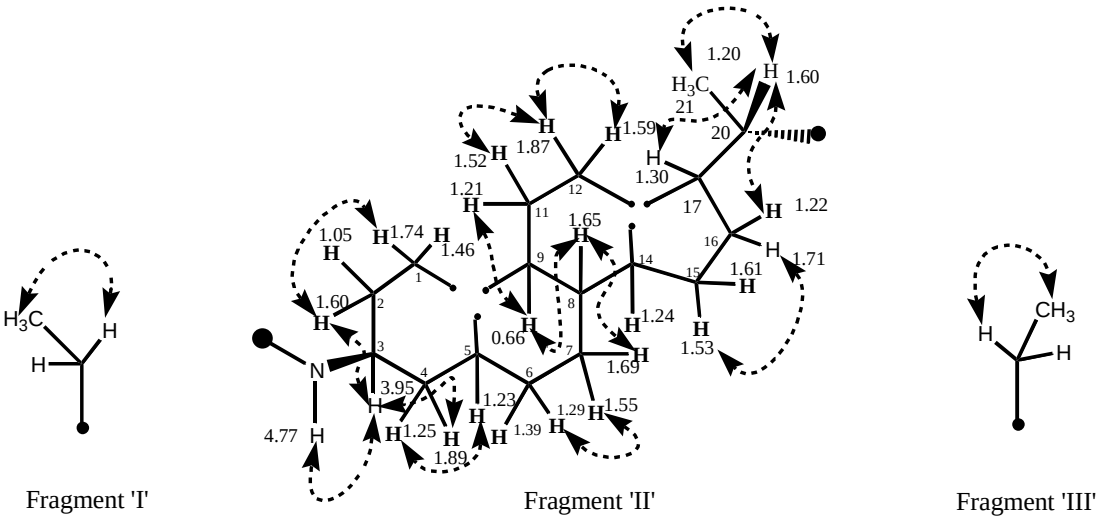
***Two-dimensional NMR Spectroscopy*:**

Two-dimensional NMR experiments such as COSY 45^{o}, TOCSY, HMQC, and HMBC were used to deduce the structure of the compound **6.** Three spin systems (I, II, III) were identified on the basis of COSY and TOCSY spectra, which are shown in Scheme-8.1.9.

Table. 8.1.6: ^{1}H- and ^{13}C- NMR chemical shift values of compound **6** (500 and 100 MHz, $CDCl_3$, ppm).

C. No	^{1}H-NMR δ (*J* and $W_{½}$ in Hz)	^{13}C-NMR δ ($J_{C\text{-}F}$ in Hz)	Multiplicity
1	1.74 , 1.46 (m)	37.4	CH_2
2	1.60 , 1.05 (m)	23.5	CH_2
3	3.95 br m ($W_{1/2}$ = 21.4 Hz)	49.9	CH
4	1.89 , 1.25 (m)	29.0	CH_2
5	1.23 (m)	45.3	CH
6	1.39 , 1.29 (m)	28.5	CH_2
7	1.69 , 1.55 (m)	31.9	CH_2
8	1.65 (m)	35.4	CH
9	0.66 (m)	54.3*	CH
10	-	35.5*	C
11	1.21 , 1.52 (m)	21.1	CH_2
12	1.59 , 1.87 (m)	39.5	CH_2
13	-	41.9	C
14	1.24 (m)	54.0	CH
15	1.53, 1.61 (m)	26.5	CH_2
16	1.22, 1.71 (m)	35.5	CH_2
17	1.30 (m)	56.6	CH
18	0.74 s	12.0	CH_3
19	0.82 s	12.2	CH_3
20	1.60 (m)	54.3*	CH
21	1.20 d (6.5)	18.3	CH_3
N_b-CH_3	2.96 d ($J_{H\text{-}F}$ = 4.0)	30.0	CH_3
N_a-H	4.77 d	-	-
2′	8.23 d ($J_{H\text{-}F}$ = 2.3)	153.3/153.2 (12.5) *	CH
4′	-	152.1/152.0(12.5) *	C
5′	-	145.2/142.7 (254.4) *	C
6′	-	155.7/155.5(15) *	C
7′	2.66 qd (6.5, 2.4)	23.9 d	CH_2
8′	1.22 t (7.5)	12.3 *	CH_3
2″	8.26 d ($J_{H\text{-}F}$ = 2.3)	152.5/152.4 *	CH
4″	-	151.6/151.4 *	C
5″	-	144.6/142.1 *	C
6″	-	152.2/152.1 *	C
7″	2.66 qd (6.5, 2.4)	23.9	CH_2
8″	1.22 t (7.5)	12.7 *	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques. * Assignments are interchangeable



Scheme- 8.1.9: Key COSY 45º and TOCSY interactions in compound **6.**

The linkages between the fragments ‘I’ and ‘II’ and ‘III’, as well as the position of various functionalities, were deduced from HMBC cross peaks. The N_a-H proton (δ 4.77) displayed HMBC interactions with C-3 (δ 49.9) and C-4′ (δ 152.1). Similarly N_b-Me protons (δ 2.96) showed HMBC correlations with C-20 (δ 54.3) and C-4″ (δ 151.6). The key HMBC interactions are shown in Fig. 8.1.18.

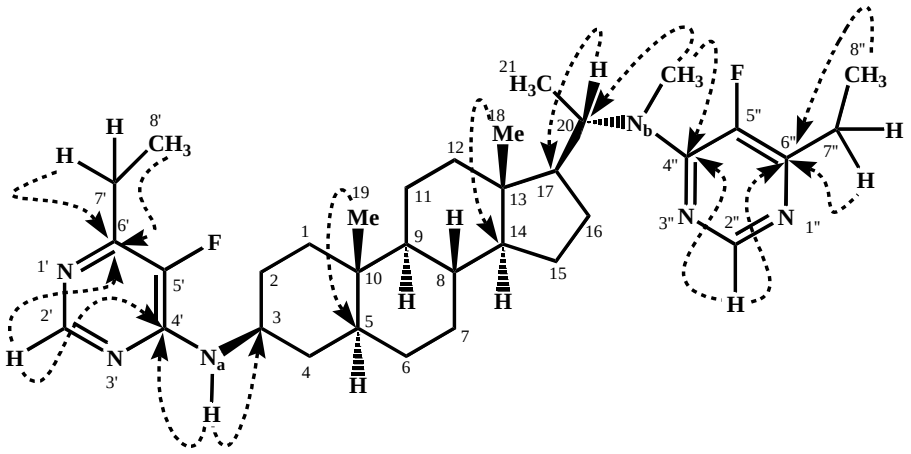


Fig. 8.1.18: Key HMBC interactions in compound **6.**

The stereochemistry of compound **6** was assigned on the biogenesis basis,[145] and NOESY correlations. The C-3 methine proton (δ 3.95, br m) was assigned to be *axial* on the basis of its

$W_{1/2}$ = 21.4 Hz, thus representing an equatorial orientation of the geminal C-3 amino group.[139] The key NOESY interactions in compound **6** are presented in Fig. 8.1.19.

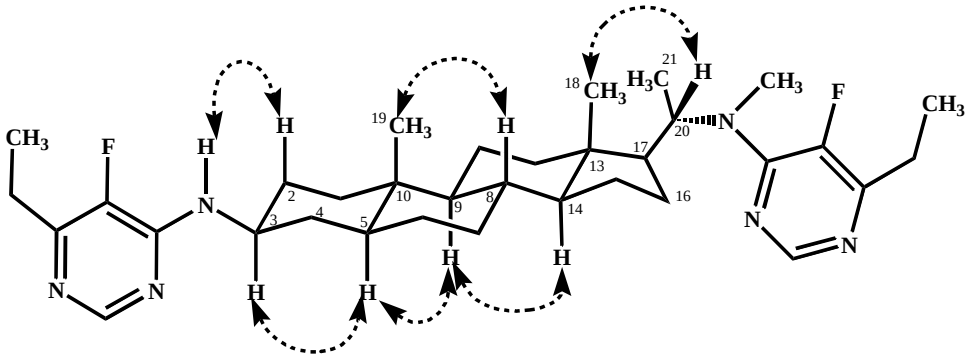


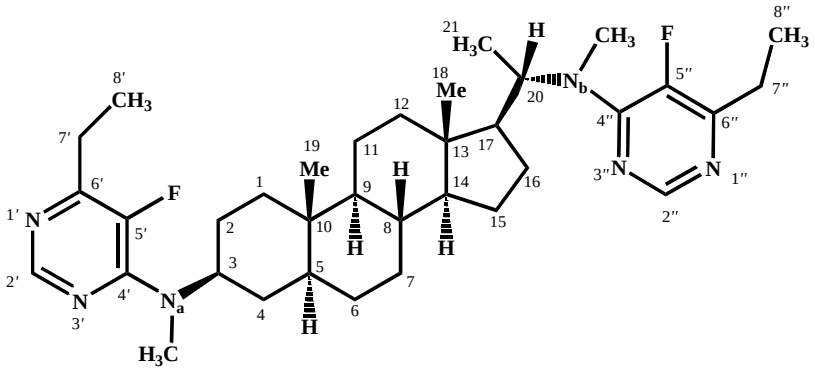
Fig. 8.1.19: Key NOESY interactions in compound **6.**

From the above discussion, the structure of compound **6** was deduced as {(3*β*, 20*S*) - 20-[(6-ethyl-5-fluoro-4-pyrimidinyl) (methyl) (amino)]-3-[(6-ethyl-5-fluoro-4-pyrimidinyl) (amino)]-5α-pregnane}, which was trivially named as adhikarimine F.

8.1.7 Adhikarimine G (7)

Adhikarimine G (**7**) was isolated as a white amorphous solid from the column chromatography of the sub-fraction A_1 (Experimental Section, Scheme-9.2, Page-132) on neutral alumina by using EtOAc/pet. ether as eluent. Compound **7** showed a positive alkaloidal test.

The optical rotation of compound **7**, $[\alpha]_D^{25}$ = + 10.6º (*c* = 0.03, MeOH), exhibited its chiral nature. The UV spectrum showed absorption maxima at 286 and 255 nm, representing a hetero-aromatic ring.[137] The IR spectrum showed characteristic absorptions at 2933 (CH), 1591 and 1493 (C=C, aromatic) cm^{-1}.



Adhikarimine G (**7**)

Molecular formula and mass spectrometry:

The EI MS exhibited the M^+ at *m/z* 594, further supported by FAB $^{+ve}$ MS (*m/z* 595). The HREI MS showed the M^+ at *m/z* 594.8343, corresponding to the formula $C_{35}H_{52}N_6F_2$ (calcd 594.8341), with twelve degrees of unsaturation. The base peak in EI MS at *m/z* 182 indicated the presence of

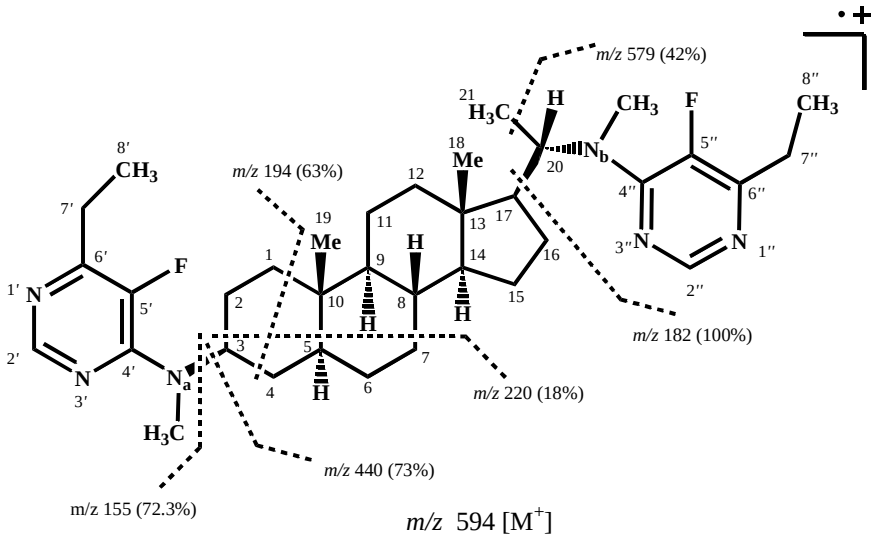


Fig. 8.1.20: Key mass fragmentation in compound **7.**

6-ethyl-5-fluoropyrimidine ring substituted at C-20 amino group, while the peak at *m/z* 155 indicated the presence of an additional 6-ethyl-5-fluoropyrimidine ring, substituted at the C-3 amino group. Key mass fragmentation pattern of compound **7** is presented in Fig. 8.1.20.

***One-dimensional NMR Spectroscopy*:**

The ^{1}H-NMR spectrum of compound **7** (Table-8.1.7) was found to be distinctly similar to that of compound **6** with the only difference being an additional methyl group, substituted at C-3 amino group. The ^{1}H-NMR spectrum displayed two 3H up-field singlets at δ 0.75 and 0.83, ascribed to the C-18 and C-19 angular methyls, respectively. A 3H doublet at δ 1.18 ($J_{21,\ 20}$ = 6.5 Hz) was assigned to C-21 secondary methyl protons. A 1H multiplet at δ 1.60 was assigned to the C-20 methine proton. Its up-field shift was due to the shielding effect of the *N*-pyrimidine ring, while two downfield 3H doublets at δ 2.96 ($J_{H\text{-}F}$ = 4.1 Hz) and 3.03 ($J_{H\text{-}F}$ = 3.8 Hz) were assigned to the N_b-CH_3 and N_a-CH_3 protons, respectively. A 1H broad singlet at δ 4.37 ($W_{1/2}$ = 17.0 Hz) was ascribed to C-3α methine proton.[139]

A 6H triplet at δ 1.23 ($J_{8',\ 7'}$ = 7.5 Hz) was attributed to the C-8′ and C-8″ methyl groups, substituted on the pyrimidine ring. A 4H quartet of a doublet at δ 2.67 ($J_{H\text{-}H}$ = 6.5 Hz, $J_{H\text{-}F}$ = 2.4 Hz) was assigned to the C-7′ and C-7″ methylene protons, while a downfield 2H doublet at δ 8.24 ($J_{H\text{-}F}$ = 2.1 Hz) was assigned to C-2′ and C-2″ protons of pyrimidine substituent, respectively.

The broad-band (BB) decoupled ^{13}C-NMR spectrum of compound **7** (Table-8.1.7) displayed resonances for all 35 carbons. The DEPT spectrum showed signals for seven methyl, eleven methylene, nine methine, and eight quaternary carbons.

Following structural features for compound **7** were deduced from spectroscopic studies:

C) a basic pregnane- type steroidal skeleton

D) Two 6-ethyl-5-fluoropyrimidine rings.

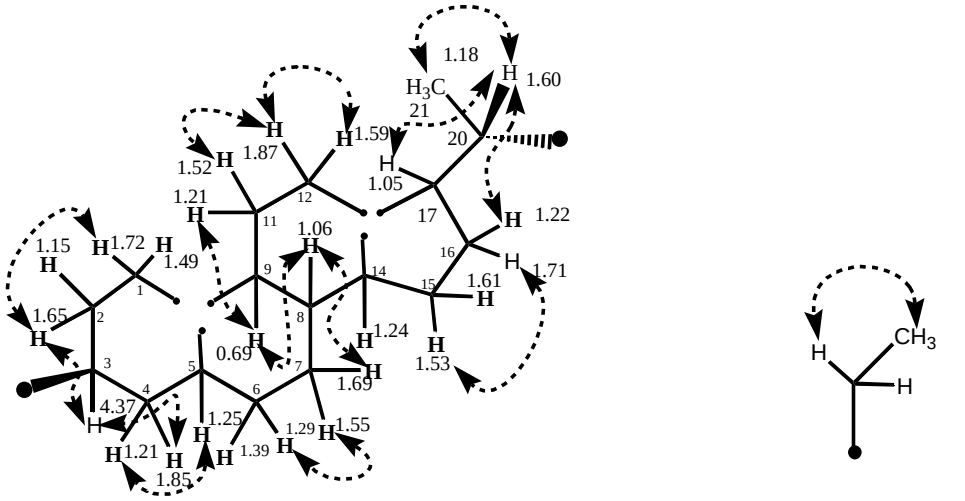
Table. 8.1.7: ^{1}H- and ^{13}C- NMR chemical shift values of compound **7** (300 and 150 MHz, $CDCl_3$, ppm)

C. No	^{1}H-NMR δ (*J* and $W_{½}$ in Hz)	^{13}C-NMR δ ($J_{C\text{-}F}$ in Hz)	Multiplicity
1	1.72 , 1.49 (m)	37.5	CH_2
2	1.65 , 1.15 (m)	25.1	CH_2
3	4.37 br m ($W_{1/2}$ = 17.0 Hz)	56.5 d	CH
4	1.85 , 1.21 (m)	31.7	CH_2
5	1.25 (m)	45.6	CH
6	1.34 , 1.26 (m)	24.7	CH_2
7	1.65 , 1.55 (m)	32.5	CH_2
8	1.06 (m)	35.4	CH
9	0.69 (m)	55.0	CH
10	-	35.5	C
11	1.21 , 1.52 (m)	21.1	CH_2
12	1.59 , 1.87 (m)	39.5	CH_2
13	-	41.9	C
14	1.24 (m)	41.3	CH
15	1.53, 1.61 (m)	26.5	CH_2
16	1.22, 1.71 (m)	31.9	CH_2
17	1.05 (m)	56.6	CH
18	0.75 s	12.3	CH_3
19	0.83 s	12.4	CH_3
20	1.60 (m)	54.2 d	CH
21	1.18 d (6.5)	18.4	CH_3
N_b-CH_3	2.96 d ($J_{H\text{-}F}$ = 4.1)	31.1	CH_3
N_a-CH_3	3.03 d ($J_{H\text{-}F}$ = 3.8)	34.4	CH_3
2′	8.24 d ($J_{H\text{-}F}$ = 2.3)	152.5/152.4 (12.5)	CH
4′	-	152.1/152.0 (12.5)*	C
5′	-	145.0/143.2 (254.4)*	C
6′	-	155.6/155.5 (15)*	C
7′	2.67 q d (6.5, 2.4)	23.9 d	CH_2
8′	1.23 t (7.5)	12.7 *	CH_3
2″	8.24 d ($J_{H\text{-}F}$ = 2.3)	152.5/152.4	CH
4″	-	152.0/151.9 *	C
5″	-	144.7/143.0 *	C
6″	-	155.5/155.4 *	C
7″	2.67 q d (6.5, 2.4)	23.8 d *	CH_2
8″	1.23 t (7.5)	12.9 *	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques. * Assignments are interchangeable.

***Two-dimensional NMR Spectroscopy*:**

Two-dimensional NMR experiments (COSY 45º, TOCSY HMQC, and HMBC) were used to deduce the structure of the compound **7.** The main spin system fragment 'I', comprising of main skeleton of compound **7** was deduced from the vicinal couplings of C-3 methine proton (δ 4.37) with C-2 (δ 1.15 and 1.65), and C-4 methylene protons (δ 1.21 and 1.85). Furthermore, the C-20 methine proton at δ 1.60, showed couplings with C-17 methine proton (δ 1.05) and C-21 methyl protons (δ 1.18). The key homonuclear couplings of fragment 'I' is presented in Scheme-8.1.10.



Fragment- I

Fragment-II

Scheme-8.1.10: Key COSY 45 º and TOCSY interactions in compound **7.**

The connectivity between the fragments 'I' and 'II', as well as the position of various functionalities, were deduced from the long- range interactions between protons and carbons (HMBC). The N_a-Me protons (δ 3.03) displayed HMBC interactions with C-3 (δ 56.5) and C-4′ (δ 152.1). Similarly N_b-Me protons (δ 2.96) showed HMBC correlations with C-20 (δ 54.2) and C-4″ (δ 152.0). The key HMBC interactions are shown in Fig. 8.1.21.

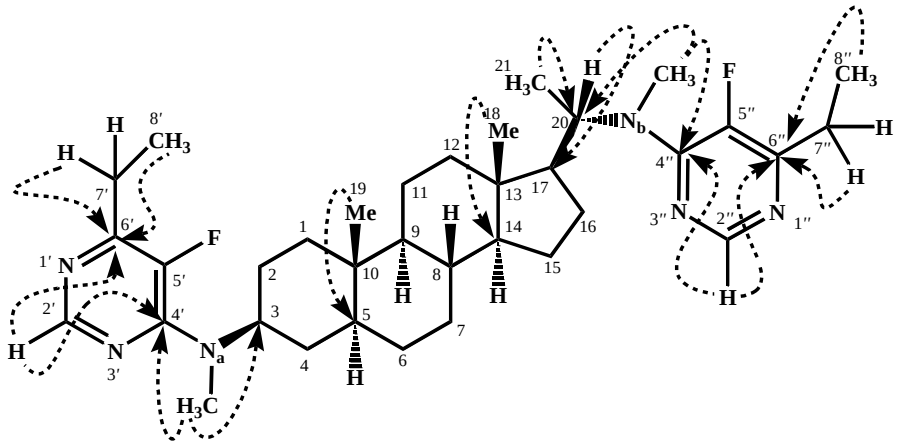


Fig. 8.1.21: Key HMBC interactions in compound **7.**

The stereochemistry of compound **7** was mainly assigned on biogenetic basis.[145] The NOESY correlations and chemical shift/coupling constant values have also contributed in the stereochemical assignment. The C-3 methine proton (δ 4.37, br m) was deduced to be *axial* on the basis of its $W_{1/2}$ = 17.0 Hz, thus indicating an equatorial orientation of geminal C-3 amino group.[139] Stereochemistry at other centers were assigned on the basis of NOESY correlations, as shown in Fig. 8.1.22.

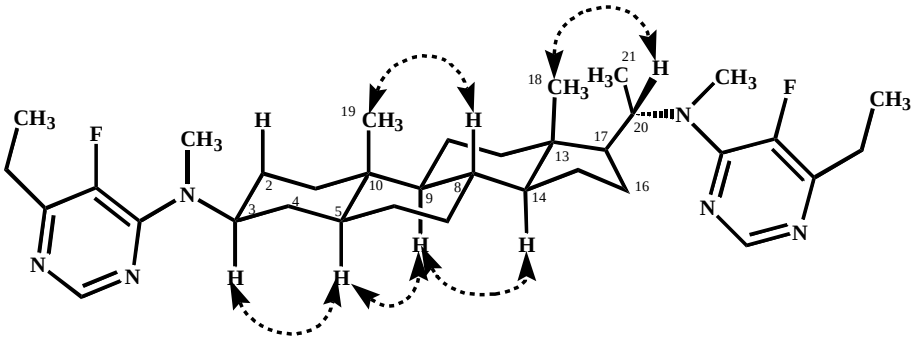
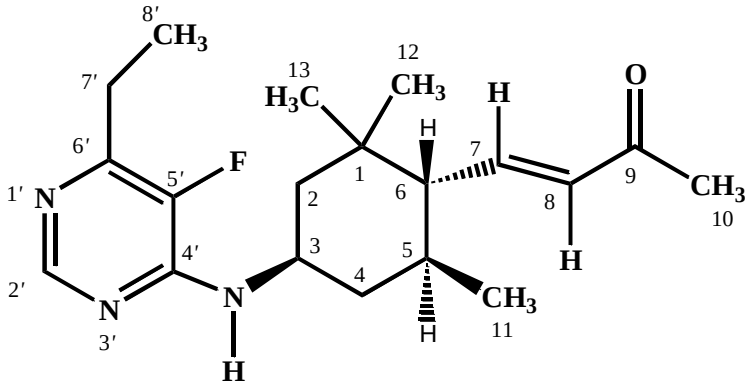


Fig. 8.1.22: Key NOESY interactions in compound **7.**

From the above spectroscopic arguments, the structure of compound **7** was deduced as {(3*β*, 20*S*)-3,20-[(6-ethyl-5-fluoro-4-pyrimidinyl)(methyl) (amino)]-5α-pregnane}, which was trivially named as adhikarimine G.

8.1.8 Adhikarione (8)

Compound **8** was isolated as a gummy material from the column chromatography of the sub-fraction D_6 (Experimental Section, Scheme-9.3, Page-133) on neutral alumina by using EtOAc/pet. ether as eluent. Compound **8** showed an orange-red color test on silica gel TLC plate when sprayed with the Dragendorff's spray reagent. The optical rotation of compound **8**, $[\alpha]_D^{25}$ = + 7.6° (*c* = 0.034, MeOH), indicated the presence of chiral centers in the molecule. The UV spectrum showed absorption maxima at 273.4 and 240.6 nm, while the IR spectrum displayed absorptions at 3346 (NH), 1669 (C=O), 1617 and 1511 (C=C, aromatic) cm^{-1}.



Adhikarione (**8**)

Molecular formula and mass spectrometry:

The M^+ of **8** was at *m/z* 333 by EI MS, which was further confirmed by FAB^{+ve} MS (*m/z* 334). The HREI MS showed the M^+ at *m/z* 333.4522, ($C_{19}H_{28}N_3OF$, calcd 333.4520), with seven degrees of unsaturation. In EI MS, the base peak at *m/z* 142 indicated the presence of 6-ethyl-5-fluoropyrimidine ring, attached to the C-3 amino group. The peak at *m/z* 318 was due to loss of a methyl group, attached with carbonyl group. The peak at *m/z* 290 was due to the cleavage of C-8/C-9 bond. The key mass fragmentation pattern of compound **8** is shown in Fig. 8.1.23.

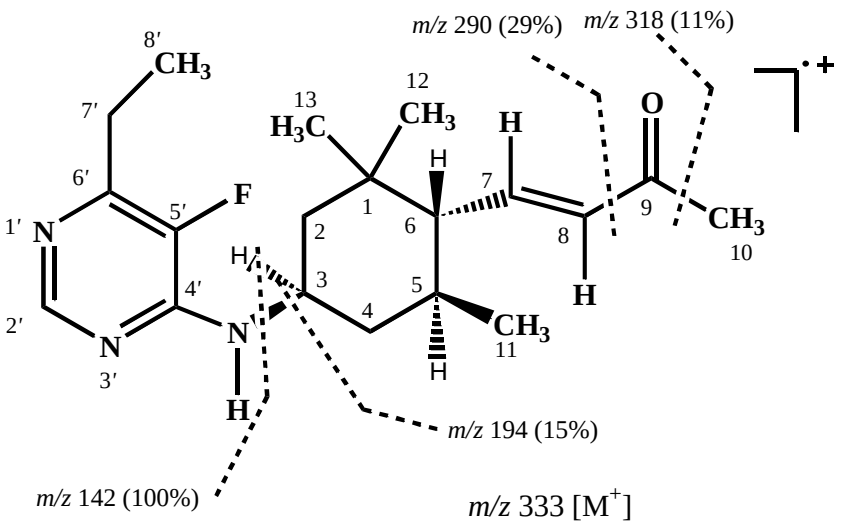


Fig. 8.1.23: Key mass fragmentation in compound **8**.

One-dimensional NMR Spectroscopy:

The ^{1}H-NMR spectrum of compound **8** was substantially different from compounds **1-7**. It displayed a 3H doublet at δ 0.81 ($J_{11,\,5}$ = 6.5 Hz), and two 3H singlets at 1.01 and 0.89, which were ascribed to the protons of C-11, C-12, and C-13 methyl groups of ionone skeleton, respectively. A 3H singlet at δ 2.24 was assigned to the C-10 methyl protons, while a 1H broad multiplet at δ 4.21 ($W_{1/2}$ = 21.8 Hz) was assigned to C-3 methine proton. A 1H downfield doublet at δ 6.03 ($J_{8,\,7}$ = 15.8 Hz) was attributed to the C-8 olefinic proton, while a double doublet at δ 6.52 ($J_{7,\,8}$ = 15.8 Hz, $J_{7,\,6}$ = 10.2 Hz) was assigned to *trans* coupled C-7 olefinic proton. Above mentioned ^{1}H NMR data are matched with previously reported data of ionone skeleton.[146]

A 3H triplet at δ 1.23 ($J_{8',\,7'}$ = 7.5 Hz) was due to the C-8′ methyl protons, substituted on the pyrimidine ring. A downfield 2H quartet of a doublet at δ 2.66 ($J_{\text{H-H}}$ = 7.5 Hz, $J_{\text{H-F}}$ = 2.5 Hz) was assigned to the C-7′ methylene protons, A 1H doublet at δ 8.27 ($J_{\text{H-F}}$ = 1.3 Hz) was assigned to H-2′ of pyrimidine substituent.

The broad-band (BB) decoupled ^{13}C-NMR spectrum of compound **8** (Table-8.1.8) displayed signals for all 19 carbons. The DEPT spectrum showed signals for five methyl, three methylene, six methine, and five quaternary carbons. This indicated that compound **8** was not a steroidal alkaloid, like **1**-**7**.

Table- 8.1. 8: ^{1}H- and ^{13}C- NMR chemical shift values of compound **8** (400 and 75 MHz, ppm in $CDCl_3$).

C. No	**^{1}H-NMR δ (*J* and W½ in Hz)**	**^{13}C-NMR δ (J_{C-F} in Hz)**	**Multiplicity**
1	-	35.3	C
2	1.15, 1.81 (m)	47.6	CH_2
3	4.21 br, m ($W_{1/2}$ = 21.8)	45.5	CH
4	0.89, 2.20 (m)	41.7	CH_2
5	0.87 (m)	30.9	CH
6	1.53 t (10.2)	57.8	CH
7	6.52 dd (15.8, 10.2)	148.6	CH
8	6.03 d (15.8)	133.9	CH
9	-	197.9	C
10	2.24 s	27.1	CH_3
11	0.81 d (6.5)	21.3	CH_3
12	1.01 s	21.0	CH_3
13	0.89 s	31.1	CH_3
N-H	4.67d (*J* = 6.7)	-	-
2′	8.27 d (J_{H-F} = 1.3)	153.3/153.2 (10.1)	CH
4′	-	151.5/151.3 (10.8)	C
5′	-	145.0/141.6 (252.5)	C
6′	-	152.4/152.2 (15)	C
7′	2.66 q d (7.5, 2.5)	23.5	CH_2
8′	1.23 t (7.5)	16.3	CH_3

All chemical shift assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC, HMBC and DEPT NMR techniques.

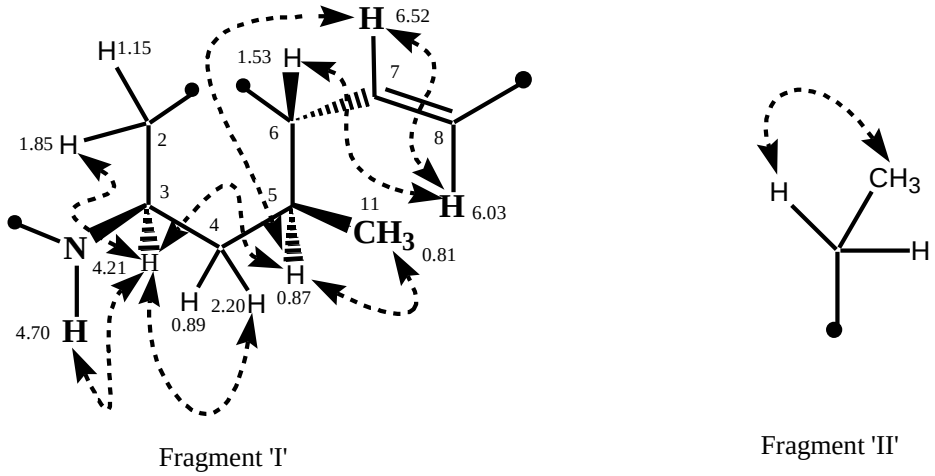
Following structural features for compound **8** were deduced from spectroscopic studies:

E) A basic Ionone-type skeleton.

F) A 6-ethyl-5-fluoropyrimidine ring, connected to ionone skeleton, through an amino group.

***Two-dimensional NMR spectroscopy*:**

The extensive review of ^{1}H-^{1}H COSY 45º and TOCSY spectra of compound **8** indicated a main spin system 'I', representing an ionone skeleton, and a small spin system 'II', comprising on the ethyl substituent of the pyrimidine ring. The main spin system 'I' was deduced from the vicinal couplings of C-3 methine proton (δ 4.21, $W_{1/2}$ = 21.8 Hz) with C-2 (δ 1.15 and 1.81) and C-4 methylene protons (δ 0.89 and 2.20). The C-3 methine proton also showed correlation with N-H (δ 4.67). Additionally C-3 methine proton also displayed cross peaks in TOCSY with C-5 methine proton (δ 0.87), which was coupled with C-6 methine proton (δ 1.53). The C-8 olefinic proton, resonated at δ 6.03, showed coupling with C-7 olefinic proton (δ 6.50), which showed cross peaks in TOCSY spectrum with C-6 and C-5 methine protons. Key COSY and TOCSY correlations of compound **8** are presented in scheme-8.1.11.



Fragment 'I' Fragment 'II'

Scheme-8.1.11: Key COSY 45º and TOCSY interactions in compound **8.**

Long-range HMBC interactions between the protons and carbons were employed to join fragments 'I' and 'II' together. The C-3 methine proton (δ 4.21) displayed the HMBC interactions with C-2 (δ 47.6) and C-4 (δ 41.7). The *N*-H (δ 4.67) displayed HMBC correlations with C-4′ (δ 151.5) and C-3 (δ 45.5). This indicated that C-4′ of 6-ethyl-5-fluoropyrimidine ring was connected to the C-3 amino group. The C-7 proton exhibited HMBC cross peaks with C-9 (δ 197.9), C-8 (δ 133.9), C-6 (δ 57.8), C-1 (δ 35.3) and C-5 (δ 30.9). Key HMBC interactions in compound **8** are presented in Fig. 8.1. 24.

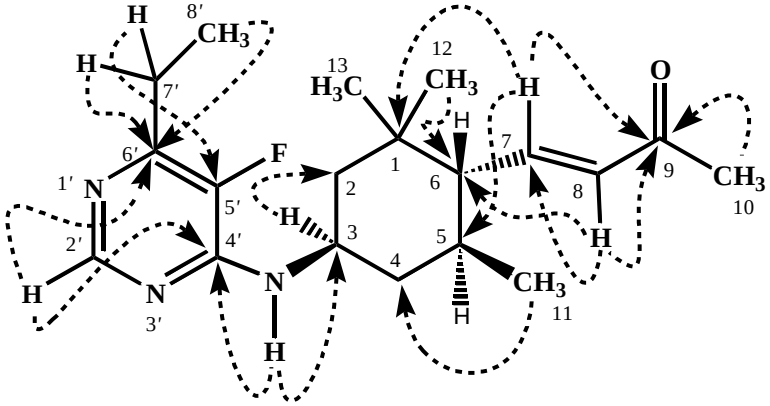


Fig. 8.1.24: Key HMBC interactions in compound **8**.

The stereochemistry and configuration in compound **8** were assigned with the help of NOESY cross peaks, and comparison of chemical shift/coupling constants data with the reported data.[146] The orientation of C-3 methine proton (δ 4.20, br m), was deduced to be *axial* on the basis of its $W_{1/2}$ = 21.8 Hz, indicating an *equatorial* orientation of the geminal C-3 amino group.[139] *Trans* orientation of C-7 and C-8 olefinic protons was deduced from their coupling constant ($J_{7,8}$ = 15.8 Hz). The *axial* orientation of C-6 proton (δ 1.53) was again deduced on the basis of coupling constant value (10.2 Hz), indicating an *equatorial* orientation of the geminal side chain.[146] The *axial* orientation of C-5 methine proton was deduced on the basis of coupling constant value ($J_{6,5}$ = 10.2 Hz). The axially oriented C-3 methine proton displayed cross peaks with C-12 methyl

protons (δ 1.01) and H-5 (δ 0.87) in NOESY spectrum. Key NOESY correlations in compound **8** are presented in Fig. 8.1.25.

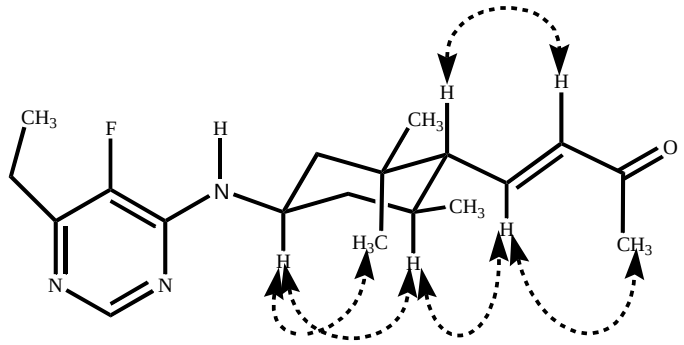
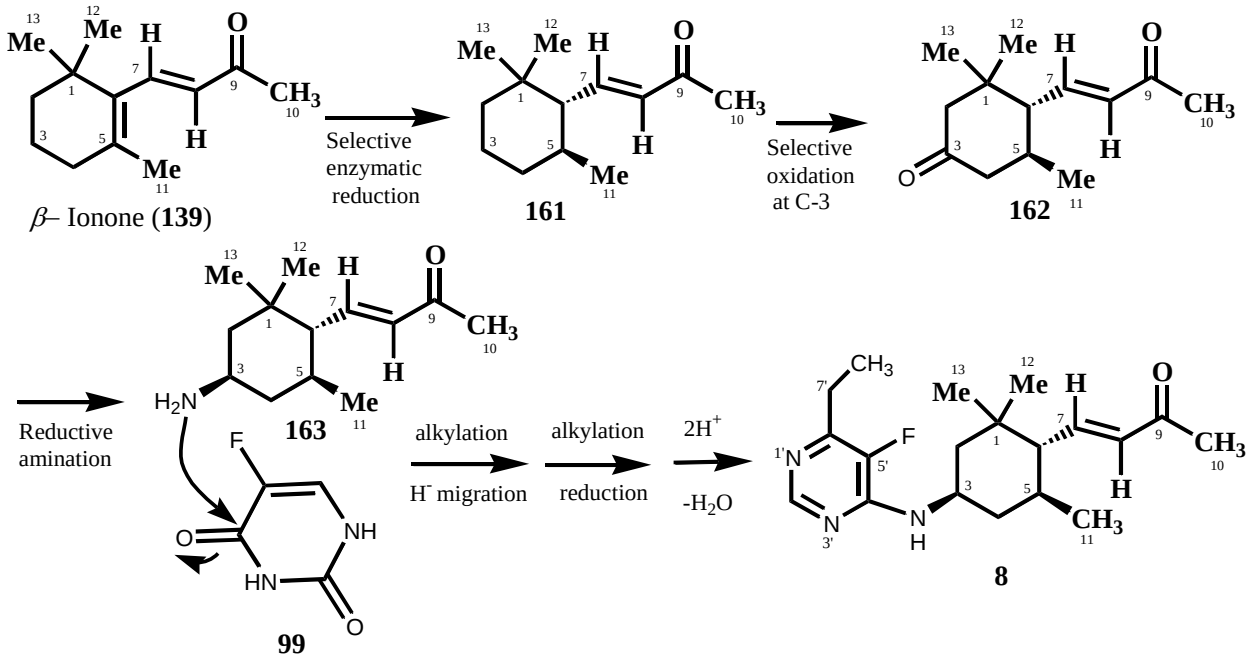


Fig. 8.1.25: Key NOESY interactions in compound **8.**

In the light of above spectral data, the structure of compound **8** was elucidated as {(3, 5 *β*), (6*α*), E-4-{4-[(6-ethyl-5-fluoro-4-pyrimidinyl) amino]-2, 2, 6-trimethylcyclohexyl}-3-buten-2-one}, which was trivially named as adhikarione.

Biogenesis of adhikarione (8):

A proposed biogenesis of compound **8** is presented in Scheme-8.1.12.



Scheme-8.1.12: Plausible biogenesis of compound **8.**

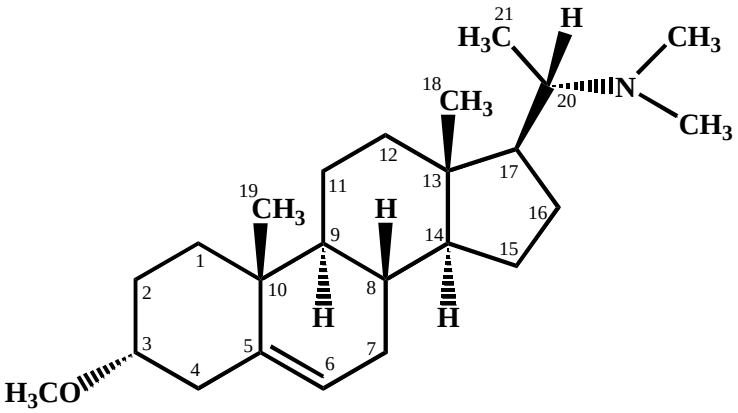
8.2 Known Steroidal Alkaloids

The following known pregnane-type steroidal alkaloids **9-17** were isolated from *S. coriacea*.

8.2.1 Alkaloid C (9)

Compound **9** was isolated as white needles from the pet. ether fraction of extract of roots of *S. coriacea* (Experimental Section, Scheme -9.5, Page-134), by repeated elution of sub fraction A on neutral alumina column with increasing polarities of EtOAc/pet. ether.

The optical rotation of compound **9** $[\alpha]_D^{25}$ = - 28° (*c* 0.03, $CHCl_3$), displayed UV absorption at 242 nm. The HREI MS of compound showed the M^+ at *m/z* 359.3128 ($C_{24}H_{41}NO$, calcd 359.3125).



Alkaloid C (**9**)

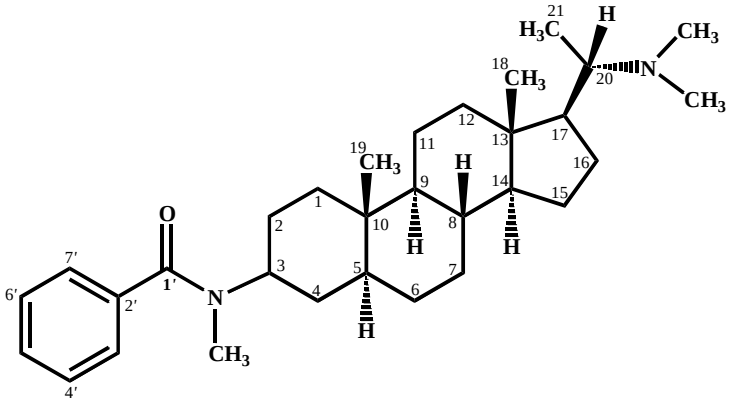
The ^{1}H-NMR spectrum of compound **9** displayed two up-field singlets at δ 0.65 and 0.98, characteristic of C-18 and C-19 angular methyls, respectively. A doublet at δ 0.86 ($J_{21, 20}$ = 6.4 Hz) was assigned to the C-21 secondary methyl, while a downfield 6H singlet at δ 2.14 was ascribed to NMe_2 protons. A 1H multiplet at δ 2.42 was assigned to the C-20 methine proton, while a multiplet at δ 3.04 was ascribed to the C-3 methine proton. A 3H singlet at δ 3.33 was assigned to the methoxy protons. A downfield signal at δ 5.34 was due to the C-6 methine proton.

The ^{13}C–NMR (broad-band and DEPT) spectra of compound **9** showed a total of 24 carbons, including six methyl, eight methylene, seven methine and three quaternary carbons. The spectroscopic data unambiguously matched with a reported compound isolated from *Sarcococca pruniformis* [22] and *Sarcococca saligna*.[19]

8.2.2 *N*$_a$-Methylepipachysamine D (10)

*N*a-Methylepipachysamine D (**10**) $[\alpha]_D^{25}$ = 60° (*c* = 0.05, $CHCl_3$) was obtained as a white amorphous solid from sub-fraction A_2 of pet. ether fraction (Experimental Section, Scheme-9.5, Page-134).

The UV spectrum displayed an absorption at 232 nm, while the IR spectrum showed absorptions at 2900 (CH), 1640 (amide C=O), and 1630-1450 (aromatic carbons) cm^{-1}. The HREI MS showed the M^+ at *m/z* 464.3787 ($C_{31}H_{48}N_2O$, calcd 464.3766).



N$_a$-Methylepipachysamine D (**10**)

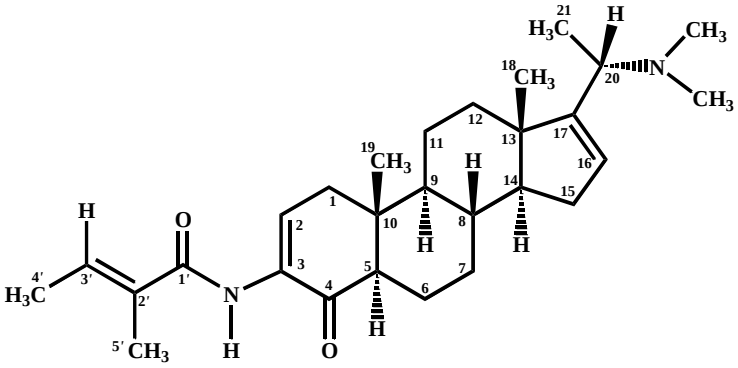
The ^{1}H-NMR spectrum of compound **10** displayed the presence of two up-field singlets at δ 0.71 and 0.83 for C-18 and C-19 angular methyls, respectively. A doublet at δ 1.16 ($J_{21,\ 20}$ = 6.5 Hz) was assigned to the C-21 methyl protons. The NMe_2 protons were resonated as a singlet at δ 2.18. The *N*-Me protons also appeared as a singlet at δ 2.68. The downfield signals between δ 7.27 -

7.33 were assigned to the aromatic protons. The ^{13}C-NMR spectra (broad-band decoupled) of compound **10** displayed resonances for all 31 carbons. The DEPT spectra showed the presence of six methyl, nine methylene, twelve methine and four quaternary carbons.

Based on these spectral studies (UV, IR, Mass, NMR), the compound was identified as N_a-methylepipachysamine D (**10**), previously obtained from *Sarcococca saligna*.[40]

8.2.3 Sarcovagenine C (11)

Sarcovagenine C (**11**) was isolated as a colorless crystalline solid from sub-fraction C_1 of the pet. ether fraction (Experimental Section, Scheme-9.5**,** Page-133). Melting point was found to be 160-162° C (Reported 160-161° C), while the optical rotation was measured as $[\alpha]_D^{25}$ = 11.6 ° (*c* = 0.04, $CHCl_3$).



Sarcovagenine C (**11**)

The UV spectrum of displayed absorption at 232 nm, while the IR spectrum showed absorptions at 3392 (NH), 2933 (CH), 1657 (amidic C=O), 1630 and 1450 (C=C) cm^{-1}.[39] The HREI MS of compound **11** showed the M^+ at *m/z* 438.3238 ($C_{28}H_{42}N_2O$, calcd 438.3246).

The ^{1}H-NMR spectrum of compound **11** exhibited up-field singlets at δ 0.82, 0.89 and 1.85 due to C-18, C-19, and C-5′ tertiary methyl protons, respectively. Two doublets at δ 1.05 ($J_{21,\,20}$ = 6.5 Hz)

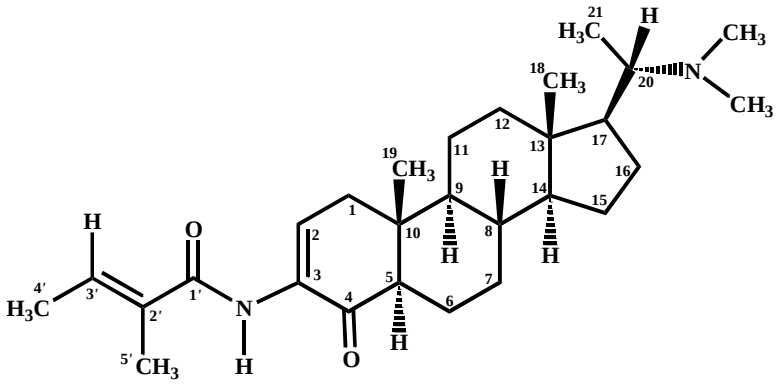
and 1.75 ($J_{4', 3'}$ = 6.7 Hz) were ascribed to the C-21 and C-4′ methyl protons, respectively. The NMe_2 protons resonated as a singlet at δ 2.18. Three downfield signals, including a broad singlet at δ 5.49, a quartet at δ 6.47 ($J_{3', 4'}$ = 6.9 Hz), and a double doublet at δ 7.64 ($J_{2, 1\alpha}$ = 6.6 Hz, $J_{2, 1\beta}$ = 2.4 Hz), were assigned to the C-16, C-2′ and C-2 olefinic protons, respectively. A 1H singlet at δ 8.16, which disappeared when sample was subjected to the deuterium exchange, was assigned to *N*-H.

The ^{13}C-NMR spectra (broad-band decoupled) of compound **11** displayed resonances for 28 carbons. The DEPT spectra indicated the presence of seven methyl, six methylene, eight methine, and seven quaternary carbons.

Based on these spectral studies (UV, IR, MS, NMR), compound **11** was identified as sarcovagenine C, previously obtained from the roots of *Sarcococca vagans*.[39]

8.2.4 Sarcovagine D (12)

Sarcovagine D (**12**) $[\alpha]_D^{25}$ = 28° (*c* = 0.03, $CHCl_3$) was isolated as a colorless crystalline solid from sub-fraction D_2 of the pet. ether fraction (Experimental Section, Scheme-9.5, Page-134).



Sarcovagine D (**12**)

The UV spectrum displayed an absorption at 212 nm, while the IR spectrum showed absorptions at 3398 (NH), 2927 (CH), 1660 (amidic C=O), 1630 (C=C) cm^{-1}. The HREI MS showed the M^+ at *m/z* 440.3394 ($C_{28}H_{44}N_2O$, calcd 440.3398).

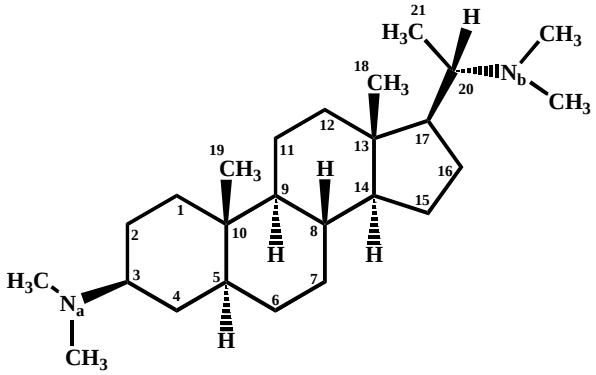
The ^{1}H-NMR spectrum of compound **12** was found to be distinctly similar to that of compound **11** with only difference being the absence of signals for the C-15/16 olefinic functionality. Three upfield singlets at δ 0.65, 0.86, and 1.87 were attributed to C-18, C-19, and C-5′ tertiary methyls, respectively. The doublets at δ 1.22 ($J_{21,\ 20}$ = 6.4 Hz) and 1.78 ($J_{4',\ 3'}$ = 6.3 Hz) were assigned to the C-21, and C-4′ methyl protons, respectively. A 6H singlet at δ 2.48 was assigned to the N-Me_2 protons. Two downfield signals at δ 6.48 as a quartet ($J_{3',\ 4'}$ = 6.6 Hz) and 7.64 as a double doublet ($J_{2,\ 1\alpha}$ = 6.7 Hz, $J_{2,\ 1\beta}$ = 2.4 Hz) were assigned to C-2′ and C-2 olefinic protons, respectively. The ^{13}C-NMR spectra (broad-band decoupled) displayed resonances for all 28 carbons, including seven methyl, seven methylene, eight methine, and six quaternary carbons.

Based on these spectral studies (UV, IR, MS, NMR), compound **12** was identified as sarcovagine D, previously reported from *Sarcococca vagans*.[41]

8.2.5 *N*-Methylpachysamine A (13)

Compound **13,** $[\alpha]_D^{28}$ = +18 ° (*c* = 0.03, MeOH), was isolated as a colorless amorphous solid from the alkaline fraction of dichloromethane extract of *S. coriacea* (Experimental Section, Scheme-9.2, Page-133). It was obtained after repeated elution of a sub-fraction E_1 on a neutral alumina column with pet. ether/EtOAc.

The UV spectrum displayed only terminal absorptions. The HREI MS showed the M^+ at *m/z* 374.3664 ($C_{25}H_{46}N_2$, calcd 374.3660).



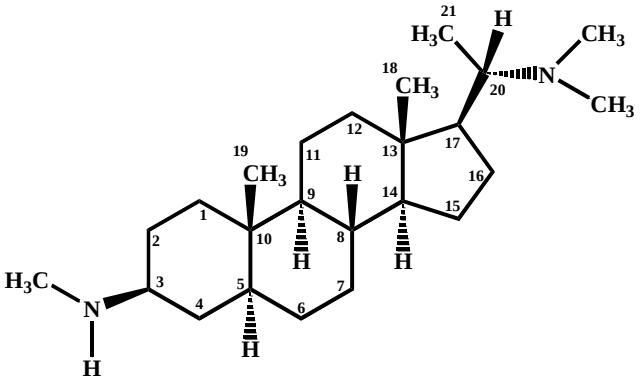
N-Methylpachysamine A (**13**)

The ^{1}H-NMR spectrum displayed two up-field 3H singlets at δ 0.61 and 0.78, assigned to the C-18 and C-19 angular methyls, respectively. A 3H doublet at δ 0.83 ($J_{21,\ 20}$ = 6.4 Hz) was assigned to the C-21 secondary methyl group. A downfield 6H singlet at δ 2.17 was ascribed to the N_b-Me_2 protons, while another 6H singlet at δ 2.19 was due to the N_a-Me_2 protons. The ^{13}C-NMR (broad-band and DEPT) spectra of compound **13** showed a total of 25 carbons, including seven methyl, nine methylene, seven methine, and two quaternary carbons.

Previously this compound was isolated from *Pachysandra terminalis* and *Sarcococca hookeriana*.[20]

8.2.6 Dictyophlebine (14)

Compound **14** $[\alpha]_D^{25}$ = + 24º (*c* = 0.04, MeOH) was isolated as colorless needles from the basic dichloromethane extract of *S. coriacea* (Experimental Section, Scheme-9.2, Page-132). It was obtained on repeated elution of a sub-fraction E_3 on neutral alumina column by pet. ether/EtOAc. The UV spectrum displayed only terminal absorptions, while the IR spectrum showed absorptions at 3350 (NH) and 2927 (CH) cm^{-1}. The HREI MS of compound **14** showed the M^+ at *m/z* 360.3035 ($C_{24}H_{44}N_2$, calcd 360.3031).



Dictyophlebine (**14**)

The ^{1}H-NMR spectrum displayed two up-field 3H singlets at δ 0.61 and 0.75, ascribed to the C-18 and C-19 angular methyls, respectively. A 3H doublet at δ 0.83 ($J_{21,\ 20}$ = 6.4 Hz) was assigned to the C-21 secondary methyl protons. A downfield 6H singlet at δ 2.13 was due to the N_b-Me_2 protons; while another 3H singlet at δ 2.39 was ascribed to the N_a-Me protons.

The ^{13}C-NMR spectra (broad-band and DEPT) showed 24 carbon signals, including six methyl, nine methylene, seven methine, and two quaternary carbons.

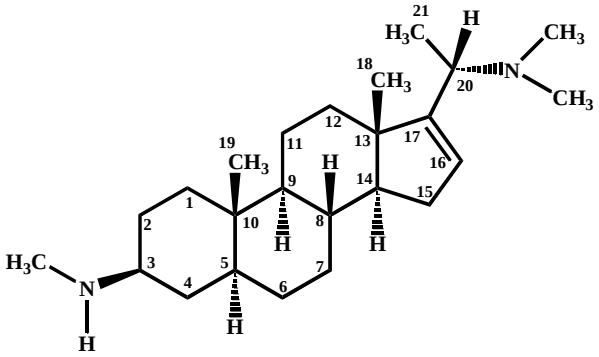
This compound was previously obtained from *Dictyophlebia lucidia*, *Funtumia latifolia*, *Sarcococca saligna* and *S. hookeriana*. [8]

8.2.7 5, 6-Dihydrosarconidine (15)

Compound **15** was isolated as colorless needles from the basic dichloromethane extract of *S. coriacea* (Experimental Section, Scheme-9.2, Page-132), by repeated elution of a sub-fraction E_3 on neutral alumina column with pet. ether/EtOAc.

The specific rotation of compound **15** was found to be $[\alpha]_D^{25}$ = -55° (*c* = 0.02, MeOH). The UV spectrum displayed only terminal absorptions, while the IR spectrum showed absorptions at 3350

(NH) and 2927 (CH) cm^{-1}. The HREI MS showed the M^+ at *m/z* 358.2875 ($C_{24}H_{44}N_2$, calcd 358.2872).



5, 6-Dihydrosarconidine (**15**)

The ^{1}H-NMR spectrum displayed two up-field 3H singlets at δ 0.65 and 0.80, which were ascribed to the C-18 and C-19 angular methyls, respectively. A 3H doublet at δ 1.03 ($J_{21,\ 20}$ = 6.4 Hz) was assigned to the C-21 secondary methyl protons. A downfield 6H singlet at δ 2.17 was due to the N_b-Me_2 protons, while another 3H singlet at δ 2.36 was ascribed to the N_a-Me protons. A 1H multiplet at δ 2.68 was assigned to the C-3 methine proton, while a downfield 1H broad singlet at δ 5.46 was assigned to the C-16 olefinic proton.

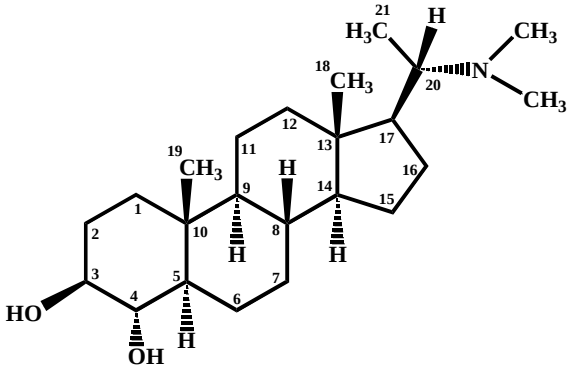
The ^{13}C- NMR spectra (broad- band decoupled) of compound **15** displayed resonances for all 24 carbons, including six methyl, eight methylene, seven methine, and three quaternary carbons. All the spectroscopic data of the compound **15** was unambiguously correlated with the reported literature values. This compound was previously isolated from *Sarcococca saligna*.[16]

8.2.8 Terminaline (16)

Compound **16** was isolated as a colorless crystalline solid from the basic dichloromethane extract of *S. coriacea* (Experimental Section, Scheme-9.4, Page-133), by repeated elution

of a sub-fraction I_1 on neutral alumina column with increasing polarities of pet. ether/EtOAc.

The optical rotation of compound **16** was found to be $[\alpha]_D^{25}$ = + 112° (*c* = 0.03, MeOH), which indicated the presence of some chiral centers in the molecule. The UV spectrum displayed an absorption at 242 nm, while its IR spectrum showed intense absorptions at 3228 (OH) and 2931 (CH) cm^{-1}. The HREI MS exhibited the M^+ at *m/z* 363.3132 ($C_{23}H_{41}NO_2$, calcd 363.3137).



Terminaline (**16**)

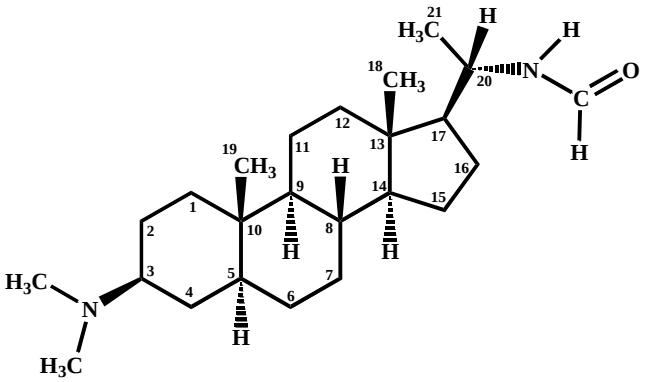
The ^{1}H-NMR spectrum displayed two up-field 3H singlets at δ 0.62 and 0.82, characteristic of C-18 and C-19 angular methyls, respectively. A doublet at δ 0.85 ($J_{21,\ 20}$ = 6.4 Hz) was assigned to the C-21 secondary methyl, while a downfield singlet at δ 2.13 was ascribed to *N*-Me_2 protons. A double doublet at δ 3.25 ($J_{4,\ 5}$ = 9.9 Hz, $J_{4,\ 3}$ = 9.1 Hz) was assigned to the C-4 methine proton, geminal to a hydroxyl group. A multiplet at δ 3.33 ($W_{1/2}$ = 17.6 Hz) was ascribed to the C-3 *axial* methine proton.[139]

The ^{13}C-NMR (broad-band and DEPT) spectra of compound **16** showed a total of 23 carbon signals, including five methyl, eight methylene, eight methine, and two quaternary carbons. All

the spectroscopic data of compound **16** was identical to the reported data of a previously obtained compound from *Pachysandra terminalis* and *Sarcococca hookeriana.*[8]

8.2.9 *Iso-N*-formylchonemorphine (17)

Compound **17** was isolated as a colorless amorphous solid from the basic dichloromethane extract of *S. coriacea* (Experimental Section, Scheme-9.4, Page-133), by repeated elution of a sub-fraction I_4 on neutral alumina column with increasing polarities of pet. ether/EtOAc.



Iso-N-formylchonemorphine (**17**)

The optical rotation of compound **17** was found to be $[\alpha]_D^{25}$ = - 20º (*c* = 0.05, MeOH), which indicated the presence of some chiral centers in the molecule. The UV spectrum displayed an absorption at 227 nm, while its IR spectrum showed intense absorptions at 3260 (NH) and 2934 (CH) and 1665 (amide) cm^{-1}. The HREI MS showed the M^+ at *m/z* 374.6155 ($C_{24}H_{42}NO$, calcd 374.6152).

The ^{1}H-NMR spectrum displayed two up-field 3H singlets at δ 0.73 and 0.81 for C-18 and C-19 angular methyls, respectively. A doublet at δ 1.13 ($J_{21,\ 20}$ = 6.4 Hz) was assigned to the C-21 secondary methyl. A 1H multiplet at δ 2.29 was assigned to the C-20 methine proton, while a singlet at δ 2.30 was ascribed to *N*-Me_2 protons. A broad multiplet at δ 3.97 ($W_{1/2}$ = 16.6 Hz) was

assigned to the C-3 *axial* methine proton,[139] geminal to an amino group, while a downfield splitted signal at δ 7.90/7.98 was characteristic of a formamide proton.

The ^{13}C-NMR (broad-band and DEPT) spectra showed 24 carbon signals, including five methyl, nine methylene, eight methine, and two quaternary carbons. Previously this compound was isolated from *Sarcococca brevifolia*.[26]

9.0 EXPERIMENTAL

9.1 General Experimental Conditions

9.1.1 Physical Constants

Melting points were determined on a Yanaco MP-S3 micro melting point apparatus. Optical rotations were measured on a JASCO digital polarimeter (model DIP-3600) in chloroform and methanol.

9.1.2 Spectroscopic Techniques

UV Spectra were recorded in methanol on Hitachi UV 3200 spectrophotometer. IR Spectra were recorded in $CHCl_3$ on a JASCO A-302 IR spectrophotometer. The mass spectra were measured on double focusing (Varian MAT 311 A) and Jeol HX 110 mass spectrometers. The ^1H – NMR spectra were recorded on Bruker AC-300, AM-400 and AMX-500 MHz instruments, while ^{13}C-NMR spectra were recorded at 75, 100, 125 and 150 MHz. Multiplicities of carbon signals were determined by using DEPT 90^o and 135^o experiments. Homonuclear ^1H-^1H connectivities were determined by using COSY 45^o experiment. One-bond ^1H-^{13}C connectivities were determined by HMQC experiment. Two- and three- bond ^1H-^{13}C connectivities were determined by HMBC experiment. ^1H-NMR chemical shifts are reported in δ (ppm) and coupling constants (*J*) were measured in Hz.

9.1.3 Chromatography and Staining

Column chromatography was performed on Merck silica gel 60 (70-230 and 240-300 mesh sizes, E. Merck), Merck alumina (70-230 mesh ASTM) and LH-20 Sephadex. Pre-coated silica gel TLC plates (E. Merck, F_{254}) were used for checking the purity of compounds. TLC plates were viewed under the ultraviolet light at 254 nm for fluorescence quenching spots and at 366 nm for

fluorescent spots. Dragendorff's spraying reagent was used for staining the compounds on TLC for alkaloidal test.

Composition of Dragendorff's Reagent- Solution-A:- 0.85 g bismuth nitrate [$Bi(NO_3)_2$ $5H_2O$] in 10 mL acetic acid and 40 L water. Solution-B:- 8 g potassium iodide in 20 mL water. Equal volume of solutions A and B were mixed and stored in a dark glass vessel as a stock solution. One mL stock solution was mixed with 2 mL acetic acid and 10 mL water before use.

9.2 Plant Material

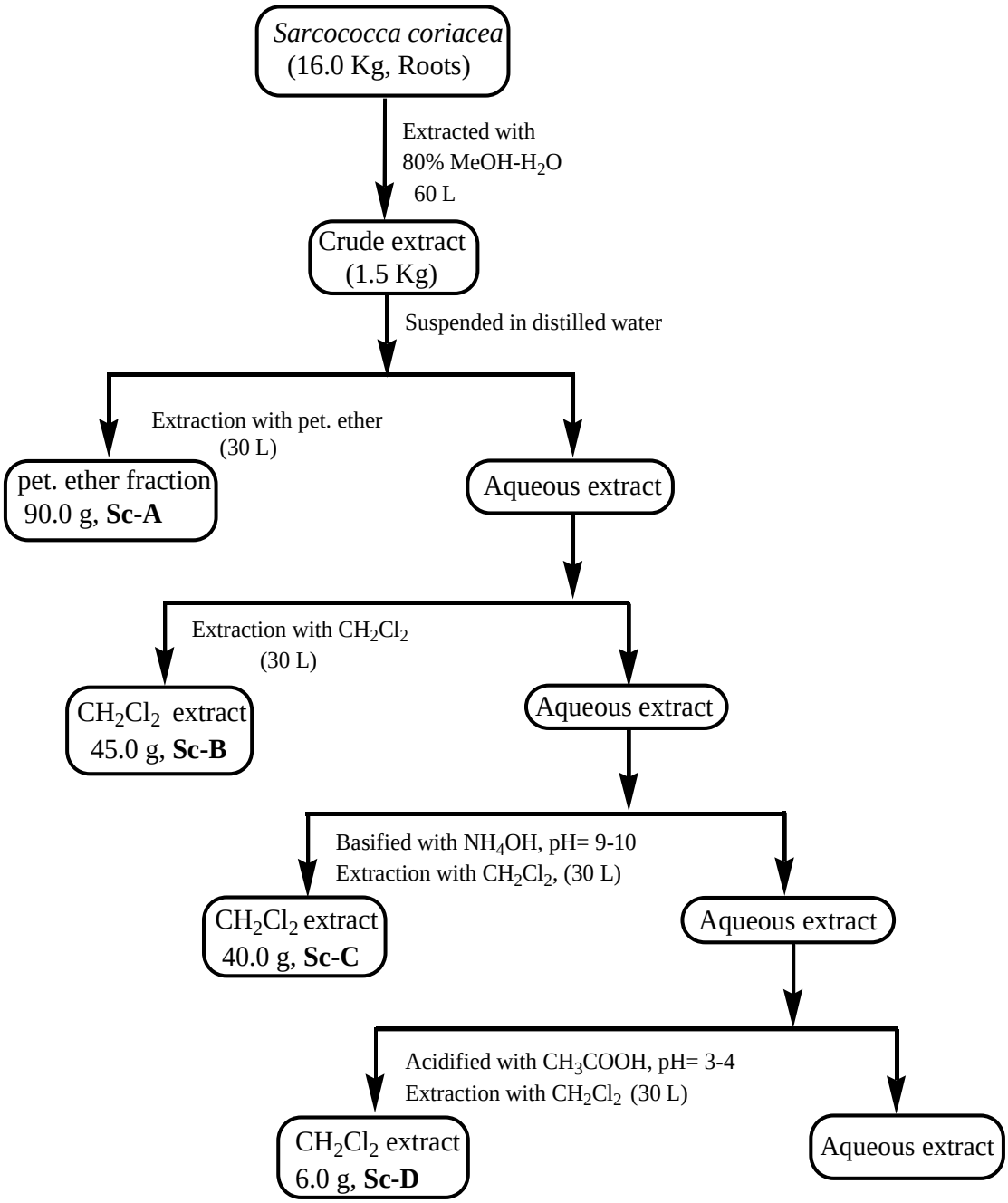
The roots of *Sarcococca coriacea* (Hook, f.) were collected from Champadevi area of Kathmandu district, Nepal, during June, 2005. Plant material was identified by Prof. Dr. Krishna Kumar Shrestha, Central Department of Botany, Tribhuvan University, Nepal. A voucher specimen (Sc-5/2005) was deposited in the same section.

9.3 Extraction, Isolation and Characterization of Compounds 1-17

Air- dried roots (16.0 kg) of *S. coriacea* were extracted with 80% methanol/water (60 L) (Scheme-9.1, Page-131). The concentrated methanolic aqueous extract (1.5 Kg) was dissolved in cold distilled water and defatted with petroleum ether (30 L), (90.0 g, **Sc-A**). The aqueous layer was then extracted with CH_2Cl_2 (30 L) to obtain "neutral fraction" (45.0 g, **Sc-B**). The aqueous layer was then made alkaline by adding ammonia solution (pH 9-10) and extracted with CH_2Cl_2 (30 L) to obtain "alkaline fraction" (30.0 g, **Sc-C**). The aqueous layer was then acidified by acetic acid to pH 3-4 and extracted with CH_2Cl_2 (30 L) to obtain an "acidic fraction" (6.0 g, **Sc-D**).

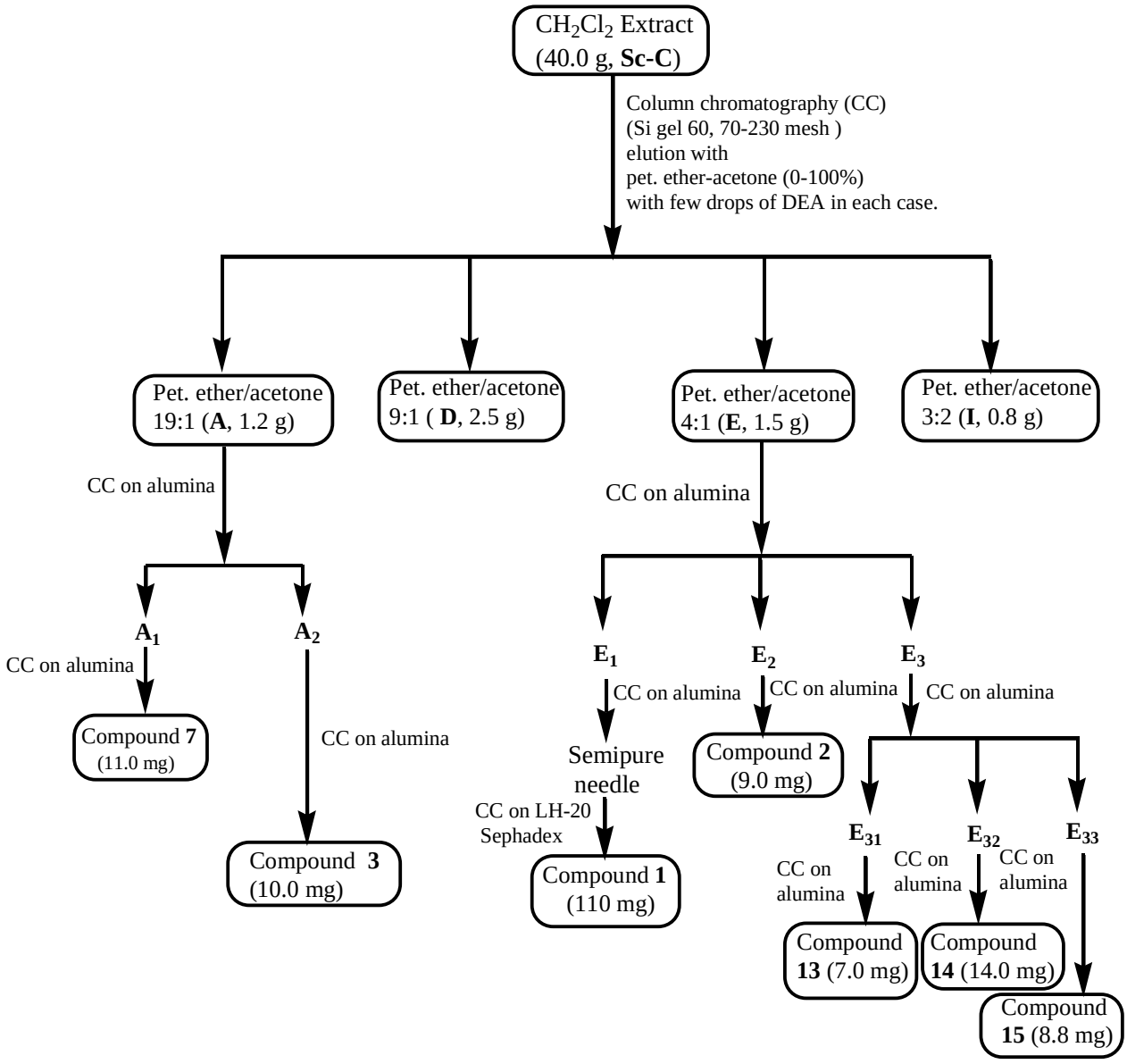
Based on the TLC results, the "alkaline" fraction **Sc-D** was initially subjected to the silica gel column chromatography, eluted with different gradients of petroleum ether and acetone (Scheme-9.2, Page-132), which afforded a number of fractions **A-K**. Elution with 20% acetone in petroleum

ether afforded a fraction **E** (1.5 g), which was further chromatographed on neutral alumina column to obtain a number of sub-fractions **E_1-E_5**. Compounds **1-2** and **13-15** were obtained from the fraction **E**. Similarly, fraction **D** (2.5 g) through the same isolation procedure (Scheme-9.3, Page-133) afforded six sub-fractions **D_1-D_6**, Compounds **4**, **6**, and **8** were obtained from the fraction **D**.

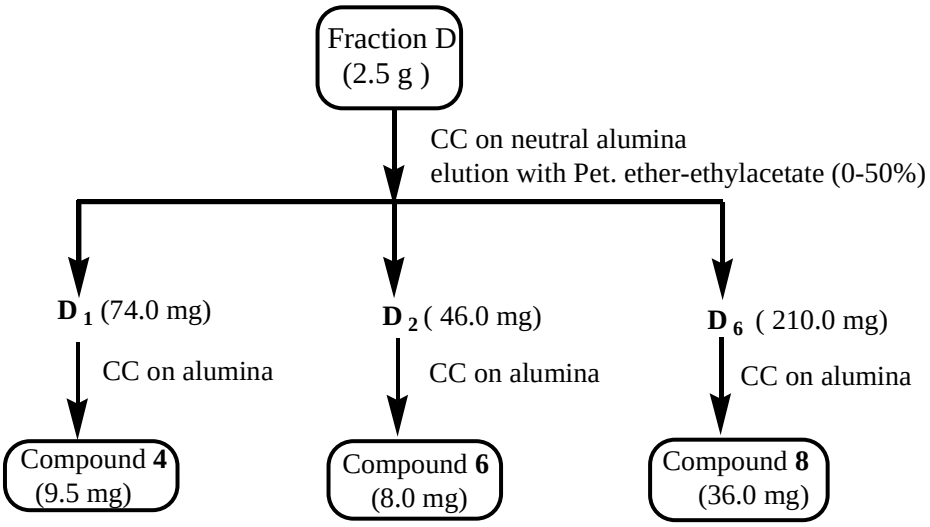


Scheme-9.1: Extraction of alkaloids from *S. coriacea***.**

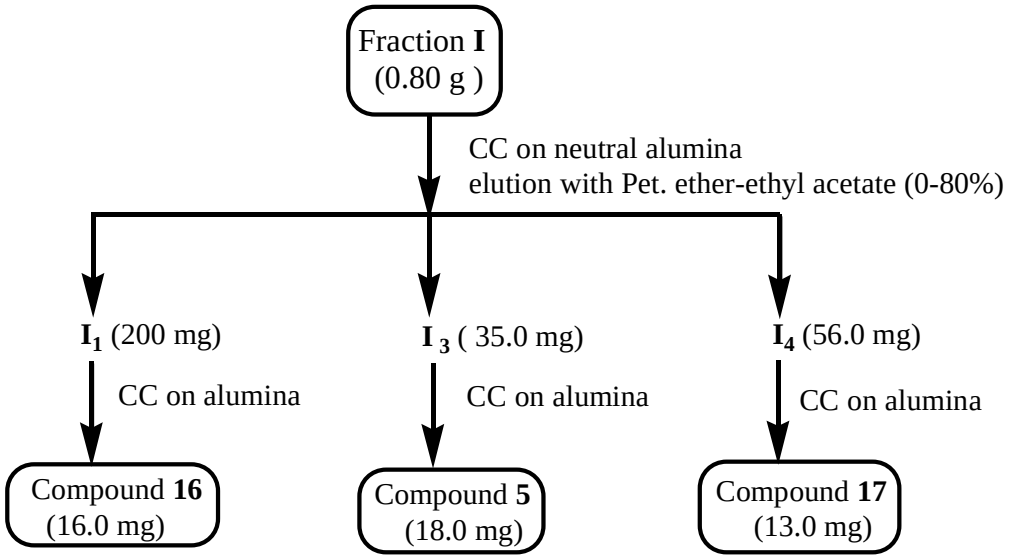
While fraction **A** also afforded four sub-fractions **A_1-A_4** (scheme-9.2, Page-132) and yielded the compounds **3** and **7.** Fraction I (800 mg) was subjected to the neutral alumina column chromatography to afford sub-fractions **I_1-I_4** (Scheme-9.4, Page-133), which yielded the compounds **5**, **16** and **17**.



Scheme-9.2: Isolation of alkaloids from *S. coriacea*.



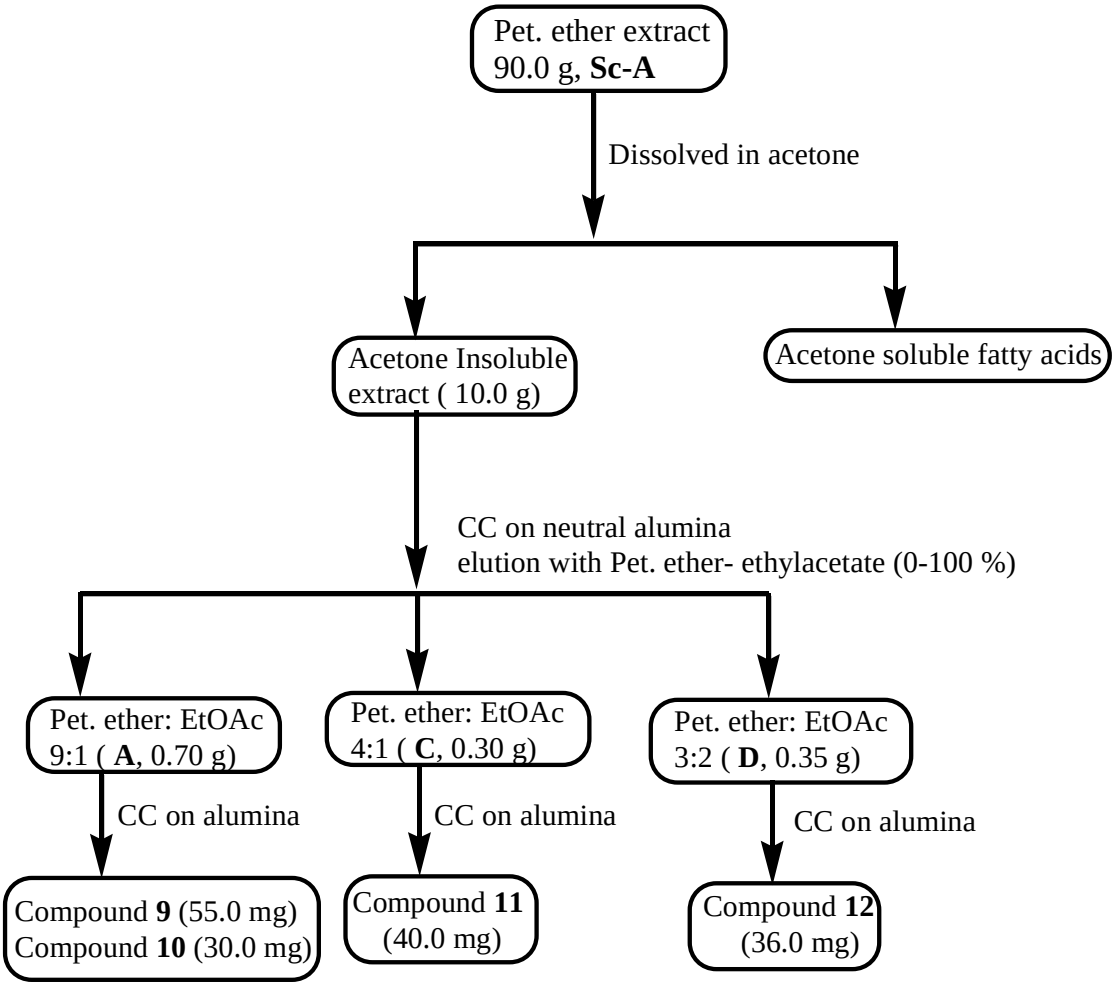
Scheme-9.3: Isolation of alkaloids from *S. coriacea*



Scheme- 9.4: Isolation of alkaloids from *S. coriacea*.

Pet. ether extract (**Sc-A**, 90g) (Scheme-9.1, Page-131) was dissolved in acetone and filtered. Acetone insoluble fraction (10 g) which showed positive alkaloidal test with Dragendorff's reagent, was subjected to column chromatography on neutral alumina by using EtOAc/pet. ether as eluent. This yielded a number of sub-fractions (Scheme-9.5, Page-134). Out of them, alkaloidal

fractions **A**, **C** and **D** were further subjected to neutral alumina column chromatography. Compounds **9** and **10** were obtained from sub-fraction **A**, Compound **11** was obtained from sub-fraction **C** while compound **12** was purified from sub-fraction **D**.



Scheme-9.5: Isolation of alkaloids from *S. coriacea*.

9.3.1 Adhikarimine A (1)

The sub-fraction $\mathbf{E_1}$ (300 mg) (Scheme-9.2, Page-132) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9:1) to afford a semi pure compound, which was further purified by Sephadex LH-20 (elution with MeOH) column, resulting in the isolation of needle type crystals of compound **1.**

Physical State: White crystal.

Yield: 110 mg, 6.8 x 10^{-4}%.

R_f : 0.55 (pet. ether/acetone/Et_2NH in 89:9:1).

M. pt.: 122 ^oC.

$[\alpha]_D^{26}$: + 26.3^o (*c* = 0.022, MeOH).

UV (MeOH) nm (log ε): λ_{max} 287 (3.1), 255 (3.4), λ_{min} 366 (1.3), 276 (5.78), 222 (2.45).

IR ($CHCl_3$) ν_{max} cm^{-1}: 2932.8 (C-H), 1591.7 and 1495.1 (C=C aromatic), 1256.4 (C-F) cm^{-1}.

EI MS *m/z* (rel. int. %): 484 (M^+, 87.6), 469 (5), 182 (97.7), 110 (62), 84 (100).

FAB^{+ve} MS: *m/z* 485.

HREI MS *m/z* : 484.7487 (calcd for $C_{30}H_{49}N_4F$).

^{1}H- and ^{13}C-NMR, δ (300 and 100 MHz, $CDCl_3$): See Table- 8.1.1

***X-Ray Diffraction Study on Compound* 1**

The X-ray diffraction analysis of compound **1** was performed by Dr. Sammer Yousuf at the School of Chemical Sciences and Food Technology, Faculty of Science and Technology, University of Kebangsaan, Malaysia.

A colorless needle (0.50 x 0.35 x 0.32 mm^3) of compound **1** was selected for data collection on Bruker Smart Apex CCD area detector diffractometer with graphite-monochromator MoKα radiations (λ = 0.71073 Å). A total of 7,727 reflections were collected by using the ω scaning technique at 298(2) ^oK.

Empirical formula	$C_{30}H_{49}FN_4$
Formula weight	484.73
Temperature	298(2) K
Wavelength	0.71073 Å

Crystal system, space group	Monoclinic, $P2_1$
Unit cell dimensions	**a** = 7.279(4) Å α = 90 °. **b** = 10.405(5) Å **β** = 91.966(11) ° **c** = 18.908(8) Å γ = 90 °
Volume	1431.2(12) $Å^3$
Z, Calculated density	2, 1.125 Mg/m^3
Absorption coefficient	0.071 mm^{-1}
F(000)	532
Crystal size	0.50 x 0.35 x 0.32 mm
Theta range for data collection	2.16 to 25.50 deg.
Limiting indices	-8<=h<=8, -12<=k<=12, -19<=l<=22
Reflections collected / unique	7727 / 2810 [R (int) = 0.0196]
Completeness to theta =	25.50 99.5 %
Max. and min. transmission	0.9777 and 0.9655
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2810 / 1 / 316
Goodness-of-fit on F^2	1.020
Final R indices [I>2sigma(I)]	R_1 = 0.0464, wR_2 = 0.1202
R indices (all data)	R_1 = 0.0608, wR_2 = 0.1304
Absolute structure parameter	10(10)
Largest diff. peak and hole	0.211 and -0.168 $e.A^{-3}$

The crystal structure data was deposited at the *Cambridge Crystallographic Data Centre*, UK (CCDC 698148).

9.3.2 Adhikarimine B (2)

The sub-fraction **E₂** (175 mg) (Scheme-9.2, Page-132) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9:1) to afford an amorphous solid compound **2.**

Physical State: White amorphous.

Yield: 9.0 mg, 5.6 x 10^{-5}%.

R_f : 0.51 (pet. ether/acetone/Et_2NH in 85:14:1).

$[\alpha]_D^{25}$: +19.2° (*c* = 0.05, MeOH).

UV (MeOH) nm (log ε): λ_{max} 286 (2.97), 253 (3.2), λ_{min} 376 (1.3), 274.6 (2.9), 223 (2.65).

IR ($CHCl_3$) ν_{max} cm^{-1}: 2929 (C-H), 1591.2 and 1494.7 (C=C aromatic), 1253 (C-F) cm^{-1}.

EI MS *m/z* (rel. int., %): 470 (M^+, 3), 465 (2), 182 (100), 96 (7), 70 (20).

FAB^{+ve} MS: *m/z* 471.

HREI MS *m/z* : 470.7221 (calcd for $C_{29}H_{47}N_4F$, 470.7217).

^{1}H- and ^{13}C-NMR, δ (500 and 100 MHz, $CDCl_3$): See Table-8.1.2.

9.3.3 Adhikarimine C (3)

The sub-fraction **A_2** (128 mg) (Scheme-9.2, Page-132) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9.5:0.5) to afford an amorphous solid **3.**

Physical State: White amorphous.

Yield: 10.0 mg, 6.2 x 10^{-5}%.

R_f : 0.57 (pet. ether/acetone/Et_2NH in 95:4:1).

$[\alpha]_D^{25}$: + 9.6° (*c* = 0.062, MeOH).

UV (MeOH) nm (log ε): λ_{max} 287 (2.8), 255 (3.0), λ_{min} 367 (0.52), 264.8 (2.8), 225 (2.17).

IR ($CHCl_3$) ν_{max} cm^{-1}: 2930 (C-H), 1591and 1489 (C=C aromatic), 1260 (C-F) cm^{-1}.

EI MS *m/z* (rel. int. %): 482 (M^+, 10.8), 467 (100), 328(57.3), 72 (26.7).

FAB^{+ve} MS: *m/z* 483.

HREI MS *m/z* : 482.7330 (calcd for $C_{30}H_{47}N_4F$, 482.7328).

^{1}H- and ^{13}C-NMR, δ (300 and 100 MHz, $CDCl_3$, MeOD): See Table-8.1.3.

9.3.4 Adhikarimine D (4)

The sub-fraction **D_1** (74.0 mg) (Scheme-9.3, Page-133) was subjected to neutral alumina column chromatography and eluted with pet. ether/EtOAc (9:1) to afford an amorphous compound **4.**

Physical State: White amorphous.

Yield: 9.5 mg, 5.9 x 10^{-5}%.

R_f : 0.49 (pet. ether/acetone/Et_2NH in 95:4:1).

$[\alpha]_D^{25}$: + 19.0^o (*c* = 0.022, MeOH).

UV (MeOH) nm (log ε): λ_{max} 275 (2.9), 243.8 (3.27), λ_{min} 328 (0.28), 217.6 (2.2).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3382.4 (N-H)**,** 2932 (C-H), 1617.8 and 1511 (C=C aromatic), 1278 (C-F) cm^{-1}.

EI MS *m/z* (rel. int. %): 468 (M^+, 4.3), 453 (100), 142(39.7), 72 (64).

FAB^{+ve} MS: *m/z* 469.

HREI MS *m/z* : 468.7061 (calcd for $C_{29}H_{45}N_4F$, 468.7058).

^{1}H- and ^{13}C-NMR, δ (400 and 150 MHz, $CDCl_3$): See Table- 8.1.4.

9.3.5 Adhikarimine E (5)

The sub-fraction **I_3** (35.0 mg) (Scheme-9.4, Page-133) was subjected to neutral alumina column chromatography, and eluted with pet. ether /EtOAc (4:1) to afford an amorphous compound **5.**

Physical State: White amorphous.

Yield: 18.0 mg, 1.1 x 10^{-4}%.

R_f : 0.49 (pet. ether/acetone/Et_2NH in 79:20:1).

$[\alpha]_D^{25}$: + 10.8^o (*c* = 0.026, MeOH).

UV (MeOH) nm (log ε): λ_{max} 286.8 (3.26), 254.2 (3.5), λ_{min} 366 (1.2), 200.6 (3.05).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3260 (N-H)**,** 2931 (C-H), 1665 (NC=O), 1593 and 1496 (C=C aromatic), cm^{-1}

EI MS *m/z* (rel. int., %): 484.3 (M^+, 8.2), 469.2 (17), 220 (20.42), 194 (43), 155 (100), 72 (64).

FAB^{+ve} MS: *m/z* 485.

HREI MS *m/z*: 484.7056 (calcd for $C_{29}H_{45}N_4OF$, 484.7052).

^1H- and ^{13}C-NMR, δ (400 and 125 MHz, $CDCl_3$): See Table-8.1.5.

9.3.6 Adhikarimine F (6)

The sub-fraction **D_2** (46.0 mg) (Scheme-9.3, Page-133) was subjected to neutral alumina column chromatography, and eluted with pet. ether /EtOAc (9:1) to afford an amorphous compound **6.**

Physical State: White amorphous.

Yield: 8.0 mg, 5.0 x 10^{-5} %.

R_f : 0.54 (pet. ether/acetone/Et_2NH in 92:7:1).

$[\alpha]_D^{25}$: + 11.0° (*c* = 0.02, MeOH).

UV (MeOH) nm (log ε): λ_{max} 285 (2.9), 248.4 (3.28), λ_{min} 367 (1.6), 219.8 (2.75).

IR ($CHCl_3$) ν_{max} cm^{-1}: 2925.8 (C-H), 1595 and 1502 (C=C aromatic).

EI MS *m/z* (rel. int., %): 580 (M^+, 11.6), 565 (9.2), 220 (20.42), 182 (100), 142 (17).

FAB^{+ve} MS: *m/z* 581.

HREI MS *m/z* : 580.8253 (calcd for $C_{34}H_{50}N_6F_2$, 580.8250).

^1H- and ^{13}C-NMR, δ (500 and 100 MHz, $CDCl_3$): See Table-8.1.6.

9.3.7 Adhikarimine G (7)

The sub-fraction **A_1** (85.0 mg) (Scheme-9.2, Page-132) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (8.5:1.5) to afford an amorphous compound **7.**

Physical State: White amorphous.

Yield: 11.0 mg, 6.8 x 10^{-5} %.

R_f : 0.56 (pet. ether/acetone/Et_2NH in 85:14:1).

$[\alpha]_D^{25}$: + 10.6° (*c* = 0.03, MeOH).

UV (MeOH) nm (log ε): λ_{max} 286.0 (2.92), 255.0 (3.16), λ_{min} 367.8 (1.5), 222.8 (2.38).

IR ($CHCl_3$) ν_{max} cm^{-1}: 2933.5(C-H), 1591 and 1493 (C=C aromatic).

EI MS *m/z* (rel. int. %): 594 (M^+, 48.5), 579 (41.7), 440 (72.9), 220 (18.2), 194 (62.5), 182 (100), 155 (72.3).

FAB^{+ve} MS: *m/z* 595.

HREI MS *m/z* : 594.8343 (calcd for $C_{35}H_{52}N_6F_2$, 594.8341).

^{1}H- and ^{13}C-NMR, δ (500 and 100 MHz, $CDCl_3$): See Table-8.1.7.

9.3.8 Adhikarione (8)

The sub-fraction **D_6** (210.0 mg) (Scheme-9.3, Page-133) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9:1) to afford a gummy compound **8.**

Physical State: gummy.

Yield: 36.0 mg, 2.25 x 10^{-4} %.

R_f : 0.58 (pet. ether/acetone/Et_2NH in 93:6:1).

$[\alpha]_D^{25}$: + 7.64° (*c* = 0.034, MeOH).

UV (MeOH) nm (log ε): λ_{max} 273.4 (2.53), 267 (2.52), 240.6 (3.07), λ_{min} 366.2 (0.70), 209.8 (2.58).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3346.8 (N-H), 2962.9 (C-H), 1669 (C=O), 1617 and 1511 (C=C aromatic), 1256 (C-F).

EI MS *m/z* (rel. int., %): 333 (M^+, 60), 318 (10.8), 290 (30), 194 (14.8), 166 (42.3), 142 (100).

FAB^{+ve} MS: *m/z* 334.

HREI MS *m/z*: 333.4522 (calcd for $C_{19}H_{28}N_3OF$, 333.4520).

^{1}H- and ^{13}C-NMR, δ (400 and 75 MHz, $CDCl_3$): See Table-8.1.8.

8.3.9 Alkaloid C (9)

The fraction **A** (700 mg) (Scheme-9.5, Page-134) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9.8:0.2) to afford a white needles like compound **9.**

Physical State: White needle.

Yield: 55.0 mg, 3.4 x 10^{-4} %.

R_f : 0.60 (pet. ether/acetone/Et_2NH in 95:4:1).

[α]$_D^{25}$: - 28^o (*c* 0.03, $CHCl_3$) {Reported: -32^o ($CHCl_3$)}.[22]

M. Pt.: 154 ^{o}C {Reported: 152-153 ^{o}C}.[22]

UV (MeOH) nm (log ε): λ_{max} 242 (2.53).

EI MS *m/z* (rel. int. %): 359 (M^+, 11), 344 (36), 72 (100).g

FAB^{+ve} MS: *m/z* 360.

HREI MS *m/z* : 359.3128 (calcd for $C_{24}H_{41}NO$, 359.3125).

^{1}H-NMR, δ (400 MHz, $CDCl_3$): 0.65 (3 H, s, Me- 18), 0.98 (3 H, s, Me-19), 0.86 (3H, d, $J_{21, 20}$ = 6.4 Hz, Me – 21), 2.14 (6 H, s, H-NMe_2), 2.42 (1 H, m, H-20), 3.04 (1 H, m, H-3), 3.33 (3H, s, OCH_3), 5.34 (1 H, d, H-6).

9.3.10 N_a-Methylepipachysamine D (10)

The fraction **A** (700 mg) (Scheme-9.5, Page-134) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9.5:0.5) to afford a white amorphous compound **10.**

Physical State: White amorphous.

Yield: 30.0 mg, 1.8 x 10^{-4} %.

R_f : 0.60 (pet. ether/acetone/Et_2NH in 94:5:1).

$[\alpha]_D^{25}$: + 60° (*c* 0.05, $CHCl_3$), {Reported: + 66° (c 0.04, $CHCl_3$)}.[39]

UV (MeOH) nm (log ε): λ_{max} 232 (1.62).

IR ($CHCl_3$) ν_{max} cm^{-1}: 2900 (C-H), 1665 (N-C=O), 1595 and 1450 (C=C aromatic) cm^{-1}.

EI MS *m/z* (rel. int., %): 464 (M^+, 4), 449 (3), 136 (49), 105 (7), 72 (100).
FAB^{+ve} MS: *m/z* 465.

HREI MS *m/z* : 464.3787 (calcd for $C_{31}H_{48}N_2O$, 464.3766).

^{1}H-NMR, δ (400 MHz, $CDCl_3$): 0.71 (3H, s, Me-18), 0.83 (3H, s, Me-19), 1.16 (3H, d, $J_{21,20}$ = 6.5, Me-21), 2.18{ 6H, s, N_b-$(CH_3)_2$}, 2.68 (3H, s, *Na*-CH_3), 3.62 (1H, m, H-3), 7.27-7.33 (5H, overlapped, H-3′-H-7′).

9.3.11 Sarcovagenine C (11)

The fraction **C** (300 mg) (Scheme-9.5, Page-134) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9.5:0.5) to afford a white needles of compound **11.**

Physical State: White needle.

Yield: 40.0 mg, 2.5 x 10^{-4} %.

R_f : 0.58 (pet. ether/acetone/Et_2NH in 93:6:1).

$[\alpha]_D^{25}$: +11.6° (*c* = 0.04, $CHCl_3$){ Reported: +5.43 ° (*c* = 0.056, $CHCl_3$)}.[38]

M. Pt.: 161-162 °C {Reported; 160-161 °C}.[38]

UV (MeOH) nm (log ε): λ_{max} 232 (1.62).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3392 (N-H), 2933 (C-H), 1657 (N-C=O), 1630 and 1450 (C=C aromatic) cm^{-1}.

EI MS *m/z* (rel. int., %): 438 (M^+, 8), 423 (100), 83 (54), 72 (91), 55 (34).

FAB^{+ve} MS: *m/z* 439.

HREI MS *m/z* : 438.3238 (calcd for $C_{28}H_{42}N_2O$, 438.3246).

^{1}H-NMR, δ (300 MHz, $CDCl_3$): 0.82 (3H, s, H-18), 0.89 (3H, s, H-19), 1.05 (3H, d, $J_{21,20}$ = 6.5 Hz, H-21), 1.75 (3H, d, $J_{4',3'}$ = 6.7 Hz, H-4′), 1.87 (3H, s, H-5′), 2.18 (6H, s, H- *N*Me_2), 5.49 (1H, br. s, H-16), 6.47 (1H, q, $J_{3',4'}$ =6.9 Hz, H-3′), 7.64 (1H, dd, $J_{2,1\alpha}$ = 6.6 Hz, $J_{2,1\beta}$ = 2.4 Hz, H-2), 8.16 (1H, s, H-*N*).

9.3.12 Sarcovagine D (12)

The fraction D (350 mg) (Scheme-9.5, Page-134) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9.4:0.6) to afford a white amorphous compound **12.**

Physical State: White amorphous.

Yield: 36.0 mg, 2.25 x 10^{-4} %.

R_f : 0.51 (pet. ether/acetone/Et_2NH in 92:7:1).

[α]$_D^{25}$: + 28° (*c* = 0.03, $CHCl_3$).

UV (MeOH) nm (log ε): λ_{max} 212 (2.5).

EI MS *m/z* (rel. int. %): 440 (4), 425 (11), 98 (4), 83 (42), 72 (100), 58 (4), 55 (23).

FAB^{+ve} MS: *m/z* 441.

HREI MS *m/z* : 440.3394 (calcd for $C_{28}H_{44}N_2O$, 440.3398).

^{1}H-NMR, δ (300 MHz, $CDCl_3$): 0.65 (3H, s, H-18), 0 .86 (3H, s, H-19), 1.22 (3H, d, $J_{21,20}$ = 6.4 Hz, H-21), 1.78 (3H, d, $J_{4',3'}$ = 6.3 Hz, H- 4′), 1.87 (3H, s, H-5′), 2.48 (6H, s, N-Me_2), 6.48 (1H, quartet, $J_{3',4'}$ = 6.6 Hz, H-3′), 7.64 (1H, dd, $J_{2,1\alpha}$ = 6.7 Hz, $J_{2,1\beta}$ = 2.4 Hz, H-2), 8.16 (1H, s, H-N).

9.3.13 *N*-Methylpachysamine A (13)

The fraction E_{31} (25 mg) (Scheme-9.2, Page-132) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (9.5:0.5) to afford a white amorphous compound **13.**

Physical State: White amorphous.

Yield: 7.0 mg, 4.3 x 10^{-5} %.

R_f : 0.55 (pet. ether/acetone/Et_2NH in 95:4:1).

$[\alpha]_D^{28}$: + 18° (*c* = 0.03, MeOH) {Reported: +16º (*c* = 0.02, MeOH)}.[20]

EI MS *m/z* (rel. int., %): 374 (M^+, 15), 359 (49), 303 (72), 110 (67), 84 (85), 72 (100), 58 (30).
FAB^{+ve} MS: *m/z* 375.
HREI MS *m/z* : 374.3664 (calcd for $C_{25}H_{46}N_2$, 374.3660).

^{1}H-NMR, δ (300 MHz, $CDCl_3$): 0.61 (3H, s, H-18), 0.78 (3H, s, H-19), 0.83 (3H, d, $J_{21,\ 20}$ = 6.4 Hz, H-21), 2.17 (6H, s, N_b-Me_2), 2.19 (6H, s, N_a- Me_2).

9.3.14 Dictyophlebine (14)

The fraction E_3 (92.0 mg) (Scheme-9.2, Page-132) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (8:2) to afford a white amorphous compound **14.**

Physical State: White amorphous

Yield: 14.0 mg, 8.7 x 10^{-5} %.

R_f : 0.48 (pet. ether/acetone/Et_2NH in 59:40:1).

$[\alpha]_D^{28}$: + 24° (*c* = 0.04, MeOH) {Reported: +26º (*c* = 0.03, MeOH)}.

IR ($CHCl_3$) ν_{max} cm^{-1}: 3350 (N-H), 2927 (C-H).

EI MS *m/z* (rel. int., %): 360 (M^+, 3), 345 (8), 289 (4), 72 (100).
FAB^{+ve} MS: *m/z* 361.
HREI MS *m/z* : 360.3035 (calcd for $C_{24}H_{44}N_2$, 360.3031).

^{1}H-NMR, δ (300 MHz, $CDCl_3$): 0.61 (3H, s, H-18), 0.75 (3H, s, H-19), 0.83 (3H, d, $J_{21,\ 20}$ = 6.4 Hz, H-21), 2.13 (6H, s, *N*-Me_2), 2.39 (3H, s, *N*-Me).

9.3.15 5, 6 - Dihydrosarconidine (15)

The fraction E_{33} (58.0 mg) (Scheme-9.2, Page-132) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (7.5:2.5) to afford a white amorphous compound **15.**

Physical State: White amorphous.

Yield: 8.8 mg, 5.5 x 10^{-5} %.

R_f : 0.53 (pet. ether/acetone/Et_2NH in 64:35:1).

$[\alpha]_D^{28}$: - 55° (*c* = 0.02, MeOH) {Reported: -60º (*c* = 0.2 $CHCl_3$)}.[16]

IR ($CHCl_3$) ν_{max} cm^{-1}: 3350 (N-H), 2927 (C-H).

EI MS *m/z* (rel. int., %): 358 (M^+, 4), 343 (100), 72 (43).

FAB^{+ve} MS: *m/z* 359.

HREI MS *m/z* : 358.3193 (calcd for $C_{24}H_{42}N_2$, 358.3198).

^{1}H-NMR, δ (300 MHz, $CDCl_3$): 0.65 (3H, s, H-18), 0.80 (3H, s, H-19), 1.03 (3H, d, $J_{21, 20}$ = 6.5 Hz, H-21), 2.17 (6H, s, N_b-Me_2), 2.36 (3H, s, N_a-Me), 2.68 (1H, m, H-3), 5.46 (1H, br. s, H-16).

9.3.16 Terminaline (16)

The fraction **I_1** (200 mg) (Scheme-9.4, Page-133) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (8:2) to afford a white crystalline compound **16.**

Physical State: White crystal.

Yield: 16 mg, 1.0 x 10^{-5}%.

R_f : 0.51 (pet. ether/acetone/Et_2NH in 59:40:1).

$[\alpha]_D^{28}$: +112º (*c* = 0.03, MeOH), {Reported: +115º (*c* = 0.16, MeOH).[35]

M. Pt.: 217-218 ºC (Reported: 216-218 ºC).[35]

IR ($CHCl_3$) ν_{max} cm^{-1}: 3228(O-H), 2931(C-H)**.**

EI MS *m/z* (rel. int., %): 363 (M^+, 5), 348 (8), 72 (100).

FAB^{+ve} MS: *m/z* 364.

HREI MS *m/z* : 363.3132 (calcd for $C_{23}H_{41}NO_2$, 363.3137).

^{1}H-NMR, δ (300 MHZ, $CDCl_3$): 0.62 (3H, s, H-18), 0.82 (3H, s, H-19), 0.85 (3H, d, $J_{21,\ 20}$ = 6.5, H-21), 2.13 (6H, s, *N*-Me_2), 3.25 (1H, d, $J_{4,\ 5}$ = 9.9 Hz, $J_{4,\ 3}$ = 9.1 Hz, H-4), δ 3.33 (1H, m, $W_{1/2}$ = 17.6 Hz, H-3*axial*).

9.3.17 *Iso-N*-formylchonemorphine (17)

The fraction I_4 (56.0 mg) (Scheme-9.4, Page-133) was subjected to neutral alumina column chromatography, and eluted with pet. ether/EtOAc (8:2) to afford a white amorphous compound **17.**

Physical State: White amorphous.

Yield: 13.0 mg, 8.1 x 10^{-5} %.

R_f: 0.55 (pet. ether/acetone/Et_2NH in 69:30:1).

$[\alpha]_D^{28}$: - 20º (*c* = 0.05, MeOH) {Reported: -14º (*c* = 0.2, $CHCl_3$).[26]

IR ($CHCl_3$) ν $_{max}$ cm^{-1}: 3260 (N-H), 2934 (C-H)**,** 1665 (C=O).

EI MS *m/z* (rel. int. %): 374 (M^+, 69.6), 359 (5), 110 (81.7), 84 (100).
FAB^{+ve} MS: *m/z* 375.
HREI MS *m/z*: 374.6155 (calcd for $C_{24}H_{42}NO$, 374.6152).

^{1}H-NMR, δ (300 MHz, $CDCl_3$): 0.73 (3H, s, H-18), 0.81 (3H, s, H-19), 1.13 (3H, d, $J_{21,\ 20}$ = 6.5 Hz, H-21), 2.30 (6H, s, *N*-Me_2), 3.97 (1H, br. m, $W_{1/2}$ = 16.6 Hz, H-3*ax*), 7.90/7.98 (1H, s, H-NCO).

10.0 BIOLOGICAL ACTIVITY

10.1 Antileishmanial Activity Study

10.1.1 Leishmaniasis

Leishmaniasis is a disease caused by the protozoan parasite of the genus *Leishmania*. Different types of protozoa are responsible for leishmaniasis, such as *L. infantum, L. donovani, L. maxicana, L. chagasi, L. amazonesis, L. major, L. aethiopica, L. brasiliensis, L. tropica,* etc. Leishmaniasis is widespread all over the tropical and sub-tropical regions of Africa, Southern Europe, South and Central America, Asian and Mediaterian regions.

There are following three types of the Leishmaniasis:

A) Cutaneous leishmaniasis

Cutaneous leishmaniasis is responsible for skin ulcers on the uncovered part of the body, such as the face, arms and legs. The disease can produce a large number of lesions which causes severe disability and invariability, leaving the patient permanently scarred that can cause serious social prejudices. Species such as *L. donovani* and *L. infantum* are mainly responsible for cutaneous leishmaniasis.

B) Mucocutaneous leishmaniasis

In this type of leishmaniasis, mucus membrane of the nose, mouth and throat cavities and surrounding tissues are partly or completely destructed. It is most often caused by *L. braziliensis*, but in some cases caused by *L. aethiopica*.

C) Visceral leishmaniasis

This, also known as "kala-azar", is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anemia. If left untreated, the fatality rate can be as high

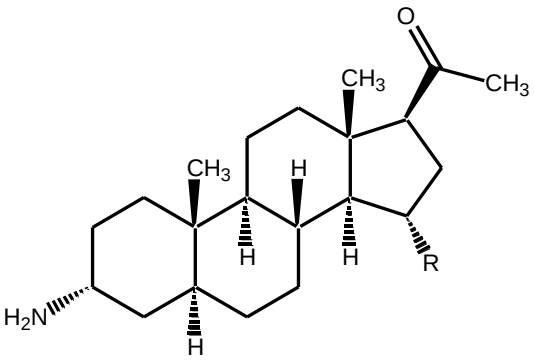
as100%. Several species of *Leishmania* are known to give rise to the visceral form of the disease such as, *L. donovani* and *L. infantum* in Africa, Asia and Europe and the *L. chagasi* in South America.

A number of synthetic drugs are used in the chemotherapy of leishmaniasis, many of which are not so effective or toxic to parasites as well as to the host. Some drugs such as tartar emetic urea, stibamine, megulamine antimoniate, sodium stibogluconate, etc cause harsh undesirable effects. Failure of treatment is also common.[148, 149] Some of the drugs which are in current use such as amphotericin B and pantamidine are toxic and nonresponsive. There is an urgent need to develop effective and nontoxic oral drugs and topical applications in order to combat the painful disease.

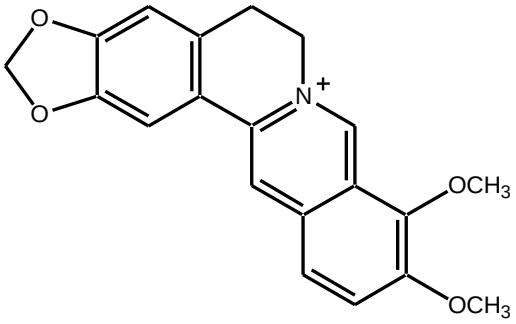
10.1.2 Natural Antileishmanial Studies

Leishmaniasis is becoming a major health challenge all around the world. Plant metabolites may play a key role to meet this challenge. The compounds quinine and emetine, isolated from the genera *Cinchona* and *Cephaelis*, respectively, have been used for the treatment of protozoanparasitic diseases, malaraia, and amoebiasis. The steroidal alkaloids, holamine **(164)** and hydroxyl holamine **(165)**, isolated from the *Holarrhena curtisii*, exhibited leishmanicidial activity against *L. donavani*.[150] Other alkaloids like berberine **(166)**, isolated from *Berberis* species, was effective against cutaneous leishmaniasis in rats,[151] and harmaline **(167)**, isolated from *Peganum harmala*, also showed an antileishmanial activity.[152] Number of quinoline alkaloids isolated from *Galipea longiflora* such as chimanine B (**168**) were found to be very effective against cutaneous leishmaniasis in mice. [153]

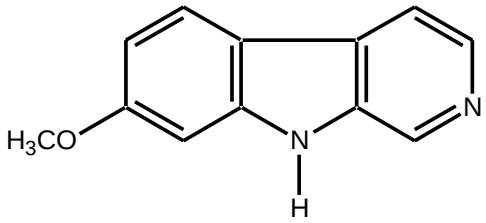
Some other classes of iridoids, coumarins, and monoterpenes, also exhibited antileishmanial activity against *L. donovani*.[154]



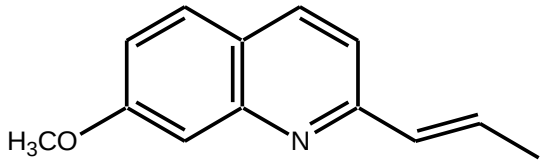
164 R = H
165 R = OH



166



167



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10.1.3 Antileishmanial Studies

The antileishmanial studies on fluorinated steroidal alkaloids was carried out by Miss Samreen at the bio-assay laboratory of the H.E.J. Research Institute of Chemistry and the results were presented in Table- 10.1

Table-10.1: *In vitro* antileishmanial activities of compounds.

Compounds	***IC*$_{50}$ (µg/mL)**	**Compounds**	***IC*$_{50}$ (µg/mL)**
1	87.08 ± 0.67	**6**	> 100
3	25.78 ± 0.45	**7**	> 100
4	25.78 ± 0.45	**8**	> 100
5	> 100	Amphotericin B *	2.0 ± 0.41

* Standard Drug

10.1.4 Assay Protocol

Leishmania promastigotes were grown in bulk early in modified NNN biphasic medium by using normal physiological saline. *Leshmania* parasite promastigotes were cultured with RPMI 1640 medium, supplemented with 10% heat inactivated foetal bovine serum (FBS). Parasites at long phase were centrifuged at 2000 rpm for 10 minutes, and washed three times with saline at same speed and time. Parasites were diluted with fresh culture medium to a final density of 1×10^6 cells/ml. The compounds to be checked were dissolved to a final concentration of 1.0 mg in 0.1 mL of PBS (Phosphate Buffered Saline, pH 7.4 containing 0.5% MeOH, 0.5% DMSO).

In a 96-well micro titer plate, 180 µL of medium was added in first row and 100 µL of medium was added in other wells. 20 µL of the experimental compound was added in medium and serially diluted. 100 µL of parasite cultures was added in all wells. Two rows were left for negative and positive controls. Negative control received medium, while the positive control contained varying concentrations of standard antileishmanial compound e.g. amphotericin B and pantamidine. The plate was incubated between 22-25 ^{o}C for 72 hours. Then the culture was examined microscopically and cells were counted on an improved neubauer counting chamber and IC_{50} values of compounds possessing antileishmanial activity were calculated by software Ezfit 5.03, Perella Scientific. All assays were run in duplicate. [154, 155]

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12.0 LIST OF PUBLICATIONS

1. M. Iqbal Choudhary, **Achyut Adhikari**, Sajan Lal Shyaula, Atta-ur-Rahman, Sammer Yousuf, Ian D. Williams, and Bohari M. Yamin, Novel Fluorine Containing Secondary Metabolites - First Time from Nature, *Nature* (Manuscript under preparation).

2. M. Iqbal Choudhary, **Achyut Adhikari**, Arif Lodhi, Zaheer-ul-Haq and Atta-ur-Rahman Mechanism of Sarcovagenine-C Binding to Acetylcholinestrase as Studied by STD, Tr-NOE NMR and Molecular Docking, (Manuscript under preparation).

3. Rehan Khan, Abdul Mallik, **Achyut Adhikari**, M. Irfan Qadir and M. Iqbal Choudhary Conferols A and B, New Anti-inflammatory 4-Hydroxyisoflavones from *Caragana conferta, Chem. Pharm. Bull.*, **2009**, 57 (4), 415-417.

13.0 GLOSSARY

AChE:

Acetylcholinestrase (AChE) is a key component of cholinergic brain synapses and neuromuscular junctions. The main role of this enzyme is the termination of impulse transmission by rapid hydrolysis of the cationic neurotransmitter acetylcholine.

a.m.u:

Atomic mass units.

Base Peak:

In the mass spectrum, the most intense peak is referred to as base peaks and is used as standard to measure the intensities of other ion-peaks.

BChE:

Butyrylcholinesterase is produced in the liver and enriched in the circulation. The exact physiological role of BChE is still elusive, but it is generally viewed as a backup for the homologous AChE.

Biogenesis:

The description of hypotheses for the synthesis of natural products within the living organism is termed as “biogenesis”.

Broad-band (BB) ^{13}C-NMR Spectrum:

It is a fully decoupled ^{13}C-NMR spectrum in which each magnetically distinct carbon appears as a singlet.

Chemical Shift:

Chemical shift corresponds to the difference between the precession frequency of the nucleus and the carrier frequency. It is expressed in a ppm value and is represented by the symbol δ.

Cosy-45º Spectrum:

A homonuclear two-dimensional NMR technique employed to determine vicinal and geminal ^{1}H-^{1}H coupling in molecules.

Coupling Constant:

In the ^{1}H-NMR spectrum, signals of individual protons are splitted into doublets, triplets, double doublets or other multiplets, etc. due to the presence of chemically nonequivalent protons on the same carbon atoms or on adjacent carbon atoms. The magnitude of splitting is known as the coupling constant "*J*" and is expressed in cycles/seconds or Hz. It is independent of the strength of the applied magnetic field but depends on the molecular stereochemistry and diminishes with, among other factors, an increasing number of bonds between the couple protons. The *J* value may be either positive or negative in sign.

Cytotoxic:

Any substance which inhibits the growth of cells.

Distortionless Enhancement by Polarization Transfer (DEPT):

DEPT is a ^{13}C-NMR experiment used to enhance the intensities of carbon signals and determine the multiplicities of carbon atoms in order to differentiate between CH_3, CH_2, and CH signals.

Electron Impact Mass Spectrum (EI MS):

The electron impact mass spectrum (EI MS) is a technique, which involves the bombardment of molecules with an electron beam (normally 70 ev) to produce ions.

Fast Atom Bombardment Mass Spectrum (FAB MS):

Fast atom bombardment mass spectrometry (FAB-MS), a soft ionization technique in mass spectrometry, which is used for the confirmation of the molecular ion of a compound as a positive or negative ion.

Heteronuclear Multiple Bond Connectivity (HMBC):

Heteronuclear Multiple Bond Connectivity experiment is an inverse, two-dimensional technique, used to determine long-range, heteronuclear interactions between carbons and protons (normally $^{1}J_{\text{C-H}}$, $^{2}J_{\text{C-H}}$, $^{3}J_{\text{C-H}}$).

Heteronuclear Multiple Quantum Coherence (HMQC):

Heteronuclear Multiple Quantum Coherence spectroscopy is a $^{1}H/^{13}C$ (single bond) Shift correlations.

High-Resolution Electron Impact Mass Spectrum (HREI MS):

High resolution electron impact mass spectrum (HREI-MS) gives information about the elemental composition through exact mass measurements. A double-focusing mass spectrometer is normally used for this purpose.

Infra-red Spectroscopy (IR):

Infrared rays cause changes in the vibrational and rotational movements of molecules which help in the detection of functional groups. Experiments are observed in the range between 400-4000 cm^{-1}.

Leishminasis:

The protozoan parasite of the genus *Leishmania* is the etiological agent of a variety of disease manifestation, collectively known as leishmaniasis.

Molecular Ion (M^+):

The molecular ion is formed by the loss of one electron from the molecule. The *m/z* ratio of the molecular ion gives the molecular weight of the compound.

Nuclear Overhauser Effect (NOE):

This is a two-dimensional NMR technique called Nuclear Overhauser Enhancement spectroscopy, which helps to determine the spatial proximity of protons to deduce the conformation and stereochemistry of organic compounds.

Optical Rotation:

It is the rotation of plane polarized light due to the presence of some asymmetric centers (chirality) in the molecule.

Proton-NMR Spectrum (^{1}H-NMR):

Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy is a one-dimensional NMR spectrum, which provides information about the electronic environment of the protons (^{1}H) in a molecule.

ROESY:

Rotating Overhauser Enhancement Spectroscopy (ROESY) is used to determine the spatial proximity of protons to deduce the conformation and stereochemistry of the molecule. It represents an exchange of transverse magnetization between the interacting nuclei.

TOCSY:

Total Correlation Spectroscopy (TOCSY) is used to determine the long-range $^{1}H/^{1}H$ correlations within a spin system.

Ultraviolet (UV) Spectrophotometry:

The ultraviolet rays (190-400 nm and 400-800 nm) are used to determine the chromophoric absorption in molecules.