# PHYTOCHEMICAL INVESTIGATIONS ON SOME *PIPER* SPECIES AND *GOUANIA MICROCARPA*

THESIS SUBMITTED TO THE UNIVERSITY OF KERALA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
**DOCTOR OF PHILOSOPHY**
IN CHEMISTRY

BY

**M. A. SUMATHYKUTTY, M.Sc.**

REGIONAL RESEARCH LABORATORY (CSIR)
TRIVANDRUM-695 019
INDIA

OCTOBER 1994

To
My Parents

वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद्
COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
क्षेत्रीय अनुसंधान प्रयोगशाला, तिरुवनन्तपुरम
**REGIONAL RESEARCH LABORATORY, TRIVANDRUM.**
तिरुवनन्तपुरम-695019
TRIVANDRUM-695 019.
भारत
INDIA

**DR. J. MADHUSUDANA RAO**
**SCIENTIST EI**

## CERTIFICATE

This is to certify that the thesis bound herewith is an authentic record of the research work carried out by Mrs. M. A. Sumathykutty, M.Sc. under my supervision in partial fulfilment of the requirements for the Degree of **Doctor of Philosophy** of the University of Kerala and further that no part thereof has been presented before for any other degree.

J. Madhusudana Rao

(J. MADHUSUDANA RAO)
SUPERVISING GUIDE

Grams : "CONSEARCH" Telex : 0435-232 Phones : PABX 76774 / 76769 / 76851 / 76852 / 70811
E-Mail : rrlt@sirnetm.ernet.in. FAX : 91-471-75186

## *DECLARATION*

*I hereby declare that this thesis is a bonafide record of research work done by me and that no part of the thesis has been presented earlier for any degree, diploma or similar title of any other University.*

*Trivandrum*
*October 1994*

*(M. A. SUMATHYKUTTY)*

# PREFACE

*I acknowledge with deep sense of gratitude and indebtedness my supervising guide, Dr. J. Madhusudana Rao, Scientist, Regional Research Laboratory, Trivandrum for his advice, valuable guidance and constant encouragement throughout the course of this investigation.*

*I am grateful to the Director, Regional Research Laboratory, Trivandrum for providing me all the facilities to carry out this work. My thanks are also due to Dr. Vijay Nair, Deputy Director for his interest in this topic. I would like to express my sincere gratitude to Dr. C. S. Narayanan, Head, Spice Processing and Flavour Technology Division for his keen interest and valuable help in carrying out this work. I am also grateful to Professor M. V. George, Emeritus Scientist, Photochemistry Research Unit for allowing me to use the IR Spectrophotometer and CHN Analyser.*

*I am thankful to Dr. N. B. Mulchandani, BARC, Bombay for providing an authentic sample of cubebin. The 500 MHz FT NMR facility provided at TIFR, Bombay and 270/400 MHz FT NMR facility at Sophisticated Instrument Facility, Indian Institute of Science, Bangalore are greatly acknowledged. I wish to express my thanks to Dr. S. Devasahayam, Scientist, National Research Centre for Spices, Calicut for conducting the bioassay experiments.*

*contd.....*

*My sincere thanks are also due to Mrs. K. P. Padmakumari for her help in carrying out the GC and GC-MS analysis and Mrs. Beena Joy for her help in recording the IR and NMR spectra. My special thanks are due to Mrs. Howa Umma for her assistance in my laboratory work. I am also grateful to all colleagues of my Division and RRL for their help and cooperation.*

*I am extremely grateful to my husband and other family members for their constant encouragement and support throughout this work.*

*Part of the work was published in the following journals and the corresponding reprints are enclosed at the end.*

1. *Lignans from Leaves of Piper nigrum Linn.*
   *M. A. Sumathykutty and J. Madhusudana Rao,*
   *Indian J. Chemistry, 1988, 27B, 388.*

2. *Higher alkanes from the fruits of Piper aurantiacum,*
   *M. A. Sumathykutty and J. Madhusudana Rao,*
   *Indian Perfumer, 1988, 32(4), 310.*

3. *Composition of Essential Oil from Piper attenuatum,*
   *M. A. Sumathykutty and J. Madhusudana Rao,*
   *Indian Perfumer, 1990, 34(3), 182.*

4. *8-Hentriacontanol and other constituents from Piper attenuatum,*
   *M. A. Sumathykutty and J. Madhusudana Rao,*
   *Phytochemistry, 1991, 30(6), 2075.*

5. *Constituents of Piper attenuatum,*
   *M. A. Sumathykutty and J. Madhusudana Rao,*
   *Fitoterapia, 1993, LXIV(3), 281.*

*(M. A. SUMATHYKUTTY)*

# C O N T E N T S

# CHAPTER I

# CHAPTER I

## A REVIEW ON THE CHEMISTRY OF GENUS PIPER

The genus Piper (Piperaceae) consists of more than 700 species distributed throughout the tropical and subtropical regions of the world[1]. Several species occur in Kerala, the most important economic spice being black pepper - Piper nigrum. Most of the species find wide application in traditional system of medicine[2]. The commercial, economic and medicinal importance of these species attracted the attention of research workers world wide. The isolation of active ingredients dates back as 1819 when piperine was isolated by Oestred[3]. Since then several species have been investigated for their chemical constituents and pharmacological properties. Several reviews have appeared on this subjects; the latest being the natural occurrence of lignans and neolignans[4]. The chemistry and technology aspects have been reviewed by Govindarajan in 1977[5]. The chemistry of Piper species has also been reviewed in two papers; one in 1975 by Atal et al[6] and the other in 1987 by Ray et al[1].

In this chapter an attempt is made to depict an overall view on the chemistry of Piper species. Emphasis is given to list out the chemical components so far isolated in literature. The Piper constituents are broadly classified as follows:

(i) Amides

(ii) Lignans

(iii) Flavonoids

(iv) Kawa-lactones and butenolides

(v) Cyclohexane derivatives

(vi) Miscellaneous compounds

(i) Amides:

Amides are the most common constituents of Piper species. This groups of compounds are subdivided into several groups depending on amine part of the molecules. These are (a) isobutyl amides (b) piperidine and pyrrolidine amides and (c) miscellaneous amides. The natural occurrence and the physical properties of these amides are recorded in Table 1.

(a) Isobutyl amides:

Thirty one isobutylamides (1-31) have so far been isolated from different parts of Piper species. Majority of

**Table 1: Natural occurrence of amides in Piper species**

| Sl No | Compound | Mol formula | Source | Part | M.P. $^{o}$C | Reference |
|---|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| | | | *ISOBUTYLAMIDES* | | | |
| 1. | N-isobutyl-octa-trans 2-trans-4-dienamide **(1)** | $C_{12}H_{21}NO$ | *P.banskii* | stem | 93-94 | 7 |
| 2. | N-isobutyl-trans-2-trans-4-decadienamide (Pellitorine) **(2)** | $C_{14}H_{25}NO$ | *P.nigrum* | berries | | 8 |
| | | | *P.ribesoides* | aerial part | | 9 |
| | | | *P.attenuatum* | root | 84 | 10 |
| | | | *P.sarmentosum* | fruit | | 11 |
| | | | *P.hanceii* | - | | 12 |
| | | | *P.longum* | - | | 13 |
| | | | *P.wallichi* | - | | 14 |
| | | | *P.sylvaticum* | - | | 15 |
| | | | *P.novae-hollandiae* | - | | 16 |
| 3. | N-isobutyl-dodeca-trans-2-trans-4-dienamide **(3)** | $C_{16}H_{29}NO$ | *P.pepuloids* | - | 130 | 17 |
| 4. | N-isobutyl-hexadeca-trans-2-trans-4-dienamide **(4)** | $C_{20}H_{37}NO$ | *P.guineense* | - | 66-68 | 18 |
| 5. | N-isobutyl-octadeca-trans-2-trans-4-dienamide **(5)** | $C_{22}H_{41}NO$ | *P.guineense* | fruit | 78-80 | 17,19,20 |
| | | | *P.argyrophyllum* | whole plant | | 50 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 6. | N-isobutyl-eicosa-<u>trans</u>-2-<u>trans</u>-4-dienamide **(6)** | $C_{24}H_{45}NO$ | *P.guineense* | fruit | 89-90 | 17,21,22 |
| 7. | N-isobutyl-eisosa-<u>trans</u>-2-<u>trans</u>-4-cis-8-trienamide **(7)** | $C_{24}H_{43}NO$ | *P.nigrum*<br>*P.officianarum* | berries<br>fruit | 67-67.5 | 8<br>23 |
| 8. | N-isobutyl-docosa-<u>trans</u>-2-<u>trans</u>-4-<u>cis</u>-10-trienamide (filfiline) **(8)** | $C_{26}H_{47}NO$ | *P.officianarum* | fruit | 66-67.5 | 24 |
| 9. | Retrofractamide A **(9)** (2E,4E,8E) | $C_{20}H_{25}NO_3$ | *P.retrofractum*<br>*P.brachystachyum* | aerial part<br>fruit | 129 | 25<br>26 |
| 10. | Retrofractamide C **(10)** (2E,4E,8E) | $C_{21}H_{27}NO_3$ | *P.retrofractum* | aerial part | | 25 |
| 11. | Retrofractamide B **(11)** (Piericide) (2E,4E,10z) | $C_{22}H_{29}NO_3$ | *P.retrofractum*<br>*P.nigrum*<br>*P.brachystachyum* | aerial part<br>berries<br>- | 114 | 25<br>27<br>26 |
| 12. | Guineensine **(12)** | $C_{24}H_{33}NO_3$ | *P.nigrum*<br>*P.guineense*<br>*P.brachystachyum*<br>*P.attenuatum*<br>*P.officianarum*<br>*P.slyvaticum* | berries<br>fruits<br>fruits<br>roots<br>roots<br>seeds | 114-15 | 8<br>18<br>26<br>10<br>28,29<br>30 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 13. | Brachystamide B **(13)** | $C_{26}H_{37}NO_3$ | *P.brachystachyum* | whole plant | – | 31 |
| 14. | Fagaramide **(14)** | $C_{14}H_{17}NO_3$ | *P.novae-hollandiae* | wood | 115-16 | 32 |
| | | | *P.hancei* | - | | 12 |
| | | | *P.amalago* | - | | 33 |
| 15. | Piperlonguminine **(15)** (2E,4E) | $C_{16}H_{19}NO_3$ | *P.novaehollandiae* | wood | 167-69 | 16 |
| | | | *P.nepalanse* | - | | 34 |
| | | | *P.attenuatum* | root | | 10 |
| | | | *P.sylvaticum* | root,stem | | 35,36 |
| | | | *P.guineense* | fruit | | 18 |
| | | | *P.hancei* | - | | 12 |
| | | | *P.longum* | root | | 37 |
| | | | *P.amalago* | - | | 33 |
| | | | *P.corcovadensis* | - | | 38 |
| 16. | Isopiperlonguminine **(16)** (2z,4z) | $C_{16}H_{19}NO_3$ | *P.corcovandensis* | - | 140-43 | 38 |
| 17. | Dihydropiper-longuminine **(17)** | $C_{16}H_{21}NO_3$ | *P.guineense* | fruit | 90-92 | 20,39 |
| | | | *P.wallichi* | - | | 14 |
| 17(a) | N-isobutyl-7-(3,4-methylendioxy-phenyl)-hepta-2-4-dienamide (2E,4E) **(17a)** | $C_{18}H_{23}NO_3$ | *P.falconeri* | stem & leaves | - | 2 14 |
| 18. | Piperlongine **(18)** | $C_{16}H_{23}NO_3$ | *P.longum* | fruit | — | 37 |
| 19. | Futoamide **(19)** | $C_{18}H_{23}NO_3$ | *P.futokadsura* | - | 128-30 | 40 |
| | | | *P.hancei* | - | | 12 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 20. | Retrofractamide C(20) (2E,8E) | $C_{20}H_{27}NO_3$ | *P.retrofractum* | aerial part | 129 | 25 |
| 21. | Pipercallosidine (21) (2E ) | $C_{18}H_{25}NO_3$ | *P.callosum* | roots | 80-82 | 41 |
| 22. | Pipercallosine (22) (2E,4E) | $C_{20}H_{27}NO_3$ | *P.callosum*<br>*P.interruptum*<br>*P.anisum* | root<br>stem<br>root, stem | 114-15 | 41<br>42<br>43 |
| 23. | Piperstachine (23) | $C_{22}H_{29}NO_3$ | *P.trichostatchyon* | - | 152 | 44 |
| 24. | Dihydropipercide (24) | $C_{21}H_{29}NO_3$ | *P.nigrum* | berries | - | 45 |
| 25. | Brachystamide A (25) | $C_{26}H_{39}NO_3$ | *P.brachystachyum* | whole plant | 101 | 31 |
| 26. | Corcovadine (26) (2E,4E) | $C_{18}H_{21}NO_5$ | *P.corcovadensis* | - | 141-44 | 38 |
| 27. | Isocorcovadine (27) (2z,4z) | $C_{18}H_{21}NO_5$ | *P.corcovadensis* | - | 80-85 | 38 |
| 28. | Piperovatine (28) | $C_{17}H_{23}NO_2$ | *P.anisum*<br>*P.corcovadensis*<br>*P.callosum*<br>*P.ovatum* | -<br>-<br>root<br>- | 120-21 | 43<br>38<br>41<br>46 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 29. | Longamide **(29)** | $C_{30}H_{61}NO$ | *P.longum* | fruit | 72 | 26 |
| 30. | Sylvamide **(30)** (2E) | $C_{14}H_{27}NO_3$ | *P.sylvaticum* | | 143-44 | 47 |
| 31. | Cyclopiperstachine **(31)** | $C_{22}H_{29}NO_3$ | *P.trichostachyon* | leaves | 220 | 48 |
| | | | *PIPERIDINE AMIDES* | | | |
| 32. | Piperine **(32)** (2E,4E) | $C_{17}H_{19}NO_3$ | *P.album* | - | 128-29 | 49 |
| | | | *P.argyrophyllum* | whole plant | | 50 |
| | | | *P.aurantiacum* | seeds | | 51 |
| | | | *P.boherifolium* | stem | | 52 |
| | | | *P.chaba* | stem, root | | 53,54 |
| | | | *P.guineense* | fruits | | 55,56,20,22 |
| | | | *P.longum* | fruit, root | | 57,58 |
| | | | *P.macropodum* | stem | | 59 |
| | | | *P.nigrum* | fruit, stem | | 60,61,5 |
| | | | *P.nepalense* | stem | | 34 |
| | | | *P.novae-hollandiae* | wood | | 16 |
| | | | *P.peepuloids* | fruit | | 17 |
| | | | *P.retrofractum* | seeds | | 62 |
| | | | *P.sylvaticum* | root, stem | | 35 |
| 33. | Piperettine **(33)** | $C_{19}H_{23}NO_3$ | *P.nigrum* | berries | 146 | 63 |
| 34. | Piperoleine A **(34)** | $C_{19}H_{25}NO_3$ | *P.nigrum* | berries | – | 60 |
| 35. | Piperoleine B **(35)** | $C_{21}H_{29}NO_3$ | *P.nigrum* | berries | – | 60 |
| 36. | Piperanine **(36)** | $C_{17}H_{23}NO_3$ | *P.nigrum* | berries | – | 64 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 37. | Piperonaline **(37)** | $C_{21}H_{27}NO_3$ | *P.longum* | fruits | - | 65 |
| 38. | Piperundecalidine **(38)** | $C_{23}H_{29}NO_3$ | *P.longum* | fruits | – | 65 |
| 39. | Dehydropiperonaline **(39)** | $C_{21}H_{25}NO_3$ | *P.longum* | fruits | – | 221 |
| 40. | Dihydropiperine **(40)** | $C_{17}H_{21}NO_3$ | *P.guineense*<br>*P.nigrum*<br>*P.novae-hollandiae*<br>*P.officianarum* | root<br>berries<br>-<br>- | 74 | 20,22,95<br>65<br>16<br>94 |
| 41. | Wisanine **(41)** (2E,4E) | $C_{18}H_{21}NO_4$<br>$C_{18}H_{21}NO_4$ | *P.guineense*<br>*P.guineense* | fruit, stem<br>root | 179-81 | 22,67<br>68 |
| 42. | Dihydrowisanine **(42)** | $C_{18}H_{23}NO_4$ | *P.guineense* | seeds | 99-100 | 96,69 |
| 43. | 3,4-Methylenedioxy cinnamoyl piperidine **(43)** | $C_{15}H_{17}NO_3$ | *P.novae-hollandiae* | wood | 80-82 | 16 |
| 44. | 2-Methoxy-4,5-methylenedioxy-cis/trans-cinnamoyl piperidide **(44)** | $C_{16}H_{19}NO_4$ | *P.peepuloids*<br>*P.amalago* | root<br>- | 98-99(cis)<br>120(trans) | 80,81,82<br>70 |
| 45. | N-trans-feruloyl-piperidine **(45)** | $C_{15}H_{19}NO_3$ | *P.nigrum* | berries | 135 | 72 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 46. | Coumaperine **(46)** (2E,4E) | $C_{16}H_{19}NO_2$ | *P.nigrum* | berries | 199.5-200 | 73 |
| 47. | Feruperine **(47)** (2E,4E) | $C_{17}H_{21}NO_3$ | *P.nigrum* | berries | 159 | 72 |
| 48. | Dihydroferuperin **(48)** (2E) | $C_{17}H_{23}NO_3$ | *P.nigrum* | berries | 78 | 72 |
| | | | *PYRROLIDINE AMIDES* | | | |
| 49. | 1-Cinnamoyl-pyrrolidine **(49)** | $C_{13}H_{18}NO$ | *P.methysticum* | - | 101-03 | 79 |
| 50. | m-Methoxy cinnamoyl **(50)** pyrrolidine | $C_{14}H_{18}NO_2$ | *P.methysticum* | - | 90-93 | 79 |
| 51. | 2-Methoxy-4,5-methylene-dioxy-trans-cinnamoyl pyrrolidide **(51)** | $C_{15}H_{17}NO_4$ | *P.guineense* | roots | 178-79 | 70 |
| 52. | Peepuloidin **(52)** | $C_{16}H_{19}NO_4$ | *P.peepuloids* | leaves | 149 | 83 |
| 53. | Piperyline (trichostachine) **(53)** (2E,4E) | $C_{16}H_{17}NO_3$ | *P.guineense* | fruits | 142-3 | 56 |
| | | | *P.nigrum* | berries | | 60 |
| | | | *P.macropodum* | stem | | 59 |
| | | | *P.trichostachyum* | leaves | | 84 |
| | | | *P.amalago* | root | | 33 |
| 54. | Wisanidine **(54)** (okolasine) (2E,4E) | $C_{17}H_{19}NO_4$ | *P.guineense* | roots | 171-73 | 22, 104 |
| | | | *P.amalago* | roots | | 33 |
| 55. | Dihydrowisanidine **(55)** | $C_{17}H_{21}NO_4$ | *P.guineense* | seeds | 82-84 | 85 |

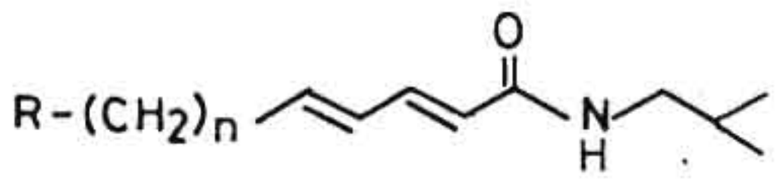
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 56. | Sarmentosine **(56)** (2E,4E) | $C_{18}H_{21}NO_3$ | *P.sarmentosum* | fruit | 77.5-79.5 | 11 |
| 57. | 1-Piperettyl pyrrolidine **(57)** | $C_{18}H_{19}NO_3$ | *P.trichostachyan* | stem | 90-3 | 86 |
| 58. | Tricholein **(58)** | $C_{20}H_{27}NO_3$ | *P.trichostachyon* | stem | - | 87,88 |
| 59. | Brachyamide B **(59)** | $C_{20}H_{25}NO_3$ | *P.brachystachyum* | fruits | - | 26 |
| 60. | Brachyamide A **(60)** | $C_{24}H_{31}NO_3$ | *P.brachystachyum* | fruits | - | 26 |
| 61. | Cyclostachine A **(61)** | $C_{22}H_{21}NO_3$ | *P.trichostachyon* | stem | 136-8 | 89 |
| 62. | Cyclostachine B **(62)** | $C_{22}H_{29}NO_3$ | *P.trichostachyon* | leaves | 135-6 | 90 |
| 63. | Trichonine **(63)** (2E,4E) | $C_{24}H_{43}NO$ | *P.trichostachyon* | leaves | 63-65 | 91 |
| 64. | Brachystine **(64)** | $C_{23}H_{41}NO$ | *P.trichystachyum* | fruits | | 26 |
| 65. | Sarmentine **(65)** (2E,4E) | $C_{14}H_{23}NO$ | *P.sarmentosum* | fruit | | 11 |
| | | | *MISCELLANEOUS AMIDES* | | | |
| 66. | 3,4,5-Trimethoxy dihydrocinnamoyl-2-pyrrolinone amide **(66)** | $C_{16}H_{19}NO_5$ | *P.demeraranum* | aerial part | 150-1 | 97 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 67. | N-3-phenyl-propanoyl pyrrole **(67)** | $C_{13}H_{13}NO$ | *P.sarmentosum* | fruit | 48.5-50 | 11 |
| 68. | 3,4-Dimethoxyphenyl propionamide **(68)** | $C_{12}H_{17}NO_3$ | *P.arboricola* | - | - | 98 |
| 69. | N-trans-feruloyl-tyramine **(69)** | $C_{18}H_{19}NO_4$ | *P.nigrum* | fruit | 144-144.5 | 73 |
| 70. | Alatamide **(70)** | $C_{16}H_{15}NO_2$ | *P.guayranum* | aerial part | 188-89 | 99 |
| 71. | Tembamide acetate **(71)** | $C_{18}H_{19}NO_4$ | *P.guayranum* | aerial part | 159 | 99 |
| 72. | Auranamide **(72)** | $C_{32}H_{30}NO_4$ | *P.aurantiacum* | fruit | 202 | 100 |
| 73. | Aurantiamide **(73)** | $C_{25}H_{26}N_2O_3$ | *P.aurantiacum* | fruit | 184 | 101,102 |
| 74. | Aurantiamide acetate **(74)** | $C_{27}H_{28}N_2O_4$ | *P.sylvaticum*<br>*P.surantiacum* | seeds<br>fruit | 188 | 108<br>101,107 |
| 75. | Aurantiamide benzoate **(75)** | $C_{32}H_{30}N_2O_4$ | *P.aurantiacum* | fruit | 211 | 115 |
| 76. | Pipermethystine **(76)** | $C_{16}H_{16}NO_4$ | *P.methysticum* | root | - | 105 |
| 77. | Sylvatine **(77)** | $C_{24}H_{33}NO_3$ | *P.sylvaticum*<br>*P.aurantiacum*<br>*P.guineense*<br>*P.longum*<br>*P.brachystachyum*<br>*P.chaba* | -<br>fruit<br>fruit<br>seeds<br>seeds<br>root | -<br>116 | 106<br>51<br>20<br>107<br>103<br>54 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 78. | Piplartine **(78)** | $C_{17}H_{19}NO_5$ | *P.longum*<br>*P.chaba*<br>*P.tuberculatum*<br>*P.aborescens* | stem<br>stem<br>root bark<br>leaves, stem | 126 | 74,75<br>56<br>76<br>77 |
| 79. | Dihydropiplartine **(79)** | $C_{17}H_{21}NO_5$ | *P.rugosum* | - | - | 78 |
| 80. | N-(3,4-dimethoxy-cinnamoyl)-pyridin-2-one **(80)** | $C_{16}H_{17}NO_4$ | *P.aborscens* | stem | 116-17 | 71 |
| 81. | N-(3-methoxy-4,5-methylene-dioxycinnamoyl)-$\Delta^3$-pyridin-2-one **(81)** | $C_{16}H_{15}NO_5$ | *P.aborscens* | stem & leaves | 157-58 | 71,77 |
| 82. | N-(3-methoxy-4,5-methylenedioxy-dihydrocinnamoyl)-$\Delta^3$ pyridin-2-one **(82)** | $C_{16}H_{17}NO_5$ | *P.aborscens* | leaves | 80-81 | 77 |
| 83. | Piplartine dimer A **(83)** | $C_{34}H_{38}N_3O_{10}$ | *P.rugosum*<br>*P.tuberculatium*<br>*P.longum*<br>*P.aborescens*<br>*P.chaba* | seed<br>root, bark<br>stem<br>leaves<br>fruits | 269-72 | 78<br>76<br>74,57<br>77<br>53 |
| | | | *ARISTOLACTAMS* | | | |
| 84. | Piperolactam A **(84)** | $C_{16}H_{11}NO_3$ | *P.boehimerifolium*<br>*P.hamiltonii*<br>*P.longum*<br>*P.attenuatum* | whole plant<br>"<br>"<br>" | | 109<br>66,109<br>109<br>" |
| 85. | Piperolactam B **(85)** | $C_{17}H_{13}NO_4$ | *P.boehimerifolium*<br>*P.longum* | whole plant | 212-14 | 66<br>66,109 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 86. | Piperolactam C (86) | $C_{18}H_{15}NO_4$ | *P.boehimerifolium* | whole plant | 187-88 | 109 |
| | | | *P.longum* | whole plant | | " |
| 87. | Piperolactam D (87) | $C_{17}H_{13}NO_4$ | *P.bachimeniforum* | whole plant | 226-27 | 110 |
| | | | *P.attenuatum* | " | | " |
| 88. | Aristolactam AII (88) | $C_{18}H_{15}NO_4$ | *P.attenuatum* | whole plant | – | 66,109 |
| | | | *P.boehimerifolium* | " | | " |
| | | | *P.hamiltoni* | " | | " |
| | | | *P.longum* | " | | 66,109 |
| 89. | Cepharanone B (89) | $C_{17}H_{13}NO_3$ | *P.attenuatum* | whole plant | – | 12,109 |
| | | | *P.boehimerifolium* | " | | " |
| | | | *P.longum* | " | | " |
| 90. | 10-Amino-4-hydroxy-2,3-dimethoxyphenanthrene-1 carboxylic acid lactum (90) | $C_{17}H_{13}NO_4$ | *P.acutisleginum* | stem | 222-24 | 111 |
| | | | *4,5-DIOXOAPORPHINES* | | | |
| 91. | Cepharadione B (91) | $C_{19}H_{15}NO_4$ | *P.boeherifolium* | whole plant | 267-68 | 66,109 |
| | | | *P.attenuatum* | " | | 66,109 |
| | | | *P.hamiltonii* | " | | 66,109 |
| | | | *P.longum* | seeds | | 66,109 |
| | | | *P.sanctum* | woody root | | 113 |
| 92. | Cepharadione A (92) | $C_{18}H_{11}NO_4$ | *P.boeherifolium* | whole plant | 341-42 | 66,109 |
| | | | *P.attenuatum* | " | | 66,109 |
| | | | *P.halmiltonii* | " | | 66,109 |
| | | | *P.longum* | seed | | 66,109 |
| | | | *P.auratum* | " | | 112 |
| | | | *P.sanctum* | woody part | | 113 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 93. | Nor-cepharadione **(93)** | $C_{18}H_{13}NO_4$ | *P.boeherifolium* | whole plant | – | 109 |
| | | | *P.attenuatum* | whole plant | | 109 |
| | | | *P.hamiltonii* | " | | 109 |
| | | | *P.longum* | root & whole plant | | 66,109 |
| 94. | Piperadione **(94)** | $C_{18}H_{13}NO_3$ | *P.attenuatum* | whole plant | – | 109 |
| | | | *P.attenuatum* | whole plant & root | | 109 |
| | | | *P.hamiltonii* | root | | 66,109 |
| | | | *P.longum* | - | | |
| 95. | 2-Hydroxy-1-methoxy-4,5-dioxoaporhine **(95)** | $C_{17}H_{11}NO_4$ | *P.attenuatum* | whole plant | – | 109 |
| | | | *P.boeherifolium* | " | | 109 |
| | | | *P.longum* | " | | 109 |
| 96. | 1,2,3-Trimethoxy-4,5-dioxo-6a,7-dehydroaporphine **(96)** | $C_{19}H_{15}NO_5$ | *P.aborscens* | stem | 199-201 | 71 |
| 97. | 1,2-Dimethoxy-4,5-dioxo-6a,7-dehydroaporphine **(97)** | $C_{18}H_{15}NO_4$ | *P.aborscens* | stem | 265-66 | 71 |



1: R = $CH_3$, n = 2

2: R = $CH_3$, n = 4

3: R = $CH_3$, n = 6

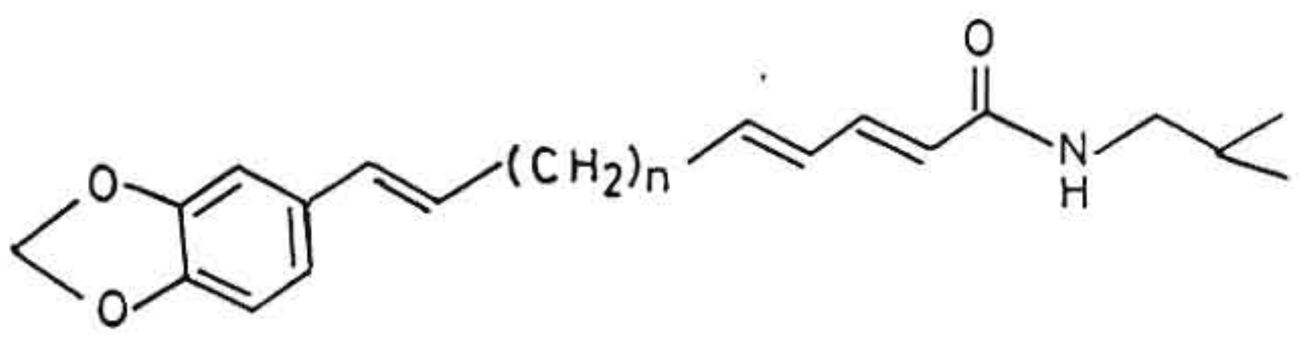
4: R = $CH_3$, n = 10

5: R = $CH_3$, n = 12

6: R = $CH_3$, n = 14

7: R = $CH_3(CH_2)_{10}CH=CH$, n = 2

8: R = $CH_3(CH_3)_{10}CH=CH$, n = 4



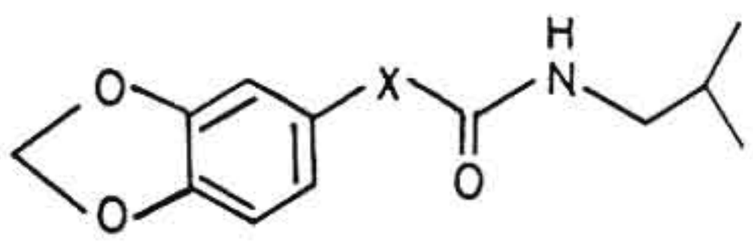
9: n = 2

10: n = 3

11: n = 4

12: n = 6

13: n = 8



14: X = CH=CH

15: X = CH=CH-CH=CH

16: X = CH=CH-CH=CH

17: X = $(CH_2)_2$-CH=CH

17.a: X = $(CH_2)_2CH=CH-CH=CH$

18: X = $(CH_2)_4$

19: X = CH=CH-$(CH_2)_2$-CH=CH

20: X = CH=CH-$(CH_2)_4$-CH=CH

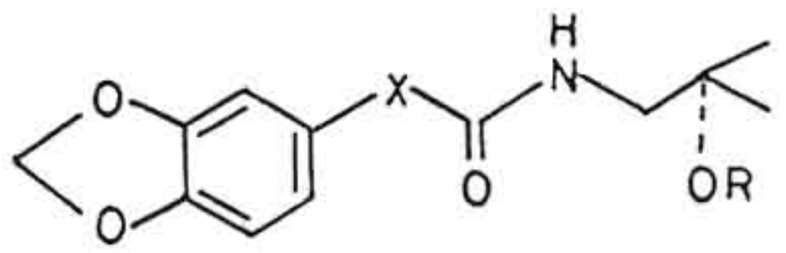
21: X = $(CH_2)_4$-CH=CH

22: X = $(CH_2)_4$-$(CH=CH)_2$

23: X = $(CH=CH)_2$-$(CH_2)_4$-CH=CH

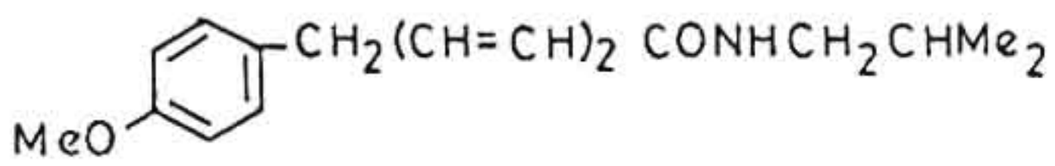
24: X = $(CH_2)_5$-$(CH=CH)_2$

25: X = $(CH_2)_{10}$-$(CH=CH)_2$



26: X = $(CH=CH)_2$, R = Ac

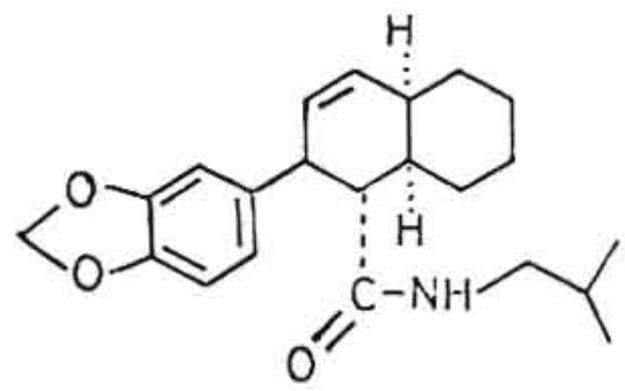
27: X = $(CH=CH)_2$, R = Ac



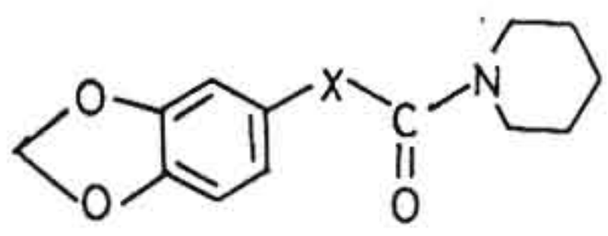
28

29. $CH_3(CH_2)_{24}$-CO-$NHCH_2CH(CH_3)_2$

30: $CH_3(CH_2)_4$-CHOH-CHOH-CH=CH-$CONHCH_2CH(CH_3)_2$



31



32: X = $(CH=CH)_2$

33: X = $(CH=CH)_3$

34: X = $CH=CH(CH_2)_4$

35: X = $CH=CH(CH_2)_6$

36: X = $(CH_2)_4$

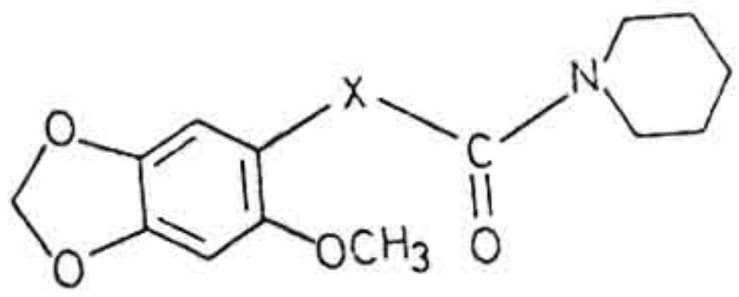
37: X = $CH=CH(CH_2)_4CH=CH$

38: X = $CH=CH(CH_2)_4(CH=CH)_2$

39: X = $CH=CH(CH_2)_2(CH=CH)_2$

40: X = $(CH_2)_2CH=CH$

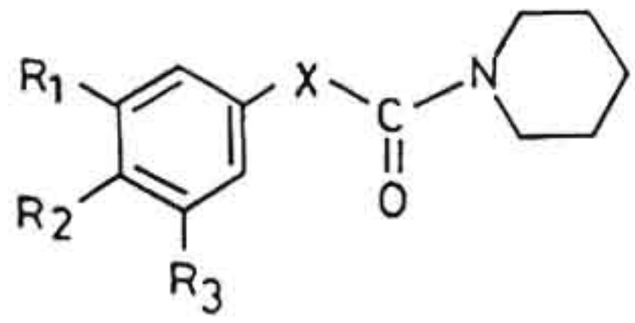
43: X = CH=CH



41: X = $(CH=CH)_2$

42: X = $(CH_2)_2CH=CH$

44. X = CH=CH

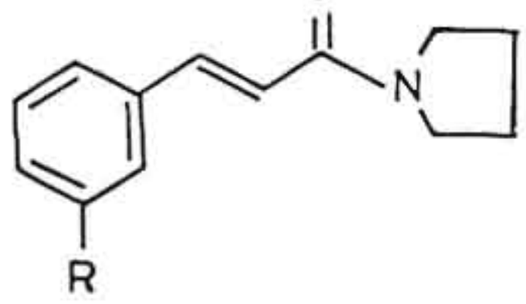


45: X = CH=CH, $R_1$ = $OCH_3$, $R_2$ = OH, $R_3$ = H

46: X = $(CH=CH)_2$, $R_2$ = OH, $R_1$ = $R_3$ = H

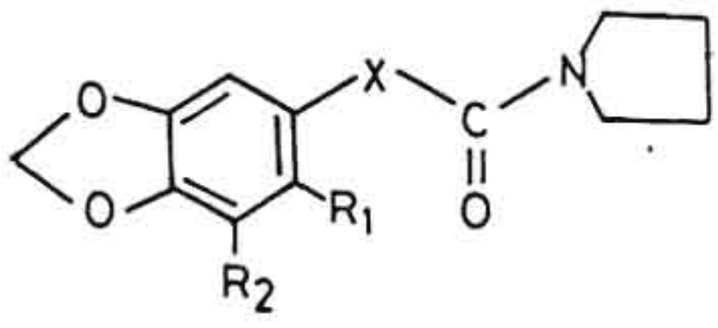
47: X = $(CH=CH)_2$, $R_1$ = $OCH_3$, $R_2$ = OH, $R_3$ = H

48: X = $(CH_2)_2CH=CH$, $R_1$ = $OCH_3$, $R_2$ = OH, $R_3$ = H



49: R = H

50: R = $OCH_3$



51: X = CH=CH, $R_1$ = $OCH_3$, $R_2$ = H

52: X = CH=CH, $R_1$ = $R_2$ = $OCH_3$

53: X = CH=CH-CH=CH, $R_1$ = $R_2$ = H

54: X = $(CH=CH)_2$, $R_1$ = $OCH_3$, $R_2$ = H

55: X = $CH_2$-$CH_2$CH=CH, $R_1$ = $OCH_3$, $R_2$ = H

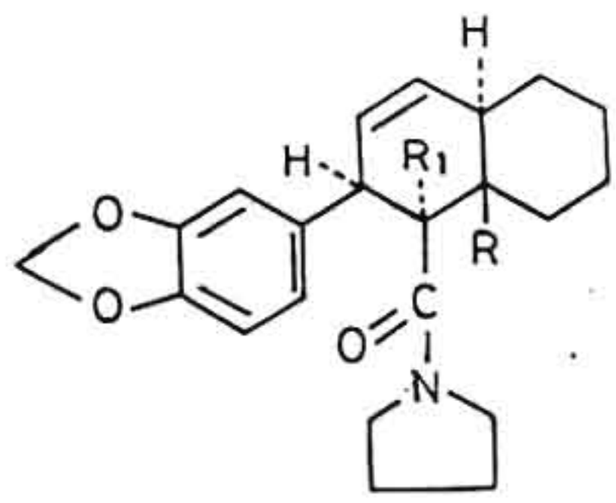
56: X = CH=CH$(CH_2)_2$CH=CH, $R_1$ = $R_2$ = H

57: X = $(CH=CH)_3$, $R_1$ = $R_2$ = H

58: X = CH=CH$(CH_2)_6$, $R_1$ = $R_2$ = H

59: X = CH=CH$(CH_2)_4$CH=CH, $R_1$ = $R_2$ = H

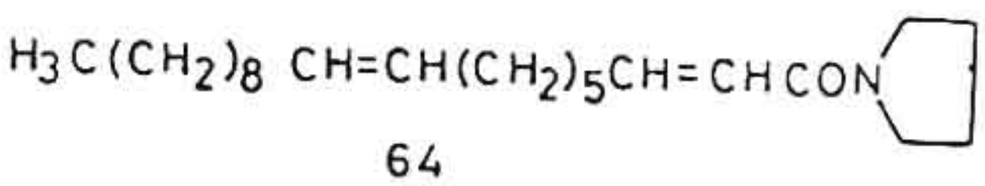
60: X = CH=CH$(CH_2)_6(CH=CH)_2$, $R_1$ = $R_2$ = H



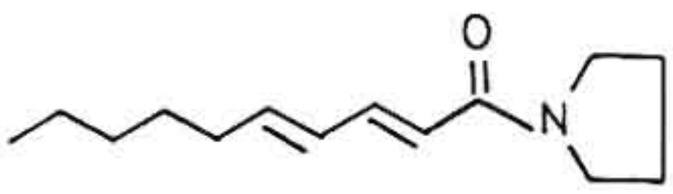
61: R = α -H, $R_1$ = β -H

62: R = β -H, $R_1$ = α -H

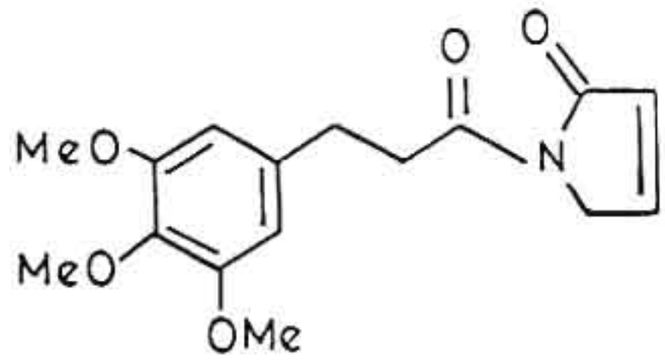
63



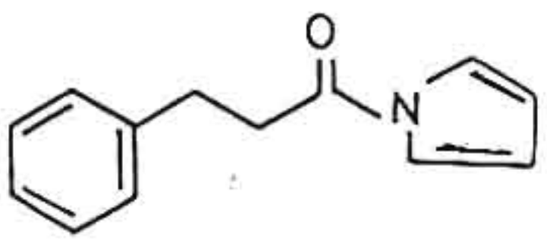
64



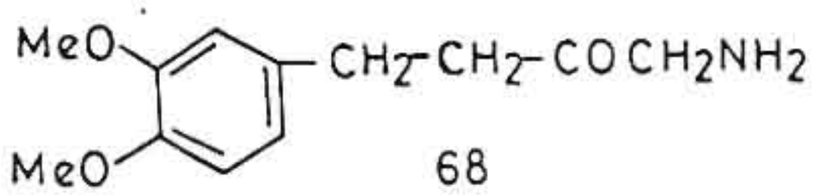
65



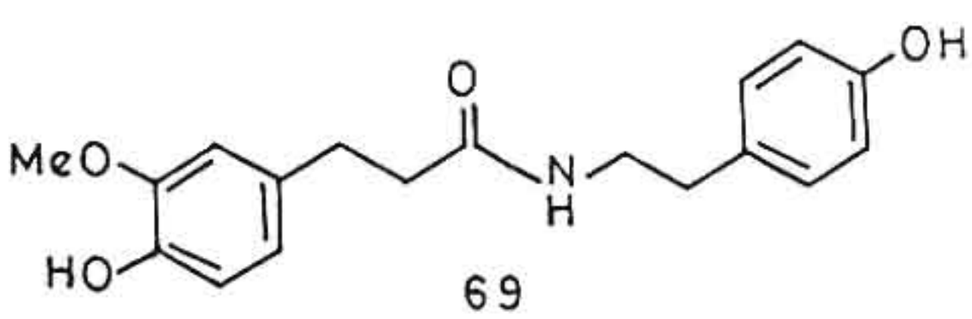
66



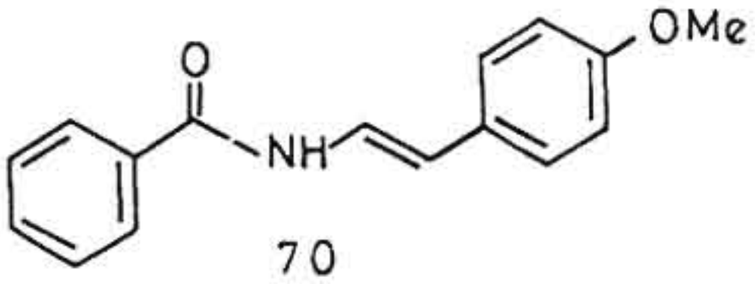
67



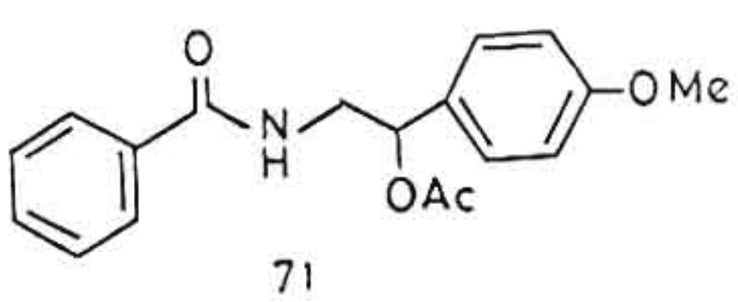
68



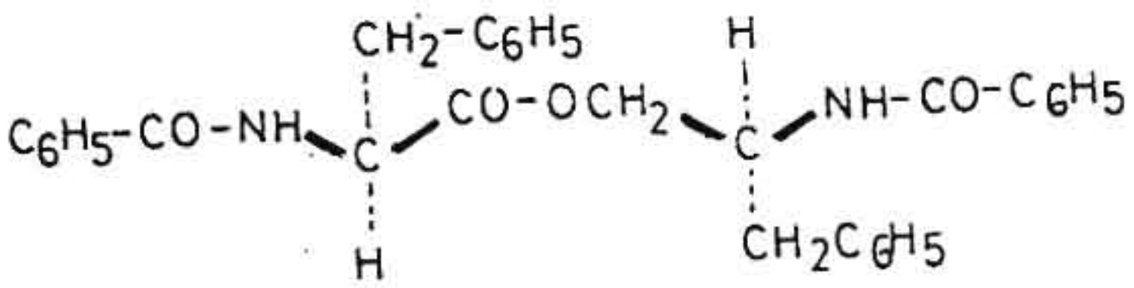
69



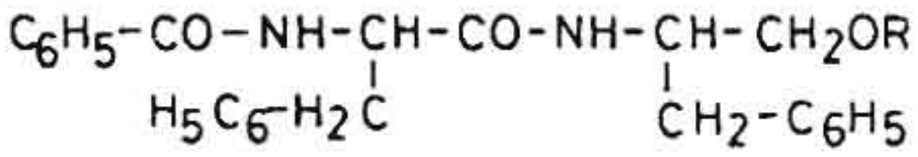
70



71



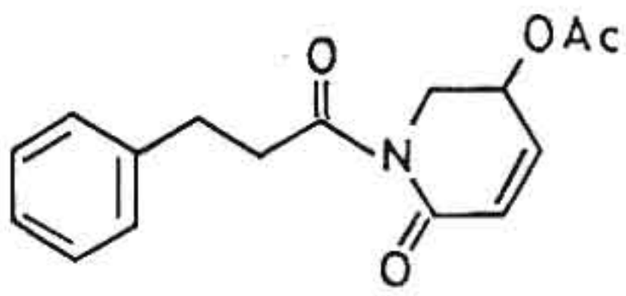
72



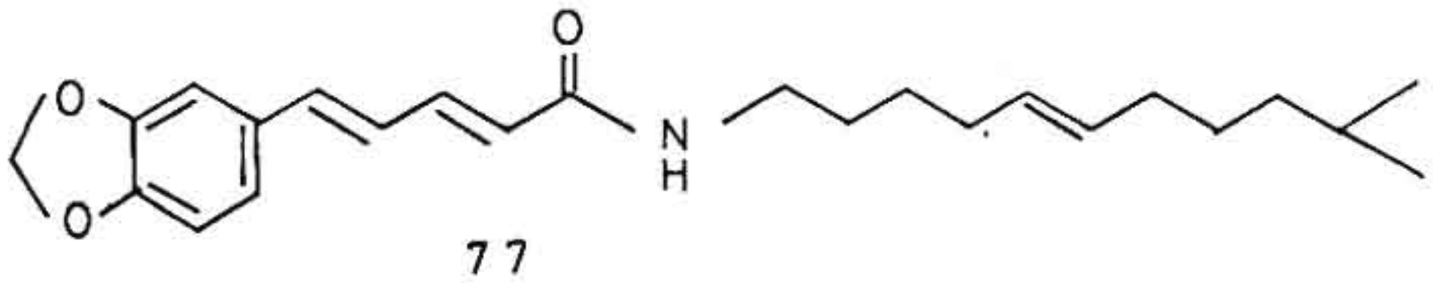
73: R = H

74: R = Ac

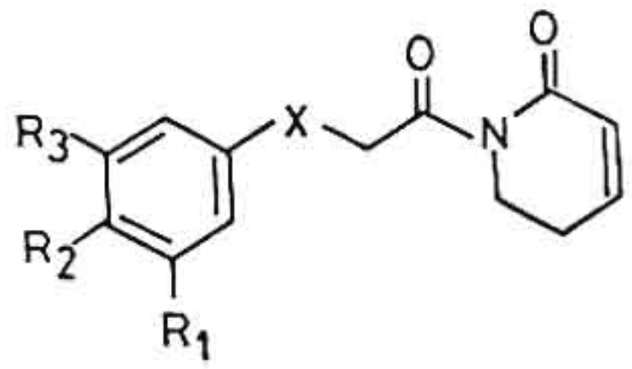
75: R = $OC_6H_5$



76



77



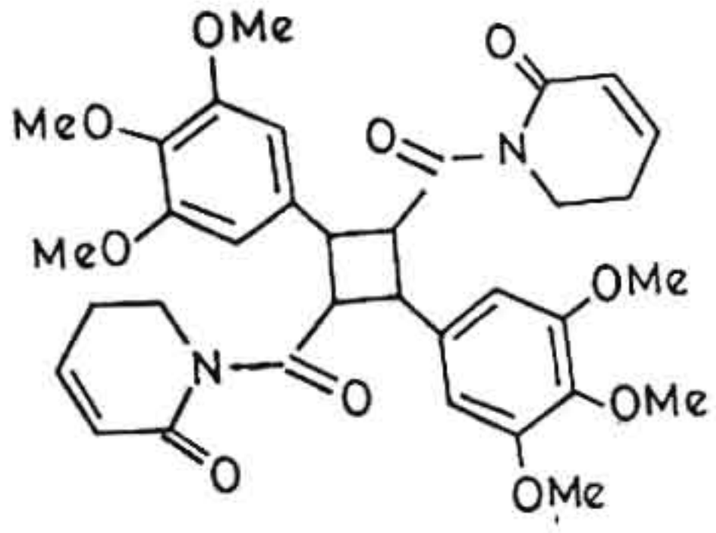
78: X = CH=CH, $R_1 = R_2 = R_3 = OCH_3$

79: X = $(CH_2)_2$, $R_1 = R_2 = R_3 = OCH_3$

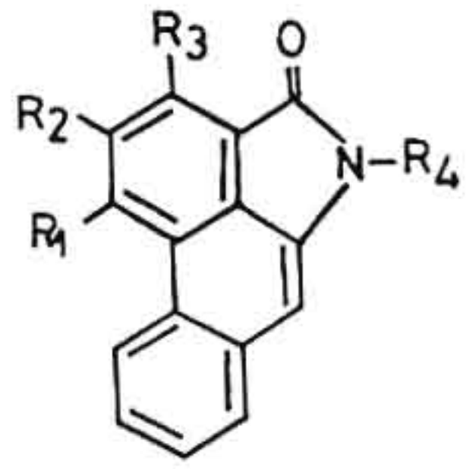
80: X = CH=CH, $R_1 = R_2 = OCH_3$, $R_3 = H$

81: X = CH=CH, $R_1 = OCH_3$, $R_2 + R_3 = -OCH_2O-$

82: X = $(CH_2)_2$, $R_1 = OCH_3$, $R_2 + R_3 = -OCH_2O-$




83




84: $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$

85: $R_1 = OH$, $R_2 = R_3 = OCH_3$

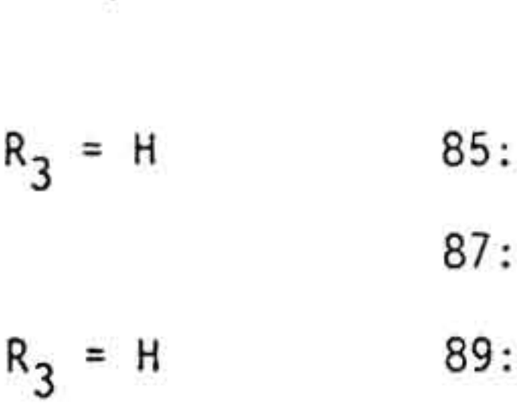
86: $R_1 = R_2 = R_3 = OCH_3$

87: $R_1 = R_2 = OCH_3$, $R_3 = OH$

88: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$

89: $R_1 = R_2 = OCH_3$, $R_3 = H$

90: $R_1 = OH$, $R_2 = R_3 = OCH_3$




91: $R_1 = R_2 = OCH_3$, $R_3 = CH_3$

92: $R_1 + R_2 = -OCH_2O$, $R_3 = CH_3$

93: $R_1 = R_2 = OCH_3$, $R_3 = H$

94: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = CH_3$

95: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$

96: $R_1 = R_2 = R_3 = OCH_3$

97: $R_1 = R_2 = OCH_3$, $R_3 = H$

them are of aromatic amides of $\Delta^{2,4}$fatty acids. The structures of these amides have most often been elucidated on the basis of spectral analysis. The identification of acids and amine part of the molecule is generally carried out by hydrolysis experiments.

Of the different isobutyl amides, special mention may be made with respect to piperovatine (28)[38,41,43,46] lognamide (29)[26] and cyclopiperstachine (31)[48]. Piperovatine is the only aromatic amide with a methoxy group in its aromatic part and longamide is perhaps the only saturated alkamide. Cyclopiperstachine (31) has got novel structural features and it could be obtained by thermal cyclisation of its open chain isomer piperstachine (23). Pellitorine (2), guineensine (12), piperlonguminine (15) and piperovatine (28) have been isolated from several Piper species.

(b) Piperidine and pyrrolidine amides:

Several piperidine and pyrrolidine amides bear common acid molecules such as 2-methoxy-4,5-methylenedioxycinnamic acid and piperic acid (4,5-methylenedioxy cinnamic acid). Wisanine (41) and wisanidine (54)[22,67] are one such pair which were isolated from P.guineense and

fully characterised from spectral, chemical and synthetic methods. A trans, cis-isomer of wisanine has also been isolated from the root bark of P.guineense[92]. Partially reduced derivatives of these compounds were also isolated from the same source and their structures were verified by synthesis[69,93].

Piperine (32), the active ingredient of black pepper (P.nigrum) occurs widely in other Piper species (Table 1). Dihydropiperine[16,94] (40), a constituent of the woody portions of P.novaehollandiae and a few other Piper species, was assigned its structure initially from spectral evidence and was later confirmed by synthesis[16]. Cinnamoyl pyrrolidine (49) and m-methoxy cinnamoyl pyrrolidine (50) were characterised from an analysis of their mass spectral data[79]. The structures of the analogous piperidine amides 43 and 44 were determined by chemical and spectral methods. The trans double bond earlier assigned to 44 was proved to be cis by the synthesis of both the isomers[81].

Piperyline (trichostachine) (53) occurs in several species. The structures of the stereo-isomers, cyclostachine A (61) and cyclostachine B (62) isolated from P.trichostachyon were resolved from their spectral analysis

and X-ray diffraction pattern[90]. 1-Piperettyl pyrrolidine (57), a pyrrolidine analogue of the previously known piperettine has also been isolated from P.trichostachyon[86].

(c) Miscellaneous amides:

Several amides other than isobutylamides, piperidine and pyrrolidine amides have been isolated in recent years. Of these the isolation of optically active aurantiamide (73) and the phenylalanine derivative, auranamide (72) by Banerji et al[101,102] from P.aurantiacum is significant. Sylvatine (77) is another interesting compound isolated first from P.sylvaticum and then later in several species. Piplartine dimer A (83) is the novel pyridone alkaloid isolated from a number of Piper species.

Cepharadione A (92) and cepharadione B (91) are the first aporphinoid alkaloids isolated from P.sanctum[114]. These two alkaloids were later isolated from a number of Piper species. Aristolactams and 4,5-dioxoaporphines are found to occur in P.attenuatum and other species only in recent years[66,109]. By chromatography and GC-MS analysis of the root extract from P.amalago[33] thirty six amides of various aliphatic and aromatic acids were detected. They were shown to be pyrrolidides and isobutyl amides. The main

constituent was found to be 5'-methoxy-3',4'-methylenedioxy cinnamic acid pyrrolidide. None of them were however isolated in pure form.

(ii) Lignans:

Next to amides, lignans occur widely in Piper species. Their occurrence in different Piper species is recorded in Table 2. The classification in this review is essentially based on a recent review on lignans and neolignans from Piperaceae by Jensen et al[4]. These compounds are divided into nine groups depending upon their common structural features.

a) 1,4-diaryl-2,3-dimethylcyclobutane lignans

b) 3,4-dibenzyl-γ-butyrolactol lignans

c) γ-butyrolactones

d) 2,3-dibenzylbutane-1,4-diol lignans

e) 2,5-bisaryl-3,4-dimethyl tetrahydrofurans

f) 2,6-bis-aryl-3,7-dioxa(3.3.0)bicylooctane lignans

g) benzofurans

h) 1,2-diarylpropanes

a) 1,4-diaryl-2,3-dimethylcyclobutane lignans:

Among this class of lignans in Piper species only

**Table 2: Natural occurrence of Lignans in Piper Species**

| Sl. No. | Compound | Mol formula | Source | Part | M.P. $^{o}$C | $[\alpha]_D$ | Ref. |
|---|---|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| | | | 1,4-diaryl-2,3-dimethylcyclobutane lignans | | | | |
| 98. | Magnosalin **(98)** | $C_{22}H_{32}O_6$ | *P.cubeba* | fruits | - | - | 116 |
| 99. | Heterotropan **(99)** | $C_{22}H_{32}O_6$ | *P.cubeba* | fruit | - | - | 116 |
| 100. | Andamanicin **(100)** | $C_{24}H_{32}O_6$ | *P.sumatranum* | stem & leaves | 110-112 | - | 117 |
| | | | 3,4-dibenzyl-Y-butyrolactol lignans | | | | |
| 101. | (-)-Clusin **(101)** | $C_{22}H_{26}O_7$ | *P.clusii* | whole plant | - | -34.5 | 118 |
| | | | *P.cubeba* | fruit | - | | 119 |
| 102. | (-)-Cubebin **(102)** | $C_{20}H_{20}O_6$ | *P.clusii* | whole plant | | | 118 |
| | | | *P.cubeba* | fruit | 131 | -17 | 119 |
| | | | *P.lacunosum* | leaves | | | 120 |
| | | | *P.ribesoides* | aerial part | | | 9 |
| | | | *P.trichostachyon* | fruits | | | 121 |
| | | | *P.nigrum* | fruits | | | 60 |
| 103. | (-)-Trichostin **(103)** | $C_{21}H_{22}O_7$ | *P.trichostachyon* | fruits | - | -62.5 | 60 |
| 104. | (-)-Clusinethyl **(104)** ether **(104)** | $C_{24}H_{30}O_7$ | *P.clusii* | fruit | | -31.6 | 123 |
| 105. | (-)-Cubebinin **(105)** (3R,4R) | $C_{24}H_{32}O_8$ | *P.cubeba* | fruits | | -23.33 | 119 |
| 106. | $\alpha$-O-Ethyl cubebin **(106)** (2S,3R,4R) | $C_{22}H_{24}O_6$ | *P.cubeba* | fruits | - | -- | 116 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 107. | β'-o-Ethyl cubebin **(107)** (2R,3R,4R) | $C_{22}H_{24}O_6$ | *P.cubeba* | fruit | - | - | 116 |
| | | | γ-butyrolactones | | | | |
| 108. | (-)-Hinokinin **(108)** | $C_{20}H_{18}O_6$ | *P.clusii* | whole plant | - | | 118 |
| | | | *P.cubeba* | fruit | - | -19.1 | 119 |
| | | | *P.ribesoids* | aerial part | | | 9 |
| | | | *P.trichostachyon* | fruit | | | 121 |
| 109. | (-)-Yatein **(109)** | $C_{22}H_{24}O_7$ | *P.cubeba* | fruit | - | - | 124,125 |
| | | | *P.clussi* | whole plant | - | | 118 |
| 110. | (-)-Cubebininolide **(110)** (2R,4R) | $C_{24}H_{30}O_8$ | *P.cubeba* | fruit | - | -17.6 | 124 |
| 111. | (2R,3R)-2-(3",4" methylenedioxy-benzyl)-3-(3',4' dimethoxybenzyl)-butyrolactone **(111)** | $C_{21}H_{22}O_6$ | *P.cubeba* | fruit | - | - | 124,126 |
| 112. | (-)-Isoyatein **(112)** (2R,3R) | $C_{22}H_{24}O_7$ | *P.cubeba* | fruit | - | -49.6 | 124 |
| 113. | (-)-Cubebinone **(113)** (2R,3R) | $C_{23}H_{26}O_8$ | *P.cubeba* | fruit | - | -36.1 | 124 |
| 114. | (-)-5"-Methoxy hinokinin **(114)** (2R,3R) | $C_{21}H_{20}O_7$ | *P.cubeba* | fruit | - | -37 | 116 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| | | | 2,3-dibenzylbutan-1,4-diol lignans | | | | |
| 115. | (-)-Dihydrocubebin **(115)** | $C_{20}H_{22}O_6$ | *P.clusii*<br>*P.cubeba*<br>*P.guineense*<br>*P.trichostachyon* | whole plant<br>fruit<br>fruit, leaves<br>fruits | 101-102 | - | 118<br>119<br>56,20<br>121 |
| 116. | Dihydrotrichostin **(116)** | $C_{21}H_{24}O_7$ | *P.trichostachyon* | fruits | | -13.3 | 121 |
| 117. | (-)-Dihydroclusin **(117)** (2R,3R) | $C_{22}H_{28}O_7$ | *P.cubeba* | fruits | 97-98 | -27.13 | 119 |
| 118. | 2R, 3R, 2-(7-methoxy 1,3-benzodioxol-5-yl) methyl 3-(3,4,5-trimethoxyphenyl) methyl butan-1,4-diol **(118)** | $C_{23}H_{30}O_8$ | *P.clusii* | whole plant | 58-60 | -24 | 123 |
| 119. | Hemiareinsin **(119)** | $C_{22}H_{24}O_7$ | *P.cubeba* | fruit | - | - | 116 |
| | | | 2,5-bisaryl-3,4-dimethyl tetrahydrofurans | | | | |
| 120. | (+)-Calopiptin **(120)** (2R,3S,4S,5S) | $C_{21}H_{24}O_5$ | *P.schmidtii* | stem | 79-83 | +30 | 127 |
| 121. | (-)-Zuionin **(121)**(2R,3R,4S,5S) | $C_{20}H_{20}O_5$ | *P.schmidtii* | stem | 119-21 | -85 | 127 |

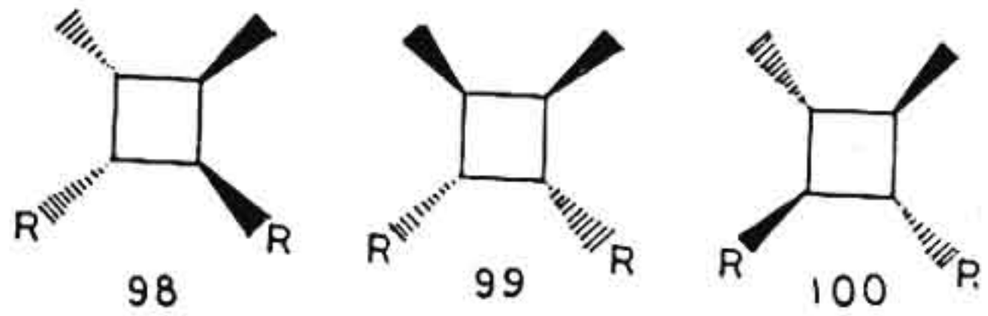
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 122. | (-)-Machilin G **(122)** (2R,3R,4S,5S) | $C_{21}H_{24}O_5$ | *P.schmidtii* | leaves | - | -12.8 | 127 |
| 123. | (-)-Galgravin **(123)** | $C_{22}H_{28}O_5$ | *P.schmidtii* | aerial part | 98-100 | -27 | 128 |
| | | | *P.wallachii* | - | | | 14 |
| | | | *P.hancei* | - | | | 14 |
| 124. | (+)-Grandicin **(124)** | $C_{12}H_{28}O_5$ | *P.polysyphorum* | | | | 117 |
| | | 2,6-bisaryl-3,7-dioxa [3,3,0] bicycloctane lignans | | | | | |
| 125. | (+)-Sesamin **(125)** | $C_{20}H_{18}O_6$ | *P.retrofractum* | aerial part | 122-24 | +64.5 | 25 |
| | | | *P.brachystachyum* | whole plant | | | 103,26 |
| | | | *P.cubeba* | fruit | | | 130 |
| | | | *P.longum* | fruit | | | 131,132,107 |
| | | | *P.peepuloids* | fruit | | | 17 |
| | | | *P.sylvaticum* | | | | 36 |
| | | | *P.guineense* | | | | 18 |
| | | | *P.clusii* | | | | 130 |
| 126. | (+)-Yangambin **(126)** | $C_{24}H_{30}O_8$ | *P.guineense* | - | - | - | 55 |
| | | | *Micropiperexcelsum* | - | | | 133 |
| 127. | Aschantin **(127)** | $C_{22}H_{24}O_7$ | *P.guineense* | - | 123 | - | 130 |
| | | | *P.clusii* | - | | | 134 |
| | | | *P.cubeba* | fruit | | | 130 |
| 128. | (+)-Asarinine **(128)** | $C_{20}H_{18}O_6$ | *P.brachystachyum* | seeds | 120 | -118.6 | 103,26 |
| | | | *P.longum* | fruits | | | 26 |

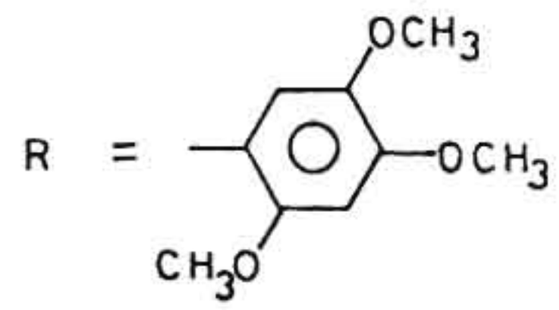
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 129. | (+)-Epiexcelsin **(129)** | $C_{22}H_{22}O_8$ | *P.aborescens* | stem | 166-67 | +120 | 71 |
| 130. | (+)-Sylvatesmin **(130)** | $C_{20}H_{24}O_6$ | *P.sylvaticum* | seeds | - | - | 135 |
| 131. | Fargesin **(131)** | $C_{21}H_{22}O_6$ | *P.brachystachyum*<br>*P.longum* | -<br>- | - | - | 26<br>26 |
| 132. | Pluviatilol **(132)** | $C_{20}H_{20}O_6$ | *P.brachystachyum*<br>*P.longum* | - | - | - | 26<br>26 |
| 133. | (+)-Diaeudesmin **(133)** | $C_{22}H_{26}O_6$ | *P.longum*<br>*P.sylvaticum*<br>*P.peepuloids* | seeds<br>seeds<br>seeds | 157-58 | +316 | 107<br>136<br>137 |
| 134. | (+)-Diayangambin **(134)** | $C_{24}H_{30}O_8$ | *P.aborescens* | stem | 153-54 | +260 | 71 |
| | | | *benzofurans* | | | | |
| 135. | Pb-53A **(135)** | $C_{21}H_{24}O_8$ | *P.clarkii* | - | - | - | 4 |
| 136. | (+)-Kadsurinone **(136)** | $C_{21}H_{24}O_5$ | *P.futokadzura*<br>*P.wallachii*<br>*P.hancei* | -<br>-<br>- | 62.5 | +3.2 | 138,139<br>14,140<br>14 |
| 137. | (-)-Kadsurin A **(137)** | $C_{21}H_{24}O_6$ | *P.futokadzuro*<br>*P.schmidtii* | -<br>- | - | -104.4 | 138<br>127 |

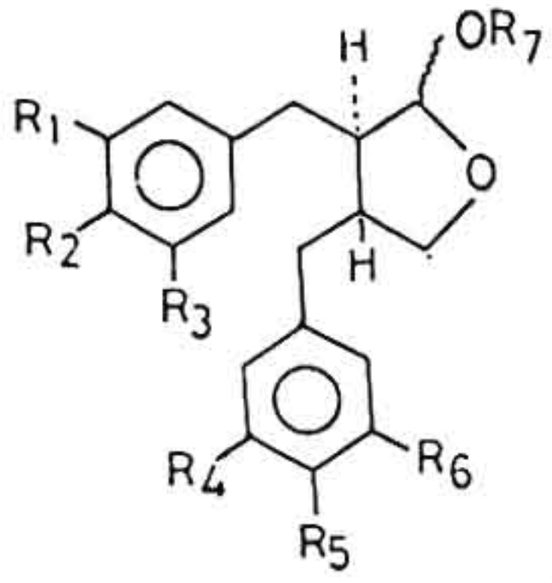
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 138. | (-)-Kadsurin B **(138)** | $C_{21}H_{26}O_6$ | *P.futokadzura* | - | 101-102 | -18.3 | 138 |
| 139. | (-)-Piperenone **(139)** | $C_{22}H_{28}O_6$ | *P.futokadzura* | leaves, stem | 86-88 | -129 | 147,148 |
| 140. | (-)-Schmiditin **(140)** | $C_{21}H_{26}O_6$ | *P.schimiditii* | aerial part | 98-100 | -21 | 128 |
| 141. | $\Delta^8$-4-Hydroxy-3-methoxy-3',4'-methylenedixoy-7.0.2'-8.3'-noeligan **(141)** (7S,8S) | $C_{20}H_{20}O_5$ | *P.capense* | roots | - | +3.40 | 141 |
| 142. | $\Delta^8$-(3,4)-(3'-4')-Bismethylenedioxy-7.0.2',8.3-neolignan **(142)** (7S,8S) | $C_{20}H_{18}O_5$ | *P.capense* | roots | - | +5.72 | 141 |
| 143. | Hancinone **(143)** | $C_{20}H_{20}O_5$ | *P.hancei* | - | - | - | 140 |
| 144. | Denudatin B **(144)** | $C_{21}H_{24}O_5$ | *P.hancei*<br>*P.wallichii* | -<br>- | | - | 140<br>14 |
| 145. | (+)-Burchellin **(145)** | $C_{20}H_{20}O_5$ | *P.hancei* | - | - | - | 12 |
| 146. | Eupomantene **(146)** | $C_{20}H_{18}O_4$ | *P.interruptum* | - | - | - | 42 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| | | | 1,2-diarylpropanes | | | | |
| 147. | $\Delta^{8}$-3',6'-Dihydro-3,4-3',4'-bismethylenedioxy-6'oxo-8.3'-neolignan **(147)** | $C_{20}H_{20}O_5$ | *P.capense* | roots | - | -132.5 | 146 |
| 148. | (-)-Isodihydro-futoquinol A **(148)** | $C_{20}H_{23}O_5$ | *P.schmidtii* | stem | - | -5.9 | 127 |
| 149. | (+)-Isodihydro-futoquinol B **(149)** | $C_{21}H_{23}O_6$ | *P.schmidtii* | stem | - | +108.5 | 127 |
| 150. | $\Delta^{8}$-1',2'-Dihydro-4-hydroxy-3-methoxy-3',4'-methylenedioxy-2'-oxo-8.1'-neolignan **(150)** | $C_{20}H_{22}O_5$ | *P.capense* | root | - | -21.8 | 146 |
| 151. | $\Delta^{8'}$-1',2'-Dihydro-3,4,3',4'-bis-methylenedioxy 2'-oxo-8.1'-neolignan **(151)** | $C_{20}H_{20}O_5$ | *P.capense* | roots | - | +3.3 | 146 |
| 152. | Iso-$\Delta^{8'}$-Dihydro-3,4,3',4'-bis-methylenedioxy-2'-oxo-8.1'-neoglinan **(152)** | $C_{20}H_{20}O_5$ | *P.capense* | roots | - | +11.2 | 146 |
| 153. | (+)-Lancifolin D **(153)** | $C_{22}H_{28}O_5$ | *P.polysyphorum* | - | - | - | 129 |
| 154. | Futoquinol (hancinone D) **(154)** | $C_{21}H_{22}O_5$ | *P.futokadzura*<br>*P.schmidtii*<br>*P.hancei*<br>*P.wallachi*<br>*P.polysyphorum* | -<br>leaves<br>-<br>-<br>- | - | - | 144<br>127<br>143<br>14<br>129 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 155. | Hancinone B **(155)** | $C_{21}H_{24}O_6$ | *P.hancei* | - | - | - | 142 |
| 156. | Hancinone C **(156)** | $C_{23}H_{28}O_7$ | *P.hancei* | - | - | - | 142 |
| | | | *P.wallachi* | - | | | 14 |
| 157. | Wallichinine **(157)** | $C_{22}H_{26}O_6$ | *P.wallachi* | - | - | - | 14 |
| | | | *P.polysyphorum* | - | | | 129 |
| 158. | Isofutoquinol B **(158)** | $C_{21}H_{22}O_5$ | *P.futokadzura* | stem, leaves | - | - | 145 |
| | | | miscellaneous lignans | | | | |
| 159. | (+)-Hancinol **(159)** | $C_{20}H_{24}O_5$ | *P.hancei* | - | - | - | 12,42 |
| 160. | Clarkinol **(160)** | $C_{19}H_{19}O_5$ | *P.clarkii* | - | - | - | 4 |
| 161. | Isofutoquinol A **(161)** | $C_{21}H_{22}O_5$ | *P.futokadzura* | leaves, stem | - | - | 145 |
| 162. | Futoenone **(162)** | $C_{20}H_{21}O_5$ | *P.futokadzura* | leaves, stem | 197 | -58 | 149 |
| 163. | $\Delta^{7'}$-3,4,5'-Trimethoxy-7-hydroxy-8.0.3'-neolignan **(163)** (7S,8R) | $C_{21}H_{25}O_5$ | *P.capense* | - | - | - | 141 |
| 164. | Polysyphorin **(164)** | $C_{23}H_{28}O_7$ | *P.polysyphorum* | - | - | - | 129 |
| 165. | (+)-Virolongin A **(165)** | $C_{23}H_{29}O_6$ | *P.polysyphorum* | - | - | - | 129 |
| 166. | (+)-Sylvone **(166)** | $C_{23}H_{28}O_8$ | *P.sylvaticum* | seeds | 138-39 | +9.6 | 150 |




101: $R_1 = R_2 = R_3 = OCH_3$, $R_4 + R_5 = -OCH_2O-$, $R_6 = R_7 = H$

102: $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = R_7 = H$

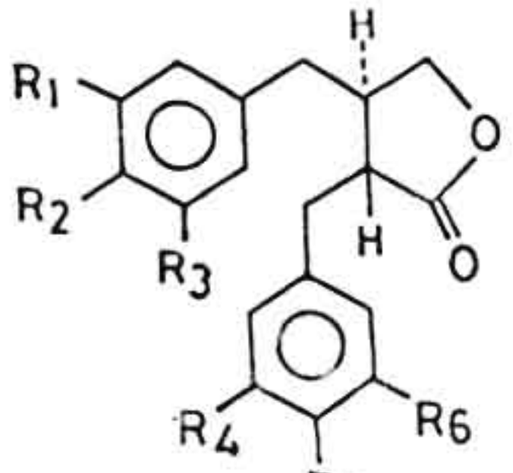
103: $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = OCH_3$, $R_6 = R_7 = H$

104: $R_1 = R_2 = R_3 = OCH_3$, $R_4 + R_5 = -OCH_2O-$, $R_6 = H$, $R_7 = C_2H_5$

105: $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3$, $R_7 = H$

106: $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = H$, $R_7 = C_2H_5$

107: $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = H$, $R_7 = C_2H_5$



108: $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = H$

109: $R_1 + R_2 = -OCH_2O-$, $R_3 = H$, $R_4 = R_5 = R_6 = OCH_3$

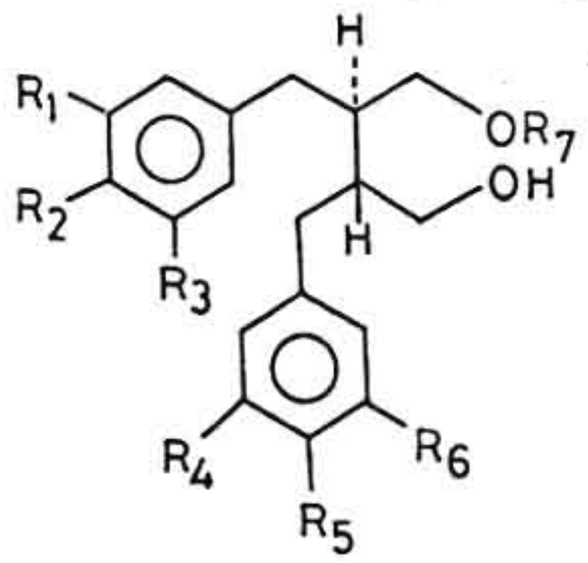
110: $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3$

111: $R_1 = R_2 = OCH_3$, $R_3 = R_6 = H$, $R_4 + R_5 = -OCH_2O-$

112: $R_1 = R_2 = R_3 = OCH_3$, $R_4 + R_5 = -OCH_2O-$, $R_6 = H$

113: $R_1 = R_2 = R_3 = R_6 = OCH_3$, $R_4 + R_5 = -OCH_2O-$

114: $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = H$, $R_6 = OCH_3$



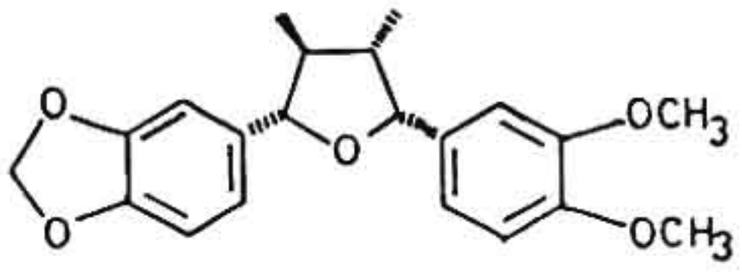
115. $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = R_7 = H$

116. $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_7 = H$, $R_6 = OCH_3$

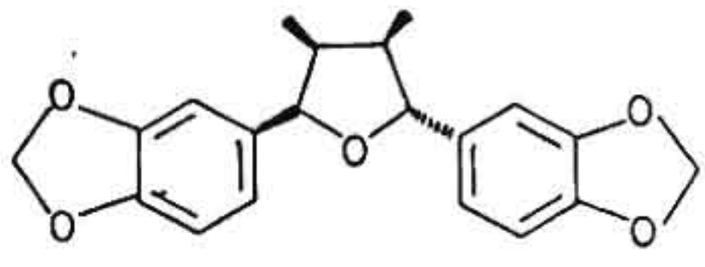
117. $R_1 + R_2 = -OCH_2O-$, $R_4 = R_5 = R_6 = OCH_3$, $R_3 = R_7 = H$

118. $R_1 = R_2 = R_3 = R_6 = OCH_3$, $R_4 + R_5 = -OCH_2O-$, $R_7 = H$

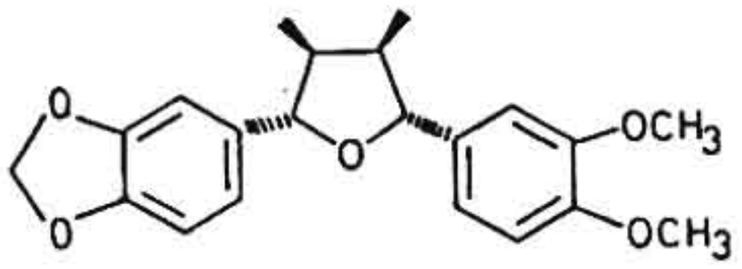
119. $R_1 + R_2 = R_4 + R_5 = OCH_2O-$, $R_3 = R_6 = H$, $R_7 = Ac$



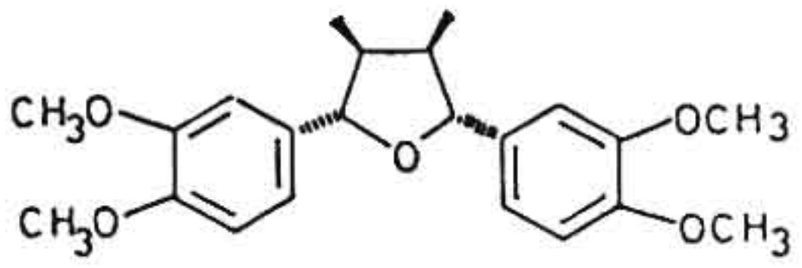
120



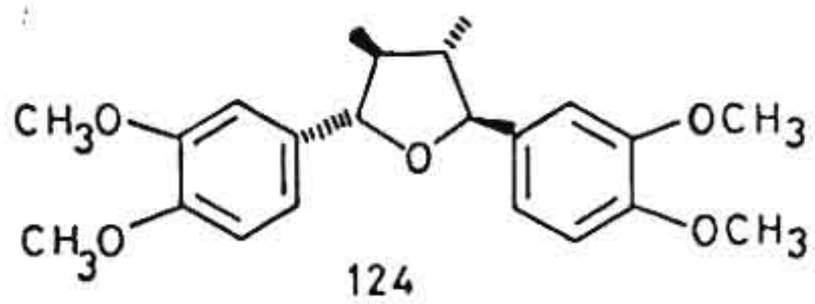
121



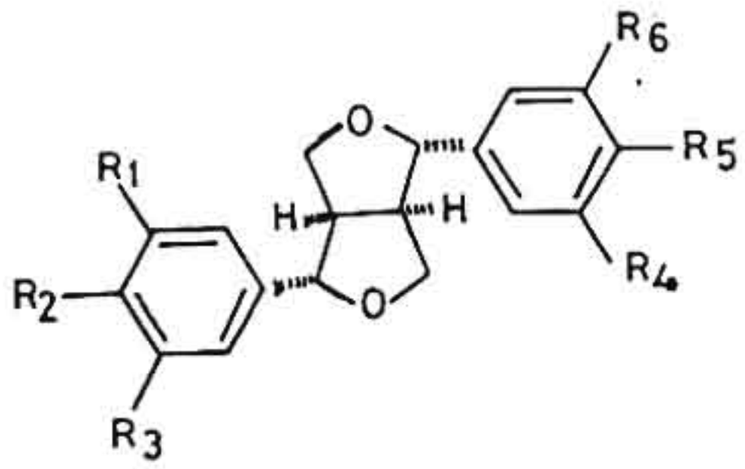
122



123



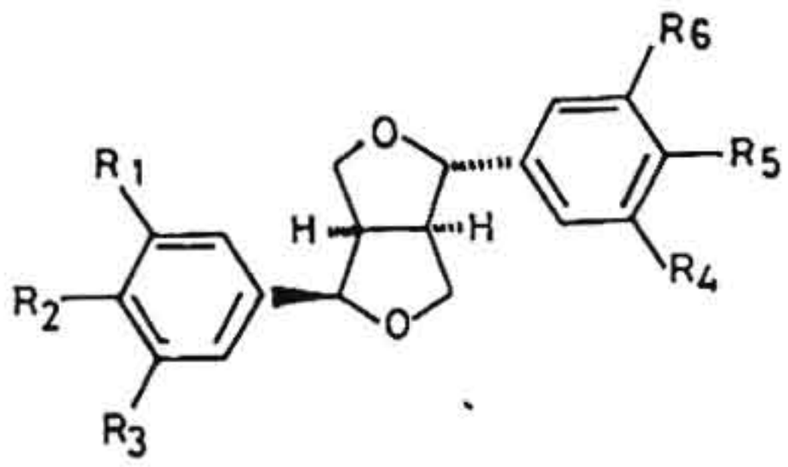
124



125. $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = H$

126. $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3$

127. $R_1 + R_2 = -OCH_2O-$, $R_3 = H$, $R_4 = R_5 = R_6 = OCH_3$




128. $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = H$

129. $R_1 + R_2 = R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = OCH_3$

130. $R_1 = R_2 = R_5 = OCH_3$, $R_4 = OH$, $R_3 = R_6 = H$

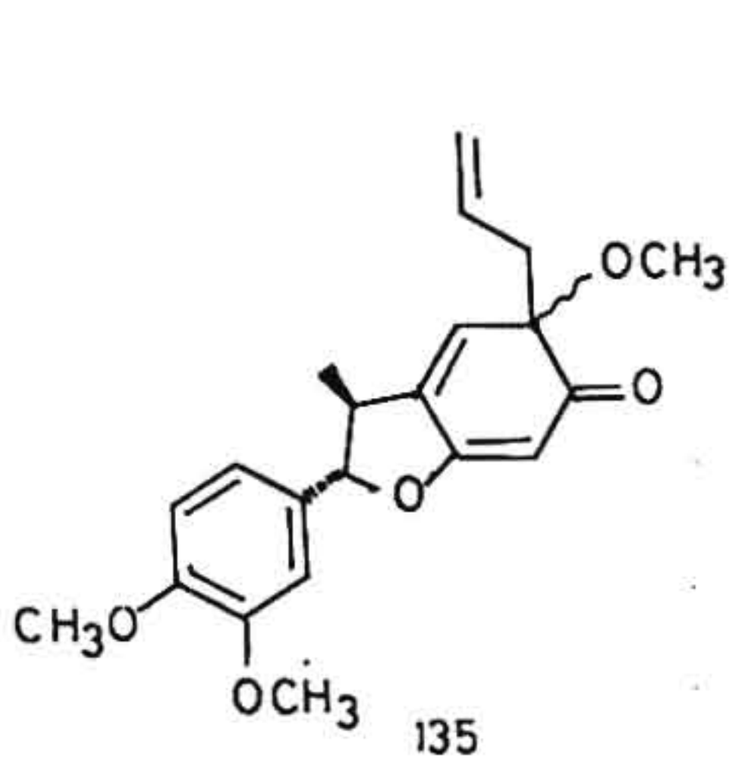
131. $R_1 = R_2 = OCH_3$, $R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = H$

132. $R_1 = OCH_3$, $R_2 = OH$, $R_4 + R_5 = -OCH_2O-$, $R_3 = R_6 = H$

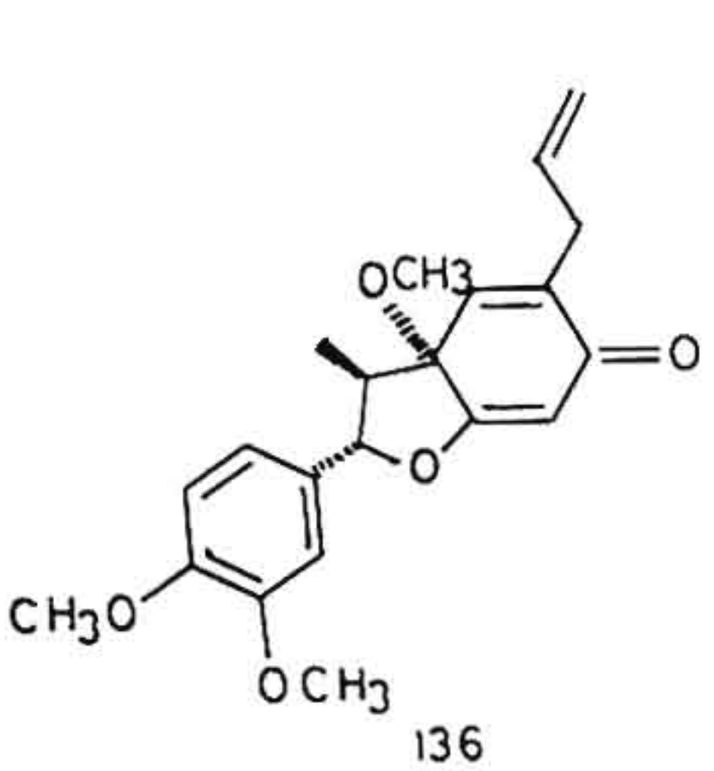



133. $R_1 = R_2 = R_4 = R_5 = OCH_3$, $R_3 = R_6 = H$

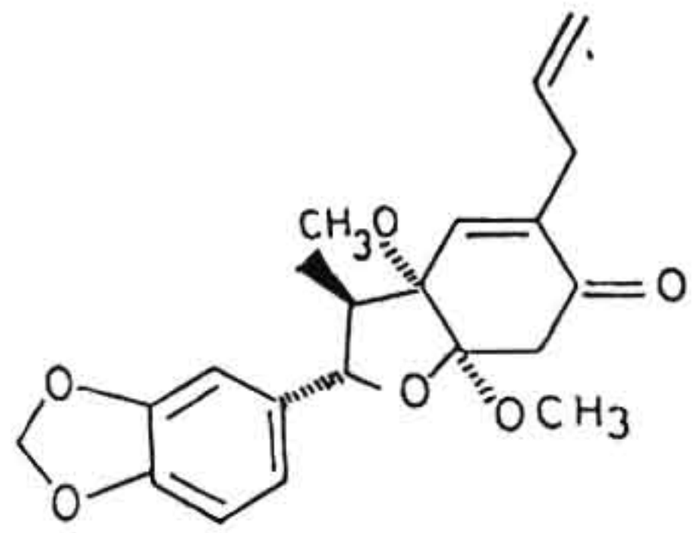
134. $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3$



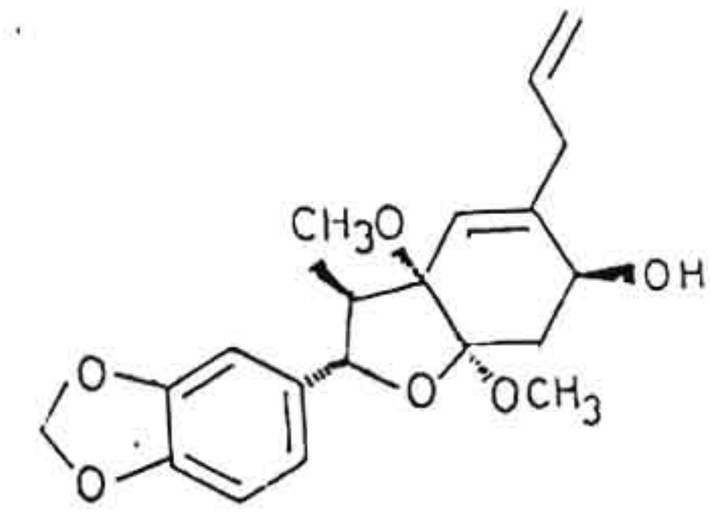

135



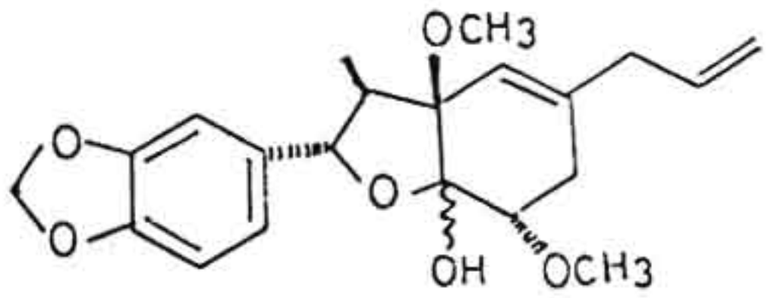

136



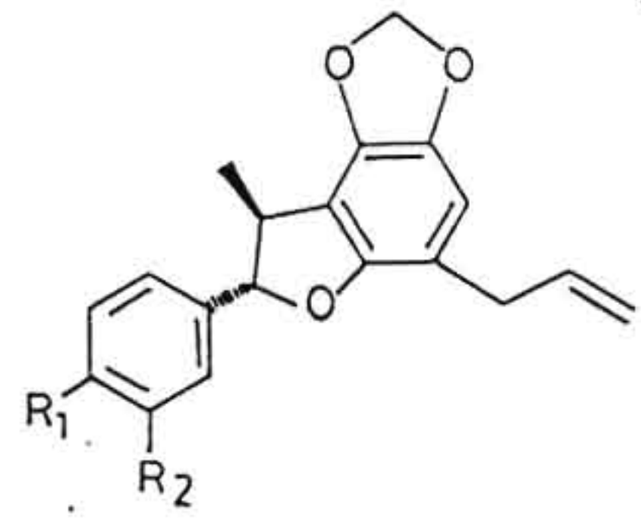
137



138

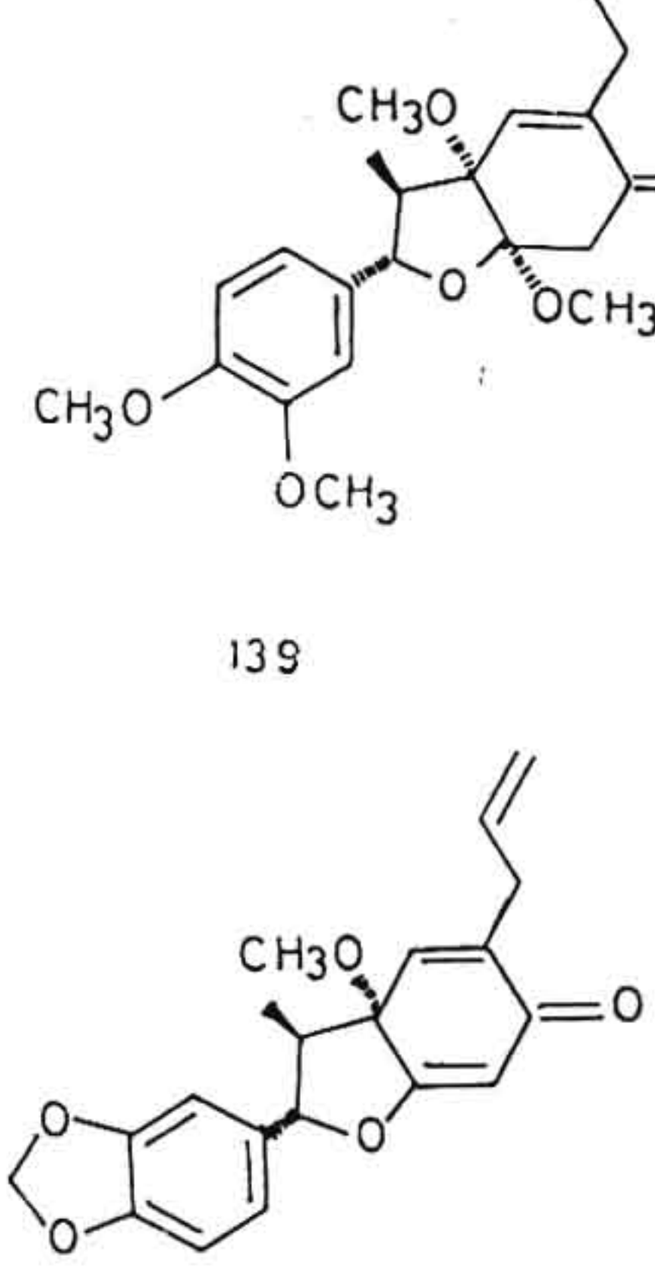


140

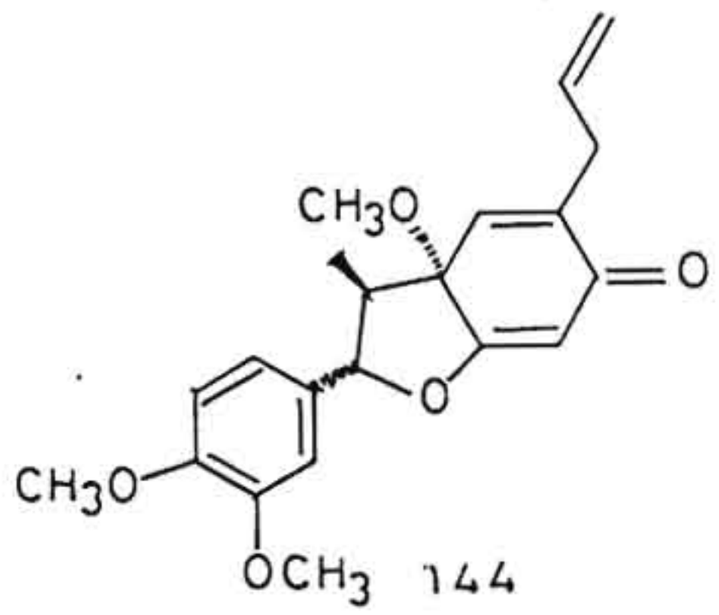


141. $R_1$ = OH, $R_2$ = $OCH_3$

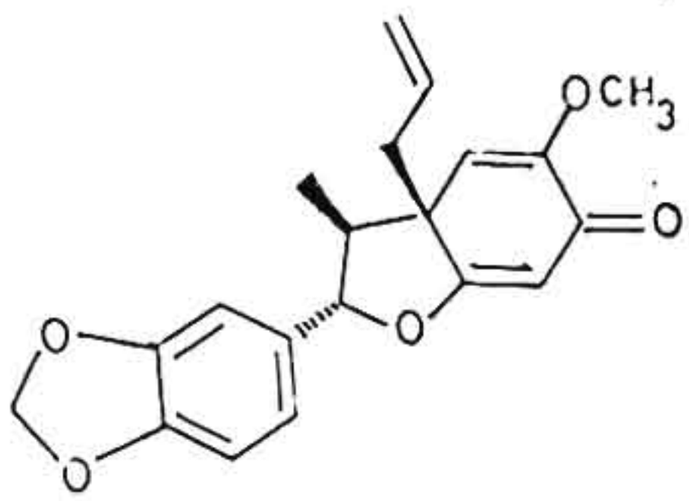
142. $R_1 + R_2$ = $-OCH_2O-$



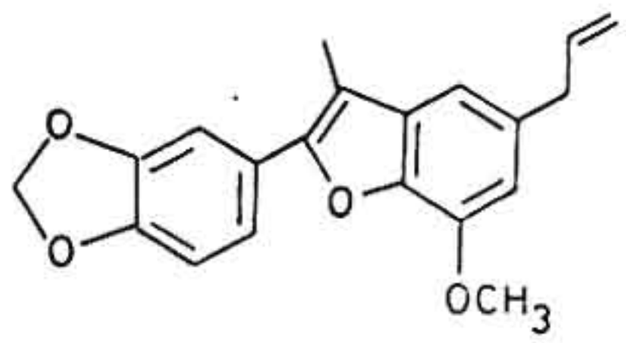
143



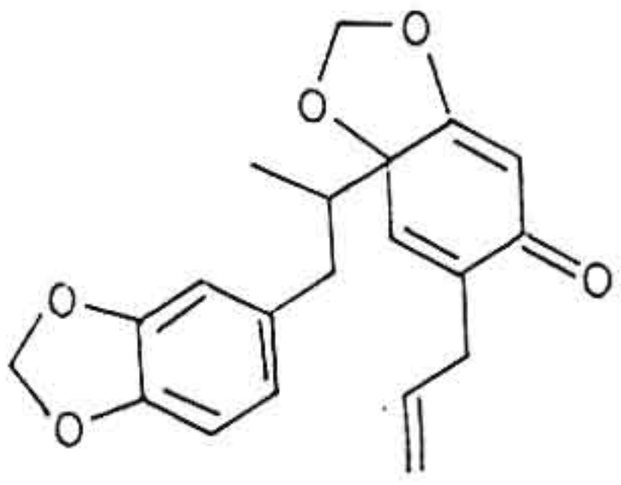
144



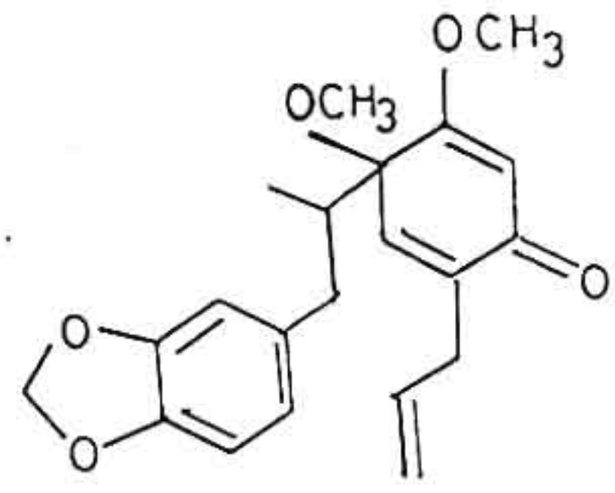
145



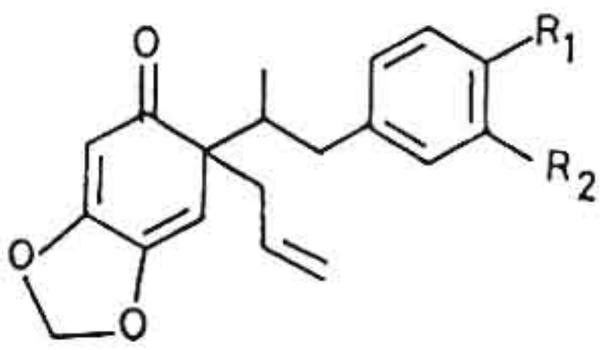
146



147

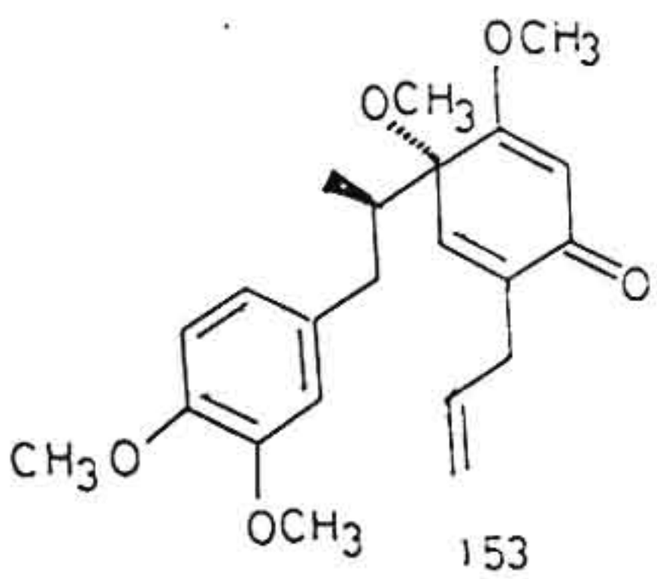


148 & 149

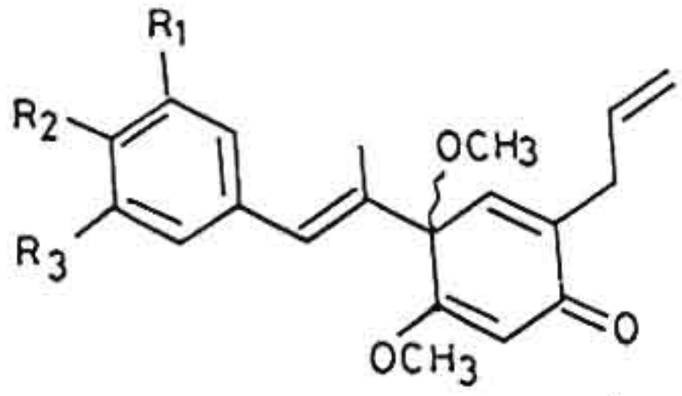


150: $R_1$=OH, $R_2$=$OCH_3$

151 & 152: $R_1$+$R_2$=-$OCH_2O$-



153

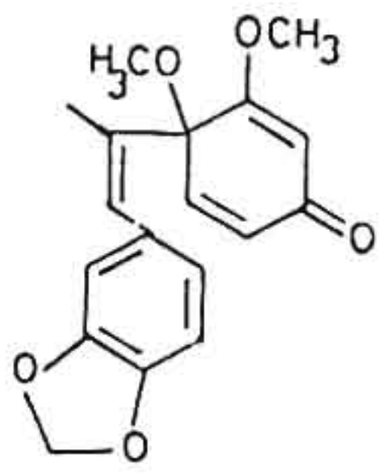


154. $R_1$ + $R_2$ = -$OCH_2O$-, $R_3$ = H

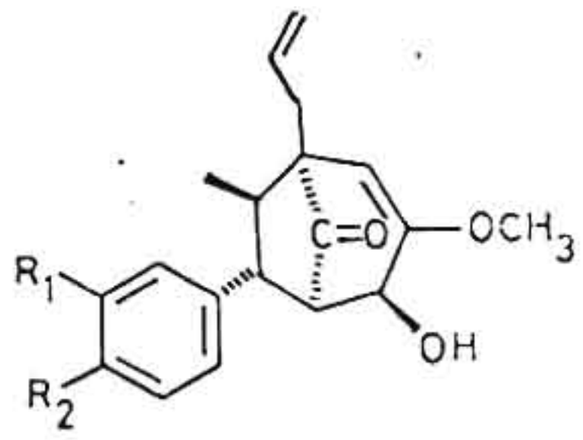
155. $R_1$ = H, $R_2$ = OH, $R_3$ = $OCH_3$

156. $R_1$ = $R_2$ = $R_3$ = $OCH_3$

157. $R_1$ = H, $R_2$ = $R_3$ = $OCH_3$

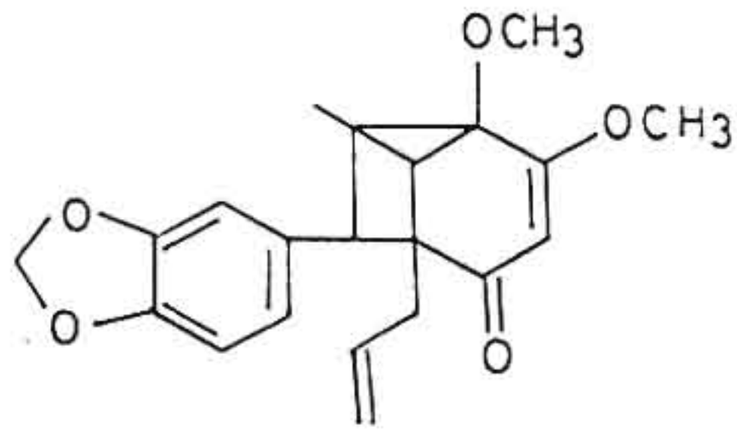


158

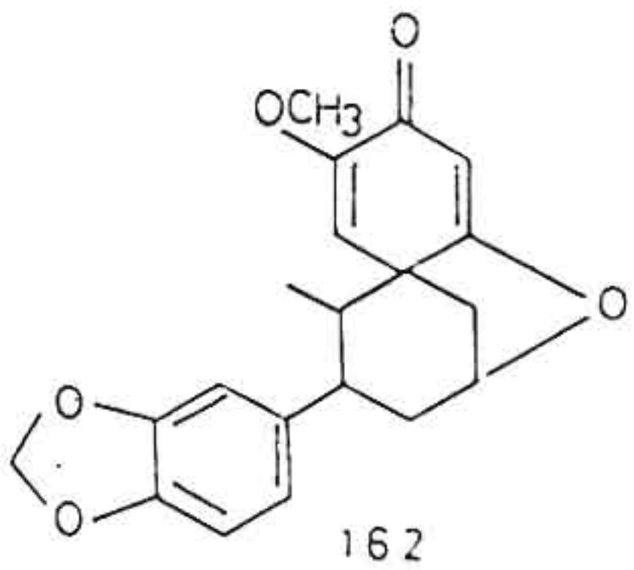


159. $R_1 = R_2 = OCH_3$

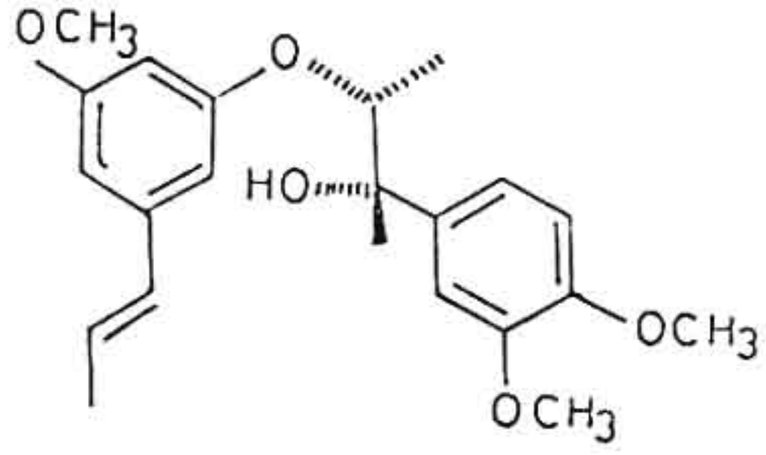
160. $R_1 + R_2 = -OCH_2O-$



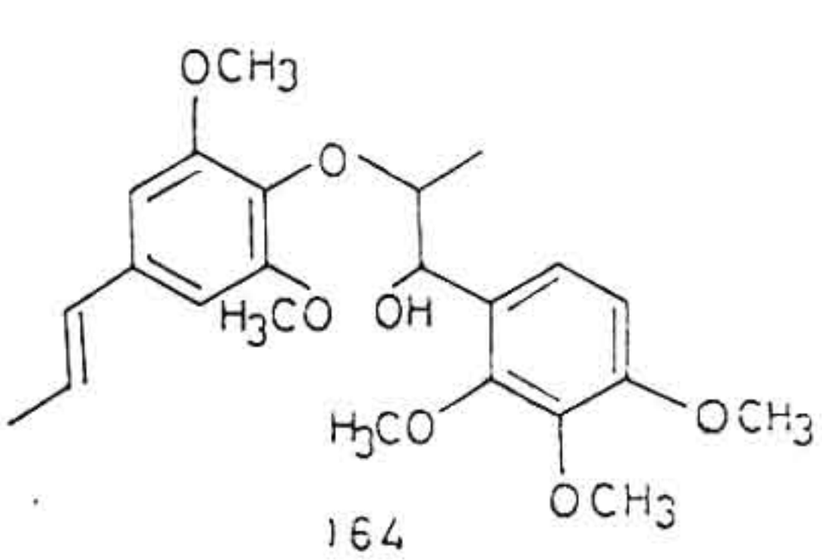
161



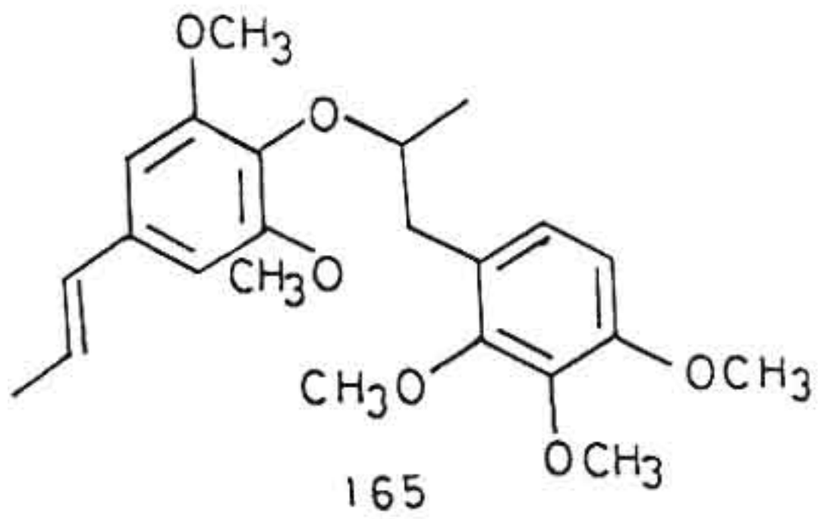
162



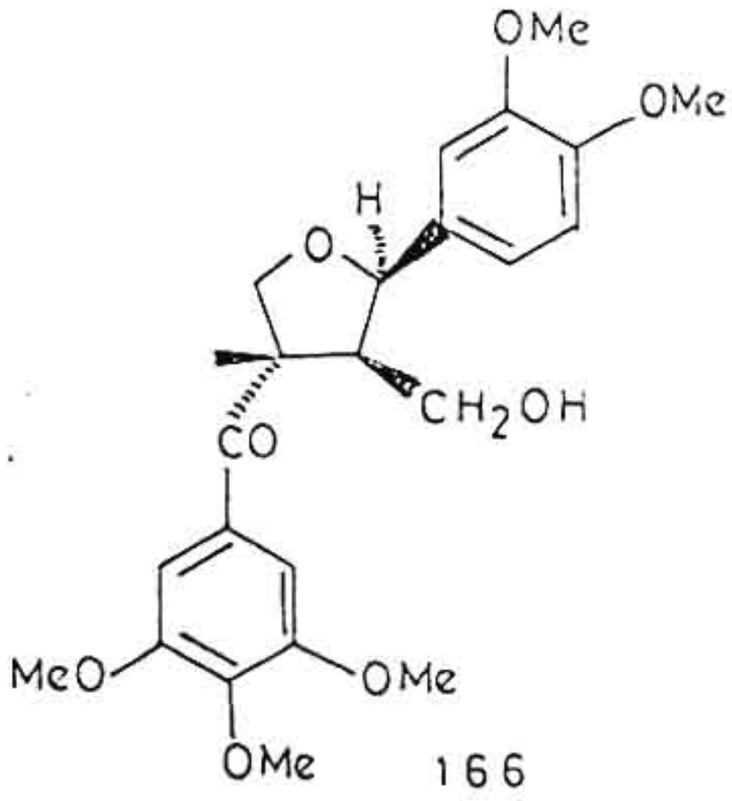
163



164



165



166

three isomers are reported to occur in P.cubeba[116] and P.sumatranum[117].

b) 3,4-dibenzyl-γ-butyrolactol lignans:

The occurrence of this class of lignans is limited to a few species such as P.cubeba, P.clusii[118] and P.trihostachyon. (-)-Cubebin[121] (102) is however reported in several species. These lignans are known to exist as a mixture of epimers[151].

c) γ-butyrolactones:

Most of these lignans are found to occur in P.cubeba. (-)-Yatein (109) is also found to occur in P.clusii.[118] (-)-Hinokinin (108) occurs in two more species, P.ribesoides[9] and P.trichostachyon[121].

d) 2,3-dibenzylbutane-1,4-diol lignans:

(-)-Dihydrocubebin (115) which was earlier known as a synthetic compound has been isolated from P.guineense[56,20]. All of these four lignans are found to co-occur along with the corresponding dibenzylbutyrolactols and lactones.

e) 2,5-bisaryl-3,4-dimethyl tetrahydrofurans:

The occurrence of this class of lignans is reported only very recently from P.schimdtii[127,128]. (-)-Galgravin

(123) is reported to occur in P.wallichi[14] and P.hancei[14] also. (+)- Gradisin (124) is isolated from P.polysyphorum[129].

f) 2,6-bisaryl-3,7-dioxa(3.3.0)bicyclooctane lignans:

2,6-Bisaryl-3,7-dioxa(3.3.0)bicyclooctane lignans occur widely in Piper species. Among these (+)-sesamin (125) is the most commonly occurring lignan.

g) benzofurans:

P.futokadsura is a rich source of benzofuran lignans. Several other Piper species are also reported to contain this type of lignans. All these lignans have trans stereochemisty of the methyl and aromatic groups. The absolute configuration has only been established for (-)-piperenone[152] (139), denudatin B[153] (144) and (+)-burchellin[154] (145).

h) 1,2-diarylpropanes:

1,2-diarylpropane lignans are the largest group of lignans isolated from Piper species and generally co-occur with benzofurans. While the stereochemistry of most of the lignans are well established, the absolute configuration[155] of 148 and 149 and 153 is determined by ORD measurements.

(iii) Flavonoids:

The natural occurrence of flavonoids in Piper species is recorded in Table 3. A careful analysis on their occurrence indicates that till recently P.methysticum[158,159] is the only Piper species from which several flavanones and chalcones have been isolated. More recently a few more species such as P.fadyenii[156], P.hispidum[160], P.aduncum[157], P.sylvaticum[136] and P.hostmannianum[161] were found to contain some more flavonoids. Flavonoid glycosides vitexin and marginotoside-6"-O-β-gentiobioside are reported to occur in the leaves of P.marginatum[169,170]. Glycosides of kaempherol, rhamnetin, quercetin and isorhamnetin are also reported in P.nigrum berries[171].

(iv) Kawa-lactones and butenolides:

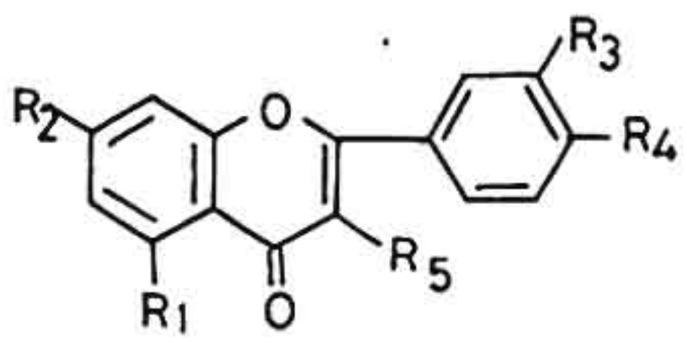
The natural occurrence of kawa-lactones and butenolides in Piper species is recorded in Table 4. The α-pyrones originally derived from kawa-kawa (P.methysticum) are known as kawa-lactones. These are generally 4-methoxy-2-pyrones with phenyl or styryl substituents at 6-position. Besides P.methysticum the only other Piper species that has yielded compounds of this group is P.sanctum. The S-configuration at C-6 position of 11-hydroxy-12-methoxy-

Table 3 : Natural occurrence of flavonoids in Piper Species

| Sl. No. | Compound | Mol formula | Source | Part | M.P. $^{o}C$ | Reference |
|---|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| 167. | 5-Hydroxy-7-methoxy flavone **(167)** (Tectochrysin) | $C_{16}H_{12}O_4$ | *P.sylvaticum*<br>*P.falconeri* | seeds<br>leaves & stem | 165<br>163 | 136<br>214 |
| 168. | 5-Hydroxy-3',4',7-trimethoxyflavone **(168)** | $C_{18}H_{16}O_6$ | *P.sylvaticum* | seeds | - | 136 |
| 169. | 3,5-Dihydroxy-4',7-dimethoxy flavone **(169)** | $C_{18}H_{16}O_6$ | *P.sylvaticum* | seeds | - | 135 |
| 170. | 7,4'-Dimethoxy-5,3'-dihydroxy flavone **(170)** | $C_{17}H_{14}O_6$ | *P.auritum* | leaves | - | 196 |
| 171. | Apigenin dimethylether **(171)** | $C_{17}H_{14}O_5$ | *P.falconeri* | leaves & stem | 163 | 214 |
| 172. | Alpinetine **(172)** | $C_{16}H_{14}O_4$ | *P.methysticum* | root | 225 | 158,159 |
| 173. | Dihydrooroxylin A **(173)** | $C_{16}H_{15}O_5$ | *P.methysticum* | root | 188-200 | 158,159 |
| 174. | Dihydrotectochrysin **(174)** (Pinostrobin) | $C_{16}H_{14}O_4$ | *P.methysticum*<br>*P.fadyenii*<br>*P.hispidum*<br>*P.aduncum* | root<br>-<br>fruit<br>fruit | 100-01 | 158,159<br>156<br>156,157<br>156,157 |
| 175. | 6-Hydroxy-5,7-dimethoxy flavanone **(175)** | $C_{17}H_{16}O_5$ | *P.hispidum* | branches<br>leaves & fruits | - | 160<br>160 |
| 176. | 8-Hydroxy-5,7-dimethoxy flavanone **(176)** | $C_{17}H_{16}O_5$ | *P.hispidum* | branches,leaves & fruits | - | 160 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 177. | 5,7,8-Trimethoxy flavanone **(177)** | $C_{18}H_{18}O_5$ | *P.hispidum* | branches, leaves & fruits | - | 160 |
| 178. | 5-Hydroxy-7-methoxy-6,8-dimethyl flavanone **(178)** | $C_{18}H_{18}O_4$ | *P.hostmannianum* | stem bark | 146-47 | 161 |
| 179. | 5,7-Dihydroxy flavanone **(179)** | $C_{15}H_{12}O_4$ | *P.hostmannianum* | stem bark | - | 161 |
| 180. | Alpinetin chalcone **(180)** | $C_{16}H_{13}O_4$ | *P.methysticum* | root | - | 158,159 |
| 181. | Flavokawain A **(181)** | $C_{18}H_{17}O_5$ | *P.methysticum* | root | 144- 6 | 162,163,164 |
| 182. | Flavokawain B **(182)** | $C_{17}H_{15}O_4$ | *P.methysticum* | root | 90-91 | 158,162,163,164 |
| 183. | Flavokawain C **(183)** | $C_{17}H_{16}O_5$ | *P.methysticum* | root | 195-96 | 164,165,166 |
| 184. | Pinostrobin chalcone **(184)** | $C_{16}H_{13}O_4$ | *P.methysticum* | root | - | 158,159 |
| 185. | 5,6-Dihydroxy-2,4-dimethoxy chalcone **(185)** | $C_{17}H_{15}O_5$ | *P.hispidum* | branches & leaves | - | 160 |
| 186. | 6-Hydroxy-2,4,5-trimethoxy chalcone **(186)** | $C_{18}H_{17}O_5$ | *P.hispidum* | branches & leaves | - | 160 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 187. | 2-Hydroxy-4,6,4'-trimethoxy chalcone **(187)** | $C_{18}H_{17}O_5$ | *P. methysticum* | rhizome | - | 167 |
| 188. | 2-Hydroxy-4,6-dimethoxy chalcone **(188)** | $C_{17}H_{16}O_4$ | *P. methysticum* | rhizome | - | 167 |
| 189. | Pinostrobin dihydrochalcone **(189)** | $C_{16}H_{15}O_4$ | *P. hispidum*<br>*P. aduncum* | fruit<br>fruit | 164-65 | 156,157<br>156,157,168 |
| 190. | Vitexin **(190)** | $C_{21}H_{20}O_{11}$ | *P. marginatum* | leaves | - | 169,170 |
| 191. | Marginotoside-6"-o-β-gentiobioside **(191)** | | *P. marginatum* | leaves | - | 170 |



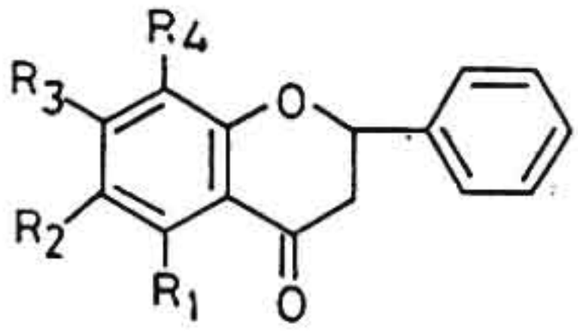
167. $R_1$ = OH, $R_2$ = $OCH_3$, $R_3$ = $R_4$ = $R_5$ = H

168. $R_1$ = OH, $R_2$ = $R_3$ = $R_4$ = $OCH_3$, $R_5$ = H

169. $R_1$ = $R_5$ = OH, $R_2$ = $R_4$ = $OCH_3$, $R_3$ = H

170. $R_1$ = $R_3$ = OH, $R_2$ = $R_4$ = $OCH_3$, $R_5$ = H

171. $R_1$ = OH, $R_2$ = $R_4$ = $OCH_3$, $R_3$ = H, $R_5$ = H



172. $R_1$ = $OCH_3$, $R_2$ = $R_4$ = H, $R_3$ = OH

173. $R_2$ = $OCH_3$, $R_1$ = $R_3$ = OH, $R_4$ = H

174. $R_1$ = OH, $R_2$ = $R_4$ = H, $R_3$ = $OCH_3$

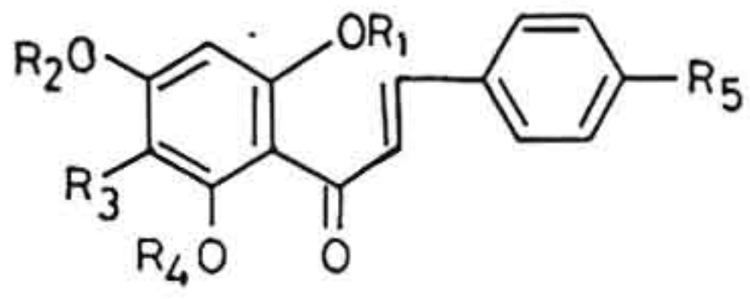
175. $R_2$ = OH, $R_1$ = $R_3$ = $OCH_3$, $R_4$ = H

176. $R_4$ = OH, $R_1$ = $R_3$ = $OCH_3$, $R_2$ = H

177. $R_1$ = $R_3$ = $R_4$ = $OCH_3$, $R_2$ = H

178. $R_1$ = OH, $R_3$ = $OCH_3$, $R_2$ = $R_4$ = $CH_3$

179. $R_1$ = $R_3$ = OH, $R_2$ = $R_4$ = H



180. $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = CH_3$

181. $R_2 = R_4 = CH_3$, $R_5 = OCH_3$, $R_1 = R_3 = H$

182. $R_2 = R_4 = CH_3$, $R_1 = R_3 = R_5 = H$

183. $R_2 = R_4 = CH_3$, $R_5 = OH$, $R_1 = R_3 = H$

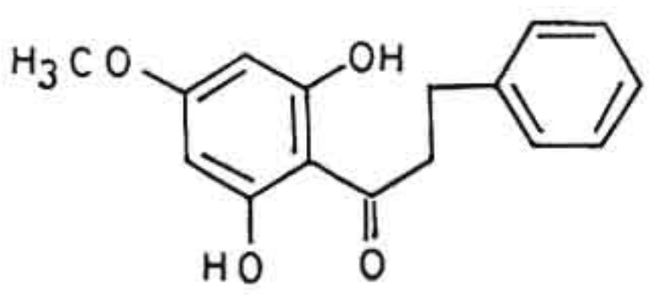
184. $R_2 = CH_3$, $R_1 = R_3 = R_4 = R_5 = H$

185. $R_3 = OH$, $R_1 = R_2 = CH_3$, $R_4 = R_5 = H$

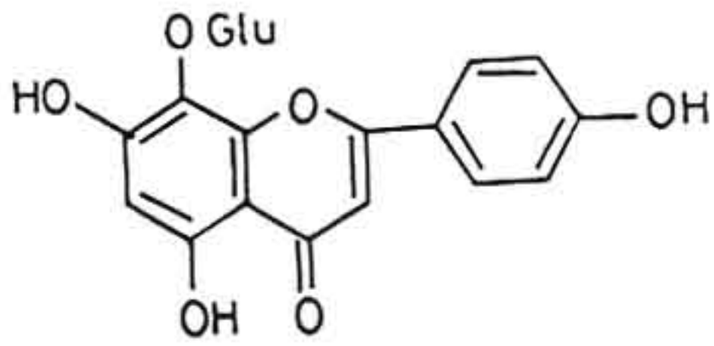
186. $R_3 = OCH_3$, $R_1 = R_2 = CH_3$, $R_4 = R_5 = H$

187. $R_1 = R_2 = CH_3$, $R_5 = OCH_3$, $R_3 = R_4 = H$

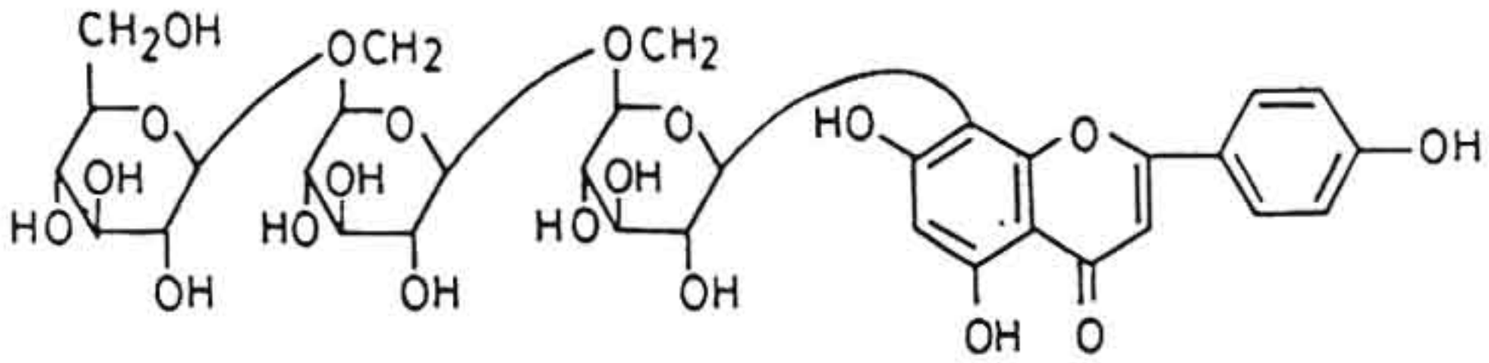
188. $R_1 = R_2 = CH_3$, $R_3 = R_4 = R_5 = H$



189



190

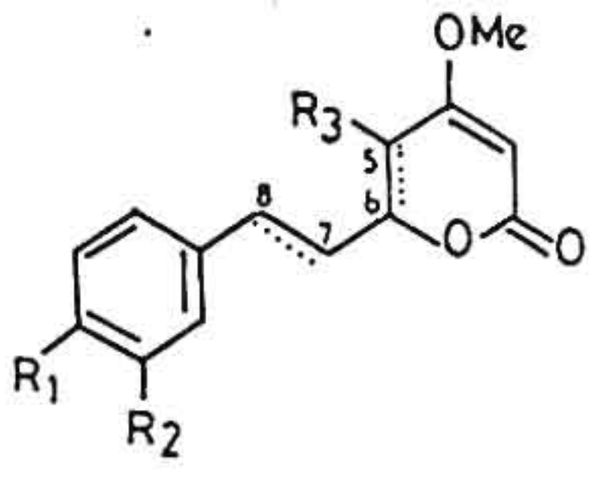


191

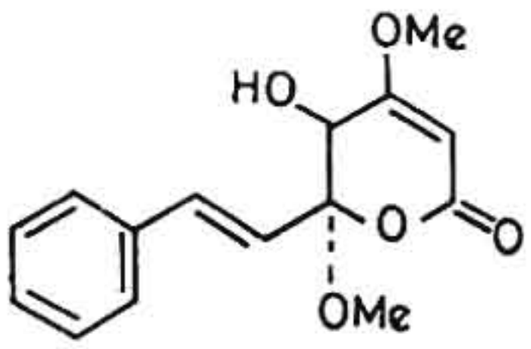
**Table 4: Natural occurrence of Kawa - Lactones and Butenolides in Piper Species**

| Sl. No. | Compound | Mol formula | Source | Part | M.P. $^{o}C$ | Reference |
|---|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| 192. | Dihydrokawain **(192)** | $C_{14}H_{16}O_3$ | *P.methysticum* | rhizome stem, leaves | 56-60 | 172,173,174 215 |
| 193. | Kawain **(193)** | $C_{14}H_{14}O_3$ | *P.methysticum* | rhizome, stem, leaves | 108-10 | 172,173,174 |
| 194. | Desmethoxy-yangonin **(194)** | $C_{14}H_{12}O_3$ | *P.methysticum* | rhizome, stem, leaves | - | 174,175 |
| 195. | Tetrahydro-yangonin **(195)** | $C_{15}H_{18}O_4$ | *P.methysticum* | rhizome, stem, leaves | - | 174,175 |
| 196. | Yangonin **(196)** | $C_{15}H_{14}O_4$ | *P.methysticum* | rhizome, stem, leaves | 153-54 | 172,173,174 |
| 197. | Dihydromethysticin **(197)** | $C_{15}H_{16}O_5$ | *P.methysticum* | rhizome, stem, leaves | - | 172,173,174 |
| 198. | Methysticin **(198)** | $C_{15}H_{16}O_5$ | *P.methysticum* | rhizome, stem, leaves | 136-37 | 172 ,173,174 |
| 199. | 11-Methoxy 12-nor yangonin **(199)** | $C_{15}H_{14}O_5$ | *P.methysticum* | stem, root | 160-61 | 158 |

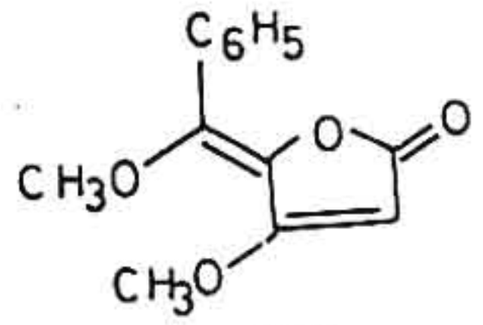
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 200. | 5-Acetoxy-6 methoxy kawain **(200)** | $C_{16}H_{16}O_5$ | *P.sanctum* | root | 176-78 | 176 |
| 201. | 5-Methoxy-5,6-dehydro-methysticin **(201)** | $C_{16}H_{14}O_6$ | *P.sanctum* | root | 255-61 | 177 |
| 202. | Dihydrokawain-5-ol **(202)** | $C_{14}H_{15}O_4$ | *P.methysticum* | root | 92 | 125 |
| 203. | 11-Hydroxy-12-methoxy-dihydrokawain **(203)** | $C_{15}H_{18}O_4$ | *P.methysticum* | root | 165-67 | 178 |
| 204. | 11,12-Dimethoxy dihydro-kawain **(204)** | $C_{16}H_{20}O_5$ | *P.methysticum* | root | 124-25 | 178 |
| 205. | (+)-5-Hydroxy-4,6-dimethoxy 6-trans-styryl-5,6-dihydro-2H-Pyran-2-one **(205)** | $C_{15}H_{16}O_5$ | *P.sanctum* | woody under-ground part | - | 183 |
| 206. | 5,6-E-Fadyenolide **(206)** | $C_{13}H_{12}O_4$ | *P.fadynii*<br>*P.aduncum*<br>*P.hispidum* | aerial part<br>"<br>" | 128-30 | 179,156<br>156<br>156 |
| 207. | 5,6-Z-Fadyenolide **(207)** | $C_{13}H_{12}O_4$ | *P.aduncum*<br>*P.hispidum*<br>*P.fadynii* | aerial part<br>"<br>" | 127-29 | 156<br>156<br>179,155 |
| 208. | Piperolide **(208)** | $C_{15}H_{14}O_4$ | *P.sanctum* | - | 110-12 | 180 |
| 209. | Methylenedioxy-piperolide **(209)** | $C_{16}H_{14}O_6$ | *P.sanctum* | stem & root | 221-24 | 181 |
| 210. | Epoxypiperolide **(210)** | $C_{15}H_{15}O_5$ | *P.sanctum* | - | - | 181,182 |



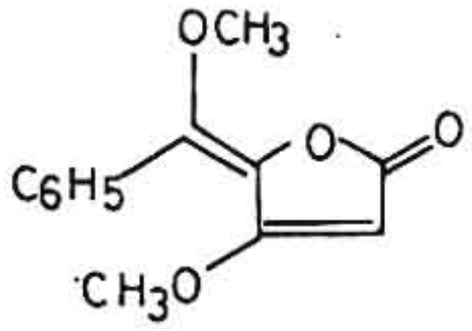

| | $R_1$ | $R_2$ | $R_3$ | $C_5$-$C_6$ | $C_7$-$C_8$ |
|---|---|---|---|---|---|
| 192. | H | H | H | - | - |
| 193. | H | H | H | - | = |
| 194. | H | H | H | = | = |
| 195. | $OCH_3$ | H | H | - | - |
| 196. | $OCH_3$ | H | H | = | = |
| 197. | $-OCH_2O-$ | | H | - | - |
| 198. | $-OCH_2O-$ | | H | - | = |
| 199. | OH | $OCH_3$ | H | = | = |
| 200. | H | H | OAC (5-methoxy) | - | = |
| 201. | $-OCH_2O-$ | | $OCH_3$ | = | = |
| 202. | H | H | OH | - | - |
| 203. | $OCH_3$ | OH | H | - | - |
| 204. | $OCH_3$ | $OCH_3$ | H | - | - |



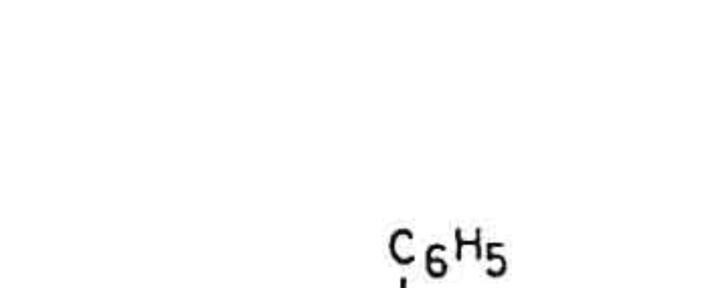
205



206

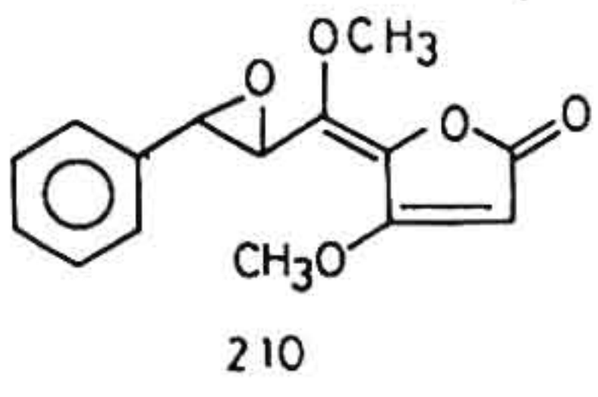


207



208. $R_1 = R_2 = H$

209. $R_1 + R_2 = -OCH_2O-$



210

dihydrokawain (203) and 11,12-dimethoxydihydrokawain (204) is also established[178].

Only five butenolides related to kawain have so far been isolated from four Piper species namely P.sanctum, P.aduncum, P.hispidum and P.fadyenii. The absolute configuration of epoxypiperolide (210) was demonstrated as 2S, 3R[182].

(v) Cyclohexane derivatives:

The natural occurrence of cyclohexane derivatives in Piper species is recorded in Table 5. Six cyclohexane derivatives are known to occur in Piper species. Among them crotepoxide (211) known to possess significant antitumour activity over Lewis lung carcinoma[195] was reported to occur in several Piper species.

(vi) Miscellaneous Compounds:

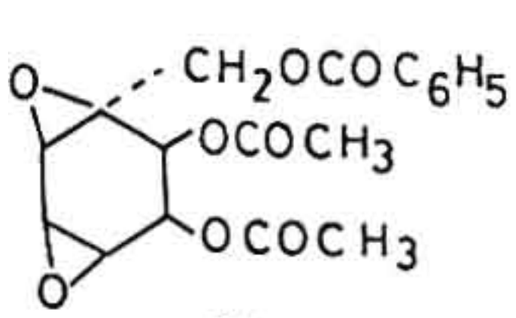
The compounds discussing under miscellaneous group is divided into the following classes:

1. Monoterpenes and Sesquiterpenes
2. Triterpenes
3. Sterols
4. Aliphatic compounds
5. Aromatic Compounds

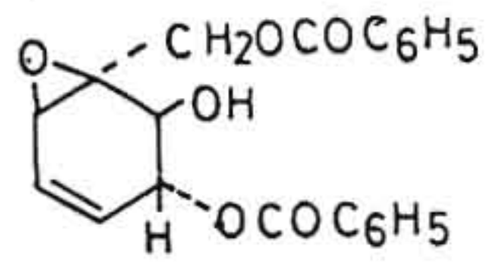
**Table 5: Natural occurrence of cyclohexane derivatives in Piper Species**

| Sl. No. | Compound | Mol formula | Source | Part | M.P. $^{o}C$ | $[\alpha]_D$ | Ref. |
|---|---|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| 211. | Crotepoxide (futoxide) **(211)** | $C_{18}H_{18}O_8$ | *P.futokadsura* | leaves, stem | 150-53 | +79.5 | 184,185 |
| | | | *P.attenuatum* | whole plant | | | 186,187 |
| | | | *P.hookeri* | stem & whole plant | | | 187,188 |
| | | | *P.brachystachyum* | whole plant | | | 189 |
| | | | *P.galcatum* | whole plant | | | 187 |
| | | | *P.clarkii* | stem & leaves | | | 190 |
| | | | *P.cubeba* | fruit | | | 191 |
| | | | *P.hancei* | fruit | | | 142 |
| | | | *P.interruptum* | stem | | | 192 |
| | | | *P.wallachi* | - | | | 14 |
| 212. | Pipoxide **(212)** | $C_{21}H_{18}O_6$ | *P.hookeri* | leaves & whole plant | 154 | -49 | 187,193, |
| | | | *P.nigrum* | whole plant | | | 187 |
| 213. | Pipoxide chlorohydrin **(213)** | $C_{21}H_{19}O_6Cl$ | *P.hookeri* | leaves & whole plant | | | 187,194 |
| | | | *P.nigrum* | whole plant | 203-106 | +93 | 187 |
| 214. | Piperenol A **(214)** | $C_{21}H_{20}O_7$ | *P.clarkii* | fruit | 48-49 | +14.6 | 191 |
| | | | *P.cubeba* | fruit | | | |
| 215. | Piperenol B **(215)** | $C_{21}H_{20}O_7$ | *P.cubeba* | fruit | - | +50 | 191 |

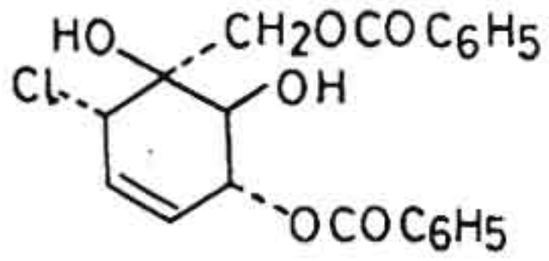
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 216. | Acetyl Piperenol A **(216)** | $C_{23}H_{22}O_8$ | *P.clarkii* | fruit | - | +12 | 191 |
| 217. | (+)-Zeylenol **(217)** | $C_{21}H_{20}O_7$ | *P.cubeba* | fruit | 131-32 | +100 | 191 |



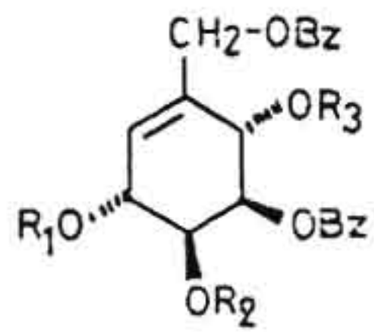
211



212

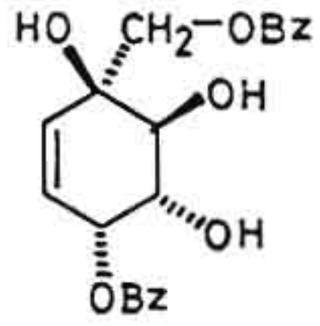


213

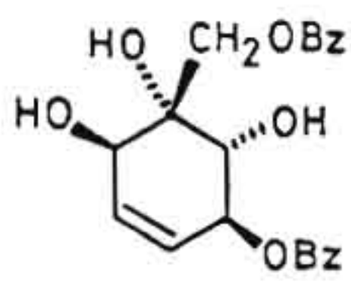


214. $R_1 = R_2 = R_3 = H$

216. $R_1 = Ac$, $R_2 = R_3 = H$



215



217

## 1. Monoterpenes and Sesquiterpenes

The chemistry of the volatile oil of the Piper species has been satisfactorily studied only in recent years. Lots of volatile constituents comprising of monoterpenes and sesquiterpenes have been identified by different workers from P.nigrum and is reviewed by Govindarajan[5] and Purseglove et al[197]. Most of these compounds have been identified as mixed compounds by GC and GC-MS. Reports on the volatiles from other species such as P.longum, P.betel, and P.cubeba are also available[5,6].

The sesquiterpene, ishwarol is isolated from P.amalago[198] and capentin from P.capense[199]. Capentin is a highly oxidised farnesene sesquiterpene with an unusual eleven-membered ether ring. Isosafrole, elemicin and sarison have been isolated from P.lenticellosum[200]. Bicyclosesquiphellandrene and 1-epicyclo-sesquiphellandrene are two more sesquiterpenes separated from P.cubeba[201]. Eupamentene is isolated from P.interruptum[42] and germacrene D from P.japonica and P.kadsura[202].

## 2. Triterpenes

There are only two reports on the occurrence of triterpenes. The first report is in P.aurantiacum from

which friedelin and epi-friedelanol were isolated by Banerji et al[203]. Friedelin is also isolated from P.schmidtii[128].

3. Sterols

The common phytosterol present in most of Piper species is β-sitosterol. It is reported to be present in about twenty Piper species. Its glycosides are also reported from a few species. Other sterols isolated from some of the species are cholestanol[204], cholesterol[204] and daucosterol[59].

4. Aliphatic compounds

Aliphatic hydrocarbons, acids, alcohols, esters and ketones are reported to be present in several Piper species. Widely distributed aliphatic compounds in this genus are n-triacontane[204], pentatriacontane[205], hentriacontane and n-triacontanol[205]. The aliphatic ketone hentriacontane-16-one is isolated from P.longum[206]. Several homologous series of aliphatic alcohols ($C_{12}$ to $C_{24}$) have been isolated from P.methysticum[207]. Some of the fatty acids present in Piper species are stearic, palmitic and caproic acids. The only unsaturated fatty acid reported is linolenic acid from P.aurantiacum[204]. 3,4-Dimethoxyphenyl

propionic acid is reported from P.arboricola[208]. The esters 2,4,6-heptatrienoate and methyl - 2E, 4E, 6E, 7-phenyl-2,4,6-heptatrienoate are also isolated from P.ribesoides and 3-(4-hydroxyphenyl)-propyl tetracosanoate from P.clarkii[190].

5. Aromatic compounds

Many aromatic organic acids such as benzoic, vanillic, cinnamic and hydroxy cinnamic acid and their derivatives are also reported to occur in several Piper species. In a few cases these acids or esters are found to contain a Y-dimethyl allyl group. Several phenolic compounds such as 3,4-dimethoxytoluene and 1-allyl-2,4,5-trimethoxybenzene[209] are also known to occur in this species. Prenylated benzoic acids[210] and phenolics are also common occurrence in several Piper species. In some cases head to tail linkage of one or more prenyl units leads to geranyl, farnesyl and geranyl-geranyl hydroxy benzoic acid or phenolic derivatives[196,211].

## Biological Activity Studies

The biological activities of many of the compounds isolated from the Piper species are reported in literature.

It has been shown that topical application of purified extract of black pepper (P.nigrum) produced high mortality in flour beetles and cowpea weevil[212]. Piperine, pellitorine and pipericide, active components of P.nigrum, have been reported to be very toxic to house flies[156]. Pipericide, dihydropipericide and guineensine are shown to possess insecticidal activity against adzuki bean weevil[27,45,213]. Piperinone, the active principle of P.futokadsura showed antifeedant activity against the larvae of Spodoptera litura[147]. Very recently an amide, (2E, 4E)-N-isobutyl-7-(3,4-methylenedioxyphenyl)-hepta-2, 4-dienamide from P.falconeri is reported to have exhibited significant insecticidal activity[214]. The prenylated benzoic acid derivatives, methyl taboganate and 2,2-dimethyl-6-carboxy-chroman-4-one from P.taboganum are recently shown as repellents of leaf cutter ants[210]. Larvicidal activity against the larvae of Toxocosara canis of the eight new piperamides has been recently studied[216].

Piperine is also shown to possess antibacterial activities against Pseudomonas aeruginosa and Alcaligenes F2518[217]. 4,5-Dimethoxy-2,3-(methylenedioxy)-1-allyl-benzene, a natural isolate of P.hispidum and P.aduncum is

found to have strong antimicrobial activity[156]. The hexane soluble fraction of ethanol extract of P.schmidtii exhibited antiamoebic activity against Entamoeba histolitica[128]. However the compounds isolated from this fraction, schmidtin, a neolignan, galgravin, a lignan as well as friedelin, 1-triacontanol, octacosanoic acid, β-sitosterol and its glucoside have not shown any anti-amoebic activity.

Alcoholic extract of the wood of P.novaehollandeae showed activity against Lewis lung carcinoma implanted in mice[16]. However this property has not attributed to any of the alkamides isolated from this plant. Crotepoxide, isolated from several Piper species is however shown to possess antitumour activity against Lewis lung carcinoma[195]. The kawalactones from P.methysticum have shown motor activity, antiserotonine activity and central muscle relaxing properties[218]. 3,4-Dimethoxyphenyl propionic acid and 3,4-dimethoxyphenyl propylamine from P.arboricola have shown analgesic activity in monkeys[219]. The neolignans, kadsurenone, kadsurin A and B from P.futokadsura[138] and P.hancei[140] and hancenone[14] from P.wallichii and P.hancei have been reported to possess PAF

activity. The flavone from P.wallichii[220] is recently shown as coronary dilator and dehydropiperonaline from P.longum having coronary vasorelaxant activity[221]. The cytotoxic pyridone alkaloids from the stems and leaves of P.aborenscens are found to display significant activity against KB cell culture system and P-388 lymphocytic leukaemia systems in cell culture[71,77].

# CHAPTER II

# CHAPTER II

# LIGNANS FROM THE LEAVES OF PIPER NIGRUM

## INTRODUCTION

The genus Piper (family: Piperaceae) consists of 700 species distributed throughout the tropical and sub-tropical regions of the world[222]. About 30 species of Piper are known in India[5]. P.nigrum from which pepper is derived is a perennial climbing vine or shrub with a smooth woody stem and alternate dark green, ovate, accuminate and thickish leaves. The vines are grown in local gardens of Kerala[5].

P.nigrum Linn. berries (black pepper) are widely used in indigenous system of medicine[222]. More than 100 terpene constituents have been reported from the essential oils[5,197] of the berries. Several alkaloids[1,5,197] have been isolated as non-volatile constituents. Piperine, β-sitosterol, hentriacontanone-16 and hentriacontanol-16 are reported in the stems of of P.nigrum[223]. Three dibenzyl butyrolactol lignans, (-)-cubebin and (-)-cubebinin[119] from

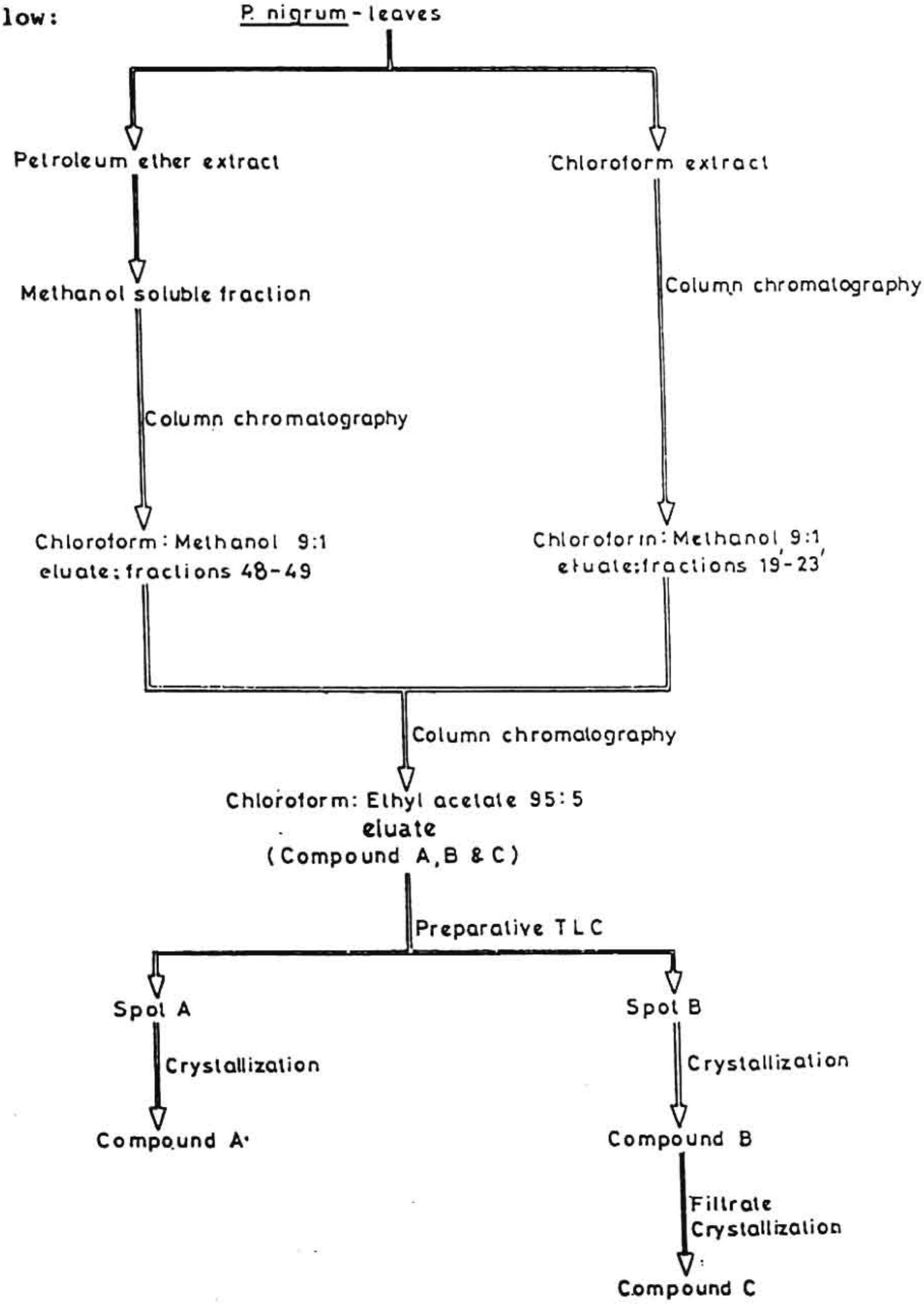
P.cubeba and (-)-clusin from P.clusii[118] are so far reported from the genus Piper.

Black, white and green pepper are three different forms of pepper products. Whole, unripe, and mature berries in the dried form constitute black pepper, while fully ripe dried fruit devoid of pericarp form the commercial white pepper. Green pepper obtained from unripe, but fully developed berries has of recent years become an important product of commerce valued in the Western market for its delicacy. Green pepper has a limited market as it undergoes blackening on storage within a short time unless preserved in brine, acetic acid or citric acid. Interestingly fresh green leaves of P.nigrum undergoes blackening during storage and drying. Further a thorough literature survey has revealed that the leaves have not been investigated chemically. Therefore, a systematic chemical investigation of the leaves is undertaken and the results are reported in this chapter.

## Chemical Examination of the Leaves of P.nigrum

The dark greenish residue from the petroleum ether and chloroform extract of the leaves of P.nigrum yielded three crystalline compounds A, B & C with the $R_f$ values

0.59, 0.32, 0.32 (solvent system benzene:ethylacetate 4:1) respectively. The bar diagram for their isolation is given below:



## Structure of Compound A

Compound A crystallized from benzene : hexane as colourless crystals, m.p.130° and analysed for $C_{20}H_{20}O_6$ ($M^+$ 356). It is identified as (-)-cubebin by direct comparison with an authentic sample, kindly provided by Dr. B. Mulchandani, BARC, Bombay.

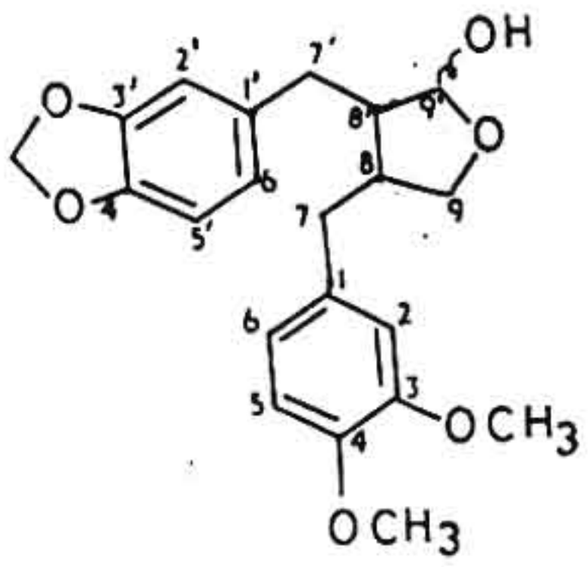
## STRUCTURE OF COMPOUNDS B & C

### Compound B

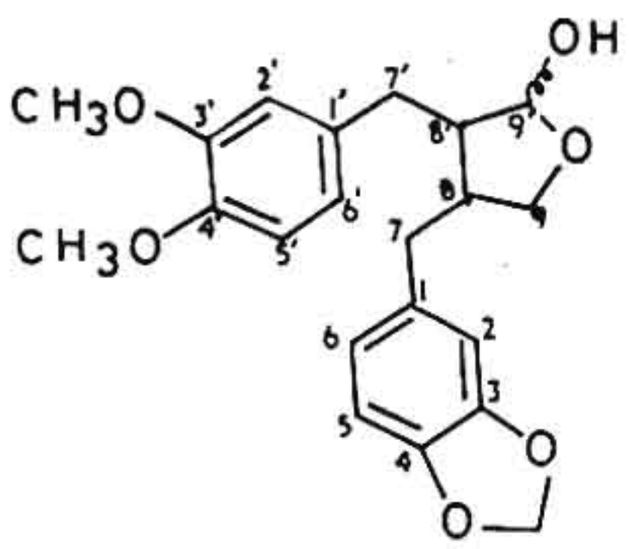
Compound B was crystallized from benzene:hexane as colourless needles m.p. 86-87°; $[\alpha]_D$ -52.86°. Elemental analysis and mass spectrum gave the molecular formula $C_{21}H_{24}O_6$ ($M^+$ 372). The molecular formula is also confirmed by the accurate mass measurement of various peaks in the mass spectrum. The IR spectrum showed the presence of hydroxyl group at 3360 $cm^{-1}$ and generally indicated its aromatic nature.

The 500 MHz $^1H$ NMR spectrum of compound B indicated the presence of two methoxyl groups at δ3.82 (3H,s) and 3.85 (3 H,s). The $^1H$ NMR spectrum also indicated a methylenedioxy group at δ5.92 (2H,s) and six aromatic protons between δ6.4 - 6.9 (6H, m). A slighly broad singlet at δ5.23 (1H,s) for a hemiacetal proton and three triplets at

δ3.59, 4.01 and 4.10 (2H, J=8Hz each) for methylene protons of furanol ring suggested that compound B is a dibenzylbutyrolactol lignan. In addition a multiplet at δ2.0 - 2.9 ( 6H, 4 benzylic and 2 methine protons) is also observed in it $^{1}$H NMR spectrum. A tentative structure (1 or 2) is thus arrived at for compound B from this data.



1



2

Compound C

Compound C was crystallized from benzene:hexane as white globulets m.p. 66° $[\alpha]_D$ - 15.88°. Elemental analysis and mass spectrum gave the molecular formula $C_{21}H_{24}O_6$ ($M^+$ 372). The molecular formula is also confirmed by the accurate mass measurement of various peaks in the mass spectrum. The IR spectrum showed the presence of hydroxyl group at 3365 $cm^{-1}$ and generally indicated its aromatic nature.

The 500 MHz $^1$H NMR spectrum of compound C also indicated the presence of two methoxyl groups at δ3.86 (3H, s) and δ3.87 (3H, s). $^1$H NMR spectrum also indicated a methylenedioxy group at δ5.92 (2H, s) and six aromatic protons between δ6.4 - 6.9 (6H, m). A slightly broad singlet at δ5.23 (1H, s) for a hemiacetal proton and three triplets at δ3.60, 4.01 and 4.11 (2H, J=8Hz each) for methylene protons of furanol ring suggested that compound C is a dibenzylbutyrolactol lignan. In addition a multiplet at δ2.0 - 2.9 (6H, 4 benzylic and 2 methine protons) is also observed in its $^1$H NMR spectrum. A tentative structure (1 or 2) is thus arrived at for compound C from the above data.

Stereochemistry of the Lignans

The dibenzylbutyrolactol lignans were known to be a mixture of epimers in ratio 1:2 at $C_9$[119]. In view of these lignans close relationship with the $^1$H NMR spectrum of 3,4-dimethoxy-3,4-desmethylenedioxy cubebin reported in literature[224], the stereochemistry of compounds B and C was determined by $CrO_3/H_2SO_4$ oxidation in acetone[118]. The oxidation products of both the compounds B and C showed the presence of carbonyl group at 1762 $cm^{-1}$, multiplets at

δ2.50 (4H, benzylic protons) and δ2.85 (2H, methine protons) in their 60 MHz $^1$H NMR spectra , thus establishing the trans-stereochemistry at $C_8$ and $C_{8'}$ positions.

The distinction between the structures of compounds B and C is made on the basis of their mass spectra. The mass spectrum of compound B (Fig.1) shows base peak at m/z 151 (100%) whereas the mass spectrum of compound C has shown the base peak at m/z 135 (100%) (Fig.2). This can be explained as shown in scheme 1. Similar observation is made in the structure establishment of 3,4-dimethoxy-3,4-desmethylenedioxy cubebin isolated by Rucker et al[224] from Aristolochia triangularis. Compound B (1) is thus identified as 3,4-dimethoxy-3,4-desmethylenedioxy cubebin (1) and compound C as 3',4'-dimethoxy-3',4'-desmethylene-dioxy cubebin (2). Rucker et al[224] also identified 3',4'-dimethoxy-3',4'-desmethylenedioxy cubebin in the petroleum ether extracts of roots and stems of Aristolochia triangularis. However, it was not isolated in pure form.

Biogenetic Considerations:

Three dibenzylbutyrolactol lignans, (-)-cubebin and (-)-cubebinin from P.cubeba[119] and (-)-clusin from P.clusii[118] are so far reported from the genus Piper. In

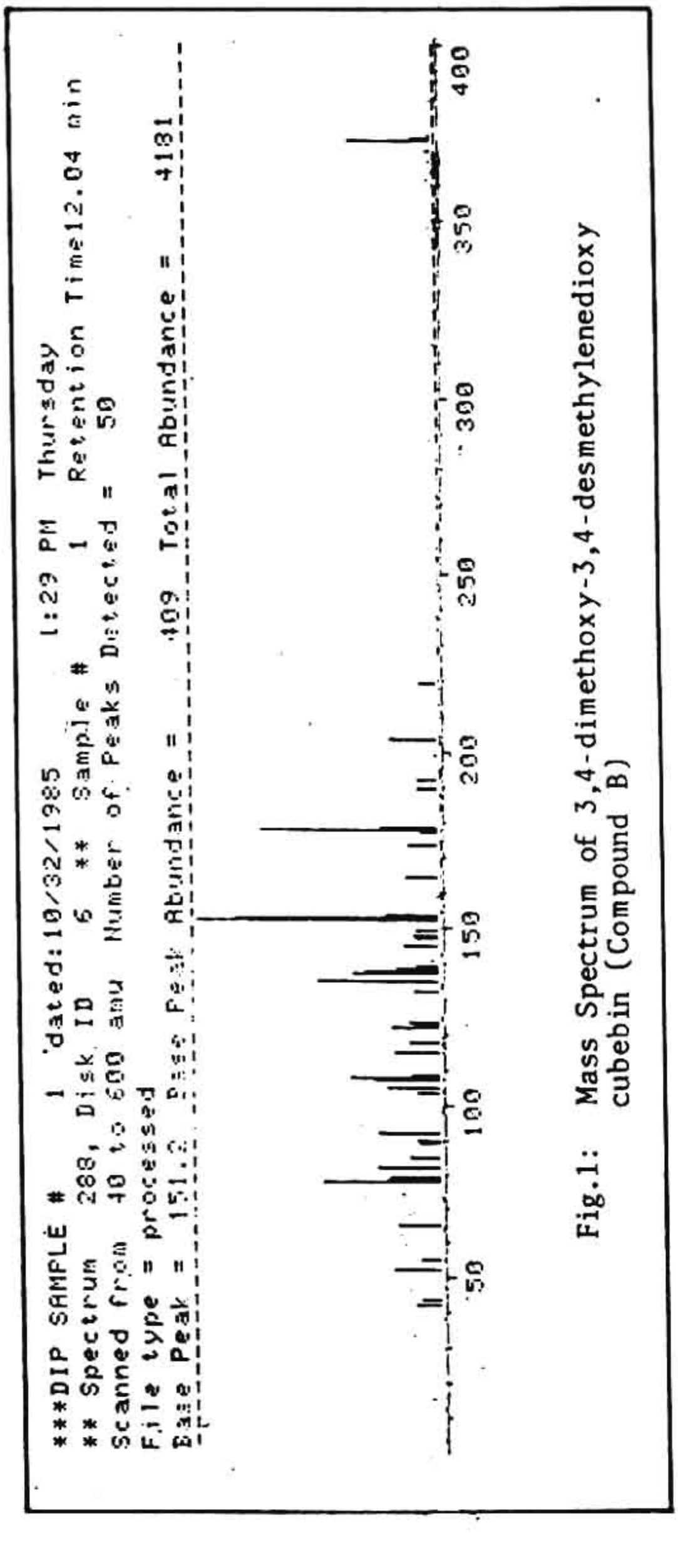



Fig.1: Mass Spectrum of 3,4-dimethoxy-3,4-desmethylenedioxy cubebin (Compound B)

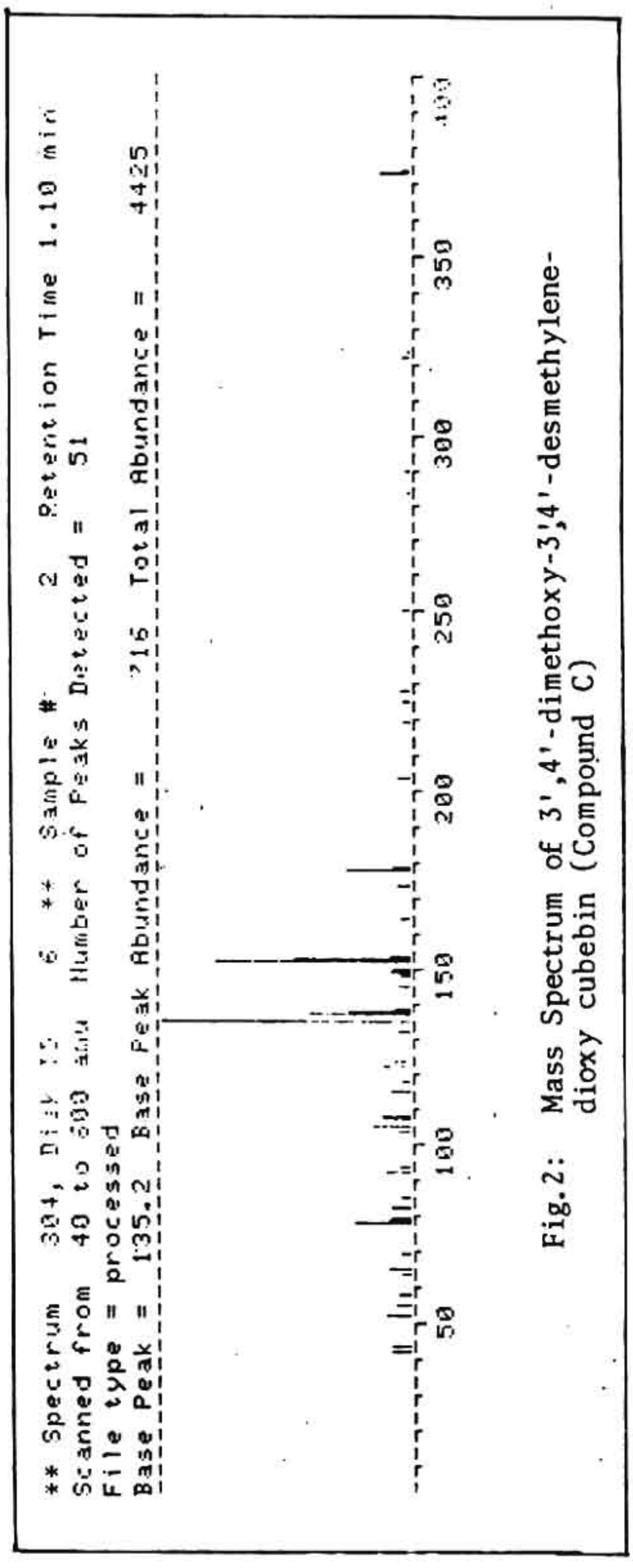
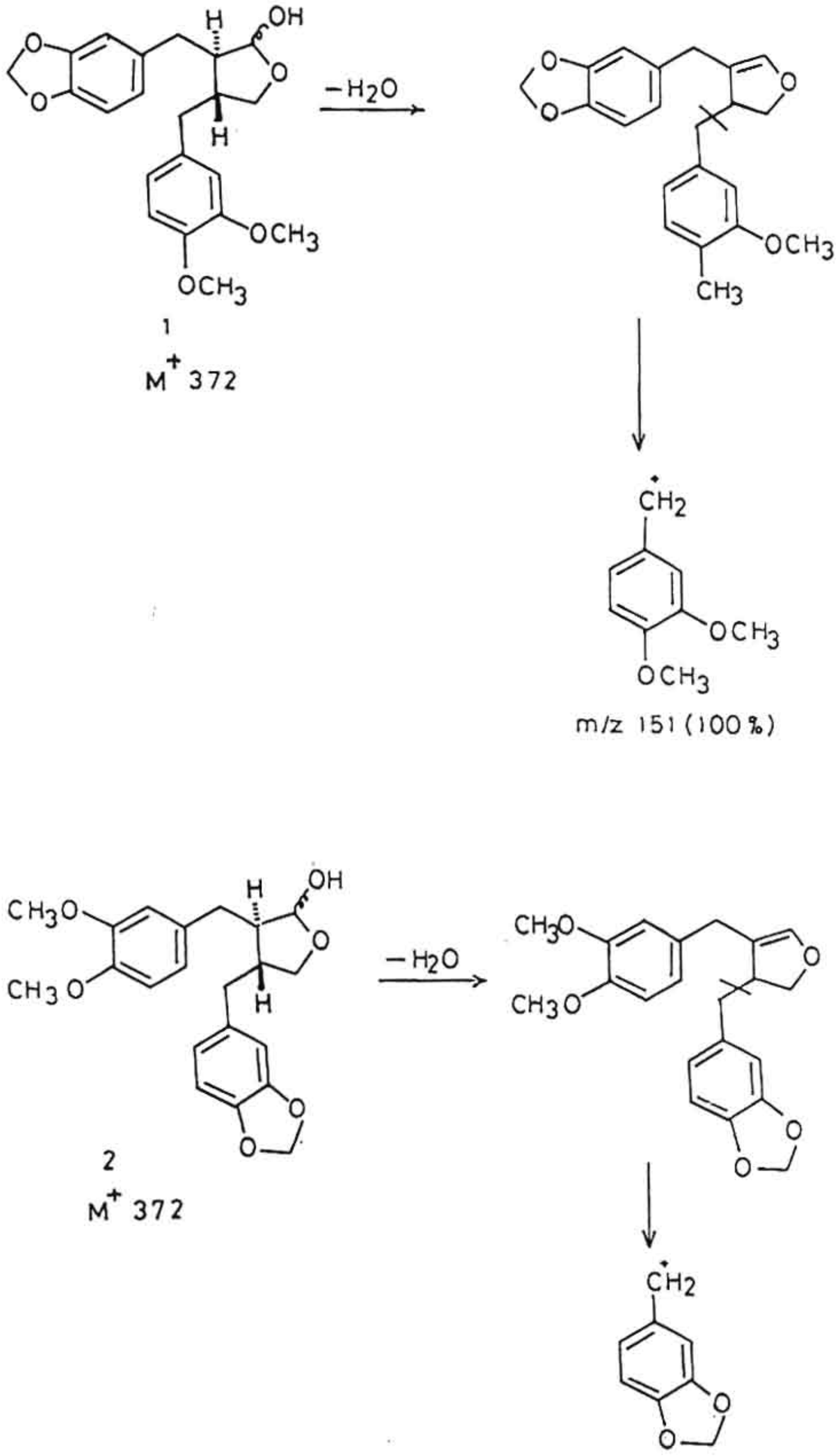



Fig.2: Mass Spectrum of 3',4'-dimethoxy-3',4'-desmethylene-dioxy cubebin (Compound C)


H
OH
O
O
H
OCH3
OCH3
−H2O
CH3
CH2
OCH3
1
M+ 372
m/z 151 (100%)
CH3O
CH3O
2
M+ 372
m/z 135 (100%)

Scheme 1

our study the two isomeric lignans, 3,4-dimethoxy-3,4-desmethylenedioxy cubebin and 3',4'-dimethoxy-3',4'-desmethylenedioxy cubebin have been isolated in pure form. The occurrence of (-)-cubebin in the berries of black pepper seems to be controversial. The first report of its identification was reported by Grewe et al in 1970[60,5]. Later Rucker et al[224] observed that cubebin is found only in P.cubeba. To sort out the controversy, a re-examination of the berries of black pepper has been undertaken. The methanol soluble portion of the petroleum ether extract of the berries in fact showed the presence of these three lignans by comparison on TLC. The occurrence of the 3,4-dimethoxy-3,4-desmethylenedioxy cubebin and 3'4'-dimethoxy and 3',4'-desmethylenedioxy cubebin along with cubebin is thus biogenetically interesting. The carbon frame work of lignan consists of a β-linkage of two $C_6$-$C_3$ building blocks which are formed by the shikimic acid path way. The build up of these carbon skeleton of the acids which form the pepper alkaloids may also begin with a $C_6$-$C_3$ building block which is then linked to the acetate units. This explains the presence of methylenedioxy or methoxy groups in the aromatic part of piperine alkaloids.

As already mentioned in the introduction, a systematical chemical investigation of the leaves is undertaken with an objective of isolating the compounds responsible for the blackening of fresh green pepper. While our work is in progress, a publication by Banerji et al[122] reported the identification of these precursors as 3,4-dihydroxyphenylethanol and its glycoside. Further investigation is therefore discontinued.

# EXPERIMENTAL

Melting points are uncorrected. Silica gel (60-120 mesh) of E-Merck grade was used for column chromatography. Silica gel G containing 13% calcium sulphate as binder was used for TLC. The plates were dried at room temperature for 12 hrs, activated for 1 hr in an air oven at 110°C. The spots were developed by spraying with 10% methanolic sulphuric acid and heating the plate in an oven at 120°C for 20 minutes. Samples for analysis were routinely dried under high vacuum. C, H analysis were performed on Perkin-Elmer 2400 CHN analyser. IR spectra were recorded on Perkin-Elmer 882 infra red spectrophotometer. Chemical shifts are in ppm (δ values) and the corresponding magnetic field is mentioned at appropriate place. Specific rotations were recorded on JASCO DIP-370 digital polarimeter and UV spectra were recorded on Hitachi 220 spectrophotometer.

# EXPERIMENTAL

## Extraction

The leaves of P.nigrum was procured from the local garden and a voucher specimen is kept at RRL, Trivandrum. The leaves (625 g) was shade dried, powdered and extracted successively with petroleum ether (60-80°), chloroform and methanol in a soxhlet extraction apparatus for 30 hrs in each case. The dark green petroleum ether extract (2.5 L) was concentrated and was then fractionated into methanol soluble and methanol insoluble fractions.

## Chromatographic Separation of the Petroleum Ether Extract

The dark green methanol soluble fraction (20 g) of the petroleum ether extract was dissolved in diethyl ether (50 ml) and silica gel (50 g) was added. The ether was removed under vacuum and the powder was transferred to a column of silica gel (250 g) set in petroleum ether. The column was eluted successively with petroleum ether, petroleum ether:chloroform 1:1, chloroform, chloroform: methanol 9:1 and chloroform:methanol 1:1 and methanol. Fractions of 100 ml were collected and concentrated. All

the fractions were monitored by TLC and grouped as shown in Table 1.

**Table 1**

| Eluant | Fraction No. | Group No. | Compound |
|---|---|---|---|
| Petroleum ether | 1 - 13 | I | - |
| Petroleum ether: chloroform 1:1 | 14 - 32 | II | - |
| Chloroform | 33 - 40 | III | - |
| Chloroform:methanol 9:1 | 41 - 47 | IV | - |
| Chloroform:methanol 9:1 | 48 - 49 | V | A, B & C |
| Chloroform:methanol 1:1 | 50 - 56 | VI | - |
| Methanol | 57 | VII | - |

Group I & II

The yellow mass obtained from the group I and II were of waxy nature and resisted crystallization. It was not examined further.

Group III & IV

The green residue obtained from these groups did not show any interesting spot. Hence it was also not examined further.

Group V

The fractions 48-49 showed two closely moving blue fluorescent spots (Rf 0.59 and 0.2) under UV. Isolation of the compounds from the dark greenish residue will be described later.

Group VI & VII

The TLC examination of the fractions from these groups in different solvent systems of increasing polarity revealed non-homogenous behaviour and no crystalline compound could be isolated.

Chromotographic Separation of Chloroform Extract

The dark green chloroform extract (14 g) was dissolved in 40 ml acetone and silica gel (40 g) was added. The solvent was removed under vacuum and the powder was transferred to a column of silica gel (200 g) set in chloroform. The column was then eluted successively with chloroform, chloroform:methanol 9:1 and methanol 50 ml and 100 ml fractions were collected and concentrated. These fractions were also monitored by TLC and grouped as shown in Table 2.

**Table 2**

| Eluant | Fraction No. | Group No. | Compound |
|---|---|---|---|
| Chloroform | 1' - 8' | I | - |
| Chloroform:Methanol 9:1 | 9' - 18' | II | - |
| Chloroform:Methanol 9:1 | 19' - 23' | III | A, B & C |
| Chloroform:Methanol 9:1 | 24' - 39' | IV | - |
| Methanol | 40' - 41' | V | - |

Group I & II

The greenish yellow residue obtained from these fractions resisted crystallization and was not examined further.

Group III

The fractions 19' - 23' were combined and concentrated. It showed two closely moving blue fluorescent spots under UV as was found in Group V of petroleum ether extract. Isolation of compounds from these fractions will be described later.

Group IV

The greenish residue from these fractions did not show any interesting compound and was not examined further.

Group V

A TLC examination of these fractions in different solvent systems revealed non-homogenous behaviour and no crystalline compound could be isolated.

Examination of Methanolic Extract

No crystalline compound could be obtained from the methanolic extract by the usual chromatographic procedure and crystallization.

Isolation of Compounds A, B & C

Since the fractions 48-49 of Group V of petroleum ether extract and the fractions 19' - 23' of Group III of chloroform extract showed similar spots, these two groups were mixed together. After removel of the solvent, the dark green residue (23 g) was subjected to column chromatography. The residue was dissolved in diethyl ether (50 ml), silica gel (50 g) was added and the solvent was removed under vacuum. This is then transferred to a column of silica gel (175 g) set up with chloroform. The column was eluted with chloroform, chloroform:ethyl acetate (95:5), chloroform:ethylacetate (9:1), and finally with methanol.

The earlier fractions of the chloroform ethyl acetate (95:5) eluate did not show the blue fluorescent

spots. The latter fractions of the chloroform;ethy acetate eluate showed the two blue fluorescent spots on TLC (solvent system benzene:ethyl acetate 4:1, UV). As these two compounds could not be separated by column chromatography, the residue (2 g) from this fractions was subjected to preparative TLC (solvent system:benzene: ethylacetate 4:1). The two bands observed under UV were separated. 30 plates of size 20 x 20 cm were done.

Upper band

The upper band of all the plates were scrapped out and extracted with ethylacetate. It was then filtered and concentrated. The solid compound obtained from this extract was crystallised 3 times from benzene:hexene to get a pure colourless crystalline compound. It was designated as Compound A (3.8 mg) m.p. 130° ($R_f$ 0.59, benzene: ethylacetate 4:1).

Lower band

The lower band was also scrapped out and extracted with ethyl acetate. On concentration of the extract a crystalline compound was obtained which was further purified by repeated crystallization from benzene:hexane. This was designated as Compound B (6.5 mg), m.p. 86-87° ($R_f$

0.32 benzene:ethylacetate 4:1). On concentration of the filtrate after the separation of Compound B, another white solid compound was obtained. It was again purified by column chromatography from benzene:hexane (4:1) and crystallized twice from benzene:hexene as white crystalline solid; designated as Compound C (5 mg), m.p. 66°.

Compound A: (-)-cubebin

Compound A is identified as (-)-cubebin, crystallised from benzene-hexane as colourless crystalline solid m.p. 130°.

IR: $\nu_{max}^{KBr}$ 3335 (OH) 2895 1490 1445 1240 965 and 820 $cm^{-1}$

MS: $M^+$ 356 (28), 203 (13), 136 (50), 135 (100), 77 (23), 31 (10), 8 (12).

Compound B: (-)-3,4-dimethoxy-3,4-desmethylenedioxycubebin

Compound B is identified as (-)-3,4-dimethoxy-3,4-desmethylenedicxy cubebin, crystallised from benzene: hexane, m.p. 86-87°C (lit, m.p. 89-91)[224], $[\alpha]_D^{24^\circ}$ - 52.86° ($CHCl_3$; c 0.35).

IR: $\nu_{max}^{KBr}$ 33360 (OH), 2945, 1605, 1528, 1500, 945 and 820 $cm^{-1}$

MS: accurate mass found

$M^+$ 372.1575 theoretical 372.1574

**Compound C: (-)-3',4'-dimethoxy,3',4'-desmethylenedioxy-cubebin**

The Compound C is characterised as (-)-3',4'-dimethoxy-3',4'-desmethylenedioxy cubebin m.p. 66°, $[\alpha]_D^{25}$ - 15.88° ($CHCl_3$; c 0.17).

UV: $\lambda_{max}^{MeOH}$ 206 and 286 nm.

IR: $\nu_{max}^{KBr}$ 3365 (OH), 2940, 1605, 1530, 1500, 940 and 820 $cm^{-1}$

MS: Accurate mass found $M^+$ 372.1576 Theoretical 372.1574

**Identification of lignans from the berries of Piper nigrum**

Piper nigrum berries (100 g) were purchased from the local market and ground to powder. The ground material was extracted with petroleum ether (60-80°) in a soxhlet extractor. The dark green residue obtained after concentration of the extracts was fractionated into methanol soluble and methanol insoluble portions. The methanol soluble portion of the petroleum ether extract of the berries showed the presence of these three lignans, (-)-cubebin; 3,4-dimethoxy-3,4-desmethylenedioxy cubebin and 3',4'-dimethoxy-3',4'-desmethylenedioxy cubebin by comparison of the $R_f$ values of these compounds on TLC.

# CHAPTER III

# CHAPTER III

## CRYSTALLINE CONSTITUTENTS FROM PIPER ATTENUATUM AND THEIR ANTIFEEDANT ACTIVITY

### INTRODUCTION:

P.attenuatum is an important species of piper genus which is much used in the Ayurvedic system of medicine[222]. It is a slender rambling climber found in the Eastern Tropical Himalayas, Assam, Khasi hills, Orissa, hills of Vishakapatnam and Godavari districts, the eastern slopes of Nilgris, the Western ghats and the hills of Tirunelveli district and Java[222,226]. The roots of P.attenuatum is reported to be used as an excellent diuretic[226]. It has an intense rubefacient effect and is used in poultices for headache and other pains[222]. In Malaysia, parts of the plant are used for washing cloths in order to scent their cloths[222,226]. Crotepoxide which is known to possess significant antitumour activity has been separated from the aerial part of the plant[186,187]. Piperine, piperlongumine,

N-isobutyl deca-trans-2-trans-4-dienamide and guineensin have been isolated from the roots of the plant by Das Gupta et al[10] . Recently Mulchandani et al[109] have isolated three aristolactams, cepharanone B, aristolactam AII and piperolactam A and five 4,5-dioxoaporphines, cepharadione B, cepharadione, nor-cepharadione B, piperadione and 2-hydroxy-1-methoxy-4,5-dioxoaporphine from the whole plant.

A thorough literature survey revealed that a systematic investigation of different parts of this plant has not been conducted. It is also observed that this plant is not attacked by pollu beetle (Longitarsus nigripennis), a devastating pest of pepper which causes about 30-40% damage of pepper gardens of Kerala. Further it was noticed that on spraying with an extract of this plant on P.nigrum it inhibited the feeding of pollu beetle. A systematic chemical investigation of different parts of the plant is therefore undertaken.

1) Crystalline constituents from P.attenuatum berries:

The petroleum ether, chloroform and methanolic extracts of the berries of P.attenuatum were prepared and bioassay of the extracts were conducted on pollu bettle. No choice experiments on feeding behaviour of pollu beetle by leaf disc technique were performed. Different

concentrations of the extractives in acetone were prepared and 1 cm leaf discs of pepper leaf were dipped in the solutions and dried. Then 3 field collected beetles were inoculated per disc. Observations were taken on the area fed in treatment and control 24 hrs after release. Four replications were maintained per treatment. The percent feeding deterrance (PFD) was calculated by using the formula $\frac{C-T}{C+T} \times 100$ where C = area fed in control and T = area fed in treatment. The results obtained is tabulated in Table 1.

These results showed that the hexane and chloroform extracts of P.attenuatum has got antifeedant activity on pollu beetle. A 100% inhibition was noticed with 6% level of both extracts. Isolation of crystalline constituents of these two extracts is therefore carried out by column chromatography over silica gel.

The green residue from the petroleum ether extract of the berries of P.attenuatum yielded five crystalline constituents A, B, C, D and E with $R_f$ values 0.68, 0.45, 0.32, 0.40, 0.42 (solvent system: benzene: ethylacetate 4:1) respectively. Their separation and purification is described in the experimental section. The bar diagram for their isolation is given below.

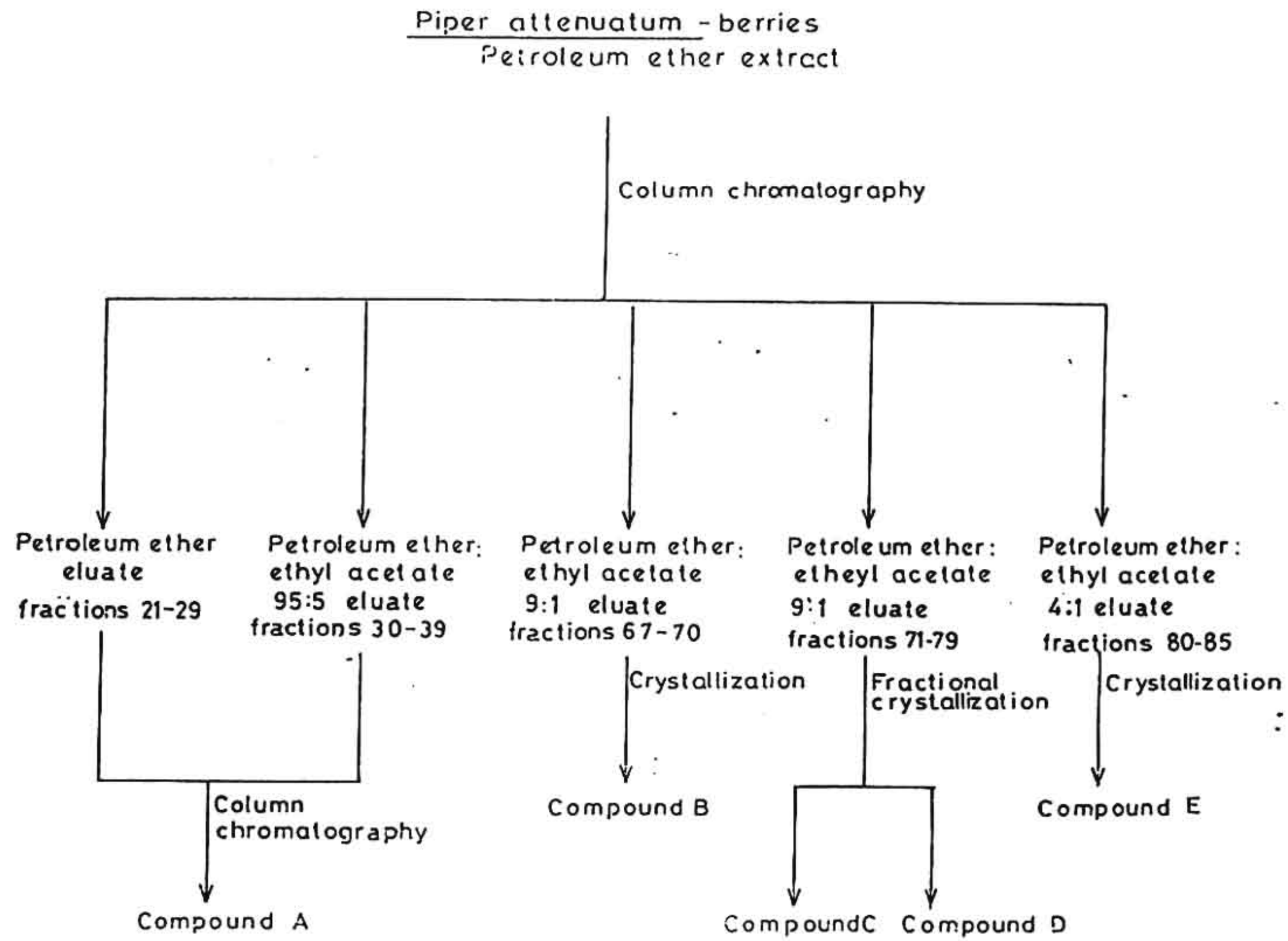

Piper attenuatum - berries
Petroleum ether extract
Column chromatography
Petroleum ether eluate fractions 21-29
Petroleum ether: ethyl acetate 95:5 eluate fractions 30-39
Column chromatography
Compound A
Petroleum ether: ethyl acetate 9:1 eluate fractions 67-70
Crystallization
Compound B
Petroleum ether: etheyl acetate 9:1 eluate fractions 71-79
Fractional crystallization
CompoundC
Compound D
Petroleum ether: ethyl acetate 4:1 eluate fractions 80-85
Crystallization
Compound E

**Table 1**

Antifeedant activity of crude extracts from P.attenuatum on pollu beetle

| Conc % | P.attenuatum petro-ether extract PFD | P.attennatum chloroform extract PFD | P.attenuatum Methanol extract PFD |
|---|---|---|---|
| 0.01 | 0.0 | 2.9 | 0.0 |
| 0.05 | 3.7 | 2.6 | 1.9 |
| 0.10 | 21.6 | 6.3 | 3.9 |
| 0.50 | 38.2 | 22.0 | 0.0 |
| 1.00 | 46.9 | 32.4 | 5.5 |
| 2.00 | 70.7 | 47.8 | 2.0 |
| 3.00 | 79.3 | 67.8 | 0.0 |
| 4.00 | 85.5 | 89.3 | 6.1 |
| 5.00 | 96.8 | 98.1 | 12.6 |
| 6.00 | 100.0 | 100.0 | 27.0 |

Structure of Compound A:

Compound A was crystallised from ethylacetate as white crystalline solid m.p. 87°. The mass spectrum showed the molecular formula as $C_{34}H_{68}O_2$ ($M^+$ 508). The IR spectrum showed a carboxyl group at 1705 $cm^{-1}$. The 200 MHz $^1H$ NMR

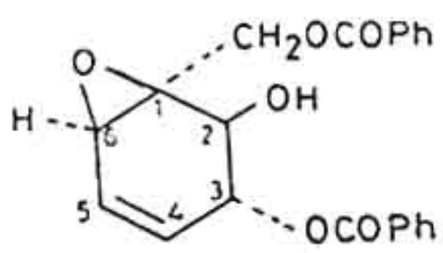
spectrum showed a triplet centered at δ2.37 (2H) for methylene protons adjacent to a carbonyl group. It also showed a methyl group at δ0.90 (3H, t) and methylene protons at δ1.30 and δ1.65 (62H, broad singlet). The mass spectrum showed a consecutive loss of fourteen and/or twenty eight mass units suggesting it to be a straight chain aliphatic compound. The IR and mass spectrum of compound A was identical with tetratriacontanoic acid reported in literature[228].

Structure of Compound B:

Compound B crystallized from ethylacetate as white crystalline needles m.p. 144-45°, $[\alpha]_D$+53.465°. Elemental analysis and mass spectra gave the molecular formula $C_{21}H_{18}O_6$ ($M^+$ 366). IR spectrum showed the presence of the hydroxyl group at 3450 $cm^{-1}$, ester carbonyls at 1725 and 1625 $cm^{-1}$, aromatic moeity at 1605 $cm^{-1}$ and epoxide at 1255, 1060 and 890 $cm^{-1}$.

The 60 MHz $^1H$ NMR spectrum of Compound B in $CDCl_3$ showed the presence of a doublet centered at δ3.25 (1H) which disappeared on $D_2O$ exchange. A doublet of a doublet centered at δ3.60 (1H) which has transformed into a doublet on $D_2O$ exchange is also noticed. The $^1H$ NMR

spectrum further showed one doublet of a doublet centered at δ4.30 (1 H) with coupling constants of J=8.0 and 6.0 Hz and also two unresolved triplets of a doublet centered at δ5.7. The aromatic region from δ7.3 to 8.1 integrated for 10 protons. An unresolved multiplet at δ6.1 (1 H) and two unresolved triplets of a doublet at δ5.9 (1 H) are accounted for the olefinic protons of a cyclohexene ring. An AB quartet [doublets at δ5-10 (1 H) and δ4.50 (1 H) respectively] with a coupling constant of J=12 Hz which is generally encountered for methylene protons attached to a benzoyl group is observed. This data coupled with its typical mass spectrum showed its identity as pipoxide[187,193] (**1**).




**1**

The occurrence of (-)-isomer is first reported from the leaves of P.hookeri[193] and P.nigrum[187] and the opposite(+) isomer is found to occur in the leaves of Uvaria purpurea[229]. This is the first time to report the occurrence of pipoxide from P.attenuatum.

Structure of Compound C:

Compound C crystallised from ethyl acetate as white crystalline globulets m.p. 200-201° $[\alpha]_D$ + 57.572°. Qualitative analysis indicated the presence of chlorine in the molecule in compound C. Elemental analysis gave the molecular formula $C_{21}H_{19}ClO_6$. The mass spectrum did not show the molecular ion, but a peak at m/z 367 for $M^+$ - Cl is noticed. The IR spectrum of compound C is very similar to that of pipoxide except for the absorption due to epoxide and hydroxyls. The hydroxyl region showed two peaks at 3537 and 3472 $cm^{-1}$. Further a peak at 789 $cm^{-1}$ for C-Cl stretching is observed.

The 60 MHz $^1H$ NMR spectrum of compound C in DMSO-d6 showed the presence of a triplet centered at δ4.15 (1 H), a broad singlet at δ4.6 (2 H), a doublet centered at δ4.8 (1 H), a multiplet between δ5.6-5.9 integrating for five protons and aromatic protons between δ7.3 to δ8.2 integrating for ten protons. This data is in excellent agreement with the data reported for pipoxide chlorohydrin (2) prepared from pipoxide by Singh et al[193]. This compound was later reported to occur in the methanolic extract of P.hookeri and P.nigrum[187]. The $^{13}C$ NMR spectrum (Fig. 1) of pipoxide chlorohydrin is not reported in literature.

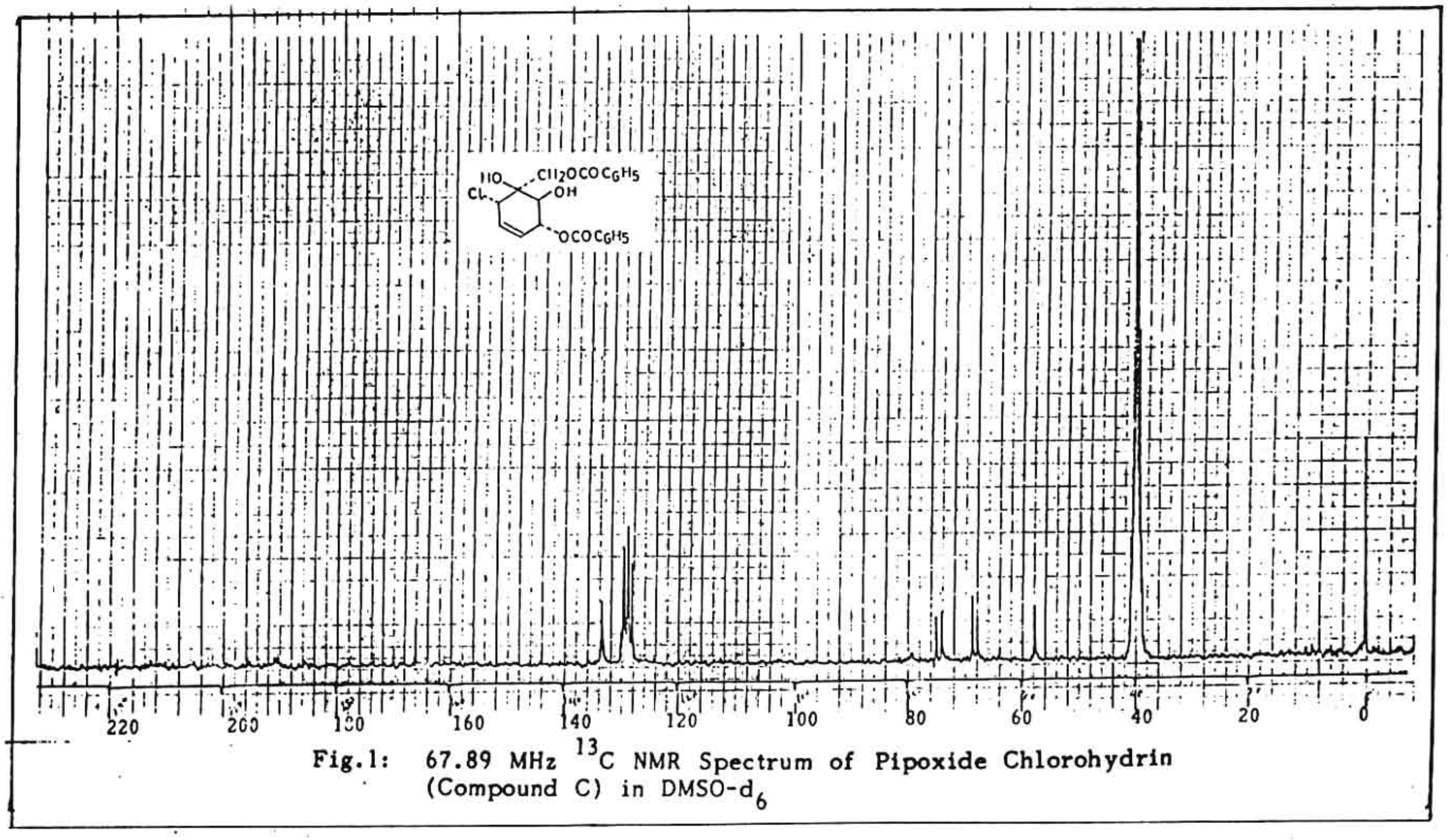
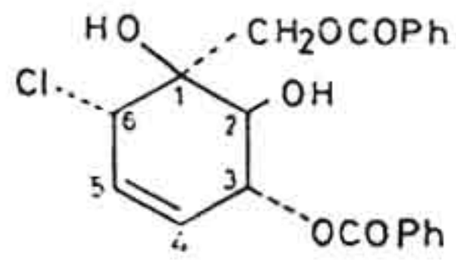


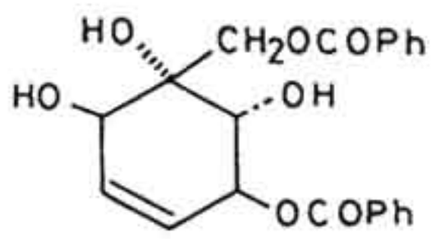

**Fig.1:** 67.89 MHz $^{13}C$ NMR Spectrum of Pipoxide Chlorohydrin (Compound C) in DMSO-$d_6$

It shows five signals at 57.53, 67.71, 68.57, 73.84 and 74.78 ppm assignable to C-7, C-3, C-2, C-6 and C-1 respectively in comparison with that of (+)-zeylenol (3) recently reported by Taneja at el[191]. The aromatic region showed six peaks at 127.88, 128.57, 129.29, 129.70, 133.25 and 165.65 ppm includes olefinic carbons C-4 and C-5 also.

The mass spectrum fragmentation pattern of pipoxide chlorohydrin is depicted in chart-1, in addition to the peaks given in the chart fragments at m/z 123(47) for protonated benzoic acid and m/z 105 (100) for $C_6H_5C{\equiv}O^+$ respectively.




2




3

Pipoxide chlorohydrin was earlier isolated from methanolic extract of P.hookeri and P.nigrum[187]. This is the second report of its occurrence from the genus Piper.

Structure of Compound D:

Compound D was crystallised from hexane:ethyl acetate as colourless shining crystals m.p. 127-28° $[\alpha]_D$- 135.5°. Elemental analysis and mass spectrum gave the

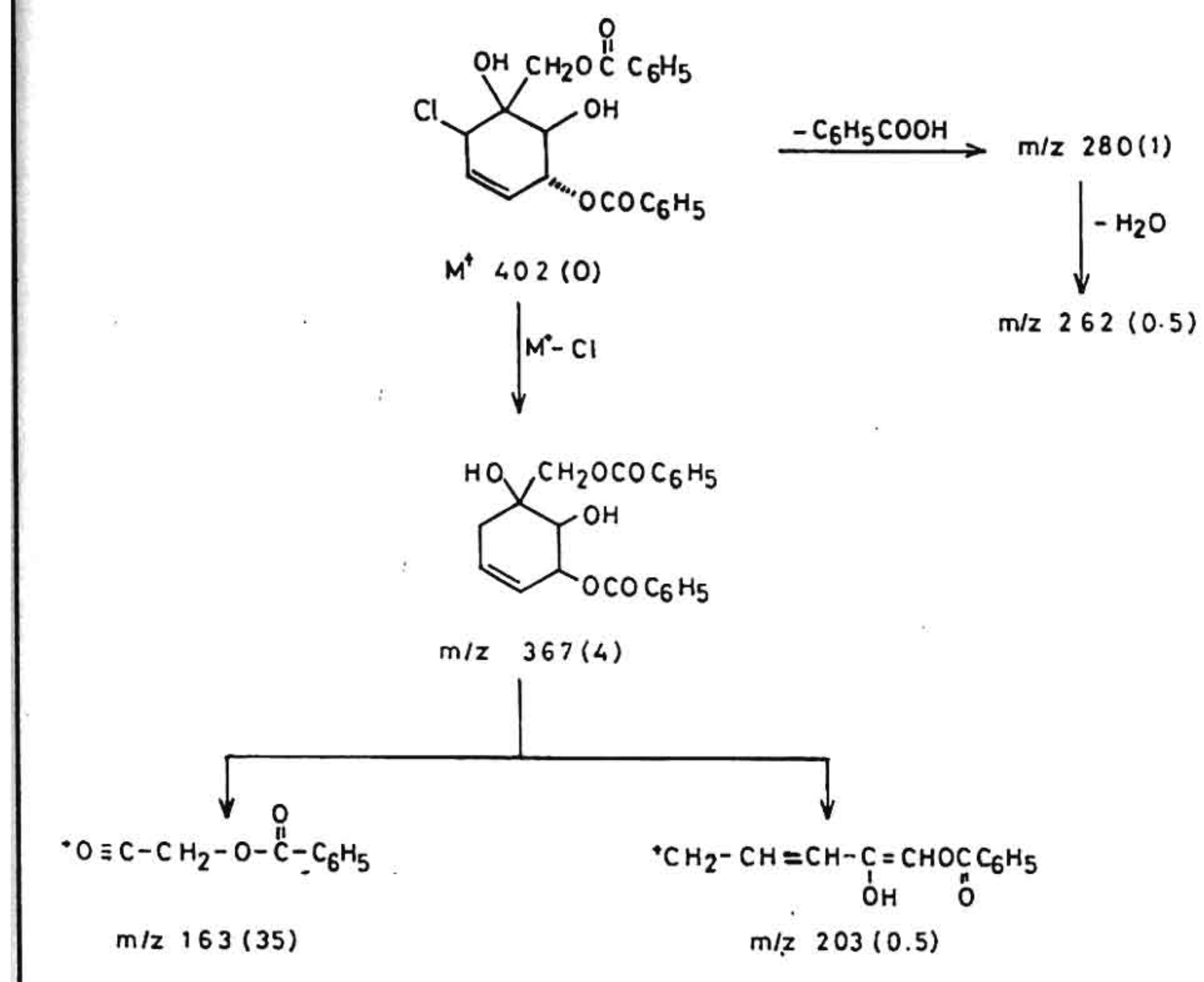
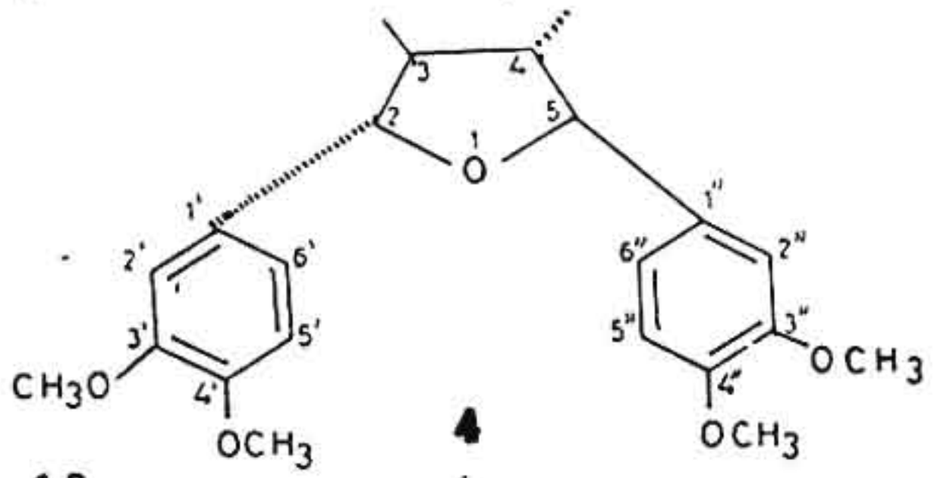



Chart I : Mass spectral fragmentation pattern of Pipoxide chlorohydrin(2)

molecular formula $C_{22}H_{28}O_5$ (M+ 372). The 300 MHz $^1H$ NMR spectrum in $CDCl_3$ showed a doublet at δ2.05 (3H, J=6.2) for methyl protons, an unresolved slightly broad singlet at δ1.80 (1 H) for a methine proton, two sharp singlets at δ3.88 (3H) and 3.91 (3 H) for aromatic methoxyl protons, a doublet at δ4.65 (1 H) for Ar-C<u>H</u> attached to a oxygen atom and aromatic protons between δ6.8 - 7.0 integrating for three protons. The 2D-COSY spectrum of compound D (Fig. 2) shows perfect symmetry in the molecule. This observation coupled with mass spectral analysis indicates two methyl groups, two methine protons, two Ar-C<u>H</u>-O protons and two 3,4-dimethoxy aromatic units. This data is in agreement with that reported for (-)-galbelgin, a 3,4-dimethyl-2,5-bisaryl tetrahydrofuranoid lignan[230,231,234] (4).




4

The $^{13}C$ NMR spectrum (Fig. 3) of (-)-galbelgin is not reported in literature. It shows ten signals, the assignments of which has been made in comparison with the reported $^{13}C$ NMR spectrum of galbacin[235] (Table 2).

This is the first report of the isolation of galbelgin from the genus <u>Piper</u>.

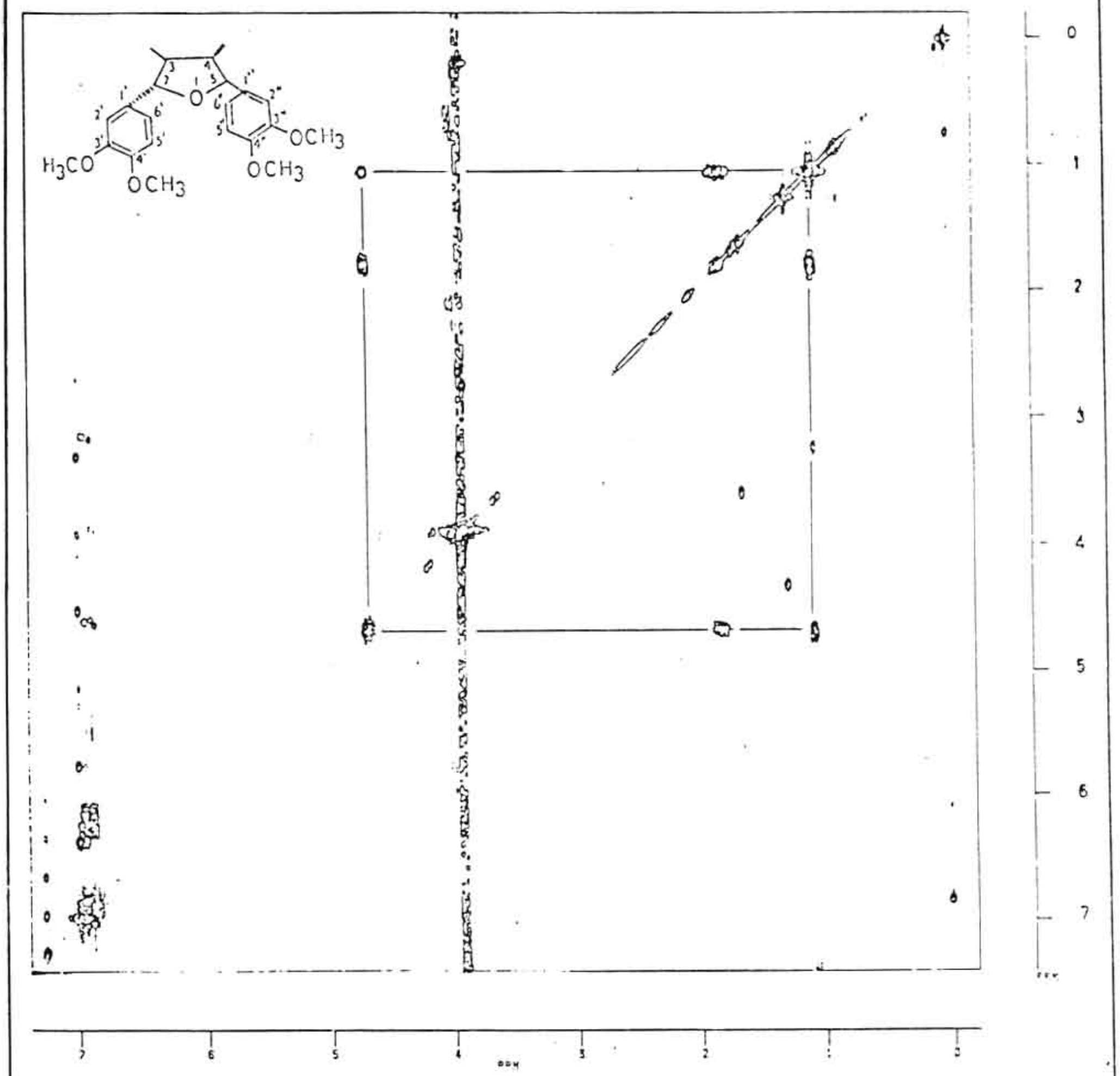



Fig.2: 270 MHz 2D-COSY ($^{1}H$-$^{1}H$) Spectrum of (-)-Galbelgin (Compound D) in $CDCl_3$

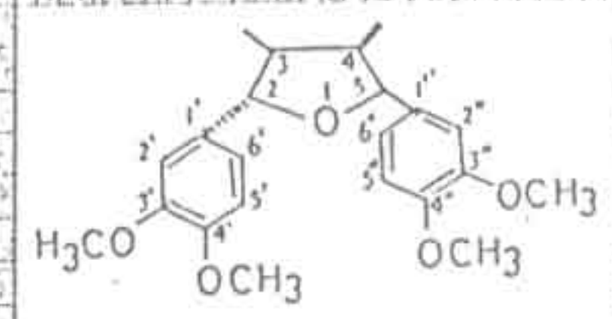






Fig.3: 67.89 MHz $^{13}C$ NMR Spectrum of (-)-Galbelgin (Compound D) in $CDCl_3$

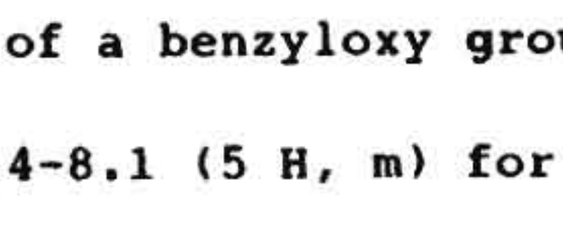
**Table 2**

**$^{13}C$ NMR Spectrum of (-)-Galbelgin (4) and Galbacin in $CDCl_3$**

| Carbon No. | (-)-Galbelgin | Galbacin |
|---|---|---|
| 1' | 135.32 | 136.1 |
| 2' | 109.91 | 106.4 |
| 3' | 149.46 | 147.5 |
| 4' | 148.91 | 146.7 |
| 5' | 111.50 | 107.7 |
| 6' | 118.82 | 119.5 |
| 2 | 88.34 | 88.1 |
| 3 | 51.09 | 50.9 |
| 4 | 51.09 | 50.9 |
| 5 | 88.34 | 88.1 |
| 1" | 135.32 | 136.1 |
| 2" | 109.91 | 106.4 |
| 3" | 149.46 | 147.5 |
| 4" | 148.91 | 146.7 |
| 5" | 111.50 | 107.7 |
| 6" | 118.82 | 119.5 |
| 2xC-Me | 14.01 | 13.7 |
| 4x-OMe | 56.13 | - |

**Structure of Compound E:**

Compound E was crystallized from ethyl acetate: hexane as white shining needles m.p. 147-48° $[\alpha]_D$+69.804°, and analysed for $C_{18}H_{18}O_8$ ($M^+$ 362). The IR spectrum showed strong carbonyl absorptions at 1769, 1754 and 1729 $cm^{-1}$. The 60 MHz $^1H$ NMR spectrum of compound E in $CDCl_3$ showed two singlets at δ2.0 (3H) and 2.1 (3H) acetoxymethyl protons, δ3.10 (1 H, q), 3.45 (1H, q), 3.65 (1 H, d), and an AB quartet centered at δ4.35 (2 H, J=12 Hz) for the methylene protons of a benzyloxy group, δ4.95 (1H, q) δ5.75 (1 H, d) and δ7.4-8.1 (5 H, m) for aromatic protons. The mass spectrum showed $M^+$ 362 and the other fragmentation peaks at 227(10), 207(7), 194(5), 163(19), 138(11), 122(3), 115(23), 105(100), 97(29) and 77(68). The compound is thus identified as (+)-crotepoxide (5)[188].



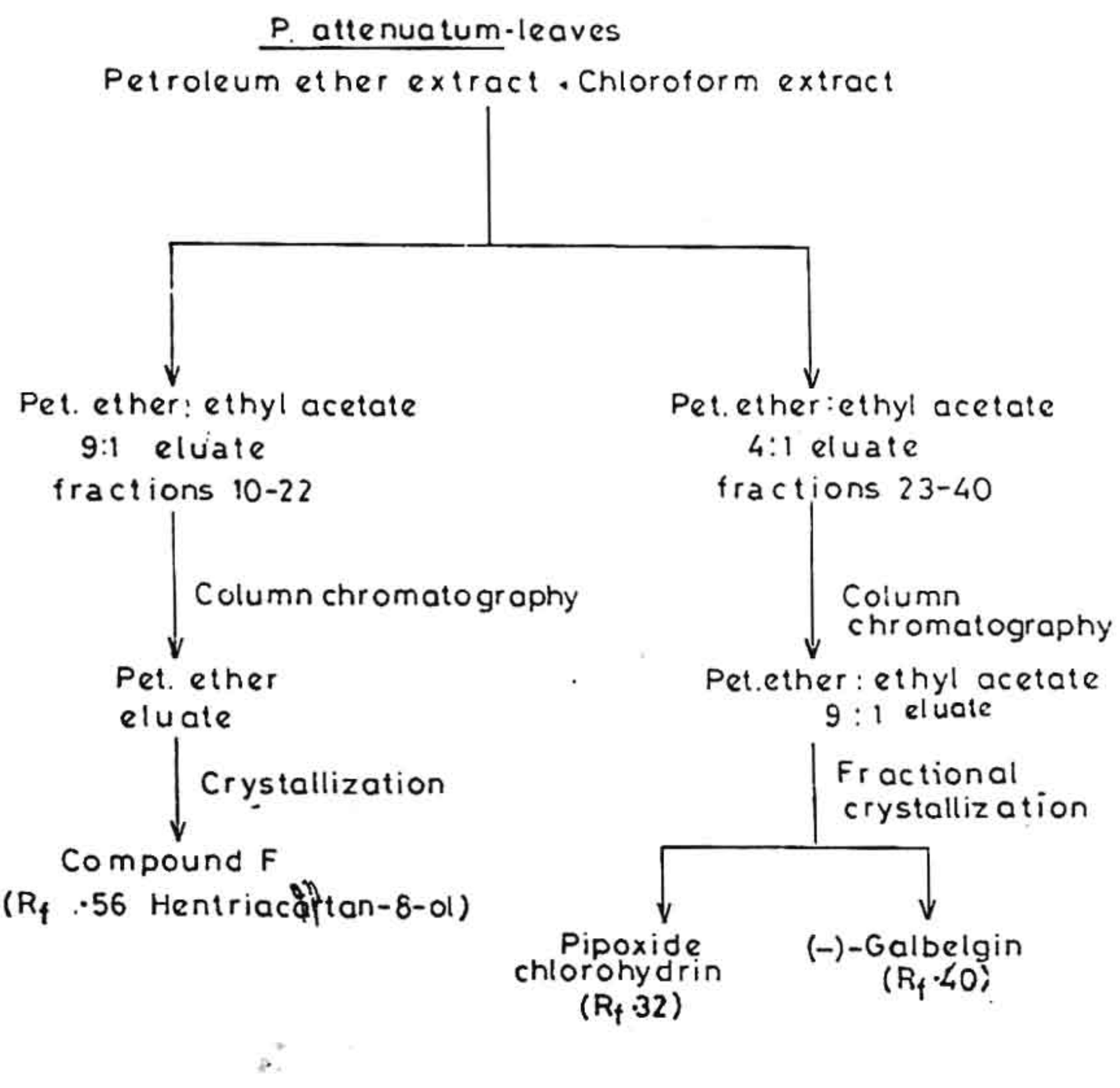

5

Crotepoxide was first isolated by Kupchan et al[195] and reported to have shown significant anticancer activity in Lewis lung carcinoma. Later it is also isolated from a few Piper species, P.futokadsura[184,185], P.hookeri[188],

P.brachystachyum[189], P.galcatum[187], P.clarkii[190], P.cubeba[191], P.hancei[142], P.interruptum[42], P.wallachi[14] and also in the whole plant of P.attenuatum[186,187]. From our study it is clear that crotepoxide occurs in commercially significant quantities in the seeds of the plant which is a renewable source (0.25%)[236].

2. Chemical examination of the leaves of P.attenuatum

The mixed green residue from the petroleum ether and chloroform extracts of the leaves of P.attenuatum yielded three crystalline compounds with $R_f$ values 0.56 (Compound F), 0.32 and 0.40 (solvent system: benzene:ethyl acetate 4:1). The bar diagram for their isolation is given below.

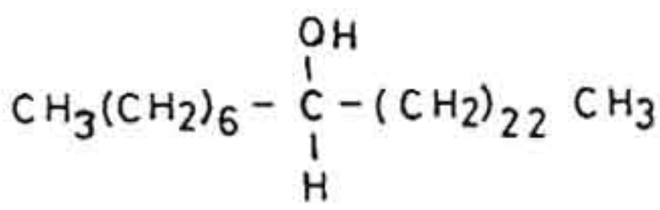

P. attenuatum-leaves
Petroleum ether extract
Chloroform extract
Pet. ether: ethyl acetate
9:1 eluate
fractions 10-22
Column chromatography
Pet. ether
eluate
Crystallization
Compound F
(Rf .56 Hentriacontan-8-ol)
Pet. ether: ethyl acetate
4:1 eluate
fractions 23-40
Column
chromatography
Pet.ether : ethyl acetate
9 : 1 eluate
Fractional
crystallization
Pipoxide
chlorohydrin
(Rf .32)
(-)-Galbelgin
(Rf .40)

Two crystalline compounds with $R_f$ values 0.32 and 0.40 were isolated from fractions 23 -40 of petroleum ether:ethyl acetate (4:1) eluate by rechromatography and fractional crystallization. They are identified as pipoxide chlorohydrin (2) and (-)-galbelgin (4) respectively by direct comparison with the samples obtained from the berries (Co-TLC, m.m.p. and super imposable IR).

Structure of Compound F:

Compound F crystallized from ethylacetate as colourless solid m.p. 77° and analysed for $C_{31}H_{64}O$ ($M^+$ 452). Its IR spectrum showed a hydroxyl at 3450 $cm^{-1}$ and generally indicated its aliphatic nature. IR spectrum of its acetate showed the carbonyl group at 1745 $cm^{-1}$. $^1H$ NMR spectrum of its acetate showed the presence of methine proton at δ5.34 (1 H, m), acetoxyl protons at δ1.94 (3H, s), two terminal methyl groups resonating between δ0.82 and 1.02 (6 H, two overlapped triplets) and 26 methylene units at δ1.25 (52 H, br s). A broad singlet at δ1.66 (4H) was attributed to two methylene units attached to the carbinolic carbon. The absence of a $[M-15]^+$ ion and the presence of a $[M+1]^+$ in its mass spectrum is characteristic of an unsymmetrical straight chain compound[237-239]. The

position of the hydroxyl group was deduced from the characteristic peaks at m/z 129 $[Me(CH_2)_6\ CHOH]^+$ and m/z 353 $[Me\ (CH_2)_{22}\ CHOH]^+$ (Scheme 1). The compound was thus characterised as 8-hentriacontanol (6).

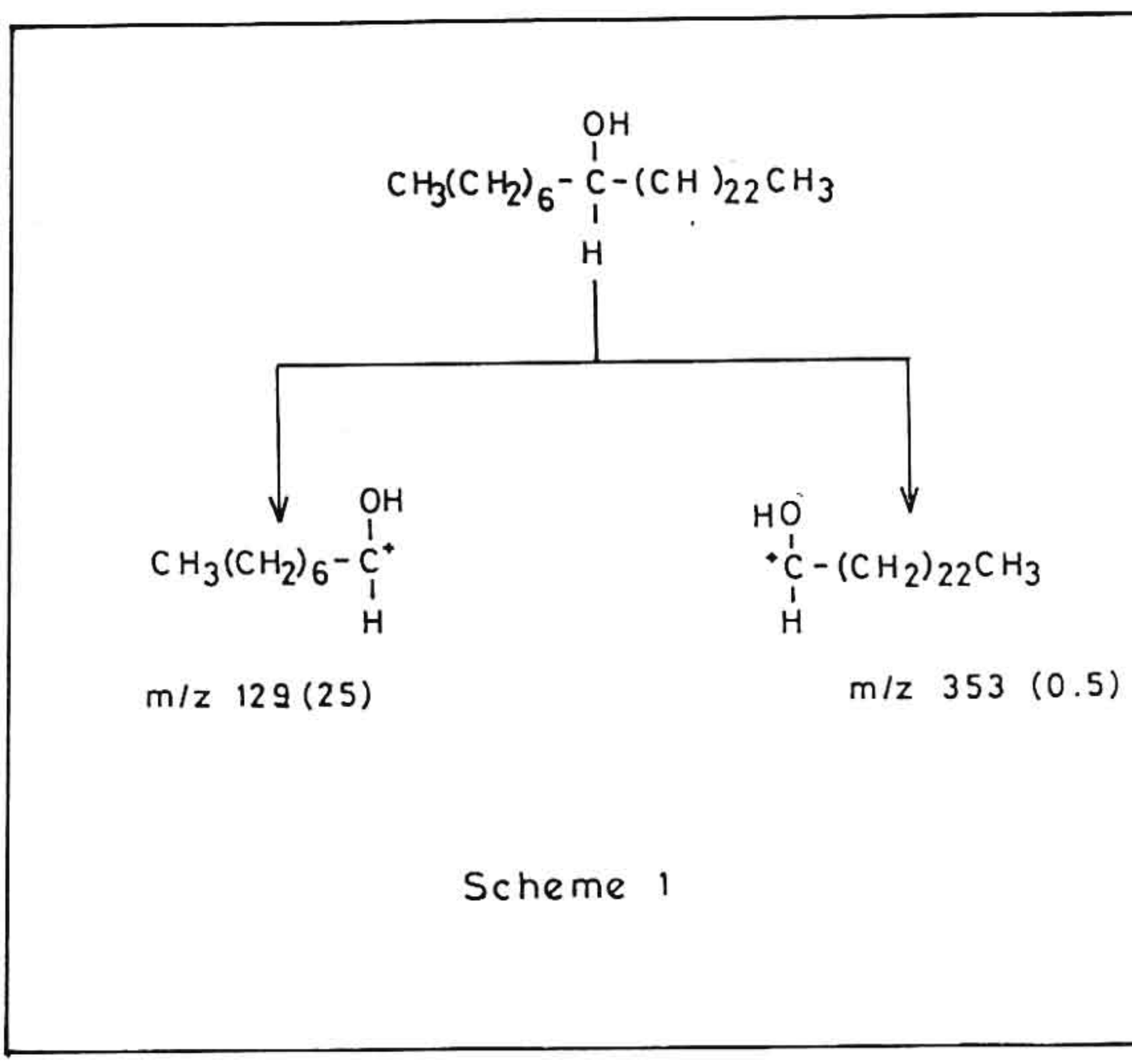


**6**

Several homologous series of aliphatic alcohols ($C_{12}$ - $C_{24}$) have been isolated from P.methysticum[207] in addition to aliphatic saturated fatty acids, unsaturated fatty acids, ketones and esters. However, it appears that no internal secondary alcohols are so far been reported from the genus Piper. As part of epicuticular waxes several other plant species have been reported to contain secondary alcohols with the hydroxyl groups at 7 to 11 and $C_{27}$ - $C_{31}$ alcohols[240]. This is the first report of the natural occurrence of 8-hentriacontanol which is likely to be a constituent of the epicuticular wax of P.attenuatum.

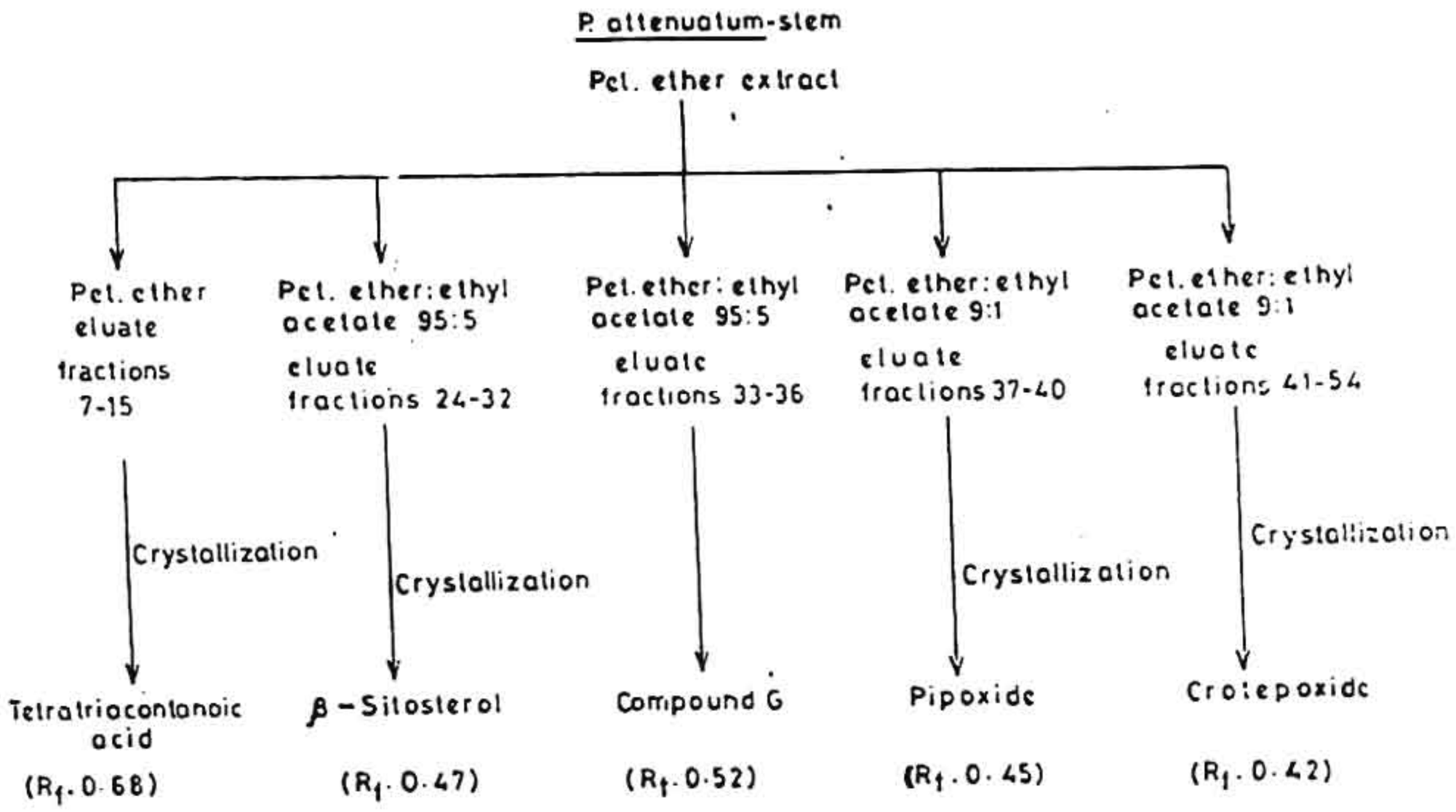
3. Chemical examination of stem of P.attenuatum

The brownish green residue obtained from the


OH
CH3(CH2)6-C-(CH)22CH3
H
OH
CH3(CH2)6-C+
H
m/z 129 (25)
HO
+C-(CH2)22CH3
H
m/z 353 (0.5)

Scheme 1

petroleum ether extract of P.attenuatum stem yielded five compounds with $R_f$ values 0.68, 0.47, 0.52(G), 0.45 and 0.42 (solvent system: benzene:ethyl acetate 4:1). The bar diagram for their isolation is given below.



The three crystalline compounds with $R_f$ values 0.68, 0.45 and 0.42 isolated from the petroleum ether extract of the stem are identified as tetratriacontanoic acid, pipoxide and crotepoxide respectively by direct comparison of their superimposable IR with authentic samples and also by Co-TLC and m.m.p. The crystalline compound with $R_f$ 0.47 gave positive Liebermann-Burchard test for steroids. It was crystallised from methanol as colourless needles, m.p. 136°. This compound was identified as β-sitosterol by m.m.p. and Co-TLC with authentic sample.

The residue from the fractions 33-36 of petroleum ether: ethylacetate 95:5 eluate on preparative TLC (UV) gave a few mg of compound G which could not be crystallised. Scarcity of the material prevented further examination.

Antifeedant activity of pure compounds

The antifeedant activity of the three cyclohexane derivatives, crotepoxide, pipoxide and pipoxide chlorohydrin on 'pollu beetle' (Longitarsus nigripennis) were studied in no-choice tests as was done in case of hexane and chloroform extracts. The results are tabulated in Table 3.

**Table 3**

Antifeedant activity of pure compounds

on pollu beetle

| Compound (conc. in ppm) | Area fed ($mm^2$) beetle/day | PFD* |
|---|---|---|
| Crotepoxide | | |
| 100 | 9.6 | 0.0 |
| 500 | 1.6 | 62.4 |
| Pipoxide | | |
| 100 | 7.0 | 0.0 |
| 500 | 7.1 | 0.0 |
| 1000 | 4.8 | 17.5 |
| Pipoxide chlorohydrin | | |
| 100 | 8.9 | 0.0 |
| 500 | 4.9 | 16.0 |
| 1000 | 5.5 | 10.3 |

* PFD - Percent Feeding Deterrance.

The results indicates that crotepoxide exhibited antifeedant activity of about 60% PFD at 500 ppm concentration in no-choice tests. Further studies are therefore suggested to use this compound as an antifeedant on pepper and other crops.

## EXPERIMENTAL

Melting points (°C) are unconnected. Silica gel (60-120 mesh) of E. Merk grade was used for column chromatography. Silica gel with 13% calcium sulphate as binder was used for TLC. The plates were dried at room temperature for 12 hrs, activated for an hour in an air oven at 100°C. The spots were developed by spraying 10% methanolic sulphuric acid and heating the plates in an air oven at 120°C for 20 minutes. Samples for analysis were routinely dried under high vacuum. C, H analysis were performed on Perkin-Elmer 2400 CHN analyser. IR spectra were recorded on Perkin-Elmer 882 infra red spectrophotometer. Chemical shifts are in ppm (δ values) and the corresponding magnetic field is mentioned at appropriate place. Specific rotations were recorded on JASCO DIP-370 digital polarimeter.

# EXPERIMENTAL

## 1) Extraction of P.attenuatum berries

The berries of P.attenuatum berries was procured from Neyyar Dam near Trivandrum. A voucher specimen is available at RRL, Trivandrum. The berries were dried in a cross flow drier at 50°C (600 gm) and was successively extracted with petroleum ether, chloroform and methanol in a soxhlet extractor for 24-30 hrs. The extracts were concentrated and the last traces of the solvent recovered under reduced pressure. The dark greenish brown residue obtained from the petroleum ether extracts showed five prominent spots with $R_f$ values 0.68, 0.45, 0.32, 0.40 and 0.42 (solvent system: benzene: ethyl acetate 4:1) corresponding to compounds A, B, C, D and E respectively. In addition to these spots the TLC plate showed dark colours at the solvent front and origin.

### Chromatographic separation of the extract

The dark residue (33 g) was dissolved in 50ml of diethyl ether and silica gel (50 g) was added. The ether was removed under vacuum.It was then transferred to a column of silica gel (400g). The column was eluted successively with petroleum ether, petroleum ether: ethyl

acetate 95:5, petroleum ether: ethyl acetate 9:1, petroleum ether: ethyl acetate 4:1, petroleum ether: ethyl acetate 1:1 and ethyl acetate. Fractions of 100 ml were collected and concentrated. The fractions were monitered by TLC and grouped as shown in Table 4.

**Table 4**

| Eluant | Fraction No. | Group No. | Compound |
|---|---|---|---|
| Petroleum ether | 1-5 | I | - |
| Petroleum ether | 6-20 | II | - |
| Petroleum ether | 21-29 | III | A |
| Petroleum ether: ethylacetate 95:5 | 30-39 | IV | A |
| Petroleum ether: ethylacetate 95:5 | 40-66 | V | - |
| Petroleum ether: ethylacetate 9:1 | 67-70 | VI | B |
| Petroleum ether: ethyl acetate 9:1 | 71-79 | VII | C&D |
| Petroleum ether: ethyl acetate 4:1 | 80-85 | VIII | E |
| Petroleum ether: ethyl acetate 1:1 | 86-89 | IX | - |
| Ethyl acetate | 90 | X | - |

Group I

The colourless oily fraction obtained contain volatile terpenic compounds and not examined further.

Group II

The yellow residue obtained from these fractions resisted crystallization and suggested waxy nature. It was not examined further.

Group III and IV

Fractions 21-39 were combined and the residue on rechromatography yielded a crystalline compound A, m.p. 87° (10 mg). $R_f$ 0.68 (benzene: ethyl acetate 4:1).

Group V

Fractions 40-66 did not show any interesting spots on TLC and no further separation is attempted.

Group VI

The residue from fractions 67-70 on crystallisation yielded a crystalline compound B ($R_f$ 0.45). It is further purified by crystallisation from ethyl acetate :hexane (140 mg), m.p. 144-45°.

Group VII

Fractions 71-79 showed two prominent spots, one violet and another black. These fractions on concentration yielded a white crystalline compound. The solid was

filtered and crystallised from ethylacetate, $R_f$ 0.32 (200 mg), m.p. 200-201° and designated as compound C. The filtrate after separation of the above compound is concentrated wherein another white crystalline compound has separated out. It is crystallised twice from ethyl acetate : hexane mixture, designated as compound D (45 mg), m.p. 127-28° ( $R_f$ 0.4).

Group VIII

Fractions 80-85 on standing gave a crystalline compound. It is filtered, washed with ether and crystallised from methanol (1500 mg), m.p. 147-148° and designated as compound E ($R_f$ 0.42).

Group IX and X

A TLC examination of these groups in different solvent systems of increasing polarity revealed non-homogenous behaviour and no crystalline compound could be isolated from these groups.

No crystalline compound could be obtained from the chloroform and methanolic extracts by usual fractionation, crystallization and chromatographic procedures.

Compound A: Tetratriacontanoic acid

Compound A is identified as tetratriacontanoic acid,

crystallised from ethyl acetate as white solid, m.p. 87° (lit 95°)[228].

IR: $\nu_{max}^{KBr}$ 2920, 2860, 1720, 1610, 1480 and 730cm$^{-1}$

MS: (Relative abundance below 10% not given).

$M^+$ 508, 494, 480 (22), 466, 452 (12), 438, 424, 410, 396, 382, 368, 354, 340, 297, 241, 185, 171, 157, 143, 129 (25), 115, 111 (15), 98 (15), 97 (27), 85 (32), 83 (31), 73 (47), 71 (52), 69 (42), 61 (30), 57 (base peak 100%), 55 (52), 43 (95) and 41 (30).

Compound B: Pipoxide

Compound B is identified as pipoxide, crystallised from ethylacetate as colourless needles, m.p. 144-45° (lit, m.p. 152-54°)[193] $[\alpha]_D^{26°}$ + 53.465 (C, 0.486, $CHCl_3$).

Analysis: Found C 68.75, H 4.82; $C_{21}H_{18}O_6$ requires:

C 68.85, H 4.95%

IR: $\nu_{max}^{KBr}$ 3450(OH), 1725, 1625, 1280, 1260, 1255, 1060 and 895 cm$^{-1}$.

MS: (Relative abundance below 10% not given)

$M^+$ 366, 244 ($M^+$ - $C_6H_5COOH$), 231, 215, 203, 163, 123, 122 (15), 106 (30), 105 (100), 102 (13), 81, 77 (88), 51 (23) and 43 (15).

$^{1}H$ NMR: (60MHz, $CDCl_3$)

3.25 (1H, d, - O<u>H</u>), 3.60 (1H, dd H-6), 4.30 (1H, dd, J=8 Hz and 6.0 Hz, H-2), 4.50 and 5.10 [2H, AB(q), J=12 Hz-$C\underline{H}_2OCOPh$], 5.70 (1H, d, t, H-3), 5.90 (1 H, d, t, H-4), 6.10 (1 H, m, H-5) and 7.3-8.1 (10 H, Ar-H).

<u>Compound C</u> : <u>Pipoxide chlorohydrin</u>

Compound C is identified as pipoxide chlorohydrin crystallised from ethylacetate as white solid, m.p. 200-201° (lit, m.p. 203-4°)[187] $[\alpha]_D^{25°}$ + 57.572 (c, 0.205 pyridine).

Analysis: Found C 62.45, H 4.55; $C_{21}H_{19}O_6$ Cl; requires C 62.67, H 4.76.

IR: $\nu_{max}^{KBr}$ 3547, 3472, 2977, 2921, 1695, 1606, 1500, 1456, 1426, 1371, 1285, 1180, 1122, 951 and 862 $cm^{-1}$.

$^{1}H$ NMR : (60 MHz, DMSO-d6)

4.15 (1H, t, -O<u>H</u>), 4.60 (2H, s, -O$C\underline{H}_2$OCOPH) 4.80 (1H, H-6), 5.6-5.9 (5H, m, H-2,3,4,5 and -OH) 7.30-8.20 (10H, Ar-H).

$^{13}C$ NMR: (67.89 MHz, DMSO-d6/TMS):

57.53, 67.71, 68.57, 73.84, 127,88, 128.57, 129.30, 133.26 and 165.65.

MS: (Relative abundance below 5% not given)

367 ($M^+$ - Cl), 262, 249 (6), 215 (6), 203, 163 (23), 158, 140, 131, 123 (43), 121 (19), 117 (17), 110, 107, 105 (100), 99, 81 and 77 (53).

Compound D: (-)-Galbelgin:

Compound D is identified as (-)-galbelgin, crystallised from hexane:ethylacetate as white crystals, m.p. 142-43° (lit, m.p. 138°)[225] $[\alpha]_D^{25°}$ -135.5 ($CHCl_3$ c, .02)

Analysis: Found C70.72, H 7.37; $C_{22}H_{28}O_5$ requires:

C 70.94, H 7.28.

IR: $\nu_{max}^{KBr}$ 3078, 1597, 1514, 1467, 1267, 1235, 1207, 1161, 1028, 967, and 803 $cm^{-1}$.

$^1H$ NMR: (300 MHz, $CDCl_3$):

1.80 (2H, s), 2.05 (6H, d, J=6.2 Hz), 3.88 (6H, S), 3.91 (6H, s) 4.65 (2H, d) and 6.8-7.0 (6H, m).

$^{13}C$ NMR:(67.89 MHz, $CDCl_3$/TMS)

149.46, 148.91, 135.32, 111.50, 109.91, 88.34, 56.13, 51.09 and 14.01.

MS: (Relative abundance below 10% not given)

$M^+$ 372 (20), 335, 287, 234, 219, 207 (10). 205 (100), 194 (14), 191 (67), 178 (24), 175 (60), 165 (20), 160 (15),

151 (11), 145, 138 (14), 131 (10), 115, 107 (13), 95 (17), 91 (37), 79 (22), 77 (35), 65.

Compound E: Crotepoxide

Compound E is identified as crotepoxide, crystallised from hexane:ethyl acetate as white needles, m.p. 147-48° (lit, m.p. 150-1°)[195], $[\alpha]_D^{25°}$ + 69.804 (c, 1.055, $CHCl_3$).

Analysis: Found C 59.94, H 5.15; $C_{18}H_{18}O_8$ requires C 59.67, H 5.01%

IR: $\nu_{max}^{KBr}$ 1769, 1754, 1729, 1454, 1374, 1284, 1236, 1215, 1121, 864 and 721 $cm^{-1}$

$^1H$ NMR: (60 MHz, $CDCl_3$)

2.0 (3H, s), 2.10 (3H, s), 3.10(1H, q), 3.45 (1H, q), 3.65 (1H, d), 4.35 (2H, AB, q, J=12Hz), 4.95 (1H, q), 5.75 (1H, d) and 7.4-8.1 (5H, m).

MS: (Relative abundance below 5% not given)

$M^+$ 362, 227 (10), ($M^+$ - $CH_2OCOPh$), 207 (7), 194 (5), 185, 163 (19), 157, 138 (11), 125, 122 (7), 115 (18), 106, 97 (29), 79, 77 (68), 75, 49, 43 (99), 32.

2) Extraction of the leaves of P.attenuatum

The leaves of P. attennatum was collected from Neyyar Dam near Trivandrum. A voucher specimen is available at RRL, Trivandrum. The shade dried powdered leaves (115g)

of P.attenuatum was extracted successively with petroleum ether (60-80°), chloroform and methanol in a soxhlet apparatus for 24, 20 and 20 hrs respectively. The extracts were concentrated and the last traces of the solvent removed under reduced pressure. The petroleum ether and chloroform extracts on TLC showed three prominent spots with $R_f$ values 0.56, 0.32 and 0.4 (solvent system: benzene: ethyl acetate 4:1).

Since the petroleum ether and chloroform extracts behaved similarly on TLC plate both the extracts were mixed. In addition to the above mentioned spots, the TLC plate showed dark colours at the solvent front and origin.

Chromatographic separation of the extracts

The mixed dark greenish residue (17.6g) from the petroleum ether and chloroform extract was dissolved in diethylether (20ml) and silica gel (25g) was added. The ether was removed under vacuum and the powder was transferred to a column of silica gel (200g). The column was eluted successively with petroleum ether, petroleum ether:ethyl acetate 95:5, petroleum ether:ethyl acetate 9:1, petroleum ether: ethyl acetate 4:1, petroleum ether: ethyl acetate 7:3, petroleum ether: ethyl acetate 1:1 and

ethyl acetate. 100 ml fractions were collected. These fractions were concentrated and monitered by TLC. The fractions were grouped as shown in Table 5.

**Table 5**

| Eluant | Fraction No. | Group No. | Compound |
|---|---|---|---|
| Petroleum ether | 1-9 | I | - |
| Petroleum ether: ethyl acetate 9:1 | 10-22 | II | F |
| Petroleum ether: ethyl acetate 4:1 | 23-40 | III | Pipoxide Chlorohydrin and (-)-Galbelgin |
| Petroleum ether: ethyl acetate 7:3 | 41-56 | IV | - |
| Petroleum ether: ethyl acetate 1:1 | 57-65 | V | - |
| Ethyl acetate | 66 | VI | - |

Group I

The yellowish-orange residue obtained from these fractions resisted crystallization and suggested waxy nature. It was not examined further.

Group II

The fractions 10-22 (4g) on rechromatography yielded an amorphous compound F, m.p. 77° (11.8mg) $R_f$ 0.56 (solvent: benzene:ethylacetate 4:1).

Group III

The fractions 23-40 showed one black and one violet spots on TLC ($R_f$ 0.32 and 0.4 resp.). The dark green residue (2.3g) was dissolved in diethyl ether (10ml) and a small amount of silica gel was added. The solvent was removed under vacuum and transferred to a column of silica gel (50g) set up with petroleum ether. The column was then eluted with petroleum ether, petroleum ether:ethyl acetate 95:5, petroleum ether:ethyl acetate 9:1, petroleum ether: ethyl acetate 4:1 and then with ethyl acetate. 50 ml fractions were collected, concentrated and monitered by TLC. The latter fractions of petroleum ether:ethyl acetate 9:1 eluate contained the two spots. On concentration of the eluate a white crystalline compound was separated out. It was filtered and further purified by crystallization from ethyl acetate (47mg) m.p. 198° ($R_f$ 0.32). This compound is identified as pipoxide chlorohydrin by direct comparison of the compound C obtained from the berries (Co-TLC, m.m.p. and superimposable IR).

The filtrate after the removal of pipoxide chlorohydrin was again concentrated wherein another white crystalline compound was obtained. It was twice crystallised from hexane:ethyl acetate. It is characterised as (-)-Galbelgin (81 mg),m.p. 127-28° by direct comparison with the compound D isolated from the berries (Co-TLC, m.m.p. and superimposable IR).

The remaining groups IV, V and VI revealed non-homogenous behaviour on TLC examination and no crystalline compounds could be isolated by repeated crystallization in different solvents.

Compound F

Compound F is identified as 8-hentriacontanol (11.8mg), m.p. 77°, a new aliphatic alcohol.

Analysis: Found C 82.41, H 14.32, $C_{31}H_{64}O$ requires;

C 82.22, H 14.25

IR: $\nu_{max}^{KBr}$ 3450, 2920, 2850, 1510, 1470 and 720 $cm^{-1}$

MS: (relative abundance below 5% not given)

$M^+$ 452, 424, 396, 368, 354, 353, 340, 339, 312, 311, 297, 283, 269, 255, 241, 227, 213, 199, 185, 171, 157, 143, 129 (25), 115, 111 (18), $[129-H_2O]^+$, 101, 97 (23), 87 (15), 85 (25), 83 (27), 71 (45), 69 (41), 57 (90), 55 (65) and 43 (100).

Compound F acetate

8-Hentriacontanol (5mg) was treated with pyridine: (0.3ml) and $Ac_2O$ (0.3ml) overnight at room temperature. After work-up it afforded a thick residue.

IR: $\gamma_{max}^{neat}$ 2930, 2860, 1745, 1465, 1260 and 725 $cm^{-1}$.

$^1H$ NMR : (270 MHz,$CDCl_3$)

0.82-1.02(6H, t), 1.25(52 H, br s), 1.66 (4H, s), 1.94(3H,s), 5.34 (1H, m).

3) Extraction of the stem of P.attenuatum

The shade dried stem of P.attenuatum was extracted successively with petroleum ether (60-80°) and chloroform in a soxhlet extractor for 20 hrs. The extracts were concentrated and the last traces of the solvent removed under reduced pressure. The greenish brown-residue from the petroleum ether extract showed five prominent spots with $R_f$ 0.68, 0.47, 0.52(G), 0.45 and 0.42 (solvent:benzene: ethylacetate 4:1). Greenish and black coloured spots are also present at the solvent front and at the bottom.

Chromatographic separation of the extract

The brownish-green residue (5g) from the petroleum ether extract was dissolved in 10 ml of diethyl ether and silica gel (5g) added. The ether was removed under vacuum

and transferred to a column of silica gel (70g). The column was eluted successively with petroleum ether, petroleum ether: ethyl acetate 98:2, petroleum ether:ethyl acetate 95:5, petroleum ether:ethylacetate 9:1, petroleum ether: ethyl acetate 4:1 and finally with ethyl acetate . 50ml fractions were collected and the fractions were monitered by TLC and grouped as shown in Table 6.

**Table 6**

| Eluant | Fraction No. | Group No. | Compound |
|---|---|---|---|
| Petroleum ether | 1-6 | I | - |
| Petroleum ether: ethyl acetate 98:2 | 7-15 | II | Tetratria-contanoic acid |
| Petroleum ether: ethyl acetate 98:2 | 16-23 | III | - |
| Petroleum ether: ethyl acetate 95:5 | 24-32 | IV | β-sitosterol |
| Petroleum ether: ethyl acetate 95:5 | 33-36 | V | G |
| Petroleum ether: ethyl acetate 9:1 | 37-40 | VI | Pipoxide |
| Petroleum ether: ethyl acetate 9:1 | 41-54 | VII | Pipoxide chlorohydrin |
| Petroleum ether: ethyl acetate 4:1 | 55-62 | VIII | - |
| ethyl acetate | 63 | IX | - |

Group I

The pale yellow coloured fractions 1-6 resisted crystallization and suggested waxy nature and not examined further.

Group II

Light yellow fractions 7-15 was concentrated. A solid compound is obtained. It is filtered and washed with petroleum ether several times. This compound was identified as tetratriacontanoic acid by direct comparison of the compound from the berries - (Co-TLC, m.p. and superimposable IR).

Group III

Fractions 16-23 were mixed together and the solvent distilled off. Since these fractions did not show any interesting spot on TLC no separation was attempted.

Group IV

Fractions 24-32 were mixed and concentrated. The solid obtained was filtered and recrystallised from methanol as colour-less needles (7mg), m.p. 131°. This compound is identified as β-sitosterol by direct comparison of its IR, m.p. and Co-TLC with authentic sample.

Group V

The residue from the fractions 33-36 showed a uv fluorescent spot. These fractions on preparative TLC gave compound G. The amount obtained was insufficient for further examination.

Group VI

Fractions 37-40 were mixed and concentrated. The white crystalline compound obtained was crystallised from ethylacetate: hexane (59mg), m.p. 148°. This compound is identified as pipoxide by direct comparison (m.p., m.m.p.and IR) with the compound isolated from berries.

Group VII

The fractions 41-54 were mixed and concentrated when a colourless crystalline solid separated out. It was further purified by repeated crystallization from methanol (37 mg), m.p. 145°. This compound is identified as crotepoxide by direct comparison with the compound (Co-TLC, m.p. and IR) obtained from the berries.

Group VIII and IX

A TLC examination of fracitons from Group VIII and IX in different solvent systems of increasing polarity did not show any interesting spots and no crystalline compound could be isolated.

No crystalline compound could be isolated from the chloroform extract by usual fractionation, crystallization and chromatographic procedures.

# CHAPTER IV

# CHAPTER IV

## ESSENTIAL OIL CONSTITUENTS OF SOME PIPER SPECIES

### INTRODUCTION

Essential oil is defined as the volatile odoriferous constituents of oily nature obtained almost exclusively from vegetable sources. Essential oils are generally liquids, but occasionally semisolids and rarely solids at ordinary temperature and volatile without decomposition[241]. Chemically they are mixtures of numerous compounds mainly composed of terpenoids. The function of the volatile oil in plant is not clearly known, but it is generally understood that in flowers they aid the natural selection by attracting or repelling certain insects and in the roots, stem and leaves, they keep the parasite away[242].

The essential oil chemistry has intrigued chemists for a long time. The pioneering work of Wallach, Semmler, Simonsen, Ruzieka and others on terpenoids paved the foundation of essential oil chemistry. The work carried out in the essential oil chemistry is so voluminous that it is intended to give a brief review[243-252] of literature in

this field. Only the major constituents of the essential oils could be identified in the latter half of the 19th century[253]. The advent of modern analytical methods like Gas Chromatography (GC), Ultraviolet (UV), Infrared (IR), Nuclear Magnetic Resonance (NMR), Spectroscopy and Mass Spectrometry (MS) have revolutionised the research on essential oils. Use of glass capillary columns, GC coulped to mass spectrometer (GC-MS) and computer techniques have added new dimensions to research in this field. High performance liquid chromatography (HPLC) and mass spectrometry - mass spectrometry (MS-MS) technique are new innovative methods adopted for analysis of natural products. There are lots of literature on the modern physical methods of analysis. These instruments are helpful in identification and characterization of even trace components present in the essential oils.

The separation and identification of monoterpenes and sesquiterpenes in plant essential oils and other natural and synthetic sources relies heavily on gas chromatography[254-255]. In some cases gas chromatography may be the sole means of identification where compounds are identified by direct comparison of retention times with

standards or precise knowledge of Kovats retention indices[256,257]. Kovats indices are the most widespread form to express relative retention times using internal standards and it plays an important role for the identification of essential oil constituents.

The fastest technique for identifying compounds by mass spectrometry involves the comparison of mass spectrum with known mass spectra from a collection of standards[258-261]. A combination of Kovats indices with mass spectral search will increase the precision of identification[262]. Jennings and Shibamoto noted that many terpenes have essentially identical mass spectra[263]. Even where combined GC-MS is used for analysis, assignments cannot be made on the basis of mass spectrometric data only[263]. The identification of sesquiterpene hydrocarbons from essential oils is also difficult and uncertain. Mass spectra of this group of hydrocarbons are closely similar. Ramaswamy et al[264] recommend that Kovats type retention indices using fatty acid ethyl esters as standards be used in conjuction with mass spectra. A combination of GC-MS and Kovats indices is the single and only tool for the analysis of flavour and fragrance materials.

Jennings and Shibamoto[263] have published a substantial set of retention indices for flavour and fragrance compounds using two different types of stationary phases viz. capillary columns with standard dimethyl polysiloxane (methylsilicone) as non-polar phase and carbowax 20 M as polar phase. Shibamoto[265] has also presented a general discussion on the use of retention indices in essential oil analysis. Anderson and co-workers have provided a significant amount of information on sesquiterpene hydrocarbons[266,267]. A series of monographs on the application of gas-liquid chromatography to the analysis of various authentic essential oils were published by the Analytical Methods Committee of the Royal Society of Chemistry[268-270].

As mentioned earlier gas chromatographic retention indices (Kovats indices) are a valuable aid in the indentification of monoterpenes and sesquiterpenes in essential oils and related natural and synthetic products. Davies[271] has compiled some 900 Kovats indices of 400 individual compounds on methyl silicone (dimethyl polysiloxane) and/or Carbowax 20 M phases from the general literature.

The chemistry of the essential oil of Piper species has been studied satisfactorily on recent years. Different workers have identified a number of compounds from P.nigrum using vapour phase chromatography and other modern techniques[272-280]. Govindarajan[5] and Purseglove et al[197] have reviewed the work carried out by different workers. The latest work on essential oil analysis from P.nigrum was carried out by Gopalakrishnan et al[281] where the composition of the oils from four new Indian genotypes were determined using Kovats retention indices combined with GC-MS analysis. Reports were also available on the essential oils of other Piper species like P.longum[5], P.betel[5,6] and P.quineense[233]. The first detailed investigation on the composition of the essential oil from the berries of P.quineense was carried out by Ekundayo et al[233] in which they have identified fifty one mono and sesquiterpenoids.

A literature survey on Piper species thus revealed that essential oil constituents of P.aurantiacum and P.attenuatum have not been investigated. Because of its importance as chewing leaves, the leaves of P.betel are investigated for its essential oil composition. Thus it is interesting to investigate the leaves of other Piper

species for their essential oil constituents. As already mentioned in Chapter III, parts of P.attenuatum is used for washing clothes to scent them[222]. A detailed investigation on different parts of P.attenuatum is therefore undertaken for their essential oil composition. In addition to this a detailed essential oil analysis is also performed on P.cubeba berries and P.nigrum leaf.

## EXPERIMENTAL

P.attenuatum plant materials were collected from a garden near Neyyar Dam, Trivandrum District. The berries of this plant was dried in a cross-flow drier (50° C) and the leaves and stem were shade dried. P.nigrum leaves were collected from the RRL campus garden and was also shade dried. P.aurantiacum seeds and berries of P.cubeba were purchased from the local market. Essential oils from all these plant materials were extracted by Clevenger distillation method[282]. Refractive index was measured in Abbe refractometer and optical rotation in DIP-370 digital polarimeter.

### Capillary GC and GC-MS Analysis

Hewlett packard 5980 A and HP 5890 FID gas chromatographs equipped with a fused silica capillary column (50 m x 0.25 mm) coated with methyl silicone (thickness 0.17 µm) were used for the GC analysis of the essential oils. GC conditions were: nitrogen as carrier gas (1 ml/min), split ratio 1:75, injection temp. 250°C, FID temp. 300° C and temp. programmed from 80° to 200°C at the

rate of 2° C/min. GC/MS analysis was conducted using a Hewlett Packard 5995 GC/MS equipped with same capillary column and under same GC conditions. MS conditions were: electron impact, ionizing voltage 70 eV, source temp. 150° C, electron multiplier at 2000 eV, scan speed 690 amu/s and scan range 40-500 amu. Compound identification was made by using a combination of Kovats indices[263,264,268] followed by co-injection with authentic samples (wherever possible) or from the MS of the compounds. In mass spectral analysis the constituents were identified by matching the mass spectra with those of authentic standards held in the NBS library on hydrocarbons and flavour and fragrances and library generated in our laboratory. Only similarity indices of 0.9 or higher were taken as proof of identity.

Retention behaviour of a compound is reported relative to that of n-paraffin hydrocarbons. Kovats retention indices[256] was calculated from the data on the retention behaviour of the n-alkane homologous series. Each n-paraffin hydrocarbon is assigned by definition an index 100 times its carbon number. The retention index KI of a compound is then calculated by using the following equation:

$$KI = 100\,N + 100\,n\,\frac{[\log Rt_A - \log Rt_N]}{[\log Rt_{(N-n)} - \log Rt_N]}$$

where KI is the Kovats index and $Rt_A$ and $Rt_{(N-n)}$ are the adjusted retention times of n-paraffin hydrocarbons of carbon numbers N and (N-n), that are respectively smaller and larger than the adjusted retention time of the unknown $Rt_A$.

It is noted that resolution of the compounds was far better in case of capillary GC programme and the identification of the compounds is based more on Kovats index values which has been supported by MS of the compounds in most cases.

## RESULTS AND DISCUSSION

1) Higher alkanes from the fruits of P.aurantiacum

P.aurantiacum[283] is a stout glabrous climber with coriaceous leaves, 7.5-10 cm long. It is found in Nepal Lakhipur and Khasi hills in Assam. It bears dropping spikes, 3.8-7.5 cm long with fruits distinctly anglular and pyramidal when ripe, about 4 mm in diameter. The fruits are reported to possess bitter, acrid and cooling properties[2].

A detailed chemical examination of the seeds of P.aurantiacum has been carried out by Rao et al[51]. β-sitosterol, piperine, piperettine, sylvatine[51], aurantiamide and its acetate[101], stearic and linoleic acids, triacontane, cholesterol and cholestanol[204], triterpenes - friedelin and epifriedelanol[136], vanillic acid and aurantiamide[100] were so far reported from the fruits.

No essential oil could be obtained by cleavenger distillation of 100 g. of the powdered P.aurantiacum seeds. In a separate experiments 50 g. of the powdered seeds were extracted continuously in a Soxhlet extractor for 10 hrs. The hexane extract was concentrated and the residue was fractionated into methanol soluble and methanol insoluble fractions. The methanol insoluble fraction was then subjected to GC and Mass Spectral Analysis.

GC analysis was carried out at isothermal temperature 300° (inj. temp. 250°, FID temp. 300°) on 10% OV-17 column with $N_2$ as carrier gas on HP 5840 A Gas Chromatograph. Three peaks were observed. Mass Spectral Analysis of the fraction was carried out at isothermal 300° using direct inlet system (HP 5995 GC-MS). The three peaks were found to correspond with $C_{31}H_{64}(M^+436)$, $C_{33}H_{68}$ $(M^+464)$, and $C_{35}H_{72}$

($M^+$ 492). There is a consecutive loss of 14 or 28 units in the mass spectra of alkanes.

2) Composition of P.nigrum leaf oil

The chemistry of pepper berry oil has been carried by various workers exhaustively. Relatively not much work has been done on leaf oil except by Rogers[284]. Rogers mentioned a high content of sesquiterpene in the oil. Previous work in our laboratory also indicated the presence of 97% sesquiterpenes and other high polar compounds. Bandopadhay et al[227] also observed a high per centage of sesquiterpenes and farnesene is the major compound. A detailed investigation of the pepper leaf oil is not conducted so far. Hence a detailed investigation of the leaf oil was conducted using capillary GC and GC/MS.

Pepper leaf yielded a clear thick setting oil (1.0%) with a pronounced heavy leafy odour. Refractive index of the oil is very high (1.4927). Capillary GC and GC-MS conditions and mode of identification were described in the experimental part.

Table 1 gives the details of components identified by capillary GC and GC-MS analysis. 63 Compounds could be identified from the essential oil of P.nigrum. Low volatile

Table 1

**Composition of P.nigrum leaf oil**

| Sl. No. | Compound | Rt. | Kovats index | | % Composition |
|---|---|---|---|---|---|
| | | | Exp. | Ref.[263,271] | |
| 1. | 3-Methyl-2-pentanol | 5.58 | 754 | 755 | 0.01 |
| 2. | 1-Hexen-3-ol | 5.79 | 776 | 770 | 0.01 |
| 3. | n-Hexan-2-ol | 5.98 | 793 | 786 | 0.04 |
| 4. | Cis-3-hexenol | 6.60 | 846 | 847 | 0.05 |
| 5. | n-Heptan-2-ol | 7.35 | 888 | 888 | 0.01 |
| 6. | α-Thujene | 8.26 | 938 | 938 | 0.01 |
| 7. | α-pinene | 8.51 | 943 | 942 | 0.03 |
| 8. | Sabinene | 9.42 | 977 | 976 | 0.02 |
| 9. | β-pinene | 9.68 | 986 | 981 | 0.07 |
| 10. | Limonene | 11.15 | 1031 | 1030 | 0.11 |
| 11. | Cis-ocimene | 11.57 | 1043 | 1039 | 0.04 |
| 12. | Linalool oxide | 12.61 | 1069 | 1068 | 0.07 |
| 13. | Terpinolene | 13.12 | 1081 | 1082 | 0.03 |
| 14. | Linalool | 13.58 | 1094 | 1092 | 0.38 |
| 15. | Dihydrolinalool | 14.87 | 1120 | 1122 | 0.08 |

| | | | | | |
|---|---|---|---|---|---|
| 16. | β-Terpineol | 15.84 | 1142 | 1137 | 0.06 |
| 17. | Terpin-4-ol | 17.42 | 1172 | 1175 | 0.02 |
| 18. | α-Terpineol | 18.01 | 1183 | 1185 | 0.28 |
| 19. | Nerol | 20.01 | 1219 | 1218 | 0.07 |
| 20. | Geraniol | 21.29 | 1244 | 1243 | 0.09 |
| 21. | Carvone oxide | 22.26 | 1261 | 1261 | 0.10 |
| 22. | Bornyl acetate | 23.26 | 1277 | 1278 | 0.21 |
| 23. | Carvacrol | 24.70 | 1299 | 1297 | 0.02 |
| 24. | Piperitenone | 25.64 | 1316 | - | 0.02 |
| 25. | Terpinyl acetate | 26.20 | 1333 | 1333 | 0.06 |
| 26. | δ-Elemene | 27.40 | 1345 | 1344 | 0.07 |
| 27. | Geranyl acetate | 28.28 | 1360 | 1364 | 0.02 |
| 28. | α-cubebene | 28.71 | 1367 | 1369 | 0.22 |
| 29. | Methyl eugenol | 29.07 | 1372 | 1376 | 0.09 |
| 30. | α-Copaene | 29.64 | 1381 | 1398 | 0.02 |
| 31. | β-Elemene | 30.61 | 1395 | 1400 | 0.07 |
| 32. | β-Bourbonene | 31.27 | 1405 | 1406 | 0.14 |
| 33. | β-Cubebene | 31.81 | 1415 | - | 1.68 |
| 34. | E, α-Farnesene | 32.21 | 1422 | - | 0.16 |
| 35. | Caryophyllene | 32.62 | 1429 | 1428 | 0.14 |
| 36. | α-Cedrene | 32.99 | 1435 | 1436 | 0.06 |
| 37. | β-Copaene | 33.50 | 1444 | 1445 | 0.59 |

| | | | | | |
|---|---|---|---|---|---|
| 38. | β-Cedrene | 33.70 | 1447 | 1446 | 0.50 |
| 39. | E,β-Farnesene | 34.10 | 1454 | 1448 | 0.29 |
| 40. | α-Humulene | 35.15 | 1471 | 1465 | 0.69 |
| 41. | ϒ-Muurolene | 35.47 | 1476 | 1475 | 1.63 |
| 42. | Alloaromadendrene | 35.72 | 1479 | 1478 | 0.32 |
| 43. | Germacrene D | 35.89 | 1482 | 1487 | 0.27 |
| 44. | β-Selinene | 36.22 | 1487 | - | 0.47 |
| 45. | E,E-α-Farnesene | 37.20 | 1502 | - | 1.55 |
| 46. | α-Murrolene | 37.41 | 1506 | 1500 | 0.12 |
| 47. | β-Bisabolene | 37.50 | 1508 | 1506 | 0.13 |
| 48. | Calamenene | 37.76 | 1512 | 1518 | 0.73 |
| 49. | δ-Cadinene | 38.09 | 1518 | 1524 | 0.17 |
| 50. | Cadina-1,4-diene | 38.85 | 1532 | 1539 | 1.35 |
| 51. | Elemol | 39.80 | 1549 | 1540 | 11.52 |
| 52. | E-Nerolidol | 40.15 | 1554 | 1553 | 1.92 |
| 53. | Caryophyllene alcohol | 40.42 | 1558 | 1559 | 4.85 |
| 54. | Caryophyllene oxide | 41.50 | 1576 | 1576 | 0.23 |
| 55. | Cedrene oxide | 42.12 | 1586 | 1585 | 0.35 |
| 56. | Cedrol | 43.68 | 1612 | 1609 | 0.36 |
| 57. | β-Eudesmol | 44.58 | 1628 | 1640 | 3.31 |

| | | | | | |
|---|---|---|---|---|---|
| 58. | α-Cadinol | 44.81 | 1632 | 1644 | 0.75 |
| 59. | Cadina-1,4-diene-3-ol | 46.21 | 1656 | 1658 | 3.20 |
| 60. | α-Bisabolol | 46.32 | 1658 | 1666 | 3.32 |
| 61. | Z,E-farnesol | 48.07 | 1686 | 1681 | 4.69 |
| 62. | E,Z-farnesol | 48.55 | 1694 | 1693 | 3.53 |
| 63. | E,E-farnesol | 49.88 | 1718 | 1714 | 0.10 |

alcohols, monoterpenes and oxygenated monoterpenes constituted 2.5% only. α-pinene, β-pipnene, sabinene and limonene are present in very small quantities. The sesquiterpene hydrocarbons constituted about 12%. The major sesquiterpenes identified are β-cubebene, ϒ-muurolene, α-humulene, calamenene, β-copaene and β-cedrene. β-caryophyllene, the major sesquiterpene hydrocarbon of pepper berry oil (16%) accounts only about 0.3% in leaf oil. The high polar oxygenated compounds account for 85.5%. The high content of oxygenated compounds may be the reason for showing a high refractive index. Most of the high polar compounds could not be identified. Of all the thirteen oxygenated sesquiterpenoid constituents identified, elemol alone constitutes about 11.5%. Other major sesquiterpene alcohols are nerolidol (2%), caryophyllene alcohol (5%), eudesmol (3%), cadina -1,4-diene-3-ol (3%), α-bisabolol (3%) and farnesols (8%). Thus we can see that the oil from the leaf contains high polar sesquiterpenes which is in agreement with the earlier reports[284].

3) Composition of P.cubeba berry oil

P.cubeba is another important species of the genus Piper. It is known as tailed pepper and is a native of Indonesia[5]. The leaves are glabrous, ovate oblongs with

cordate or rounded bases; the fruits are borne five or more on spikes and are subglobose, somewhat apiculate and stalked. Dry cubebs are almost globular and are 3 to 6 mm diameter; a stalk like portion about 5 to 7 mm long attached to the base gives it the name 'tailed pepper'.

The characteristic constituent of the spice is the volatile oil which varies from 12.5-20%. Early analysis of the volatile oil indicated the presence of α-pinene/camphene, 1-cadinene, azulene and 'cubeb camphor', which may be an odourless sesquiterpene alcohol ($C_{15}H_{26}O$, m.p. 105-106°) found only in old samples[285]. Recent investigation of the oil showed copaene, a cadaline type sesquiterpene, a new tricyclic sesquiterpene, a sesquiterpene alcohol, a new sesquiterpene, alcohol of cadalene type and an azulene alcohol[286]. Recently Ikeda et al[274] analysed the oil by GC and found presence of α-pinene, α-thujene , β-pinene, sabinene, α-phellandrene, α-terpinene, myrcene, d-limonene, β-phellandrene and ϒ-terpinene. Masada[232] reported that the oil contains α-pinene, β-pinene, limonene, cineole, ϒ-terpinene, citronellal caryophyllene, citronellyl acetate, methyl salycilate and hydroxy citronelall. Isolation of bicyclosesquiphellandrene

from this oil is also reported[201]. Ramaswamy et al[264] have reported a few sesquiterpene hydrocarbons. Lots of lignans have also been isolated from this plant and summarised in the review part of this thesis. This part of the thesis deals with the first detailed capillary GC analysis of the essential oil from P.cubeba berries.

The oil from the berries is subjected to capillary GC analysis. GC conditions are described in the experimental section. Initial fractionation of the oil into hydrocarbon and oxygenated fractions are conducted using silica gel column and eluting with petroleum ether and methylene chloride respectively.

Chemical composition of the oil is presented in Table 2. Out of the eighty components present in the oil, almost fifty seven compounds could be identified. It contains about 32% of monoterpenes and 68% of sesquiterpenes and other high polar compounds. Among the monoterpenes β-pinene alone constitute about 18%. α-pinene and limonene content are 2% each. Monoterpene hydrocarbon accounts for about 25% and the rest being oxygenated compounds. Although lots of oxygenated monoterpenes are present, their percentage composition is relatively very low. The major oxygenated monoterpenes are linalool, α-terpineol and linalool oxide.

## Table 2

Composition of P.cubeba berry oil

| Sl. No. | Compound | Rt. | Kovats index | | % Composition |
|---|---|---|---|---|---|
| | | | Exp. | Ref.[263,271] | |
| 1. | α-Thujene[a] | 8.86 | 931 | 938 | 0.007 |
| 2. | α-Pinene[a] | 9.04 | 939 | 942 | 2.20 |
| 3. | β-Pinene[a] | 10.16 | 984 | 981 | 18.19 |
| 4. | Dihydro ocimene | 10.44 | 994 | 995 | 0.74 |
| 5. | α-Phellandrene[a] | 10.78 | 1007 | 1002 | 0.21 |
| 6. | 1,4-Cineol | 10.94 | 1014 | 1014 | 0.03 |
| 7. | $\Delta^3$-Carene | 11.06 | 1018 | 1018 | 0.33 |
| 8. | P-Cymene | 11.15 | 1022 | 1020 | 0.36 |
| 9. | Limonene[a] | 11.49 | 1035 | 1030 | 2.01 |
| 10. | Trans-Ocimene | 11.63 | 1041 | 1038 | 0.04 |
| 11. | γ-Terpinene[a] | 12.10 | 1055 | 1057 | 0.72 |
| 12. | Linalool oxide | 12.45 | 1070 | 1068 | 1.31 |
| 13. | Terpinolene | 12.88 | 1084 | 1082 | 0.18 |
| 14. | Linalool | 13.24 | 1095 | 1092 | 1.50 |
| 15. | Myrcenol | 13.37 | 1099 | 1103 | 0.58 |

| | | | | | |
|---|---|---|---|---|---|
| 16. | Fenchol | 13.60 | 1108 | 1110 | 0.007 |
| 17. | Dihydrolinaool | 13.86 | 1118 | 1122 | 0.13 |
| 18. | Citronellal[a] | 14.32 | 1134 | 1137 | 0.10 |
| 19. | Terpinen-4-ol | 15.20 | 1165 | 1174 | 0.06 |
| 20. | α-Terpineol | 15.61 | 1178 | 1175 | 1.67 |
| 21. | Dihydrocarvone | 15.81 | 1184 | 1183 | 0.29 |
| 22. | Trans-carveol | 16.57 | 1209 | 1209 | 0.01 |
| 23. | Neral | 17.10 | 1228 | 1227 | 0.01 |
| 24. | Geraniol | 17.56 | 1245 | 1243 | 0.02 |
| 25. | Geranial | 17.91 | 1256 | 1252 | 0.003 |
| 26. | Sabinyl acetate | 18.21 | 1266 | 1262 | 0.001 |
| 27. | Safrole | 18.45 | 1274 | 1277 | 0.08 |
| 28. | Thymol | 18.80 | 1285 | 1287 | 0.05 |
| 29. | Terpinyl acetate | 20.01 | 1328 | 1333 | 0.03 |
| 30. | Citronellyl acetate[a] | 20.31 | 1338 | 1335 | 0.54 |
| 31. | Methyl eugenol | 20.76 | 1354 | - | 2.27 |
| 32. | β-Copaene[a] | 21.65 | 1384 | 1398 | 0.91 |
| 33. | β-Elemene[a] | 22.16 | 1401 | 1400 | 7.20 |
| 34. | β-Cubebene[a] | 22.41 | 1411 | - | 5.59 |
| 35. | β-Caryophyllene | 22.75 | 1424 | 1428 | 0.36 |
| 36. | β-Copaene | 23.19 | 1441 | 1444 | 3.34 |

| | | | | | |
|---|---|---|---|---|---|
| 37. | β-Cedrene | 23.51 | 1452 | 1446 | 0.03 |
| 38. | E-β-Farnesene | 23.65 | 1458 | 1448 | 0.14 |
| 39. | α-Humulene | 23.85 | 1465 | 1465 | 0.31 |
| 40. | Alloaro-madendrene[a] | 24.16 | 1476 | 1478 | 2.33 |
| 41. | Germacrene D[a] | 24.30 | 1480 | 1488 | 1.51 |
| 42. | β-bisabolene | 24.71 | 1495 | 1506 | 3.12 |
| 43. | Calamenene[a] | 24.95 | 1504 | 1518 | 2.57 |
| 44. | δ-Cadinene[a] | 25.30 | 1517 | 1524 | 4.74 |
| 45. | Caryophyllene alcohol | 26.48 | 1560 | 1559 | 23.64 |
| 46. | Caryophyllene oxide | 26.82 | 1572 | 1576 | 1.29 |
| 47. | Cedrene epoxide | 27.09 | 1582 | 1585 | 0.01 |
| 48. | Humulene oxide (T) | 27.56 | 1598 | - | 0.98 |
| 49. | Isocedrol (T) | 27.70 | 1603 | - | 0.19 |
| 50. | Cedrol | 27.78 | 1605 | 1609 | 0.25 |
| 51. | β-Eudesmol | 28.50 | 1629 | 1640 | 2.43 |
| 52. | Cadinol | 28.83 | 1640 | 1644 | 0.88 |
| 53. | β-Bisabolol | 29.19 | 1651 | 1662 | 0.98 |
| 54. | Z,Z-Farnesol | 29.61 | 1664 | 1666 | 0.64 |
| 55. | Z,E-Farnesol | 30.05 | 1678 | 1681 | 0.04 |

| | | | | | |
|---|---|---|---|---|---|
| 56. | E,Z-Farnesol | 30.57 | 1693 | 1693 | 0.07 |
| 57. | E,E-Farnesol | 31.41 | 1718 | 1714 | 0.007 |

a = previously identified

T = tentatively identified

Among the sesquiterpene hydrocarbons β-elemene and β-cubebene constitute 7% and 6% respectively. Other major sesquiterpenes are β-copaene, δ-eadinene, β-bisabolene, α-copaene, alloaromadendrene calamenene and germacrene D. A sesquiterpene alcohol constitute about 23% of the oil. this compound is tentatively identified as caryophyllene alcohol since its Kovats index matches with it. High polar compounds are present in very small quantities and have yet to be identified.

4) Composition of P.attenuatum berry, stem and leaf oils

As already mentioned there is no systematic chemical investigation on P.attenuatum. Chapter III of this thesis deals with the crystalline constituents from different parts of this plant. This part of the chapter deals with the composition of the essential oils from the berry, stem and leaf of P.attenuatum.

Optical rotation, GC and GC-MS conditions are described in the experimental section. Table 3 shows the percentage yield, optical rotation and refractive indices of the oils from berry, stem and leaf of P.attenuatum. Berries showed 1.6% oil. Refractive index of stem oil was lower than that of oils from berries and leaves. The

monoterpene content of the leaf oil is slightly higher than the other two oils.

**Table 3**

Physico-Chemical Characteristic of the Oils from P.attenuatum

| Characteristics | Berry | Leaf | Stem |
|---|---|---|---|
| Volatile Oil % | 1.6 | 0.75 | 0.08 |
| Refractive index | 1.496 | 1.497 | 1.485 |
| Optical rotation | -0.644° | -2.094° | -1.153° |
| Monoterpene % | 2-3 | 3-4 | ~ 1 |
| Sesquiterpenes and other polar compounds | 97-98 | 96-97 | 98-99 |

(a) Berry Oil

Table 4 shows the composition of the essential oil from berries along with Kovats indices. The oil contains 2-3% monoterpenes, 97% sesquiterpenes and other high polar compounds (Fig.1). About 66 constituents could be identified from this oil. The major monoterpene is found to be β-pinene and α-pinene. Other monoterpenes, like

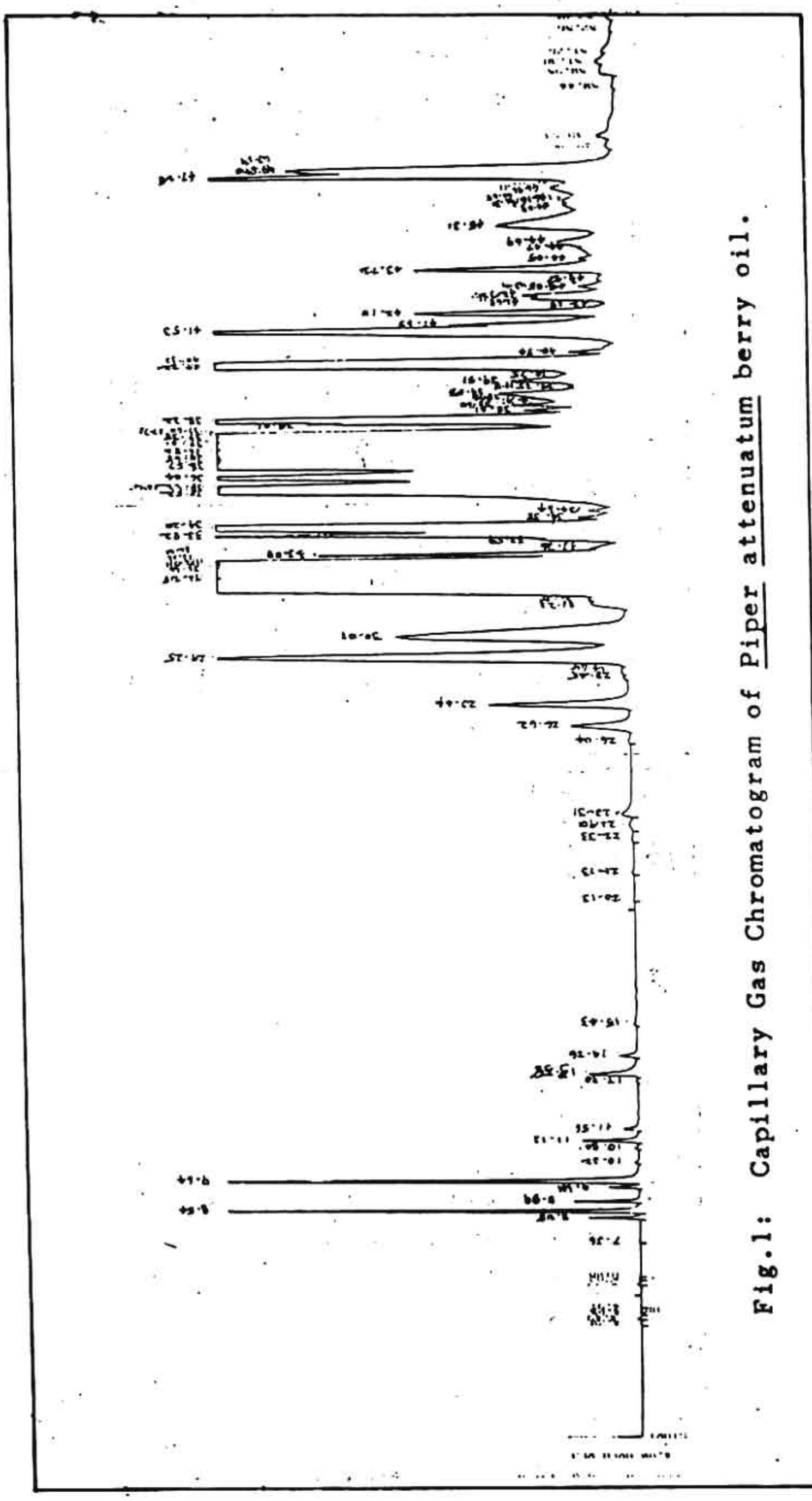


Fig.1: Capillary Gas Chromatogram of Piper attenuatum berry oil.

Table 4

Composition of P.attenuatum berry oil

| Sl. No. | Compound | Rt. | Kovats index | | % composition |
|---|---|---|---|---|---|
| | | | Exp. | Ref.[263,271] | |
| 1. | 1-Hexen-3-ol | 5.77 | 774 | 770 | 0.004 |
| 2. | n-Hexan-2-ol | 5.97 | 792 | 786 | 0.003 |
| 3. | n-Hexanol | 6.88 | 861 | 858 | 0.002 |
| 4. | n-Heptan-2-ol | 7.36 | 888 | 888 | 0.005 |
| 5. | α-Thujene | 8.28 | 933 | 938 | 0.06 |
| 6. | α-Pinene | 8.54 | 944 | 942 | 0.75 |
| 7. | Camphene | 8.89 | 958 | 954 | 0.08 |
| 8. | Sabinene | 9.41 | 977 | 976 | 0.04 |
| 9. | β-Pinene | 9.64 | 984 | 981 | 0.98 |
| 10. | α-Phellandrene | 10.32 | 1006 | 1002 | 0.01 |
| 11. | P-Cymene | 10.84 | 1022 | 1020 | 0.01 |
| 12. | Limonene | 11.12 | 1030 | 1030 | 0.10 |
| 13. | trans-ocimene | 11.56 | 1043 | 1038 | 0.04 |
| 14. | Terpinolene | 13.30 | 1085 | 1082 | 0.01 |
| 15. | Linalool | 13.58 | 1091 | 1092 | 0.15 |

| | | | | | |
|---|---|---|---|---|---|
| 16. | Myrcenol | 14.26 | 1106 | 1103 | 0.05 |
| 17. | Sabinol | 15.43 | 1133 | 1135 | 0.03 |
| 18. | Citral | 20.13 | 1222 | 1222 | 0.004 |
| 19. | Geraniol | 21.13 | 1241 | 1243 | 0.01 |
| 20. | Carvone oxide | 22.33 | 1262 | 1261 | 0.01 |
| 21. | Safrole | 22.90 | 1271 | 1277 | 0.03 |
| 22. | Bornyl acetate | 23.31 | 1278 | 1278 | 0.13 |
| 23. | Dihydrocarvyl acetate | 26.04 | 1323 | 1319 | 0.02 |
| 24. | Terpinyl acetate | 26.62 | 1333 | 1333 | 0.26 |
| 25. | δ-Elemene | 27.44 | 1347 | 1344 | 0.58 |
| 26. | Geranyl acetate | 28.45 | 1363 | 1364 | 0.04 |
| 27. | α-Cubebene | 28.64 | 1366 | 1369 | 0.04 |
| 28. | Methyl eugenol | 29.25 | 1375 | 1376 | 2.53 |
| 29. | α-Copaene | 30.01 | 1386 | 1398 | 2.06 |
| 30. | β-Elemene | 31.23 | 1405 | 1400 | 0.17 |
| 31. | β-Bourbonene | 31.35 | 1406 | 1406 | 0.13 |
| 32. | β-Cubebene | 32.28 | 1423 | - | 10.32 |
| 33. | β-Caryophyllene | 32.60 | 1430 | 1428 | 13.1 |
| 34. | α-Cedrene | 32.85 | 1434 | 1436 | 4.67 |
| 35. | α-Bergamotene | 33.08 | 1437 | 1439 | 0.63 |
| 36. | β-Copaene | 33.59 | 1445 | 1444 | 0.20 |

| | | | | | |
|---|---|---|---|---|---|
| 37. | β-Cedrene | 33.82 | 1449 | 1446 | 1.19 |
| 38. | E-β-Farnesene | 34.20 | 1455 | 1448 | 3.15 |
| 39. | ϒ-Elemene | 34.38 | 1457 | - | 0.15 |
| 40. | α-Humulene | 34.64 | 1462 | 1465 | 0.11 |
| 41. | ϒ-Muurolene | 35.52 | 1476 | 1475 | 2.78 |
| 42. | Alloromadendrene | 35.64 | 1478 | 1478 | 1.34 |
| 43. | Germacrene D | 36.04 | 1487 | 1488 | 2.57 |
| 44. | β-Selinene | 36.57 | 1492 | - | 3.95 |
| 45. | α-Selinene | 37.01 | 1499 | - | 4.00 |
| 46. | α-Muurolene | 37.38 | 1505 | 1500 | 4.14 |
| 47. | β-bisabolene | 37.60 | 1509 | 1506 | 4.68 |
| 48. | Elemicin | 37.73 | 1512 | 1516 | 0.82 |
| 49. | Calamenene | 38.01 | 1517 | 1518 | 0.82 |
| 50. | δ-Cadinene | 38.22 | 1522 | 1524 | 2.41 |
| 51. | Z-Nerolidol | 38.41 | 1524 | 1524 | 0.26 |
| 52. | β-Sesquiphellandrene (T) | 38.86 | 1532 | - | 0.24 |
| 53. | Cadina-1,4-diene | 39.03 | 1534 | 1539 | 0.60 |
| 54. | Elemol | 39.51 | 1543 | 1540 | 0.62 |
| 55. | E-Nerolidol | 40.22 | 1555 | 1553 | 3.02 |
| 56. | Caryophyllene alcohol | 40.44 | 1559 | 1559 | 1.00 |
| 57. | Cedrene epoxide | 42.10 | 1586 | 1585 | 0.89 |

| | | | | | |
|---|---|---|---|---|---|
| 58. | Cedrol | 43.27 | 1605 | 1609 | 0.10 |
| 59. | T-Muurolol (T) | 44.05 | 1619 | 1630 | 0.14 |
| 60. | β-Eudesmol | 44.47 | 1626 | 1640 | 0.24 |
| 61. | α-Cadinol | 44.69 | 1630 | 1644 | 0.47 |
| 62. | Cadina-1,4-diene-3-ol | 46.16 | 1655 | 1658 | 0.16 |
| 63. | β-Bisabolol | 46.31 | 1657 | 1662 | 0.12 |
| 64. | Z,Z-Farnesol | 46.91 | 1667 | 1666 | 0.08 |
| 65. | Z,E-Farnesol | 47.54 | 1678 | 1681 | 0.52 |
| 66. | E,Z-Farnesol | 48.63 | 1695 | 1693 | 0.33 |

T = Tentatively identified

limonene, terpinyl acetate, linalool and safrole are present in small quantities. This oil contains low volatile alcohols in trace amounts.

The sesquiterpene hydrocarbon of this oil constitute about 75% of the oil. The major sesquiterpene constituents are β-caryophyllene (13%) and β-cubebene (10%). Other major sesquiterpenes between 3-5% concentration are α-cedrene, β-bisabolene, α-muurolene, α-selenene and β-farnesene. The berry oil contains about 22% of other oxygenated polar compounds. About fifteen compounds could be identified from this part. Nerolidol and caryophyllene alcohol constitute about 3.0 and 2.6% respectively. Other oxygenated sesquiterpenes identified are elemol, β-eudesmol, α-cadinol, cadina -1,4-diene-3-ol, β-bisabolol and farnesols. Lots of unidentified compounds are also present in this oil.

(b) Leaf Oil

Table 5 shows the composition of P.attenuatum leaf oil. The oil contains 3.5% monoterpenes, 43% sesquiterpenes and the rest being high polar constituents. The chromatogram (Fig.2) shows about 117 constituents and only 67 could be identified. About thirty three monoterpene

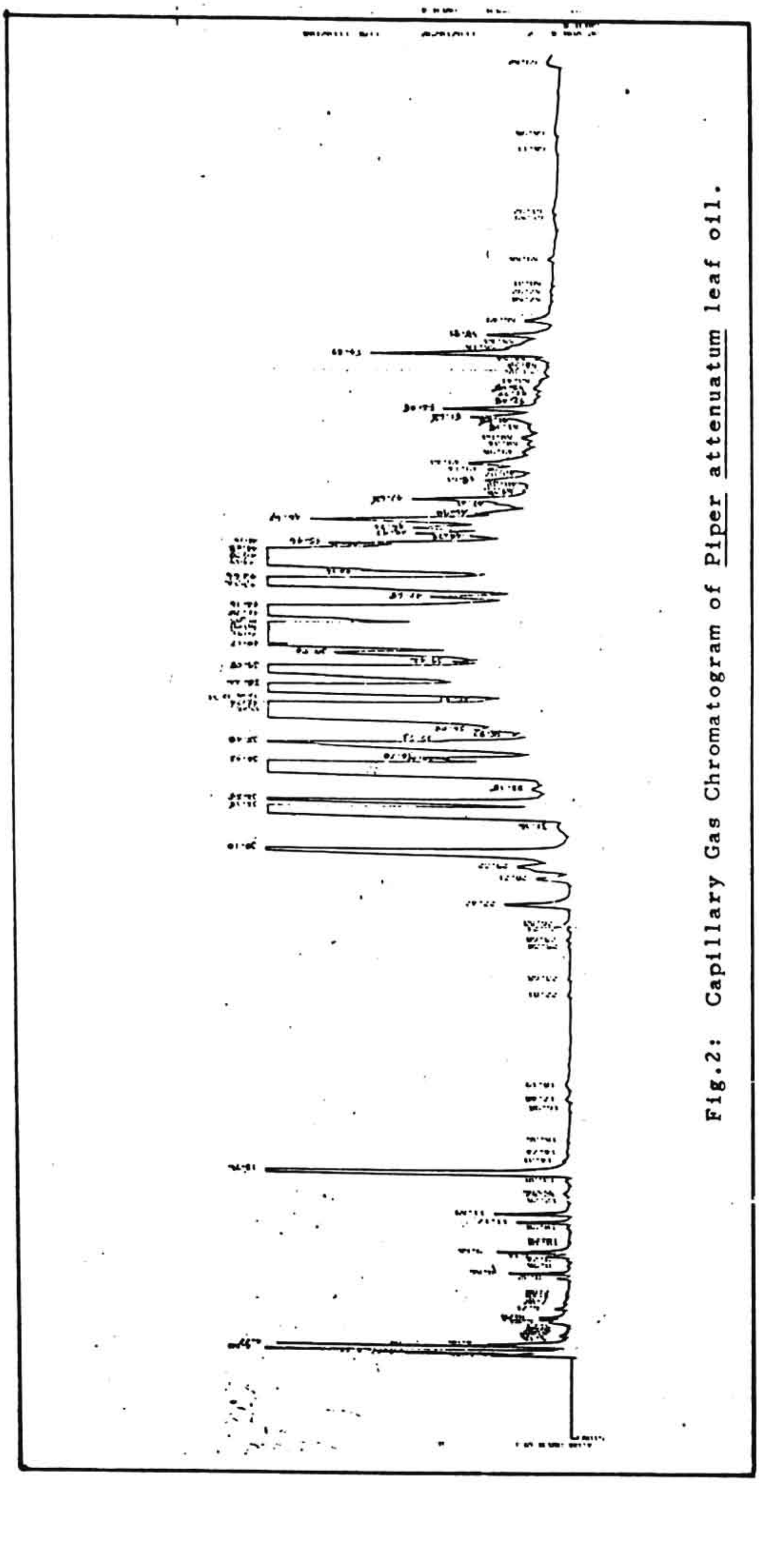


Fig.2: Capillary Gas Chromatogram of Piper attenuatum leaf oil.

Table 5

Composition of P. attenuatum leaf oil

| Sl. No. | Compound | Rt. | Kovats index | | % Composition |
|---|---|---|---|---|---|
| | | | Exp. | Ref[263,271] | |
| 1. | Methyl isobutyl ketone | 5.33 | 723 | - | 0.02 |
| 2. | 3-Methyl-2-pentanol | 5.56 | 752 | 755 | 0.006 |
| 3. | 4-Methyl-2-pentanol | 5.64 | 760 | 758 | 0.007 |
| 4. | 1-Hexen-3-ol | 5.79 | 775 | 770 | 0.01 |
| 5. | n-Hexan-2-ol | 6.02 | 796 | 786 | 0.06 |
| 6. | Furfural | 6.25 | 816 | 815 | 0.01 |
| 7. | Cis-3-Hexenol | 6.71 | 850 | 847 | 0.10 |
| 8. | 1-Hepten-3-ol | 7.02 | 869 | 868 | 0.03 |
| 9. | n-Heptanal | 7.26 | 883 | 883 | 0.01 |
| 10. | n-Hepten-2-ol | 7.40 | 890 | 888 | 0.01 |
| 11. | α-Thujene | 8.32 | 935 | 938 | 0.34 |
| 12. | α-Pinene | 8.56 | 944 | 942 | 0.09 |
| 13. | Camphene | 8.95 | 960 | 954 | 0.02 |
| 14. | Sabinene | 9.44 | 977 | 976 | 0.05 |
| 15. | β-Pinene | 9.65 | 985 | 981 | 0.15 |

| | | | | | |
|---|---|---|---|---|---|
| 16. | n-decane | 10.10 | 999 | 1000 | 0.007 |
| 17. | α-Phellandrene | 10.25 | 1004 | 1002 | 0.02 |
| 18. | P-Cymene | 10.90 | 1024 | 1020 | 0.02 |
| 19. | Limonene | 11.17 | 1032 | 1030 | 0.11 |
| 20. | trans-ocimene | 11.59 | 1044 | 1038 | 0.17 |
| 21. | ϒ-Terpinene | 12.25 | 1061 | 1057 | 0.02 |
| 22. | Linalool oxide | 12.57 | 1068 | 1068 | 0.01 |
| 23. | Terpinolene | 13.30 | 1085 | 1082 | 0.03 |
| 24. | Linalool | 13.76 | 1094 | 1092 | 1.67 |
| 25. | Myrcenol | 14.31 | 1107 | 1103 | 0.04 |
| 26. | Dihydrolinalool | 14.74 | 1117 | 1122 | 0.04 |
| 27. | Sabinol | 15.36 | 1131 | 1135 | 0.10 |
| 28. | Borneol | 16.95 | 1164 | 1164 | 0.03 |
| 29. | Terpinen-4-ol | 17.48 | 1174 | 1175 | 0.04 |
| 30. | α-Terpineol | 18.19 | 1186 | 1185 | 0.16 |
| 31. | Safrole | 22.81 | 1270 | 1277 | 0.13 |
| 32. | Piperitenone | 25.60 | 1315 | - | 0.03 |
| 33. | Terpinyl acetate | 26.53 | 1331 | 1333 | 0.05 |
| 34. | δ-Elemene | 27.47 | 1347 | 1344 | 0.29 |
| 35. | α-Cubebene | 28.71 | 1367 | 1369 | 0.16 |
| 36. | Methyl eugenol | 29.32 | 1376 | 1376 | 0.34 |

| | | | | | |
|---|---|---|---|---|---|
| 37. | α-Copaene | 30.10 | 1388 | 1398 | 1.92 |
| 38. | β-Elemene | 31.26 | 1405 | 1400 | 0.14 |
| 39. | (E)-2-Farnesene | 32.18 | 1421 | - | 4.67 |
| 40. | β-Caryophyllene | 32.55 | 1427 | 1428 | 1.30 |
| 41. | α-Bergamotene | 33.15 | 1438 | 1439 | 0.27 |
| 42. | (E)-β-Farnesene | 34.43 | 1459 | 1448 | 7.85 |
| 43. | α-Humulene | 34.65 | 1463 | 1465 | 0.60 |
| 44. | γ-Muurolene | 35.40 | 1474 | 1475 | 1.91 |
| 45. | Alloaromadendrene | 35.53 | 1476 | 1478 | 0.29 |
| 46. | Germaerene D | 35.83 | 1481 | 1488 | 0.18 |
| 47. | β-Selinene | 36.08 | 1484 | - | 0.38 |
| 48. | ar-Curcumene | 37.07 | 1499 | - | 6.21 |
| 49. | E,E,α-Farnesene | 37.21 | 1502 | - | 2.25 |
| 50. | α-Muurolene | 37.24 | 1503 | 1500 | 0.76 |
| 51. | β-Bisabolene | 37.28 | 1504 | 1506 | 1.78 |
| 52. | δ-Cadinene | 38.22 | 1521 | 1524 | 4.94 |
| 53. | Cadina-1,4-diene | 39.42 | 1542 | 1539 | 0.30 |
| 54. | Elemol | 39.84 | 1549 | 1540 | 1.36 |
| 55. | E-Nerolidol | 40.17 | 1554 | 1555 | 1.08 |
| 56. | Caryophyllene alcohol | 40.93 | 1567 | 1559 | 5.95 |
| 57. | Caryophyllene oxide | 41.35 | 1574 | 1576 | 4.98 |

| | | | | | |
|---|---|---|---|---|---|
| 58. | α-Cedrene epoxide | 42.16 | 1587 | 1585 | 4.22 |
| 59. | Cedrol | 43.52 | 1609 | 1609 | 4.64 |
| 60. | T-Muurolol (T) | 44.06 | 1619 | 1630 | 0.66 |
| 61. | β-Eudesmol | 44.64 | 1629 | 1640 | 4.26 |
| 62. | α-Cadinol | 44.88 | 1633 | 1644 | 1.92 |
| 63. | β-Bisabolol | 46.23 | 1656 | 1662 | 0.70 |
| 64. | Z,Z-Farnesol | 46.90 | 1667 | 1666 | 0.45 |
| 65. | Z,E-Farnesol | 47.68 | 1680 | 1681 | 0.77 |
| 66. | E,Z-Farnesol | 48.63 | 1695 | 1693 | 0.30 |
| 67. | E,E-Farnesol | 49.95 | 1719 | 1714 | 0.30 |

T = Tentatively identified.

constituents have been identified from this oil. The concentration of all these monoterpenes are very low in this oil except linalool (1.67%). Other constituents present are α-terpineol, terpinyl acetate, bornyl acetate β-pinene, limonene etc. The concentration of these constituents are comparatively very less. The oil shows more lower alcohols than in berry oil.

About twenty sesquiterpene constituents could be identified from the leaf oil. Among the sesquiterpene hydrocarbons β-farnesene , ar-curcumene and δ-cadinene constitute about 8%, 6% and 5% respectively. Other major constituents are α-farnesene,γ-muurolene, β-elemene, β-caryophyllene and β-bisabolene. ar-Curcumene is found only in the leaf oil. The leaf oil contains a high percentage (53%) of oxygenated sesquiterpenoids. Among these high polar constituents, caryophyllene alcohol, caryophyllene oxide, cedrol, cedrene epoxide and β-eudesmol are the major constituents. Other constituents are elemol, nerolidol, cadinol, β-bisabolol and farnesols.

(c) Stem Oil

Capillary GC of the P.attenuatum stem oil could not be conducted for Kovats indices determination. However, GC-

MS analysis of the oil is conducted in a Hewlett Packard Model 5995 B 50m flexible silica capillary column. Here the identification of the compounds are mainly based on Rt and MS (Fig.3).

Table 6 shows the analysis of stem oil from P.attenuatum along with MS fragmentation data. The oil shows a very low concentration of monoterpene ( 1%) and a relatively high percentage of sesquiterpenes and other polar constituents.

Among the sesquiterpenes, δ-cadinene ( 13%) is the major constituent. Other major constituents are β-caryophyllene and α-humulene which are present to the extent of 7-8%. It also shows a comparatively high concentration of β-bisabolene, β-elemene and α-copaene. Other constituents are α-cubebene, γ-muurolene, gurgunene and calarene. A major sesquiterpene hydrocarbon at Rt 23.40 is present to the extent of 18%, but it could not be identified. The sesquiterpene alcohols identified are elemol, nerolidol and caryophyllene alcohol. Several sesquiterpene hydrocarbons and alcohols are also present in this oil which could not be identified.

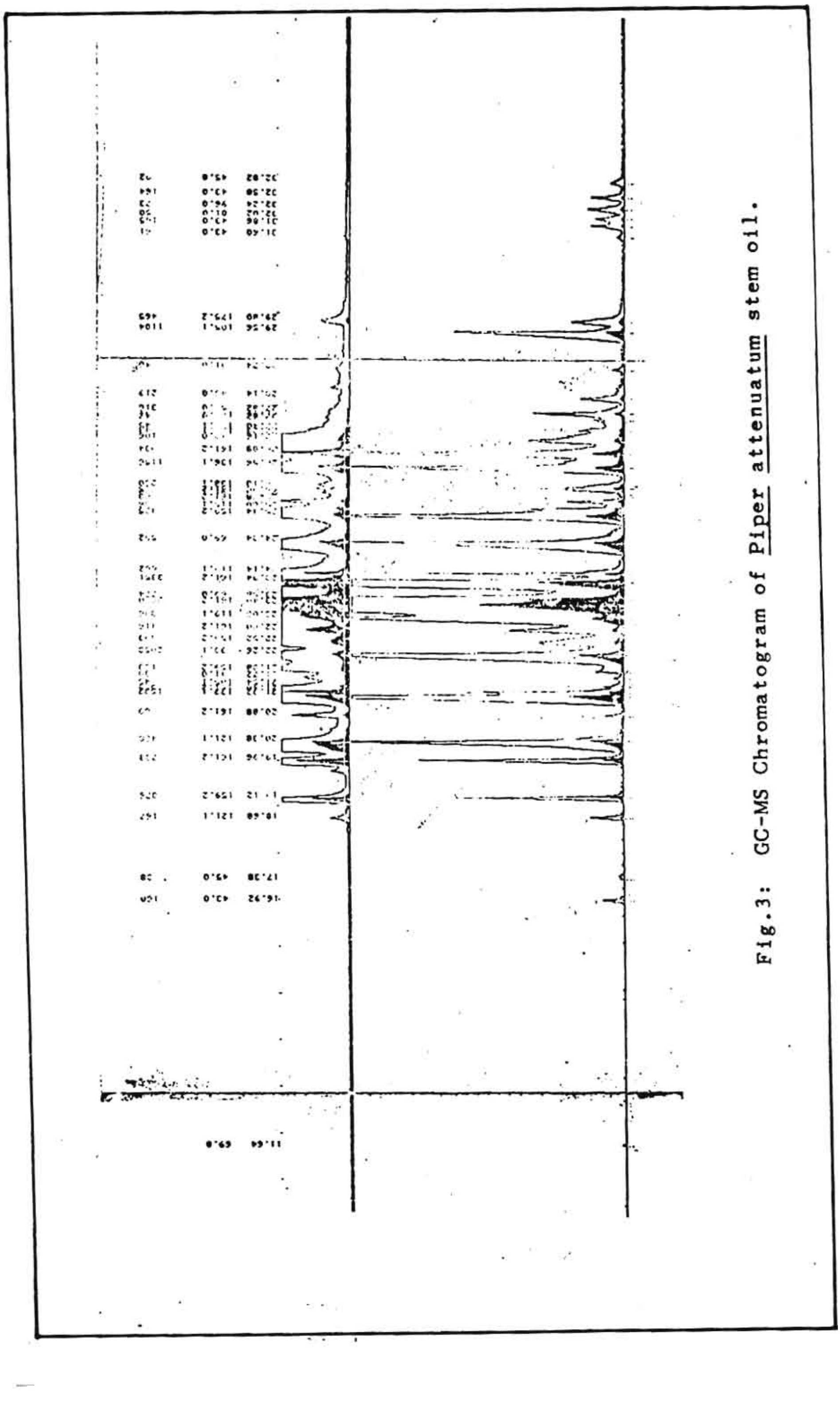


Fig.3: GC-MS Chromatogram of Piper attenuatum stem oil.

Table 6

**Composition of <u>P.attenuatum</u> stem oil**

| Sl. No. | Compound | Rt. | % | MS fragmentation data in order of abundance |
|---|---|---|---|---|
| 1. | Limonene | 11.64 | 0.05 | 68,93,79,136,121, 107,53. |
| 2. | Undecanone | 11.92 | 0.26 | 43,58,71,59,41,170 |
| 3. | Terpinolene | 18.68 | 0.49 | 121,93,79,91,136 |
| 4. | α-Cubebene | 19.12 | 2.44 | 159,161,119,105,41, 91,204. |
| 5. | α-Copaene | 19.96 | 3.07 | 161,159,119,105,204, 91,93,41,120 |
| 6. | β-Elemene | 0.38 | 4.86 | 121,147,93,161,133,41, 119,91,105,67 |
| 7. | α-Gurgunene | 20.88 | 0.23 | 161,204,189,41,119, 133,91,147,105. |
| 8. | β-Caryo-phyllene | 21.32 | 8.16 | 133,41,93,91,120,161, 119,105,79,107. |
| 9. | Calarene | 21.40 | 0.60 | 161,159,119,105,204, 162,43,91,77. |
| 10. | β-Farnesene | 21.88 | 0.35 | 159,41,161,69,93,119, 133,105,120. |
| 11. | α-Humulene | 22.26 | 7.46 | 93,121,147,80,91,41, 92,67,107,122. |

| | | | | |
|---|---|---|---|---|
| 12. | ϒ-Muurolene | 22.80 | 1.23 | 161,159,204,133,162, 119,115,189,105,91 |
| 13. | Sesquiterpene hydrocarbon (UI) | 23.40 | 18.38 | 161,159,105,41,133, 128,121,115,43 |
| 14. | β-Bisabolene | 23.56 | 4.11 | 69,93,41,109,67,79, 109,91,53,94,121. |
| 15. | δ-Cadinene | 23.94 | 12.84 | 161,119,134,204,159, 105,91,41,128 |
| 16. | Z-Nerolidol | 24.84 | 4.23 | 69,93,41,107,119,133, 123,136,43,91,121,109 |
| 17. | Elemol | 25.44 | 5.62 | 159,131,119,91,105,43, 133,145,41,205,220 |
| 18. | Caryophyllene alcohol | 26.88 | 3.82 | 161,119,204,121,105, 43,79,41,91,93. |

Compared to the oils from other parts of the plant, the stem oil is found to be different. It is devoid (except few compounds) of low volatile monoterpenoid constituents whereas the berry and leaf oil contains about 25-30 constituents.

## CONCLUSION

P.aurantiacum does not contain any essential oil. P.nigrum leaf oil contains about 100 constituents and only 63 constituents could be identified. P.cubeba berry oil contains about 80 components and 37 more components could be identified in addition to the constituents already identified. Capillary GC chromatogram of P.attenuatum berry oil indicated the presence of 100 constituents and could identify about 66 constituents. P.attenuatum leaf oil showed the presence of 117 constituents and identified about 67 constituents. GC-MS of P.attenuatum stem oil showed about 46 constituents and only 17 could be identified. So it can be seen that lots of unidentified constituents mostly sesquiterpenes and other high polar constituents are present in different parts of P.attenuatum

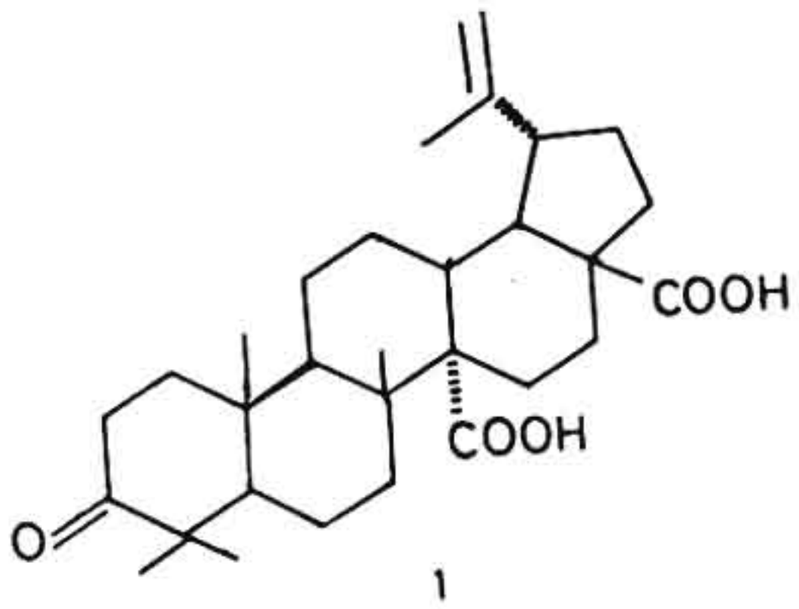
and also in P.nigrum leaf and P.cubeba berry oil. Isolation and characterisation of these constituents may lead to identification of new sesquiterpenes.

# CHAPTER V

# CHAPTER V

## TRITERPENES FROM *GOUANIA MICROCARPA*

The genus *Gouania* belongs to the family *Rhamnaceae* consisting of forty five species distributed in tropical and subtropical regions[287]. Two species *G.leptostachya* and *G.maderaspatana* are reported to occur in India[288]. The leaves of *G.leptostachya* are used by the Lepchas to make poultices for sores and the genus has febrifugal properties[287]. Recently a new species *G.microcarpa* was discovered in the local forest by TBGRI, Palode, Trivandrum. Recent investigations in our laboratory on the leaves of this plant has led to the isolation of a new triterpene, Gouanic acid (1): a first report on the chemical examination of the genus *Gouania*[289]. A reinvestigation of the leaves resulted in the isolation of two more triterpenes. The isolation and structure determination of these two triterpenes forms the subject matter of this chapter.




1

The petroleum ether and chloroform extracts of the dried leaves of G.microcarpa were mixed and on column chromatography gave four crystalline compounds designated as A, B, C and D with $R_f$ values 0.86, 0.54, 0.36 and 0.30 (solvent system: benzene:ethyl acetate:methanol 75:23:2) respectively.

Structure of Compound A:

Compound A was crystallised from ethyl acetate as white crystalline solid, m.p. 93°. The mass spectrum gave the molecular formula as $C_{34}H_{68}O_2$ ($M^+$508). The IR spectrum showed a carboxyl group at 1705 $cm^{-1}$ and generally indicated its aliphatic nature. The 200 MHz $^1H$ NMR spectrum showed a triplet centred at δ2.37 (2H) for methylene protons adjacent to a carboxyl group. It also showed a methyl group at δ0.90 (3H, t) and methylene protons at

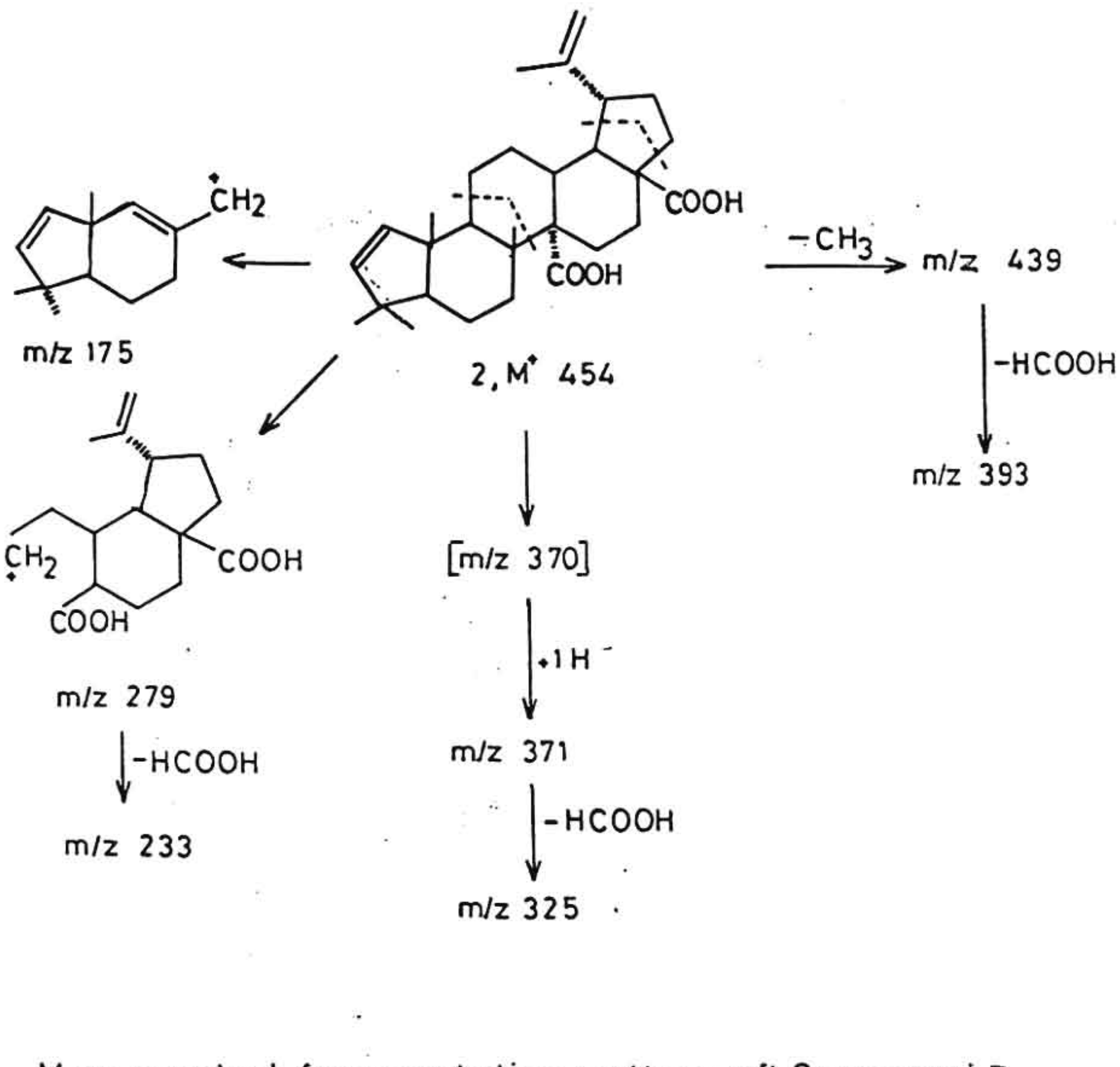
δ1.30 and 1.65 (62H, broad singlets). The mass spectrum showed a consecutive loss of fourteen and/or twenty eight mass units suggesting it to be a straight chain aliphatic compound. The IR and mass spectrum of Compund A was identical with tetratriacontanoic acid reported in literature[228].

Structure of Compound B:

Compound B was crystallised from ethyl acetate as colourless micro crystals, m.p. >310°. Elemental analysis and mass spectral analysis gave the molecular formula $C_{29}H_{42}O_4$ ($M^+$ 454). It gave positive Libermann - Burchard test for triterpenes and tetranitromethane test for double bond. The IR spectrum showed a strong carbonyl absorption at 1689 $cm^{-1}$ for one or more carboxyl groups. It further exhibited two bands at 895 and 758 $cm^{-1}$. The former band was in the position expected for the methylene out-of-plane deformation in an isopropenyl group. Compound B formed a dimethyl ester with diazomethane as shown by two methoxyl groups at δ3.68 (3H, s) and 3.67 (3H, s) in its 400 MHz $^1H$ NMR spectrum. The 400 MHz $^1H$ NMR spectrum further showed two slightly broad singlets at δ4.75 (1H), δ4.63 (1H) and

a sextet centered at δ3.04 (1H) for the two vinylic protons at C-29 and 19β-H protons for lup-20 (29)-ene class of triterpenes respectively. The latter assignment has recently been made by Casadevall et al in connection with the structural establishment of cylicodiscic acid[290]. The $^1$H NMR further showed five methyl groups at δ1.69 (3H, s) for C-30 methyl protons and δ0.89 (3H, s), 0.96 (3H, s), 0.97 (3H, s) and 1.01 (3H, s) for C-26, C-25, C-24 and C-23 methyl protons respectively. The $^1$H NMR also showed two characteristic doublets centred at δ5.91 (1H, d, J = 5.7 Hz) and 5.39 (1H, d, J = 5.7 Hz) respectively for the vicinal hydrogen atoms of cis disubstituted ethylenic linkage of a five membered carbocyclic ring system. Further the mass spectrum of compound B showed a base peak at m/z 175 (Chart I) indicating that probably ring-A is in a contracted form and the methyl groups at C-27 and C-28 are in oxidised form. This data is in agreement with that reported for ceanothenic acid (2) isolated from Ceanothus americanus[294]. The mass spectrum is however not reported in literature and therefore its mass spectral fragmentation pattern is depicted in Chart I.

CHART 1




Mass spectral fragmentation pattern of Compound B
(Ceanothenic acid)

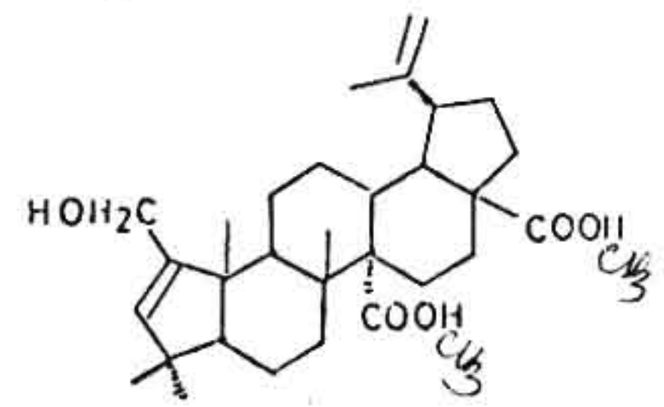
**Structure of Compound C:**

Compound C was crystallised from ethyl acetate as colourless crystals, m.p. 305-7°. Mass spectral analysis gave the molecular formula $C_{30}H_{44}O_5$ ($M^+$ 483). It gave positive Liebermann-Burchard test for triterpenes and tetranitromethane test for double bond. The IR spectrum showed a strong carbonyl absorption at 1692 $cm^{-1}$ for one or more carbonyl and/or carboxyl groups. Compound C formed a dimethyl ester with diazomethane as shown by two methoxyl groups at δ3.69 (3H, s) and 3.67 (3H, s) in its 90 MHz $^1H$ NMR spectrum. The $^1H$ NMR spectrum further showed two slightly broad singlets at δ4.73 (1H), 4.63 (1H), and a multiplet centred at δ3.00 (1H) for the two vinylic protons at C-29 and 19 β-H protons for lup-20 (29)-ene class of triterpenes respectively. The five methyl groups appeared at δ1.69 (3H, s) for C-30 methyl protons and δ0.90 (3H, s), 0.96 (6H, s), 1.02 (3H, s) for C-26, C-25, C-24 and C-23 methyl protons respectively. This data is in excellent agreement with that of Gouanic acid (1)[289] recently isolated in our laboratory. The identity is

further confirmed by direct comparison with an authentic sample of gouanic acid (TLC, Co-TLC and super-imposable IR).

Structure of Compound D:

Compound D was crystallised from ethyl acetate as amorphous powder, m.p. >310°. Elemental analysis and mass spectral analysis gave the molecular formula $C_{30}H_{44}O_5$ ($M^+$ 484) establishing the isomeric nature with gouanic acid. It gave positive Libermann-Burchard test for triterpenes and tetranitromethane test for double bond. The IR spectrum showed a strong carbonyl absorption of 1695 $cm^{-1}$ and also strong hydroxyl absorption at 3350 $cm^{-1}$. Compound D formed a dimethyl ester with diazomethane as shown by two methoxyl groups at δ3.69 (3H, s) and 3.67 (3H, s) in its 400 MHz $^1H$ NMR spectrum (Fig.1). The 400 MHz $^1H$ NMR in $CDCl_3$ also showed two slightly broad singlets at δ4.75 (1H), 4.63 (1H) and a sextet centred at δ3.02 (1H) for two vinylic protons at C-29 and 19 β-H protons for lup-20(29)-ene class of triterpenes respectively. Further five methyl groups are also observed at δ1.69 (3H, s) for C-30 methyl protons and



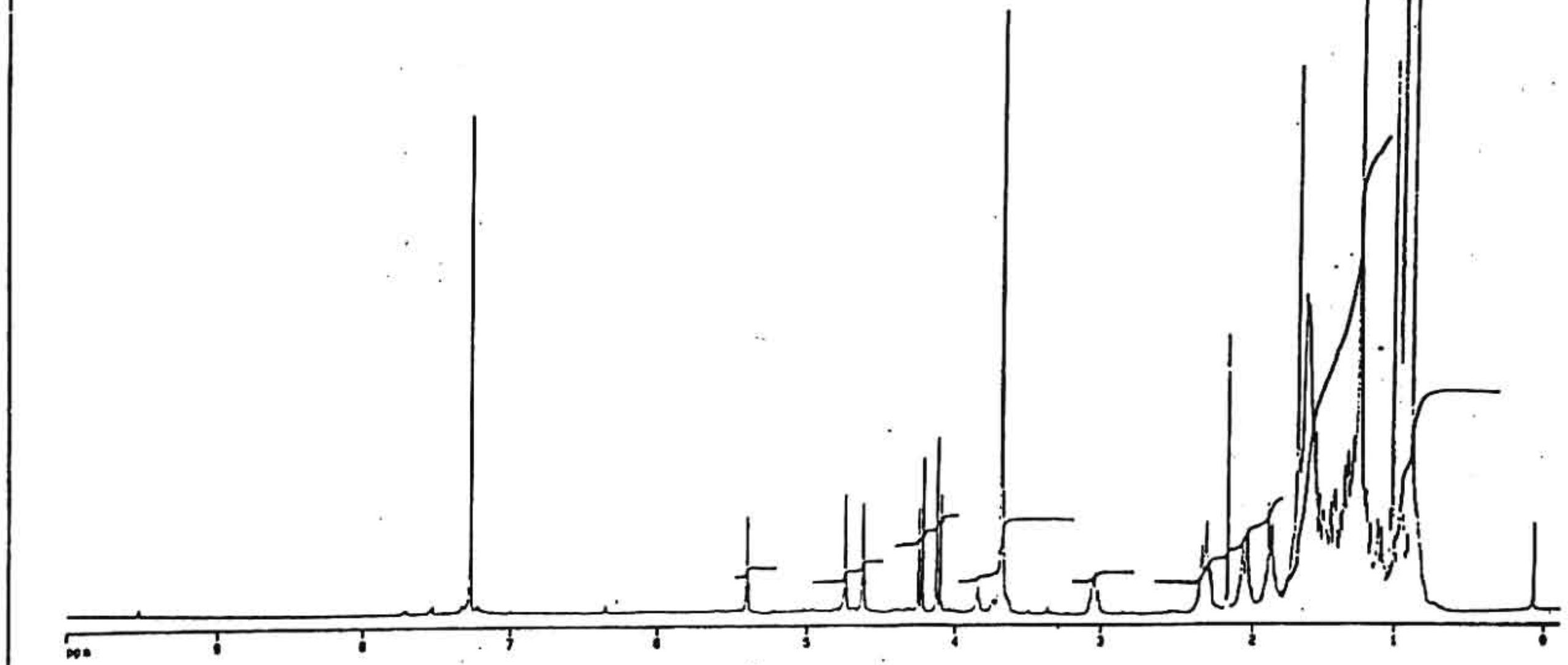
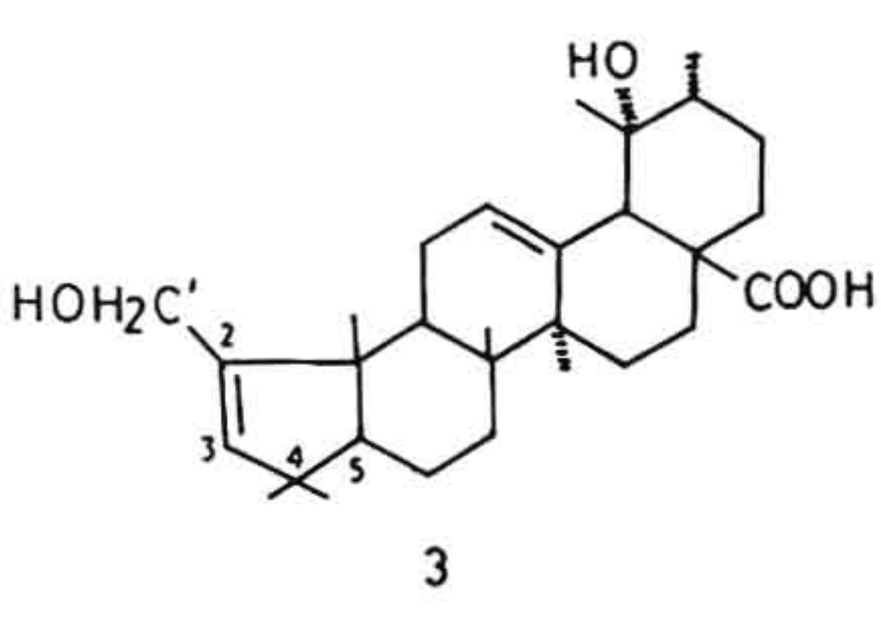
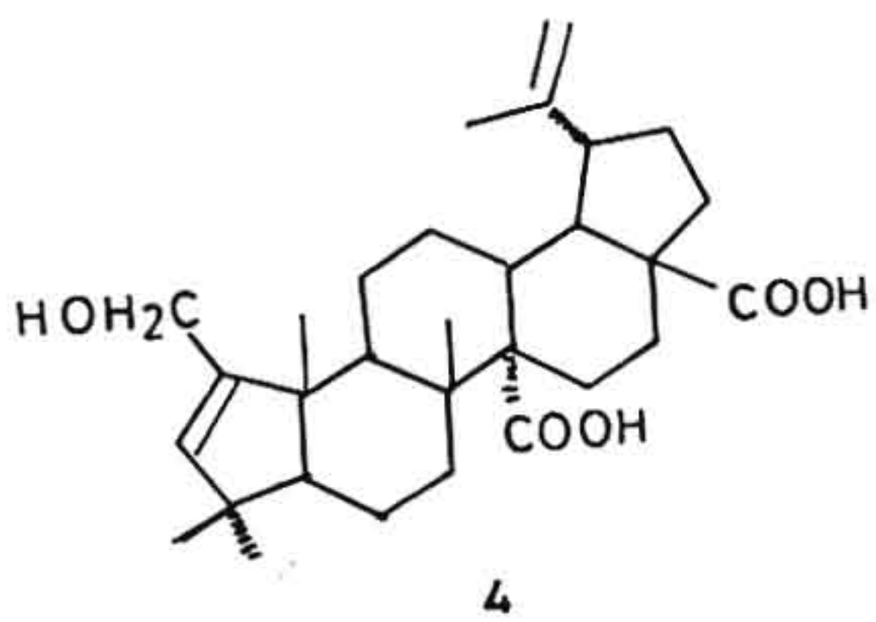



Fig.1: 400 MHz $^{1}H$ NMR Spectrum of Gouaninic Acid Dimethyl Ester in $CDCl_3$

δ0.89 (3H, s), 0.96 (3H, s), 0.98 (3H, s) and 1.03 (3H, s) for C-26, C-25, C-24 and C-23 methyl protons respectively. In addition to these signals a slightly broad singlet at δ5.40 (1H) and a quartet centred at δ4.25 (2H) with a coupling constant of J = 11Hz are also noticed. A comparison of the $^{1}$H NMR spectra of ceanothenic acid and compound D reveals that the slightly broad singlet at δ5.40 (1H) is probably uncoupled because of a substituent at C-2 position. The two proton quartet at δ4.25 (2H) is thus assignable to the methylene protons of a hydroxymethylene group situated at C-2. Further the position and the coupling constant of the hydroxymethylene protons at δ4.25 (2H, q, J = 11 Hz) and also the chemical shift of the olefinic proton at δ5.40 (1H, s) is identical with that reported for ring A of hyptadienic acid (3) recently isolated by Prakasa Rao et al[291].




3



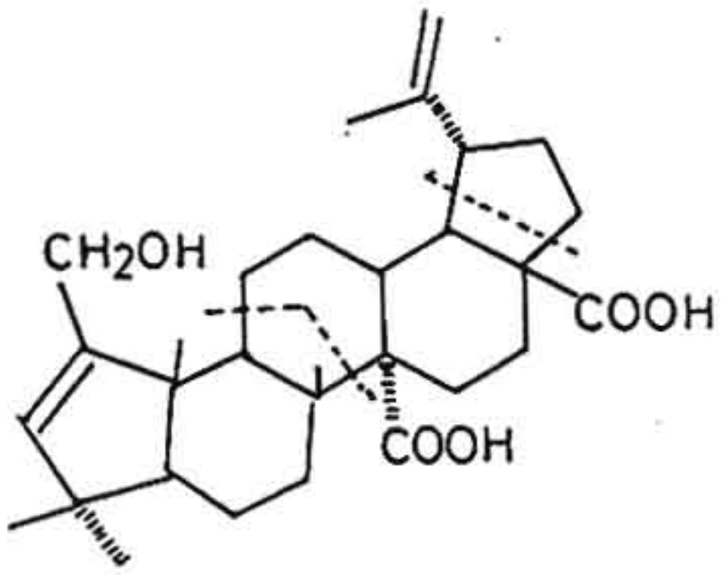

4

The mass spectrum of compound D showed peaks at m/z 205 and m/z 297 (chart 2) which are also present in the mass spectrum of gouanic acid. The latter peak corresponding to rings D and E of both gouanic acid and ceanothenic acid indicates that compound B is also a lup-20(29)-ene-27,28-dicarboxylic acid. This conclusion coupled with the $^{1}H$ NMR spectral data suggests structure 4; A (1)-1-hydroxy-lup-2(3),20(29)-diene-27,28-dioic acid for compound D, a new triterpene named as gouaninic acid. The mass spectral fragmentation pattern is shown in chart 2.

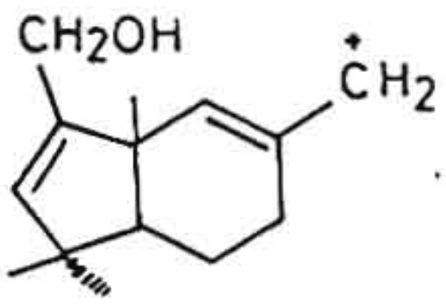
Biogenetic considerations:

Ceanothenic acid is the first report of its occurrence in the genus Gouania and also second report of its natural occurrence. A-ring contracted triterpenes are rare. So far only nine such compounds are reported from natural sources. All these nine compounds are lupane derivatives and were isolated from Rhamnaceae[292-299] and Alangiaceae[300-302]. Only one ursene class of A-ring contracted triterpene hyptadienic acid from Hyptis suaveolens (Labiatae)[291] is so far isolated. Gouaninic acid

CHART 2



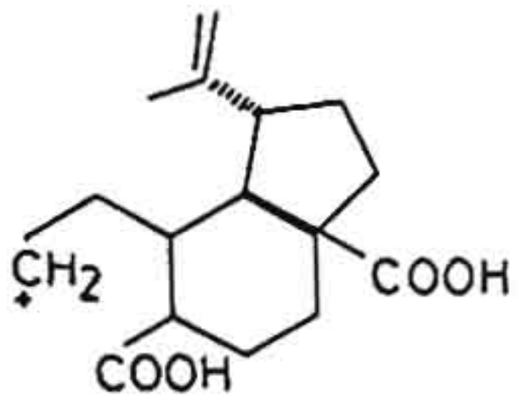

4, M⁺ 484




m/z 205

$-CH_2OH$

m/z 174




m/z 279

$-HCOOH$

m/z 233

$-CH_2OH$ → m/z 453 $\xrightarrow{-C_3H_7}$ m/z 410

$-C_3H_7$ → m/z 441 $\xrightarrow{-HCOOH}$ m/z 395

[m/z 402 $\xrightarrow{+1H}$ m/z 403]

$-H_2O$

m/z 385

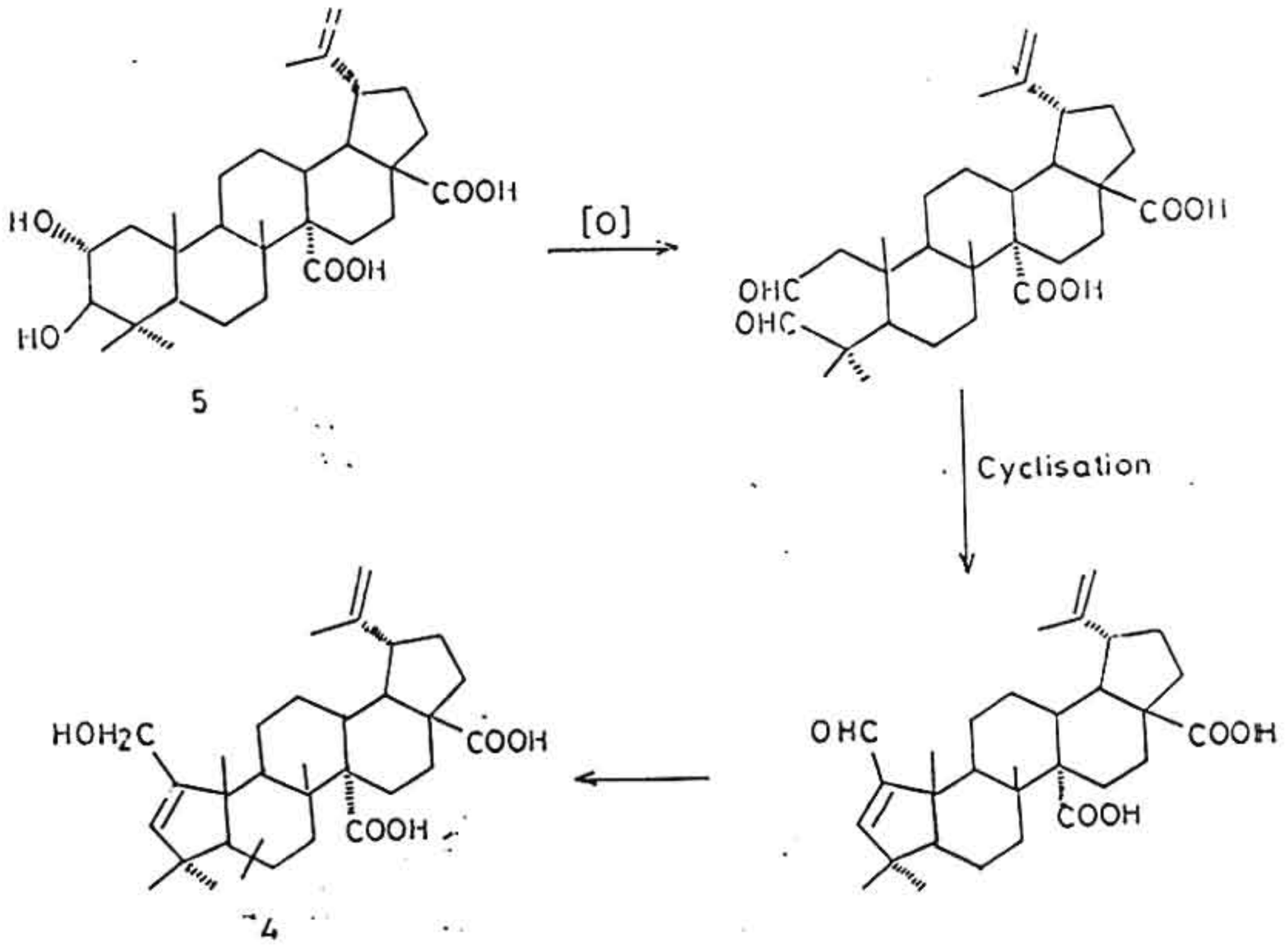
$-HCOOH$

m/z 339

Mass spectral fragmentation pattern of Compound D
(gouaninic acid)

is yet another example of A-ring contracted lupene triterpene from Rhamnaceae family.

The biogenetic pathway for ring A-ring contracted lupenes is already depicted by Halsall et al[303]. By analogy with hyptadienic acid a biogenetic sequence (Scheme 1) can be formulated in which oxidation and reduction reactions of an hypothetical dihydroxy triterpene 5 give rise to gouaninic acid.




Scheme 1

## EXPERIMENTAL

Melting points (°C) are uncorrected. Silica gel (60-120 mesh) of E. Merck grade was used for column chromatography. Silica gel with 13% calcium sulphate as binder was used for TLC. The plates were dried at room temperature for 12 hours, activated for one hour in an air oven at 100°C. The spots were developed by spraying 10% methanolic sulphuric acid and heating the plates in an air oven at 120°C for 20 minutes. Samples for analysis were routinely dried under high vacuum. C,H analysis for all the samples were performed on Perkin-Elmer 2400 CHN analyser. IR spectra were recorded on Perkin-Elmer 882 in-frared spectrometer. Chemical shifts are in ppm (δ values) and the corresponding magnetic field is mentioned at appropriate place.

## EXPERIMENTAL

1. Chemical examination of the leaves of Gouania Microcarpa:

Extraction:

The leaves of G.microcarpa was procured from the local forest and identified by TBGRI, Palode, Trivandrum. A voucher specimen is available at TBGRI. The shade-dried powdered leaf (200 g) was extracted successively with petroleum ether (60-80°) and chloroform in a soxhlet apparatus. The petroleum ether extract (1.5 lit) was concentrated and the last traces of the solvent removed under reduced pressure. The dark green residue thus obtained resisted crystallisation from common organic solvents. It showed three prominent spots with $R_f$ values 0.86, 0.54 and 0.36 (solvent system: benzene:ethylacetate: methanol 75:23:2) corresponding to compounds A, B and C respectively. In addition to these spots it showed a minor spot with $R_f$ value 0.30 (solvent system: benzene:ethyl acetate:methanol 75:23:2) corresponding to compound D. The chloroform extract also showed similar behaviour

with increased intensity of the spot corresponding to compound D. Hence it was mixed with the residue from the petroleum ether extract and chromatographed.

Chromatographic separation of the extract:

The combined dark green residue (8 g) was dissolved in diethyl ether (25 ml) and silica gel (15 g) was added. The ether was removed under vacuum and the powder was transferred to a column of silica gel (180 g). The column was set up in petroleum ether: ethyl acetate 95:5 and eluted successively with petroleum ether:ethyl acetate 95:5, petroleum ether:ethyl acetate 9:1, petroleum ether: ethyl acetate 3:1 and ethyl acetate. Fractions of 100 ml were collected and concentrated. Monitoring by TLC the fractions were grouped as shown in Table 1.

**Table 1**

| Eluant | Fraction | Group | No. | Compound |
|---|---|---|---|---|
| Petroleum ether: ethyl acetate 95:5 | 1-6 | I | | - |
| Petroleum ether: ethyl acetate 9:1 | 7-10 | II | | A |
| Petroleum ether: ethyl acetate 9:1 | 11-17 | III | | B |
| Petroleum ether: ethyl acetate 3:1 | 18-25 | IV | | C |
| Petroleum ether: ethyl acetate 3:1 | 26-35 | V | | D |
| Ethyl acetate | 36-40 | VI | | - |

Group I

The yellow residue obtained from these fractions suggested waxy nature. It was not examined further.

Group II

The fractions 7-10 were combined and concentrated. The solid separated was crystallised from ethyl acetate as white crystalline solid (8 mg) m.p. 93°. It was designated as compound A. ( $R_f$ 0.86).

Group III

The fractions 11-17 were combined and the solvent removed where a colourless crystalline solid separated out. It was crystallised from ethyl acetate as colourless microcrystals (4 mg), m.p. >310°. It was designated as compound B ($R_f$ 0.54).

Group IV

The fractions 18-25 were combined and the solvent was removed. The solid was filtered and was recrystallised from ethyl acetate as white crystalline solid (15 mg), m.p. 305-8°. It was designated as compound C ($R_f$ 0.36).

Group V

The fractions 26-35 were combined and concentrated to a small volume when a colourless powder has separated out. It was filtered and recrystallised from ethyl acetate as white solid (5 mg) m.p. >310°. It was designated as compound D.

Group VI

The fractions 36-40 were mixed and the solvent distilled off. It was found to be a mixture of several compounds by TLC. The amount of the mixture obtained was insufficient for further examination.

Compound A: Tetratriacontanoic acid

Compound A is identified as tetratriacontanoic acid. It was crystallised from ethyl acetate as white solid, m.p. 93° (lit[188], m.p. 95°).

IR: $\nu_{max}^{KBr}$ 2920, 2840, 1705, 1460, and 720 $cm^{-1}$

MS: (relative abundance below 10% not given)

$M^+$ 508, 494, 480 920), 466, 452 (20), 438 (10), 424, 410, 396, 382, 368, 354, 340, 297, 241, 185, 171, 157, 143, 129 (30), 115, 111 (15), 98 (18), 97 (30), 83 (36), 73 (50), 71 (54), 69 (44), 57 (base peak 100%) 55 (56), 43 (98) and 41 (36).

Compound B: Ceanothenic acid

Compound B is identified as ceanothenic acid. It was crystallised from ethyl acetate as colourless micro-crystals, m.p. >310° [lit[294], 350-354° (decomposition)]

Analysis: Found C 76.35, H 9.30

$C_{29}H_{42}O_4$ requires C 76.61, H 9.31%

1R: $\nu_{max}^{KBr}$ 3451 (br), 2956, 2931, 2873, 1689, 1640, 1510, 1450, 1239, 1106, 1022, 890 and 758 $cm^{-1}$.

MS: (Relative abundance below 10% not given)

$M^+$ 454 (18), 439 (30), 394 (15), 393 (50), 372 (18),

371 (67), 327, 325 (20), 279 (11), 233, 205, 204, 203, 201, 197, 189 (32), 188, 187 (12), 177 (50), 176 (32), 175 (base peak 100%), 174 (20), 173 (42), 171, 161 (33), 159 (28), 157, 147 (18), 145 (18), 135 (34), 134 (15), 133 (33), 131 (20), 123, 122 (40), 121 (88), 120 (20), 119 (52), 117, 109 (47), 108 (37), 107 (99), 105 (53), 95 (28), 93 (47), 91 (58), 83 (17), 81 (42), 79 (41), 77 (22), 69 (40), 67 (33), 57 (17), 55 (53), 43 (40), and 41 (63).

Colour reaction:

Ceanothenic acid gave the characteristic Liebermann-Burchard test for triterpenes producing violet colour. It gave yellow colour with tetranitromethane.

Compound B dimethyl ester: Ceanothenic acid dimethyl ester

Compound B (5 mg) in dry ether (5 ml) was treated with diazomethane prepared from nitrosomethyl urea (1 g) at 0° and left overnight in a refrigerator. Ether was carefully evaporated and the residue was passed through a small column of silica gel using petroleum ether: ethyl acetate 4:1 as eluant. The dimethyl ester thus formed resisted crystallisation.

IR $\gamma_{max}^{neat}$: 2960, 2930, 2875, 1720, 1640, 1440, 1205, 1160, 1105, 940, 900, 880, and 715 $cm^{-1}$.

Compound C: Gouanic acid

Compound C is identified as gouanic acid. It was crystallised from ethyl acetate as colourless crystals, m.p. 305-8° (lit[289], 305-8°).

IR: $\nu_{max}^{KBr}$ 3600-3000, 2954, 2873, 1692, 1652, 1453, 1238, and 899 $cm^{-1}$.

MS: (relative abundance below 10% not given)

$M^+$ 484, 466 (20), 451, 438 (18), 424, 422 (16), 420 (12), 410, 405, 395 922), 385 (base peak 100%), 375, 339 (30), 293 (12), 279, 261, 233 (18), 219 (18), 217 (16), 205 (50), 191 914), 187 (28), 177 (62), 175 (30), 173 (23), 163 (18), 161, 159 (28), 145 928), 133 (34), 121 (60), 119 (58), 109 (40), 107 (60), 105 (44), 95 (52), 93 (46), 91 (44), 83 (26), 81 (62), 79 (40), 77 (20), 69 (54), 67 (42), 57 (26), 55 (72), 43 (60) and 41 (70).

Compound C dimethyl ester: Gouanic acid dimethyl ester

Compound C (5 mg) in dry ether 95 ml) was treated with diazomethane prepared from nitrosomethyl urea (1 g) at 0° and left overnight in a refrigerator. Usual work up followed by column chromatography over silica gel using

petroleum ether: ethyl acetate 3:1 as eluant gave dimethyl ester as semisolid.

Compound D: Gouaninic acid:

Compound D is a new triterpene named gouaninic acid. It was crystallised from ethyl acetate as white powder, m.p. >310°.

Analysis: Found C 73.68; H 9.20%

$C_{30}H_{44}O_5$ requires C 74.34; H 9.15%

IR: $\nu_{max}^{KBr}$ 3350 (br), 2950, 2927, 2875, 1695, 1640, 1510, 1460, 1245, 1105, 1020, 890 and 755 $cm^{-1}$.

MS: (Relative abundance below 10%) not given.

$M^+$ 484, 466, 453, 451, 441, 438, 424, 422 (11), 420, 410, 395 (15), 385 (52), 377, 375, 339 (15), 293 (17), 279, 261, 233, 219 (21), 217 (19), 216 (18), 215 (17), 207 (15), 206 (11), 205 (38), 203 (12), 201 (11), 197, 188, 187 (10), 177 (35), 176 (17), 175 (47), 174 (40), 173 (42), 171, 161 (33), 159 (25), 157, 147 (16), 145 (15), 135 (30), 134 (14), 133 (31), 131 (18), 123, 122 (41), 121 (55), 120 (20), 119 (base peak 100%), 117, 109 (45), 108 (35), 107 (87), 105 (48), 95 (40), 93 (46), 91 (51), 83 (22), 81

(52), 79 (40), 77 (21), 69 (47), 67 (38), 57 (21), 55 (63), 43 (50) and 41 (66).

Colour reaction

Gouaninic acid gave characteristic Liebermann-Burchand test for triterpenes. It gave bright yellow colour with tetranitromethane.

Compound D dimethyl ester: Gouaninic acid dimethyl ester:

Compound D (3 mg) in dry ether (5 ml) was treated with diazomethene prepared from nitrosomethyl urea (1 g) at 0° and left overnight in a refrigerator. Ether was carefully evaporated and the residue was passed through a small column of silica gel using petroleum ether: ethyl acetate 3:1 as eluant. The dimethyl ester thus obtained could not be crystallised.

# CHAPTER VI

# CHAPTER VI

## SUMMARY

The thesis describes the isolation and structural determination of several crystalline substances from a few Piper species and Gouania microcarpa. Four Piper species have also been examined for their essential oil constituents.

The first chapter deals with a review of the natural occurrence of crystalline constituents from the genus Piper.

The second chapter describes the isolation and characterization of three compounds designated as A, B and C from the combined petroleum ether and chloroform extracts of P.nigrum leaves. Compound A is identified as (-)-cubebin. Compound B is a lignan m.p. 86-87°, $[\alpha]_D$ - 52.86° and has molecular formula $C_{21}H_{24}O_6$ ($M^+$ 372). The 500 MHz $^1H$ NMR spectrum of compound B indicated the presence of two methoxyl groups at δ3.82 (3H, s), 3.85 (3H, s); a methylenedioxy group at δ5.92 (2H, s); a slightly broad

singlet at δ5.23 (1H, s) for a hemiacetal proton; three triplets at δ3.59, 4.01, 4.10 (2H, J = 8Hz each) for methylene protons of furanol ring; a multiplet between δ2.0-2.9 (6H, 4 benzylic and 2 methine protons) and six aromatic protons between δ6.4-6.9 (6H, m). Compound C has m.p. 66°, $[\alpha]_D$-15.88° and molecular formula $C_{21}H_{24}O_6$ ($M^+$ 372). The 500 MHz $^1H$ NMR spectrum of compound C indicated the presence of two methoxyl groups at δ3.86 (3H, s), 3.87 (3H,s); a methylenedioxy group at δ5.92 (2H, s); a slightly broad singlet at δ5.23 (1H, s) for a hemiacetal proton; three triplets at δ3.60, 4.01, 4.11 (2H, J = 8 Hz each) for methylene protons of furanol ring; a multiplet between δ2.0 - 2.9 (6H, 4 benzylic and 2 methine protons) and six aromatic protons between δ6.4 - 6.9 (6H, m). Compounds B and C are thus identified as isomeric dibenzylbutyrolactol lignans. The oxidation ($CrO_3/H_2SO_4$) products of both the compounds B and C showed the presence of carbonyl group at 1762 $cm^{-1}$ in their IR spectra, multiplet at δ2.50 (4H, benzylic protons) and δ2.85 (2H, methine protons) in their 60 MHz $^1H$ NMR spectra, thus establishing the trans stereochemistry at $C_8$ and $C_8'$ positions. A base peak at m/z 151 in the mass spectrum of compound B establishes its identity as 3,4-dimethoxy-3,4-desmethylenedioxy cubebin. A

base peak at m/z 135 in the mass spectrum of compound C establishes its identity as 3',4'-dimethoxy-3',4'-desmethylenedioxy cubebin. The two isomeric lignans are thus isolated in pure form for the first time. The methanol soluble portion of the petroleum ether extract of the berries showed the presence of all the three lignans, thus settling the doubtful presence of (-)-cubebin in black pepper.

A systematic bioassay-guided chemical examination of different parts of P.attenuatum has led to the isolation of six compounds A, B. C, D, E and F and the results are reported in chapter III. Compounds A, B, C, D and E isolated from the berries are identified as tetratriacontanoic acid, (+)-pipoxide, pipoxide chlorohydrin, (-)-galbelgin and (+)-crotepoxide respectively from their spectral characteristics. This is the first time to report the occurrence of pipoxide from P.attenuatum. Pipoxide chlorohydrin was earlier isolated from the methanolic extract of P.hookeri and P.nigrum. This is the second report of its occurrence from the genus Piper and its $^{13}C$ NMR spectrum is depicted. (-)-Galbelgin is isolated first time from the genus Piper and its $^{13}C$ NMR assignments have

been made. (+)-Crotepoxide known to possess significant anticancer activity in Lewis lung carcinoma occur in commercially significant quantities in the berries of P.attenuatum. From the combined petroleum ether and chloroform extracts of the leaves of P.attenuatum, pipoxide chlorohydrin, (-)-galbelgin and compound F were isolated. Compound F has m.p. 77° and analysed for $C_{31}H_{64}O$ ($M^+$ 452). The IR spectrum of its acetate showed the carbonyl group at 1745 $cm^{-1}$ and 270 MHz $^1H$ NMR spectrum showed the presence of methine proton at δ5.34 (1 H, m), acetoxyl protons at δ1.94 (3H, s), two terminal methyl groups resonating between δ0.82 and 1.02 (6H, two overlapped triplets), 26 methylene units at δ1.25 (52 H, br, s) and a broad singlet at δ1.66 (4H), attributed to two methylene groups attached to the carbinolic carbon. The position of the hydroxyl group was deduced from the characteristic peaks at m/z 129 for $[Me(CH_2)_6 CHOH]^+$ and m/z 353 for $[Me(CH_2)_{22}CHOH]^+$. Compound F is thus identified as hentriacontan-8-ol, a new aliphatic alcohol which is likely to be a constituent of the epicuticular wax of P.attenuatum. In the petroleum ether extract of the stem of P.attenuatum, tetratriacontanoic acid, β-sitosterol, pipoxide and crotepoxide were identified. Finally (+)-crotepoxide was shown to possess

moderate antifeedant activity on pollu beetle (Longitarsus nigripennis), a serious pest of P.nigrum.

The results of investigations on the essential oil constituents of the fruits of P.aurantiacum, P.nigrum leaf, P.cubeba berries, P.attenuatum berry, stem and leaf form the subject matter of chapter IV. Three higher alkanes $C_{31}H_{64}$ ($M^+$ 436), $C_{33}H_{68}$ ($M^+$ 464) and $C_{35}H_{72}$ ($M^+$ 492) are identified in the methanol insoluble portion of the petroleum ether extract of the fruits of P.aurantiacum by a combination of GLC and MS. 63 Terpene compounds could be identified from the essential oil of P.nigrum leaf by Kovats indices and GC-MS analysis. The major sesquiterpene hydrocarbons identified are β-cubebene, γ-muurolene, humulene, calamenene, β-copaene and β-cedrene. Elemol (11.5%) constitute the major sesquiterpene alcohol and other major sesquiterpene alcohols identified are nerolidol (2%), caryophyllene alcohol (5%), eudesmol (3%), Cadina-1,4-diene-3-ol (3%), α-bisabolol (3%) and farnesols (8%). From the P.cubeba berry oil 37 more components could be identified in addition to the compounds already reported. P.cubeba berry oil contains 32% monoterpenes and 68% of sesquiterpenes and other high polar compounds. The physico-

chemical characteristics of essential oils from P.attenuatum berries, leaf and stem have been determined. Capillary gas chromatogram of P.attenuatum berry oil indicated the presence of about 100 constituents out of which 66 constituents could be identified. The major sesquiterpene constituents are β-caryophyllene (13%) and β-cubebene (10%). P.attenuatum leaf oil showed the presence of 117 constituents out of which 67 constituents are identified. 33 Monoterpene constituents have been identified, the concentration of them being very low except for linalool (1.67%). Among the 20 sesquiterpene constituents, the hydrocarbons β-farnesene, ar-curcumene and δ-cadinene constitute about 8%, 6% and 5% respectively. P.attenuatum stem oil showed about 46 constituents out of which 17 compounds could be identified by their retention times and mass spectra. In general the essential oils from P.attenuatum berries, leaf and stem contain minor amounts of monoterpenes (1-4%) while the sesquiterpene and other polar compounds constitute more than 96%. Several sesquiterpene and other polar compounds are yet to be identified and it may be necessary to isolate them in pure form.

Chapter V describes the chemical examination of the leaves of Gouania microcarpa. The combined petroleum ether

and chloroform extracts of the dried leaves gave four crystalline compounds A, B, C and D. Compound A and C are identified as tetratriacontanoic acid and gouanic acid [3-oxolup-20(29)-ene-27,28-dioic acid ] respectively by their spectral characteristics and by direct comparison with authentic samples. Compound B is identified as ceanothenic acid from its mass spectral fragmentation pattern and by a study of the 400 MHz $^{1}H$ NMR spectrum of its dimethyl ester. This is the first report of its occurrence in the genus Gouania and second report of its natural occurrence. Compound D is a new triterpene dicarboxylic acid named as gouaninic acid. Gounaninic acid, m.p. >310° has molecular formula $C_{30}H_{44}O_5$ ($M^+$ 484). It formed a dimethyl ester with diazomethane as shown by two methoxyl groups at δ3.69 (3H, s) and 3.67 (3H, s) in its 400 MHz $^{1}H$ NMR spectrum. The 400 MHz $^{1}H$ NMR spectrum further showed two singlets at δ4.75 (1H), 4.63 (1H) and a sextet centered at δ3.02 (1H) for two vinylic protons of C-29 and 19 β-H protons for lup-20(29)-ene class of triterpenes respectively. The NMR spectrum also showed five methyl groups at δ1.69 (3H, s) for C-30 methyl protons and δ0.89 (3H, s), 0.96 (3H, s), 0.98 (3H, s) and 1.03 (3H, s)

for C-26, C-25, C-24 and C-23 methyl protons respectively. In addition to these signals, a singlet at δ5.40 (1H) and a quartet centered at δ4.25 (2H, J = 11 Hz) are also noticed. By a comparison of $^{1}H$ NMR spectra of hyptadienic acid [A(1)-1,19 α-dihydroxy-urs-2(3), 12-dien-28-oic acid] and its derivatives with that of gouaninic acid dimethyl ester, the structure of compound D is established as A(1)-1-hydroxy-lup-2(3),20(29)-diene-27,28-dioic acid. The structure of compound D is also confirmed by its mass spectral fragmentation pattern. By analogy with hyptadienic acid a biogenetic sequence for the formation of gouaninic acid is also formulated.

## REFERENCES

## References


1. Sengupta, S. and Ray, A. B.; Fitoterapia, 1987, 58(3), 147.

2. Chopra, R. N., Nayer, S. L. and Chopra, I. C.; Glossary of Indian Medicinal Plants, CSIR, New Delhi, India, 1956, 194.

3. Oestred; Schweigger's Journal, 1819, 29, 80.

4. Soren, J., Jesper, H. and Per M. Boll; Phytochemistry, 1993, 33(3), 523.

5. Govindarajan, V. S.; CRC Critical Reviews in Food Science and Nutrition, 1977, 9(2), 115.

6. Atal, C. K., Dhar, K. L. and Singh, J.; Lloydia, 1975, 38, 256.

7. Loder, J. W. and Nearn, R. H.; Phytochemistry, 1972, 11(8), 2645.

8. Nakatani, N. and Inatani, R.; Agric. Biol. Chem., 1981, 45(6), 1473.

9. Kiijoa, A., Pinto, M. M., Tantisewie, B. and Herz, W.; Planta Med., 1989, 55(2), 193.

10. Dasgupta, S. and Ray, A. B.; Indian J. Chem., 1979, 17B(5), 538.

11. Likhitwitayawuid, K., Ruangrungsi, N., Lange, G. L. and Decicco, C. P.; Tetrahedron, 1987, 43(16), 3689.

12. Li Shuming, Han. Guiqiu, Arison, B. H. and Chang, M. N.; Yaoxue Xuebao, 1987, 22(3), 196; CA:107:20765 g.

13. Dhar, K. L. and Atal, C. K.; Indian J. Chem., 1967, 5(11), 588.

14. Han, G. O., Wei, L. H., Li, C. L., Qiao, L., Jia, Y. Z. and Zhing, Q. T.; Yaoxue Xuebo, 1989, 24(6), 438; CA:112:91459 b.

15. Banerji, A., Raj, R. N. and Ghosh, P. C.; Experimentia, 1974, 30(3), 223.

16. Loder, J. W., Anne Moorhouse and Russell, G. B.; Aust. J. Chem., 1969, 22(7), 1531.

17. Dhar, K. L. and Raina, M. L.; Planta Medica, 1973, 23(3), 295.

18. Okogun, J. I. and Ekong, D. E. U.; J. Chem. Soc. Perkin Trans. I, 1974, 19, 2195.

19. Tackie, A. N., Dwuma-Badu, D., Ayim. J. S. K., Elsohly, H. N., Knapp, J. E., Slatkin, D. J. and Schiff, Jr. P. L.; Phytochemistry, 1975, 14(8), 1888.

20. Dwuma-Badu, D., Ayim, J. S. K. and Dabra, T. T.; Lloydia, 1976, 39(1), 60.

21. Addae-Mensah, I., Torto, F. G., Oppong, I. V., Baxter, I. and Sanders, J. K. M.; Phytochemistry, 1977, 16(4), 483.

22. Addae-Mensah, I., Torto, F. G., Dimonyeka, C. I., Baxter, I. and Sanders, J. K. M.; Phytochemistry, 1977, 16(6), 757.

23. Gupta, O. P., Gupta, S. C., Dhar, K. L. and Atal C. K.; Phytochemistry, 1977, 16(9), 1436.

24. Gupta, O. P., Gupta, S. C., Dhar, K. L. and Atal, C. K.; Indian J. Chem. Sect. B, 1976, 14B(12), 912.

25. Banerji, A., Bandopadhayay, D, Sarkar, M., Siddhanta, A. K., Pal, S. C., Ghosh, S., Abraham, K. and Shoolery, J. N.; Phytochemistry, 1985, 24(2), 279.

26. Koul, S. K., Taneja, S. C., Agarwal, V. K. and Dhar, K. L.; Phytochemistry, 1988, 27(11), 3523.

27. Miyakado, M., Nakayama, I., Yoshioka, K. and Nakatani, N.; Agric. Biol. Chem., 1979, 43(7), 1609.

28. Kiuchi, F., Nakamura, N., Tsuda, Y., Kondo, K. and Yoshimura, H.; Chem. Pharm. Bull., 1988, 36(7), 2452.

29. Gupta, O. P.; Dhar, K. L. and Atal, C. K.; Phytochemistry, 1976, 15(3), 425.

30. Banerji, A., Banerji, J., Chatterjee, A. and Shoolery, J. N.; Ind. J. Chem., 1980, 19B(5), 346.

31. Banerji, A. and Das C.; Phytochemistry, 1989, 28(11), 3039.

32. Viswanathan, N., Balakrishnan, V., Joshi, B. K. and Philipsborn, W. V.; Helv. Chim. Acta., 1975, 58(7), 2026.

33. Achenbach, H., Fietz, W., Worth, J., Waibel, R. and Portecop, J.; Planta Medica, 1986, 1, 12.

34. Gupta, O. P., Atal, C. K. and Gaind, K. N.; Phytochemistry, 1972, 11(8), 2646.

35. Mahanta, P. K., Ghanim, A. and Gopinath, K. W.; J. Pharm. Sci, 1974, 63(7), 1160.

36. Banerji, A. and Dhar, K. L.; Phytochemistry, 1974, 13(10), 2327.

37. Chatterjee, A. and Dutta, C. P.; Tetrahedron, 1967, 23(4), 1769.

38. Soares Costa Sonia and Mors Walter, B.; Phytochemistry, 1981, 20(6), 1305.

39. Dwuma-Badu, D., Ayim, J. S. K., Dabra, T. T., Elsohly, H. N., Elsohly, M. A., Knapp, J. E., Slatkin, D. J. and Schiff Jr., P. L.; Phytochemistry, 1976, 15(5), 822.

40. Vig, O. P., Lal, M., Trehan, I. R. and Singh, S.; Indian J. Chem., 1978, 16B, 458.

41. Pring Brian, G.; J. Chem. Soc.Perkin Trans. I, 1982, 7, 1493.

42. Thebpatiphat, S., Peng Precha, S. and Ternai, B.; J. Sci. Soc. Thailand, 1988, 14(3), 225.

43. Giesbrecht, A. M., Dc Alvarenga, N. A., Gottilieb, O. R. and Gottilich, H. R.; Planta Medica, 1981, 43(4) 375.

44. Joshi, B. S., Viswanathan, N., Gawad, D. H., Balakrishnan, V. and Phillipshorn, W. V.; Helv. Chim. Acta, 1975, 58, 1551.

45. Miyakado, M., Nakayama, I., Ohno, N. and Yoshioka, H.; Curr. Themes Trop. Sci., 1983, 2, 369.

46. Mukapugay, H. C., Soejarto, D. D., Kinghorn, A. D. and Bordas, E.; J. Ethnopharmacol., 1983, 7(2), 235.

47. Banerji, A., Paul, S. and Sudhir, C.; Phytochemistry, 1982, 21(6), 1321.

48. Joshi, B. S., Viswanathan, N., Gawad, D. H., Balakrishnan, V. and Phillipsborn, W. V.; Helv. Chim. Acta, 1975, 58(8), 2295.

49. Glasl, H., Borup-Grochtmann, L. and Wagner, H.; Dtsch. Apoth. Ztg., 1976, 116(43), 1638.

50. Banerji, A. and Nandi, G.; Indian J. Chem. Sect. B., 1988, 27B(2), 163.

51. Rao, J. M., Subrahmanyam, K. and Rao, K. V.; Curr. Sci., 1974, 43(3), 76.

52. Mahanta, P. K., Ghanim, A. and Gopinath, K. W.; J. Pharm. Sci., 1974, 63(7), 1160.

53. Mishra, S. S. and Tewari, J. P.; J. Pharm. Sci., 1964, 53(11), 1423.

54. Patra, A. and Ghosh, A.; Phytochemistry, 1974, 13(12), 2889.

55. Haensel, R., Leuokert, C. and Schultz. G.; Z. Naturforch, 1966, 21(6), 530.

56. Dwuma-Badu, D., Ayim, J. S. K., Dahra, T. T., Elsohly, H. N., Knapp, J. E., Slatkin, D. J. and Schiff, Jr. P. L.; Lloydia, 1975, 38(4), 343.

57. Joshi, B. S., Kamat, V. N. and Saksena, A. K.; Tetrahedron Lett., 1968, 2395.

58. Dutta, C. P., Banerjee, Noton, Sil., Ajoy, K. and Roy, D. N.; Indian J. Chem., Sect. B., 1977, 15B(6), 583.

59. Hou, C. Y.; Zhang, J. Q., Zhang, Y. M. and Liu, Y. L.; Yaoxue Xuebao, 1989, 24(10), 789.

60. Grewe, R., Freist, W., Newmann, H. and Kerston, S.; Chem. Ber., 1970, 103(12), 3572.

61. DcClyne, R. and Verzele, M.; Bull. Soc. Chim. Belg., 1975, 84(5), 435.

62. Shin, Kuk Hyun Yun Hye Sook, Woo Won Sick and Lee Chung Kyu; Soul Taehakkyo Saengyak Yonguso Opjukjip, 1979, 18, 87, CA:93:215484 s.

63. Spring, F. S. and Stark, J.; J. Chem. Soc., 1950, 1177.

64. Taxler, J. T.; J. Agric. Food. Chem., 1971, 19, 1135.

65. Tabuneng, W., Bando, and H. Amiya, T.; Chem. Pharm. Bull., 1983, 31(10), 3562.

66. Desai, S. J., Prabhu, B. R. and Mulchandani, N. B.; Phytochemistry, 1988, 27(5), 1511.

67. Crombie, L., Pattenden, G. and Stemp, G.; Phytochemistry, 1977, 16(9), 1437.

68. Addae-Mensah, I., Torto, F. G. and Baxter, I.; Tetrahedron Lett., 1976, 35, 3049.

69. Sondengam, B. L. and Kimbu, S. F.; Tetrahedron Lett., 1977, (1), 69.

70. Dominquez, X. A., Verde, S. J., Sucar, S. S. and Trevino, Rosa; Phytochemistry, 1986, 25(1), 239.

71. Duh Chang, Yih and Wu, Yang Chang; Phytochemistry, 1990, 29(8), 2689.

72. Inatani, R. Nakatani, N. and Fuwa, H.; Agric. Biol. Chem., 1981, 45(3), 667.

73. Nakatani, N., Inatani, R. and Fuwa, H.; Agric. Biol. Chem., 1980, 44(12), 2831.

74. Atal, C. K. and Banga, S. S.; Indian J. Pharm., 1962, 24, 105.

75. Atal, C. K. and Banga, S. S.; Curr. Sci., 1963, 32(8), 354.

76. Filho, B. R., De Souza, M. P. and Mattos, M. E. O.; Phytochemistry, 1981, 20(2), 345.

77. Duh Chang Yih, Wu Yang Chang and Wang, Shang Kwei; J. Natl. Prod., 1990, 53(6), 1575.

78. Maxwell, A. and Ramprasad; J. Natl. Prods., 1991, 54(4), 1150.

79. Achenbach, H. and Karl, W.; Chem. Ber., 1970, 103(8), 2535.

80. Gupta, O. P., Gupta, S. C., Dhar, K. L. and Atal, C. K.; Phytochemistry, 1978, 17(3), 601.

81. Sehgal, C. K., Kachroo, P. L., Sharma, R. L., Taneja, S. C., Dhar, K. L. and Atal, C. K.; Phytochemistry, 1979, 18(11), 1865.

82. Sharde, S., Ashok, K. and Dhar, K. L.; Phytochemistry, 1986, 25(8), 1997.

83. Atal, C. K., Moza, P. M. and Andrew, P.; Tetrahedron Lett., 1969, 11, 1397.

84. Singh, J., Dhar, K. L. and Atal, C. K.; Tetrahedron Lett., 1969, 16, 4975.

85. Sondengam, B. L., Kimbu, S. F. and Connolly, Joseph, D.; Phytochemistry, 1977, 16(7), 1121.

86. Singh, J., Potdar, M. A., Atal, C. K. and Dhar, K. L.; Phytochemistry, 1974, 13(3), 677.

87. Singh, J. and Santani, D. D.; Indian J. Pharm., 1975, 37(6), 172.

88. Singh, J., Santani, D. D. and Atal, C. K.; Phytochemistry, 1976, 15(12), 2018.

89. Joshi, B. S., Viswanathan, N., Gawad, D. H., Balakrishnan, V., Phillipsborn, W. V. and Quick, Q.; Experimentia, 1975, 31(8), 880.

90. Joshi, B. S., Viswanathan, N., Gawad, D. H., Balakrishnan, V. and Phillipsborn, W. V.; Helv. Chim. Acta, 1975, 58(8), 2295.

91. Singh, J., Dhar, K. L. and Atal, C. K.; Tetrahedron Lett., 1971, 24, 2119.

92. Woode, K. A., Phillips, F. L., Addae-Mensah, I., Bart, J. C. J. and Chaudhuri, S.; J. Natl. Prod., 1984, 47, 1024.

93. Linke, S., Kurz, J. and Zeiler, H.; Liebigs Ann. Chem., 1982, 1142.

94. Gupta, S. C., Gupta, O. P., Dhar, K. L. and Atal, C. K.; Indian J. Pharm., 1975, 37(6), 172.

95. Okogum, J. I., Sondengam, B. L. and Kimbu, S. F.; Phytochemistry, 1977, 16(8), 1295.

96. Addae-Mensah, I., Torto, F. G. and Torto, B.; Planta Medica, 1981, 41(2), 200.

97. Maxwell, A. and Ramprasad, D.; J. Natl. Prod., 1989, 52(4), 891.

98. Ho Chi-Fen, Lu, Yung-Chuan and Tsai Ting-Kuo; Yao Hsuch Tung Pao, 1981, 16(5), 63.

99. Maxwell, A. and Ramaprasad, D.; J. Natl. Prod., 1989, 52(2), 411.

100. Banerji, A. and Ray, R.; Indian J. Chem., 1981, 20B(7), 597.

101. Banerji, A. and Das, R.; Indian J. Chem., 1975, 13(11), 1234.

102. Banerji, A. and Pay, R.; Phytochemistry, 1981, 20(9), 2217.

103. Datta, C. P. and Banerjee, N.; Indian J. Chem., 1976, 14B(5), 389.

104. Sondengam, B. L., Kimbu, S. F., Njimi, T., T. Okogun, J. I. and Ekong, D. E. U.; Tetrahedron Letters, 1977, 4, 367.

105. Smith, Roger, M.; Tetrahedron, 1979, 35(3), 437.

106. Banerji, A. and Ghosh P.C.; Tetrahedron, 1973, 29(7), 977.

107. Dutta, C. P., Banerji N. and Roy, D. N.; Phytochemistry, 1975, 14(9), 2090.

108. Banerji A., Ray,R., Sidhanta, A. and Pal, S.; Indian J. Chem., 1979, 17B(5), 538.

109. Desai, S. J., Chaturvedi, R., Badheka, L. P. and Mulchandani, N. B.; Indian J. Chem., 1989, 28B, 775.

110. Desai, S. J., Chaturvedi, R. and Mulchandani, N. B.; J.Natl. Prod., 1990, 53(2), 496.

111. Olsen, C. E., Tyagi, O. D., Boll, P. M. Hussaini, F. A., Parmar, V. S., Sharma, N., Taneja, P. and Jain, S. C.; Phytochemistry, 1993, 33(2), 518.

112. Haensel, R., Leuschke, A., and Gomez-Pompa, A.; Lloydia, 1975, 38(6), 529.

113. Haensel, R. and Leuschke, A.; Phytochemistry, 1976, 15(8), 1326.

114. Akasu, M., Itokawa, H. and Fujita, M.; Tetrahedron Lett., 1974, 3609.

115. Banerji, A., Ray, R., Bandopadhay, D., Basu, S., Maitri, S., Bose, A. and Majumdar, P. L.; Indian J. Chem., 1993, 776.

116. Badheka, L. P., Prabhu, B. R. and Mulchandani, N. B.; Phytochemistry, 1987, 26, 2033.

117. Malhotra, S., Kaul, S. K., Taneja, S. C., Pusphpangadan, P. and Dhar, K. L.; Phytochemistry, 1990, 29(8), 2733.

118. Koul, S. K., Taneja, S. C., Dhar, K. L. and Atal, C. K.; Phytochemistry, 1983, 22(4), 999.

119. Prabhu, B. R. and Mulchandani, N. B.; Phytochemistry, 1985, 24(2), 329.

120. Vanegas, T. M., Cabezas, F. F., Gottlieb, O. R., Sandoval, L. and Montero, E. O.; Rev. Latinoamre Quim., 1984, 14, 139, CA:100:188783 t.

121. Koul, S. K., Taneja, S. C., Pushpangadan, P. and Dhar, K. L.; Phytochemistry, 1988, 27(5), 1479.

122. Variyar, P. S., Pendhakar, M. B., Banerjee, A. and Bandyopadhay, C.; Phytochemistry, 1988, 27(3), 715.

123. Koul, S. K., Taneja, S. C., Dhar, K. L. and Atal, C. K.; Phytochemistry, 1984, 23(9), 2099.

124. Bhadeka, L. P., Prabhu, B. R. and Mulchandani, N. B.; Phytochemistry, 1986, 25(2), 487.

125. Achenbach, H., Wittmann, G.; Tetrahedron Letters, 1970, 37, 3259.

126. Lopes, L. M., Yoshida, M. X. and Gottlieb, O. R.; Phytochemistry, 1983, 22, 1516.

127. Tyagi, O. D., Jensen, S., Boll, P. M. Sharma, N. K., Bisht, K. S.and Parmar, V. S.; Phytochemistry, 1993, 32(2), 445.

128. Neeraja, J., Garg, H. S. and Bhakuni, D. S.; J. Natl. Prod., 1990, 53(2), 479.

129. Ma, Y., Han, G. Q., Li, C. L., Cheng, J. L., Arison, B. H. and Hwang, S. B.; Acta Pharm. Sin., 1991, 26, 345.

130. Haensel, R. and Zander, D.; Arch. Pharm., 1961, 294, 699.

131. Atal, C. K., Girotra, R. N. and Dhar, K. L.; Sci. Cult., 1963, 29(11), 568.

132. Govindachari, T. R., Jadhav, S. J., Joshi, B. S., Kamat, V. N., Mohamed, P. A., Parthasarathy, P. C., Patankar, S. J., Prakash, D., Rane, D. F. and Viswanathan, N.; Indian J. Chem, 1969, 7, 308.

133. Briggs, L. H., Combie, R. C. and Couch, R. A. R.; J. Chem. Soc. C., 1968, 3042.

134. Haensel, R. and Zander, D.; Arch. Pharm., 1969, 302, 904.

135. Banerji, A. and Pal, S.; J. Natl. Prod., 1982, 45, 672.

136. Banerji, A. and Das, R.; Indian J. Chem. Sect. B., 1977, 15(5), 495.

137. Atal, C. K., Dhar, K. L. and Pelter, A.; J. Chem. Soc. C., 1967, 2228.

138. Chang, M. N., Han, G. Q., Arison, B. H., Springer, J. P., Hwang, S. B. and Shen, T. Y.; Phytochemistry, 1985, 24(9), 2079.

139. Strickler, M. P., Stone M. J., Kinnington, A. S. and Goldstein, D. M.; J. Chromatog., 1989, 484, 369.

140. Han, G. Q., Li, S., Li, C., Springer, J. P., Hwang, S. B. and Chang, M. N.; Yaoxue Xuebao, 1986, 21(5), 361, CA:105:111982 c.

141. Green, T. P., Galinis, D. L. and Wiemer, D. F.; Phytochemistry, 1991, 30(5), 1649.

142. Li, S. M. and Han, G. Q.; Zhiwa Xuebao, 1987, 29(33), 293, CA:108:3404 u.

143. Han, G. Q., Wei, L. H., Li, C. L., Qiao, L., Jia, Y. Z. and Zheng, Q. T.; Acta Pharm. Sin., 1989, 24, 438.

144. Takahashi, S. and Ogiso, A.; Chem. Pharm. Bull., 1970, 18(1), 100.

145. Matsui, K. and Mukanata, K.; Tetrahedron Letters., 1976, 4371.

146. Green, T. P. and Wiemer, D. F.; Phytochemistry, 1991, 30(11), 3759.

147. Matsui, K. and Mukanata, K.; Tetrahedron Letters, 1975, 24, 1905.

148. Matsui, K., Wada, K. and Munakata, K.; Agric. Biol. Chem., 1976, 40(5), 1045, Ibid, 1976, 1113.

149. Ogiso, A., Kurabayashi, M., Mishima, M. and Woods, M. C.; Tetrahedron Letters, 1968, 16, 2003.

150. Banerji, A., Sarkar, M., Ghosal, T., Paul, S. C. and Shoolery, J. N.; Tetrahedron, 1984, 40(24), 5047.

151. Rucker, G., Langmaan, B. and De Siqueira, N. S.; Planta Medica, 1981, 41, 143.

152. Matsui, K., Fukuyama, K., Tsukihara, K., Tsukihara, T., Katsube, Y. and Mukanata, K.; Bull. Chem. Soc. Jap., 1976, 49, 62.

153. Iida, T., Ichino, K. and Ito, K.; Phytochemistry, 1982, 21, 2939.

154. Lima, O. A., Gottlieb, O. R. and Magalhaes, M. T.; Phytochemistry, 1972, 11, 2031.

155. Diaz, D. P., Yoshida, M. and Gottlieb, O. R.; Phytochemistry, 1980, 19, 285.

156. Muraleedharan Nair, G., Mansingh, A. P. and Burke Basil, A.; J. Agric. Biol. Chem., 1986, 50(12), 3053.

157. Burke, B. and Nair, M. G.; Phytochemistry, 1986, 25(6) 1427.

158. Haensel, R., Sauer, H. and Rimpler, H.; Arch. Pharm., 1966, 299(6), 507.

159. Saucer, V. H. and Haelsel, R.; Planta Med., 1967, 15, 443.

160. Vieria, P. C., De Alvarenga, M. A., Gottlieb, O. R. and Gottlieb, H. E.; Planta Med., 1980, 39(2), 153.

161. Diaz, D., Pedro, P., Aries, C., Tiberio and Joseph-Nathan, Pedro; Phytochemistry, 1987, 26(3), 809.

162. Dutta, C. P. and Som, U. K.; J.Indian Chem. Soc., 1978, 55(9), 932.

163. Keller, F. and Klohs; Lloydia, 1963, 26, 1.

164. Som, U. K., Dutta, C. P., Sarkar, G. M. and Banerjee, R. D.; Natl. Acad. Sci. Lett., 1985, 8, 109.

165. Dutta, C. P., Ray, L. P. K., Chatterjee, A. and Roy, D. N.; Indian J. Chem., 1973, 11(5), 509.

166. Dutta, C. P., Ray. L. P. K., Chatterjee, A. and Roy, D. N.; J. Indian Chem. Soc., 1976, 53, 1194.

167. Haensel, R., Ranft, G. and Bachr, P.; Z. Naturforsch., 1963, 186(5), 370.

168. Achenbach Hans, Calle, A., Jairo Mansa, D., Domez and Poveda, C. N.; Rev. Mex. Ciene. Farm., 1984, 14(1), 2, CA:100:188774 r.

169. Foungbe, S., Tellequin, F. Paris, M., Jacquemin, H. and Paris, R. R.; Ann. Pharm. Fr., 1976, 34, 339.

170. Tellequin, F., Paris M., Jacquemin, H. and Paris, R. R.; Planta Medica, 1978, 33(1), 46.

171. Voesgen, B. and Herrmann, K.; Z. Lebensum-Unters-Forsch; 1980, 170(3), 204.

172. Hansel, R. and Beiersolorff, H. V.; Naturwissenschaften, 1958, 45, 573, CA:53:11758 a.

173. Klohs, M. W., Keller, F. and Williams, R. E.; J. Med. Pharm. Chem. I, 1959, 95.

174. Roger, M. Smith; Phytochemistry, 1983, 22(4), 1055.

175. Meyer, Hans, J.; Ethnopharmacol. Search Psychoact Drugs, Proc. Sym., 1967, (Pub. 1979, 133), CA:92:121862 r.

176. Haensel, R., Pelter, A., Schulz, J. and Hille, C.; Chem. Ber., 1976, 109, 1617.

177. Haensel, R., Beer, C. and Schulz, J.; Chem. Ber., 1973, 106, 3119.

178. Achenbach, H., Karl, W. and Regel, W.; Chem. Ber., 1972, 105, 2182.

179. Pelter, A., Albayati, R., Haensel, R., Dinter, H. and Burke; Tetrahedron Lett., 1981, 22, 1545.

180. Haensel, R. and Pelter, A.; Phytochemistry, 1971, 10, 1627.

181. Pelter, A. and Haensel, R.; Z. Naturforsch, 1972, 27, 1186.

182. Haensel, R. and Schulz, J.; Z. Naturforsch, 1978, 33, 688.

183. Haensel, R. and Schulz, J.; Arch. Pharm., 1982, 315, 147.

184. Ogiso, A., Kurabayashi, M., Mishima, M. and Woods, M. C.; Tetrahedron Lett., 1968. 16, 2003.

185. Takabashi, S.; Phytochemistry, 1969, 8, 321.

186. Desai, H. K., Gawad, D. H., Govindachari, T. R., Joshi, B. S., Kamat, V. N., Parthasarathy, P. C., Ramachandran, K. S., Shanbhag, M. N., Sidhaye, A. K. and Viswanathan, N.; Indian J. Chem., 1975, 13, 97.

187. Joshi, B. S., Gawad, D. H. and Fuhrer, H.; Tetrahedron Lett., 1979, 2427.

188. Singh, J., Dhar, K. L. and Atal, C. K.; Curr. Sci., 1969, 38, 471

189. Singh, J. and Atal, C. K.; Indian J. Pharm., 1969, 31, 129.

190. Boll, P. M., Hald, M., Parmar, V. S., Tyagi, O. D., Bisht, K. S., Sharma, N. K. and Hansen, S.; Phytochemistry, 1992, 31, 1035.

191. Taneja, S. C., Koul, S. K., Pushpangadhan, P., Dhar, K. L., Daniewski, W. M. and Schilf, W.; Phytochemistry, 1991, 30, 871.

192. Thebpatiphat, S., Pengprecha, S. and Ternai, B.; J. Sci. Soc. Thailand 1988, 14, 225.

193. Singh J., Dhar, K. L., and Atal, C. K.; Tetrahedron, 1970, 26, 4403.

194. Singh, J., Dhar, K. L. and and Atal, C. K.; Indian J. Pharm., 1971, 30, 50.

195. Kupchan, S. M., Hemingway, R. J., Coggon, P., McPhail, A. T. and Sim, G.A.; J. Am. Chem. Soc., 1968, 90, 2982.

196. Ampofo, S. A., Roussis, V. and Wiemer, D. F.; Phytochemistry, 1987, 26, 2367.

197. Purseglove, J. W., Brown, R. G., Green, C. L. and Robbins, S. R. J.; Spices, 1981, Vol. I, 51, Longman, London and New York.

198. Achenbach, H., Gross J. and Portecop, J.; Planta Med., 1984, 50(6), 528.

199. Chen, T. B.; Green, T. P. and Wiemer, D. F.; Tetrahedron Letters, 1992, 33(39), 5673.

200. Diaz, D. P. P., Dorado, V. and Jorge; Rev. Lutinoam, Quim, 1986, 17, 58.

201. Terbune, S. T., Hogg, J. W. and Lawrence, B. M.; Phytochemistry, 1974 13(7), 1183.

202. Yoshihara, K. O., Yoshimoto, S. and Tsutomu, H. Y.; Tetrahedron Lett., 1969, 27, 2263.

203. Banerji, A. and Das, R., Indian J. Chem., 1977, 15B, 395.

204. Singh, J., Santani, D. D. and Pani, R.; Indian J. Pharm., 1976, 38(2), 58.

205. Deshpande, S. M., Upadhyay, R. R. and Singh, R. P.; Curr. Sci., 1970, 39(16), 372.

206. Manavalan, R. and Singh, J.; Indian J. Pharm. Sci., 1979, 41(5), 190.

207. Graeza Lajos and Ruff Peter; Arch. Pharm., 1986, 319(5), 475.

208. Hi Qifen, Lu Yongquan, Cai Dingquo, Cui Yingjuan, Lai Jing and Wang Housheng; Zhongcaoyao, 1981, 12(10), 433.

209. Masuda, T., Inazumi, A., Yamada, Y., Pandolina, W. G., Kikuzaki, H.and Nakatani, N.; Phytochemistry., 1991, 30, 3227.

210. Vassilios, R., Stephen, A. A. and David, F. W.; Phytochemistry 1990, 29, 1787.

211. Anderson, M. and Ramprasad, D.; J. Natl. Prod., 1989, 52, 614.

212. Su, C. F.; J. Econ. Ent., 1977, 70, 18.

213. Miyakado, M., Nakayama, I. and Yoshioka, H,; Agric. Biol. Chem. 1980, 44(7), 1701.

214. Parmar, V. S., Sinha, R. and Shakil, N. A.; Indian J. Chem., 1993, 32B, 392.

215. Roger, S. M., Thakrar, H., Arowolo, T. A., Shafi, A. A.; J. Chromatog., 1984, 283, 303.

216. Kiuchi, F., Nakamura, N., Tsuda, Y., Kondo, K. and Yoshimura, H.; Chem. Pharm. Bull, 1988, 36(7), 2452.

217. Yamaguchi, I. and Ozeki, S.; Kenkyu Kiyo Tokyo Kasei Daigaku, 1985, 25, 201.

218. Hara, M. J., Kinnavd, W. J. and Buckley, J. P.; J. Pharm. Sci., 1965, 54(7), 1021.

219. Ho Chi-Fen, Lu, Yung-Chuan and Tsai, Ting-Kuo; Yao Hsuch Tung Pao, 1981, 16(5), 63, CA:95:138470 w.

220. Li, Xinfang and Huang Yuehua; Zhongyao Tongbao, 1985, 10(6), 277, CA:103:171692 b.

221. Shoji, Noboru; J. Pharm. Sci., 1986, 75(12), 1188.

222. Kirthikar and Basu; Indian Medicinal Plants, Periodical Export Book Agency, Vivek Vihar, Delhi, India; 1981, 2133.

223. Singh, J., Vasudevan, K. and Roy, R. S.; J. Indian Chem. Soc., 1976, 53, 1162.

224. Rucker, G. and Langman, B.; Tetrahedron Letters, 1978, 5, 457.

225. Birch, A. J., Milligan, B., Smith, E. and Speake, R. N.; J. Chem. Soc., 1958, 4471.

226. The Wealth of India, A dictionary of Indian Raw Materials and Industrial Products, CSIR, New Delhi, 1969, Vol.VIII, p.117.

227. Bandhopadhyay, C. and Variyar, P. S.; PAFAI J., Jan-March, 1988, 25.

228. Umar Mohamood, Shukla, Y. N. and Thakur, R. S.; Phytochemistry, 1983, 22, 167.

229. Holbert, G. W., Ganem, B., Engen D. Van, Clardy, J., Borsub, L., Chantrapromma, K., Sadavongvivad, C. and Thebtaranonth, Y.; Tetrahedron Letters, 1979, 715.

230. Sarkanen, K. V. and Wallis, A. F. A.; J. Chem. Soc. Perkin, 1, 1973, 1869.

231. Takaoka, D., Watanabe, K. and Hiroi, M.; Bull. Chem. Soc. Jpn., 1976, 49, 3564.

232. Masada, Y.; Analysis of Essential Oils by Gas Chromatography and Mass Spectroscopy, John Wiely and Sons, Inc., New York, 1976, 244.

233. Ekundayo, O., Laakso, I., Adegbola, R. M., Oguntimein, B., Sofowora, A. and Hiltunen, R.; J. Agric. Food. Chem., 1988, 36, 880.

234. Stevenson, R. and Williams, J. R.; Tetrahedron, 1977, 33(3), 285.

235. Fonseca, S. F., Barata, L. E. S., Ruveda, E. A. and Baker, P. M.; Canadian J. Chem., 1979, 57(4), 441.

236. A process for the isolation of anticancer agent (crotepoxide) from the berries of piper attenuatum; Patent No.NF 181/90, Sumathykutty, M. A. and Madhusudana Rao, J., RRL, Trivandrum, Kerala.

237. Stoianova-Ivanova, B. and Hadjieva, P.; Phytochemistry, 1969, 9, 1549.

238. Benon, J. H., Lester, G. R., Saunders, R. A. and Williams, A. E.; Trans Faraday Soc., 1961, 57, 1259.

239. Chakravarthi, D. and Debnath, N. B.; J. Indian Chem. Soc., 1974, 51, 260.

240. Holloway, P. J., Jeffree, C. E. and Baker, E. A.; Phytochemistry, 1976, 15, 1768.

241. Parry, E. J.; Chemistry of Essential Oils and Artificial Perfumes, Scott Green Wood, London, 1922, Vol.II.

242. Buttery, R. G., Maddox, D. M., Light, D. M. and Ling, L. C.; J. Agric. Food Chem., 1986, 34, 786.

243. Simonsen, J. L and Owen, L. N. The Terpenes, Cambridge University Press, Cambridge, 1947, Vol.I, 1949, Vol.II. Simonsen, J. L. and Barton, D. H. R., 1952, Vol.III. Simonsen, J. L. and Ross, W. C. J., 1957, Vol.IV and Vol.V.

244. Barter, D. H. R. and Harper, S. H.; in Chemistry of Carbon Compounds, Rodd, E. H.; Ed. Elsevier Publishing Co., Amsterdam, 1953, Vol.II B., p.489.

245. Mayo, de P.; in The chemistry of Natural Products, Bentley, K. W., Ed., Interscience Publishers, Inc., New York, 1959, Vol.II.

246. Pinder, A. R.; The chemistry of Terpenes, Chapman and Hall Ltd., London, 1960.

247. Barton, D. H. R. and Mayo, de P.; Recent advances in sesquiterpenoid chemistry, Quart. Reviews, The Chemical Society, 1962, 16, 101.

248. Barton, D. H. R.; in Progress in Organic Chemistry, Rodd, E. H., Ed. Elsevier Publishing Co., Amsterdam, 1963, 26, 489.

249. Mellor, J. M. and Munavalli, S.; Quart. Reviews, The Chemical Society, London, 1964, 18, 270.

250. Newman, A. A.; Chemistry of Terpenes and Terpenoids, Academic Press, London, 1972.

251. Devon, T. K. and Scott, A. I.; Handbook of naturally occuring compounds, Academic Press, New York, 1972, Vol.II

252. Terpenoids and Steroids, Overton, K. H., Sr. Reporter, The Chemical Society/Royal Scoeity of Chemistry, London, 1971-76, Vol.I-VI, Hanson, J. R.; Sr. Reporter, 1977-81, Vol.VII-X.

253. Guenther, E.; The Essential Oils, D. Van Nostrand Co., New York, 1950, Vol. I-VI.

254. Littlewood, A. B.; Gas Chromatography-Principles, Techniques and Applications, Academic Press Inc., New York, 1970.

255. Teranishi, R., Lundin, R. E., Mc Fadden, W. H. and Scherrer, J. R. in The Practice of Gas Chromatography, Ettre, L. S. and Zlatkis, Z. Eds. Interscience Publishers, Inc., New York, 1967.

256. Kovats, E.; Helv. Chim. Acta., 1958, 41, 1915.

257. Kovats. E.; In Advances in Chromatography, Ed. by J. C. Giddings and R. A. Keller, New York, Academic Press, 1965.

258. Chapman, R.; "Computers in Mass Spectrometry", London, Academic Press, 1978, 101-149.

259. Alencar, J. W. and Craveiro, A. A.; in "Essential Oils", Ed. by B. D. Mookherjee and C. J. Mussinan, Wheaton, Allured Publishing Co., 1981, 229-246.

260. Ulthman, A. P., Koontz, J. P., Hindesliter Smith, J., Woodward, W. S. and Reilley, C. H.; Anal. Chem., 1982, 54, 1772.

261. Milne, G. W. A.; in Natural Products as Medicinal Agents, Ed. by J. L. Beal and E. Reinhard, Berlin, Verlarger, 1981, 55.

262. Hamming, M. C. and Foster, G. N.; "Interpretation of Mass Spectra of Organic Compounds", New York, Academic Press, 1965.

263. Jennings, W. and Shibamoto, T.; Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Column Gas Chromatography, Academic Press, New York, 1980.

264. Ramaswamy, S. K., Briscese, P., Gargiullo, R. J. and Von Geldern, T.; in Flavours and Fragrances, A World Perspective, Proceedings of the 10th International Congress of Essential Oils, Fragrances and Flavours, Washington, D.C., USA, 16-20 November, 1986, 951.

265. Shibamoto, T., Sandra, P. and Bicchi, C. (Editors); Capillary Gas Chromatography in Essential Oil Analysis, Huthig, Heidelberg, 1987, p.259.

266. Andersen, N. H., Bissonette, P., Liu, C. B., Shunk, B., Ohta, Y., Tseng, C. L. W., Moore, B. and Huneck, S.; Phytochemistry, 1977, 16, 1731.

267. Anderson, N. H., Costin, C. R., Kramer Jr., C. M., Ohta, Y. and Hunek, S.; Phytochemistry, 1973, 12, 2709.

268. Analytical Methods Committee, Analyst, 1984, 109, 1339.

269. Analytical Methods Committee, Analyst, 1984, 109, 1343.

270. Analytical Methods Committee, Analyst, 1988, 113, 1125.

271. Davies, N. W.; J. Chromatography, 1990, 503, 1.

272. Hasselstrom, T. E., Hewitt, E. J. and Ritter, J. J.; J. Agric. Food Chem., 1957, 5, 53.

273. Jennings, W. G. and Wrolstad, R. E.; J. Food Sci., 1961, 26, 499.

274. Ikeda, R. N., Stanley, W. L., Vannier, S. H. and Spitler, E. M.; J. Food Sci., 1962, 27, 455.

275. Nigam, M. C. and Handa, K. I.; Indian Perfumer, 1964, 8, 15.

276. Jennings, W. G. and Wrolstad, R. E.; J. Food Sci., 1965, 30, 274.

277. Jennings W. G. and Muller, C. J.; J. Agric. Food Chem., 1967, 15, 762.

278. Jennings, W. G., Creveling, R. K. and Muller, C. J.; J. Agric. Food Chem., 1968, 16, 113.

279. Debrauwere, J. and Verzele, M.; J. Sci. Food Agric., 1975, 26, 1887.

280. Debrauwere, J. and Verzele, M.; J. Chrom. Sci., 1976, 14, 296.

281. Gopalakrishnan, M., Nirmala Menon, N., Padmakumari, K. P., Jayalekshmi, A. and Narayanan, C. S.; J. Essent. Oil Res., 1993, 5, 247.

282. Official Methods of Analysis, 14th Ed., 1984, p.563-572, Association of Official Analytical Chemists, Inc., Arlington, VA, USA.

283. The wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, CSIR, New Delhi, 1969, Vol.III, 177.

284. Rogers, J. A.; Flavour Chemistry, American Chemical Society, Washington, D.C, 1966, 212.

285. Guenther, E., The Essential Oils, Vol.5, Van Nostrand, New York, 1952, 138.

286. Razdan, R. K. and Bhattacharya, S. C.; Perfum. Essent. Oil Rec. 1954, 45, 181.

287. Kirthikar, K. R., Basu, B. D. and An, I. C. S.; Indian Medicinal Plants, Periodical Expert Book Agency, Vivek Vihar, Delhi, India, 1981, I, 600.

288. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, CSIR, New Delhi, 1956, Vol.IV, 252.

289. Sheela, P. Nair and Madhusudana Rao, J.; Phytochemistry, 1993, 33, 711.

290. Pambou Tchivounda, H., Koudogbo, B. Besace, Y. and Casadevall, E.; Phytochemistry, 1990, 29, 3255.

291. Raja Rao, K. V., Rao, L. J. M. and Prakasa Rao, N. S.; Phytochemistry, 1990, 29, 1326.

292. Julian, P. K., Pikl, J. and Dawson, R.; J. Am. Chem. Soc., 1938, 60, 77.

293. Mayo, P. de and Starrt, A. N.; Can. J. Chem., 1962, 40, 788.

294. Mayo, P. de and Starrt, A. N.; Can. J. Chem., 1962, 40, 1632.

295. Guise, G. B., Ritchie, E. and Taylor, W. C.; Aust. J. Chem., 1962, 15, 314.

296. Eade, R. A., Ellis, J., Harper, P. and Simes, J. J. H.; J. Chem. Commun., 1969, 579.

297. Branch, G. B., Burgess, D. V., Dunstan, P. J., Foo, L. Y., Green, G. H., Mack, J. P. G., Ritchie, E. and Taylor, W. C.; Aust. J. Chem., 1972, 25, 2209.

298. Roitman, J. N. and Jurd, L.; Phytochemistry, 1978, 17, 491.

299. Baxter, R. L. and Walkinshaw, D. M.; Phytochemistry, 1988, 27, 2350.

300. Eade, R. A., Ellis, J. and Simes, J. J. H.; Aust. J. Chem., 1967, 20, 2737.

301. Burbage, M. B., Jewers, K., Eade, R. A., Harper, P. and Simes, J. J. H.; J. Chem. Commun., 1970, 814.

302. Burbage, M. B., Jewers, K., Eade, R. A., Harper, P. and Simes, J. J. H.; J. Chem. Commun., 1971, 195.

303. Halshall, T. G. and Aplin, R. T.; in Progress in the Chemistry of Organic Natural Products, (Zechmeister, R. L., Ed.), Springer, New York, 1964, Vol. 22, 153.

# PUBLICATIONS

Indian Journal of Chemistry
Vol. 27B, April 1988, pp. 388-389

# Lignans from Leaves of *Piper nigrum* Linn.

M A SUMATHYKUTTY & J MADHUSUDANA RAO*
Regional Research Laboratory, Trivandrum 695 019

*Received* 3 *August* 1987: *accepted* 7 *September* 1987

From the petrol and chloroform extracts of the leaves of *Piper nigrum* Linn. two isomeric lignans (−)-3,4-dimethoxy-3,4-desmethylenedioxycubebin and (−)-3',4'-dimethoxy-3',4'-desmethylenedioxycubebin have been isolated in pure form along with (−)-cubebin.

*Piper nigrum* Linn. berries (black pepper) are widely used in indigenous system of medicine[1]. More than 100 terpene constituents have been reported from the essential oil[2] of the berries. Several alkaloids[2] have been isolated as non-volatile constituents. Piperine, β-sitosterol, hentriacontane, hentriacontanone-16 and hentriacontanol-16 are reported in the stems of *P. nigrum*[3]. Three dibenzylbutyrolactol lignans, (−)-cubein and (−)-cubebinin[4] from *P cubeba* and (−)-clusin[5] from *P clusii* are so far reported from the genus *Piper*. Herein, we report the isolation and identification of three dibenzylbutyrolactol lignans from *P. nigrum* leaves. We also took this opportunity to completely characterise the two isomeric lignans.

The petroleum ether and the chloroform extracts of dried powdered *P nigrum* leaves on repeated column chromatography and preparative TLC gave three crystalline compounds A-C. Compound-A was identified as (−)-cubein by its superimposable IR, PMR and mass spectra with that of an authentic sample.

The 500 MHz PMR spectra of compounds B and C were very similar and indicated their identity as dibenzylbutyrolactol lignans. The *trans*-stereochemistry at 8 and 8' positions was established by the characteristic PMR spectra[5] (60 MHz; δ 2.50, *m*, 4H, benzylic protons and 2.85, *m*, 2H, methine protons) of the lactones obtained by oxidation with $CrO_3/H_2SO_4$ in acetone[7]. The mass spectrum of B showed base peak at *m/z* 151 as observed by Rucker *et al.*[6] and established its identity as 3,4-dimethoxy-3,4-desmethylenedioxycubebin. Compound-C showed base peak at *m/z* 135, thus establishing[6] its structure as 3',4'-dimethoxy-3',4'-desmethylenedioxycubebin. These two lignans were earlier isolated as the corresponding lactones from *Aristolochia triangularis* by Rucker *et al.*[7].

The methanol soluble portion of the petroleum ether extract of the berries also showed the presence of these three lignans by co-TLC.

*Extraction and separation of compounds*

The leaves of *P nigrum*, obtained from the local gardens, were shade dried, powdered (625 g) and extracted successively with petroleum ether (60-70°) and chloroform in a Soxhlet apparatus for 40 hr and 30 hr respectively. The methanol soluble fraction of the petroleum ether extract (20 g) and the chloroform extract (14 g) were separately subjected to column chromatography (silica gel, 250 g and 200 g respectively). The mixed residue (23 g) from 9:1 chloroform-methanol eluates was rechromatographed over silica gel (175 g). A fraction from chloroform - ethyl acetate (95:5) eluate showed the presence of two closely moving spots [solvent system benzene - ethyl acetate 9:1 (UV)]. The residue from this fraction (2 g) was subjectd to preparative TLC (UV), upper band gave the compound-A (3.8 mg), which recrystallised from benzene-hexane.

The extract from the lower band on fractional crystallisation followed by further recrystallisations from benzene-hexane gave compound B (11.5 mg) and C (5 mg)

(−)-3,4-Dimethoxy 3,4-desmethylenedioxycubebin (B), white needles, m.p. 86-87° (lit.[6] 89-91°), $[\alpha]_D^{25°}$ −52.86 ($CHCl_3$; *c* 0.35) (Found: $M^+$, 372.1575. $C_{21}H_{24}O_6$ requires 372.1574); MS *m/z* (rel. int.): 372 ($M^+$, 33.7), 203 (19.1), 177 (73.3), 152 (82), 151 (100), 145 (13.9), 135 (50.4), 123 (11.7), 121 (19.1), 81 (24.9).

(−)-3',4'-Dimethoxy-3',4'-desmethylenedioxycubebin (C), white globulets, m.p. 66°, $[\alpha]_D^{25°}$ −15.88 ($CHCl_3$, *c* 0.17); UV (MeOH): 236 amd 286 nm; IR (KBr) 3365 (OH), 2940 1605, 1530, 1500, 940 and 820 $cm^{-1}$; PMR (500 MHz, $CDCl_3$/TMS): δ 2.0-2.9 (6H, *m*, 4-benzylic and 2-methine protons), 3.82 and 3.85 (6H, *s*, Ar-OC$H_3$), 3.59, 4.01 and 4.10 (2H, triplets, *J* = 8Hz each, methylene protons of furanol ring), 5.23 (1H, *s*, hemiacetal proton), 5.92 (2 H, *s*, OC$H_2$O), 6.4-6.9 (6H, *s*, ArH); (Found: $M^+$, 372.1576. $C_{21}H_{24}O_6$ requires 372.1574); MS. *m/z* (rel, int.): 372 ($M^+$, 11.6), 203 (5.4), 177 (25.8), 152 (46.9), 151 (77.7), 145 (4.9), 135 (100), 123 (75), 121 (11), 81 (7.7) (Found. C, 67.5, H, 6.4. $C_{21}H_{24}O_6$ requires C 67.7; H, 6.5%).

We thank Dr N B Mulchandani, Bio-Organic Division, BARC, Bombay for an authentic sample of (−)-cubebin. We are also thankful to Dr M M Dhingra, TIFR, Bombay for the 500 MHz PMR spectra of compounds B and C.

## References


1 Kirtikar & Basu, *Indian Medicinal Plants*, (Periodical Export Book Agency, Vivek Vihar, Delhi, India) 1981, 2133.

2 Purseglove J W, Brwon E G, Green G L & Robins S R J, *Spices*, Vol 1 (Longman, London and New York) 1981, p 10.

3 Singh J, Vasudevan K & Roy R S, *J Indian chem Soc*, **53** (1976) 1162.

4 Prabhu B R & Mulchandani N B, *Phytochemistry*, **24** (1985) 329.

5 Koul S K, Taneja S C, Dhar K L & Atal C K, *Phytochemistry*, **22** (1983) 999.

6 Rucker G & Longmann B, *Tetrahedron Lett*, **5** (1978) 457.

7 Rucker G, Longmann B & De Siqueira N S, *Planta medica*, **41** (1981) 143.

INDIAN PERFUMER 32(4)1988 P.P. 310-311

# HIGHER ALKANES FROM THE FRUITS OF PIPER AURANTIACUM

M.A.Sumathykutty and J.Madhusudana Rao

ABSTRACT

Three higher alkanes $C_{31}H_{64}$, $C_{33}H_{68}$ and $C_{35}H_{72}$ were identified in the methanol insoluble portion of the petroleum ether extract by a combination of GLC and MS.

PIPER AURANTIACUM[1] is a stout glabrous climber with coriaceous leaves, 7.5 - 10 cm long, and is found in Nepal, Lakhipur and Khasi hills in Assam. It bears dropping spikes, 3.8 - 7.5 cm long with fruits distinctly angular and pyramidal when ripe, about 4 mm in diameter. The fruits are reported to possess bitter, acrid and cooling properties[2].

While the chemistry of volatile and non-volatile constituents of the genus PIPER is age old, detailed chemical examination of the seeds of PIPER AURANTIACUM has been reported only recently[3]. β-Sitosterol, piperine, piperettine, sylvatine[3], aurantiamide and its acetate[4], stearic and linoleic acids, triacontane, cholesterol and cholestanol[5], triterpenes friedelin and epifriedelanol[6], vanillic acid and auranamide[7] were so far reported from the fruits. We did not find any essential oil in the fruits. However, three higher alkanes were identified by a combination of GLC and MS.

No essential oil could be obtained by Clevenger distillation of 100 g of the powdered PIPER AURANTIACUM seeds. In a seperate experiment 50 g of the powdered seeds were extracted continously in a Soxhlet with hexane for 10 hrs. The residue from the hexane extract

Regional Research Laboratory (CSIR) Trivandrum-695019

was fractionated into methanol soluble and insoluble fractions. The methanol insoluble fraction was subjected to GLC and Mass Spectral analysis.

GLC analysis was carried at isothermal temperature 300°C (inj. temp. 250°, FID temp. 300°) on 10% OV-17 column with $N_2$ as carrier gas on HP 5840A Gas Chromatograph. Three peaks were observed at retention times 6.00, 8.93 and 13.48 minutes, respectively. Mass spectral analysis was carried out at isothermal 300° using direct inlet system (HP 5995 GC-MS). The three peaks were found to correspond with $C_{31}H_{64}$ ($M^+$436), $C_{33}H_{68}$($M^+$464) and $C_{35}H_{72}$($M^+$492), respectively. There is a consecutive loss of 14 and/or 28 units in the mass spectra of alkanes.

## REFERENCES

1. The Wealth of India 1969. CSIR Publication, New Delhi, 8:177 & 118.

2. Chopra, R.N.; Nayar, S.L. and Chopra, I.C. 1956. Glossary of Indian Medicinal Plants, CSIR Publication, New Delhi: 194.

3. Madhusudana Rao, J.; Subrahmanyam, K. and Jagannadha Rao, K.V. 1974. Cur. Sci., 45: 76-77.

4. Banerjee, A. and Das R. 1975. Indian J. Chem., 13: 1234-6.

5. Singh, J.; Santani, D.D. and Pani Rajini 1976. Indian J. Pharm. 38: 58-9.

6. Banerjee, A. and Das, R. 1977. Indian J. Chem., 15B: 395-6.

7. Banerjee, A. and Avijit, R.R. 1981. Indian J. Chem., 20: 597-8.

INDIAN PERFUMER 34(3) 1990 P.P. 182-185

# COMPOSITION OF ESSENTIAL OIL FROM PIPER ATTENUATUM

M.A. SUMATHYKUTTY AND J. MADHUSUDHANA RAO


## *ABSTRACT*

*Chemical composition of volatile oil from different parts of piper attenuatum was determined by GC and Capillary GC-MS. 17 constituents were identified. β- carryophyllene is the major constituent of berry oil where as β-bisabolene is the major constituent of stem oil.*


## INTRODUCTION

*Piper attenuatum* (wild pepper family piperaceae) is a slender, rambling climber with stout but soft flexuous branches distributed in the eastern tropical Himalayas, Sikkim, Butan, Assam, Sylhet, Khasia hills, Orissa, hills of Visakhapatnam and Godavari, eastern slopes of Nilgiris and the western ghats and hills of Thirunelveli district [1,2].

The root of piper attenuatum is reported to be used as an excellent diuretic [2]. In Malaysia, parts of the plant are used for washing clothes in order to scent them. It has an intense rubefacient effect and is used in poultices for headache and other pains [1,2]. Crotepoxide possessing significant antitumor activity was separated from the aerial part of the plant [3,4]. Piperine, piperlongumine N-isobutyl deca-trans-2-trans 4-dienamide and guineensin [5] were isolated from the roots of the plant. Isolation of aristolactams and 4,5-dioxoaporphins have also been reported from *P. attenuatum* [6].

So far no investigation of the volatile oil of this plant has been carried out. This paper reports the GC study of the oils from berries, stem and leaf and capillary GC-MS study of stem oil.

## MATERIALS AND METHODS

The plant material was collected from a garden near Neyyar Dam, Trivandrum district. The leaves and stem were air dried and the berries were dried in the cross-flow drier. Volatile oils were obtained by hydro-distillation method. Refractive index was measured in Abbe refractometer and optical rotation in DIP-370 digital polarimeter. GC analysis of the oils were carried out in a Hewlett Packard Model 5840 A with 1/6"x6' OV-17 column, temperature programmed from 80 to 200°C at the rate of 5°/minute, injection port temperature 250°C and $N_2$ as carrier gas with flow rate of 20ml/min. Components of the oil were identified by comparison

Regional Research Laboratory (CSIR), Trivandrum 695019

of retention time with authentic samples and also by co-injection.

Capillary GC-MS was carried out in a Hewlett packard Model 5995 B 50 m. flexible silica capillary column with 0.02 m.m. i.d. was used. Carrier gas was helium at 1 ml/min., split ratio 75:1 and temperature programming from 90-200° at the rate of 5°/min. The constitutents were identified by matching the mass spectra with NBS library on hydrocarbons and flavour and fragrances and library generated in our laboratory. Sensory evaluation of the oils is also conducted by a panel of 10 judges.

## RESULTS AND DISCUSSIONS

Table 1 shows the percentage yield optical rotation, refractive index etc. of the oils obtained from the berries, stem and leaves of *P. attenuatum* plant. Berries showed highest oil content. Refractive index of stem oil was lower than those of oils from berries and leaves. The monterpene content in the leaf oil was slightly higher 4-5%.

TABLE 1. Physico Chemical Characteristics of Oils from P. Attenuatum

| *Characteristics* | *Berry* | *Leaf* | *Stem* |
|---|---|---|---|
| Volatile Oil % (DWB) | 1.6 | 0.75 | 0.08 |
| Refractive Index | 1.496 | 1.497 | 1.485 |
| Optical rotation | -0.644° | -2.094° | -1.153° |
| Monoterpenes % | 1 - 1.5 | 4 - 5 | 1 - 2 |
| Sesquiterpenes and other polar compound % | 98.5 - 99 | 95 - 96 | 98 -99 |

Table 2 shows the GC analysis of the oils. 17 Constituents were identified from these oils. β-caryophyllene (28%) was the major constituent of berry oil. Other constituents present in significant amounts in berry oil were β-bisabolene (18.6%) δ-cadinene (13.5%) and α-humulene (7.6%). An unidentified sesquiterpene hydrocarbon (Rt 25.05) was found to be the major constituent (33.5%) of leaf oil. It was absent in other two oils. Ar-curcumene was found only in the leaf oil β-Bisabolene (20%) was the major constituent of stem oil.

TABLE 2. GC Analysis of oils from different parts of P.attenuatum

| S.N. Constituents | Berry (%) | Leaf (%) | Stem (%) |
|---|---|---|---|
| 1. α-pinene | 0.09 | 0.62 | - |
| 2. Camphene | 0.05 | 0.05 | - |
| 3. β-pinene | 0.12 | 0.68 | - |
| 4. Δ-Carene | - | 0.03 | 0.003 |
| 5. d-limonene | 0.19 | 0.14 | 0.08 |
| 6. Linalool | 0.25 | 0.42 | 0.29 |
| 7. α-cubebene | 1.72 | - | 0.72 |
| 8. Copaene | 4.05 | 1.39 | 1.53 |
| 9. Farnesene | 2.08 | 0.51 | 2.39 |
| 10. β-elemene | 1.37 | - | 3.48 |
| 11. β-caryophyllene | 28.02 | 2.50 | 6.60 |
| 12. α-humulene | 7.60 | 2.15 | 6.46 |
| 13. β-bisabolene | 18.59 | 5.97 | 19.96 |
| 14. δ-cardinene | 13.56 | 0.63 | 4.15 |
| 15. α-muurolene | 2.51 | - | 1.66 |
| 16. ar-curcumene | - | 3.50 | - |
| 17. α-elemol | 1.48 | - | 4.91 |

Percentage composition of the unidentified high boiling sesquiterpene hydrocarbons and oxygenated compounds are presented in Table 3 as the mass spectra of these compounds showed ($M^+$) at M/Z 204 and 222. Most of the sesquiterpenes present in the leaf oil at high Rt could not be identified although they are present in consid-

crable amounts. These constituents are almost absent in berry and stem oils. Two unidentified sesquiterpene alcohols are present in the stem oil in 5.2% and 10.4% level.

MS data of the compounds identified by capillary GC-MS is shown in table 4. about 12 constituents could be identified from the stem oil.

Sensory evaluation shows that oil from the berry is mild, pleasant, aromatic piney with medicinal odour. Stem oil has a pleasant, herbal with mild sweet odour. The leaf oil has terpenic, earthy, leafy smell and also not pleasant. All the three oils have got a penetrating odour. The essential oils from berries and stems may find use in perfumery application with retention of bottom-note for longer periods.

## ACKNOWLEDGEMENT

We thank Mrs. K.P. Padmakumari for GC-MS analysis and Mrs. Beena Symon for GC analysis.

## REFERENCES

1. Wealth of India, A dictionary of Indian Raw materials and Industrial Products; CSIR, New Delhi, 1969, Vol. VIII, p.117.

2. Kirthikar and Basu, Indian Medicinal Plants, (Periodical Export Book Agency, Vivek Vihar, Delhi, India), 1981, Vol. III, p.2135.

3. Desai, H.K.; Gawad, D.H.; Govindachari, T.R.; Joshi, B.S.; Kamat, V.N.; Chemical Investigation of some Indian Plants, Part III. *Indian J. Chem. B*; 97,1975.

4. Joshi, B.S. and Gawad, D.H.; Revised structure of pipoxide and pipoxide chlorohydrin. *Tetrahedron* Lett. *26*, 2427, 1979.

5. Dasgupta, S. and Ray, A.B.; Amides of piper attenuatum; *Indian J. Chem. Sect. B; 17B* (5), 538-40, 1979.

6. Desai, S.J.; Chaturvedi, R.N.; Badheka, L.P. and Mulchandani, N.B.; Aristolactams and 4, 5- dioxoaporphines from Indian Piper Spices, *Indian J. Chemistry, 28B*, 775-777, 1989.

TABLE 3. Unidentified sesquiterpene and other polar compounds.

| S.NO. | Constituents | Leaf % | Stem % |
|---|---|---|---|
| 1. | Sesquiterpene hydrocarbon (Rt 22.9) | 2.87 | 1.66 |
| 2. | Sesquiterpene alcohol (Rt 23.16) | - | 5.21 |
| 3. | Sesquiterpene alcohol (Rt 24.76) | - | 10.41 |
| 4. | Sesquiterpene hydrocarbon (Rt 25.05) | 33.56 | - |
| 5. | Sesquiterpene hydrocarbon (Rt 25.73) | - | 2.38 |
| 6. | Sesquiterpene hydrocarbon (Rt 25.81) | 5.34 | |
| 7. | .Sesquiterphydrocarbon (Rt 26.51). | 4.32 | - |
| 8. | Sesquiterpene hydrocarbon (Rt 27.42) | 14.42 | 1.34 |
| 9. | Sesquiterpene hydrocarbon (Rt 28.02) | 2.75 | - |
| 10 | Sesquiterpene hydrocarbon (Rt 28.97) | 2.74 | - |
| 11. | Sesquiterpene hydrocarbon (Rt 29.94) | - | 2.24 |
| 12. | Sesquiterpene hydrocarbon (Rt 30.22) | 3.72 | - |
| 13. | Sesquiterpene hydrocarbon (Rt 32.16) | 2.50 | - |

Berry %
2.08
0.06
2.15

TABLE 4. GC-MS Analysis of Piper attenuatum oils

| S.NO. | Name of the Compounds | RT | Method of Identification | MS data fragmentation in order of abundance |
|---|---|---|---|---|
| 1 | Undecanone | 16.92 | M.S. | 43, 58, 71, 170 |
| 2 | Linalool | 18.68 | R.T. | |
| 3 | α-Cubebene | 19.12 | MS/RT | 159, 161, 119, 105, 41, 91, 204 |
| 4 | α-copaene | 19.96 | MS/RT | 161, 159, 119, 105, 204, 91, 93, 41, 120 |
| 5 | β-farnesene | 20.38 | MS/RT | 121, 147, 93, 161, 133, 41, 119, 91, 105, 67 |
| 6 | α-elemene | 20.88 | MS | 161, 204, 189, 41, 119, 133, 91, 147, 105 |
| 7 | β-Caryophyllene | 21.32 | MS/RT | 133, 41, 93, 91, 120, 161, 119, 105, 79, 107, 147 |
| 8 | α-humulene | 22.26 | MS/RT | 93, 121, 147, 80, 91, 92, 67, 41, 107, 122 |
| 9 | β-Bisabolene | 23.56 | MS | 69, 93, 41, 109, 67, 53, 39, 94, 121, 91, 119 |
| 10 | δ-cadinene | 23.94 | MS/RT | 161, 119, 134, 204, 159, 105, 91, 41, 128, 189 |
| 11 | α-muurolene | 24.14 | MS | 119, 159, 161, 105, 204, 121, 120, 91, 129, 41 |
| 12 | Sesquiterpene hydrocarbon | 24.84 | MS | 69, 43, 41, 133, 131, 136, 107, 119, 161, 43, 133, 41, 145, 205, 121 |
| 13 | Sesquiterpene alcohol | 25.44 | MS | 159, 131, 119, 91, 105, 43, 133, 41, 145, 205, 121 |
| 14 | Sesquiterpene alcohol | 26.56 | MS | 136, 119, 161, 122, 105, 147, 121, 91, 41, 43, 133 |
| 15 | Elemol | 26.88 | MS | 161, 119, 204, 121, 43, 105, 79, 41, 91, 162 |

*Phytochemistry*, Vol. 30, No. 6, pp. 2075–2076, 1991
Printed in Great Britain.

0031-9422/91 $3.00 + 0.00




# 8-HENTRIACONTANOL AND OTHER CONSTITUENTS FROM *PIPER ATTENUATUM*

M. A. SUMATHYKUTTY and J. MADHUSUDANA RAO*

Regional Research Laboratory (CSIR) Trivandrum 695019, India

(*Received in revised form* 23 *October* 1990)

**Key Word Index**—*Piper attenuatum*; Piperaceae; leaves; pipoxide chlorohydrin; galbelgin; 8-hentriacontanol.

**Abstract**—Pipoxide chlorohydrin, (−)-galbelgin and a new aliphatic alcohol, 8-hentriacontanol have been isolated from the leaves of *Piper attenuatum*.

## INTRODUCTION

*Piper attenuatum* is an important species which is much used in the Ayurvedic system of medicine [1]. Crotepoxide, known to possess significant antitumour activity for Lewis lung carcinoma [2], has been reported from the whole plant of *P. attenuatum* [3]. Recently Mulchandani *et al.* [4] have reported the occurrence of several aristolactams and 4,5-dioxoaporphines from the aerial parts of this plant. We report the isolation and identification of three compounds from *P. attenuatum* leaves.

## RESULTS AND DISCUSSION

The petrol and chloroform extracts of dried powdered *P. attenuatum* leaves on repeated column chromatography gave three crystalline compounds **1**–**3**. Compound **2** was identified as pipoxide chlorohydrin [3] by its mp, IR, $^1$H NMR and mass spectral properties. It was earlier isolated from methanolic extracts of *P. hookeri* and *P. nigrum* [3]; this is the second report of its occurrence in the genus *Piper*.

Compound **3** was identified as (−)-galbelgin [5] by comparison of its mp, IR, $^1$H NMR and mass spectra with literature data [6]. This is the first report of the isolation of a 3,4-dimethyl-2,5-bisaryltetrahydrofuranoid lignan from the genus *Piper*.

Compound **1** had a molecular formula $C_{31}H_{64}O$ ([M]$^+$ *m/z* 452). Its IR spectrum showed hydroxyl absorption at 3450 cm$^{-1}$ and generally indicated its aliphatic nature. The $^1$H NMR of its acetate showed the presence of a methine proton at $\delta$5.34 (1H, *m*), acetoxyl protons at $\delta$1.94 (3H, *s*), two terminal methyl groups resonating between $\delta$0.82 and 1.02 (6H, two overlapped triplets) and 26 methylene units at $\delta$1.25 (52H, *br s*). A broad singlet at $\delta$1.66 (4H) was attributed to two methylene units attached to the carbinolic carbon. The absence of a [M−15]$^+$ ion and the presence of a [M+1]$^+$ in its mass spectrum is characteristic of an unsymmetrical straight chain compound [7–9]. The position of the hydroxyl group was deduced from the characteristic peaks at *m/z* 129 [Me(CH$_2$)$_6$CHOH]$^+$ and *m/z* 353 [Me(CH$_2$)$_{22}$CHOH]$^+$. The compound was thus characterized as 8-hentriacontanol, a new aliphatic alcohol. It is likely to be a constituent of the epicuticular wax of this plant as was observed in other plant species by Holloway *et al.* [10].

## EXPERIMENTAL

Mps: uncorr. Silica gel (60–120 mesh) was used for CC. The homogeneity of all compounds was checked by TLC in several solvent systems.

*Extraction and isolation.* Leaves of *P. attenuatum* obtained from local gardens (a voucher specimen is available at TBGRI, Palode, Trivandrum) were shade-dried, powdered (115 g) and extracted successively with petrol (60–70°) and CHCl$_3$ in a Soxhlet apparatus for 24 and 20 hr, respectively. The combined petrol and CHCl$_3$ extracts (17.6 g) were subjected to silica gel CC (200 g) using *n*-hexane, hexane–EtOAc (9:1) and hexane–EtOAc (4:1) as eluants. The hexane–EtOAc eluate (4 g) on rechromatography (silica gel, 40 g) gave **1** (5 mg), mp 77°, analysing for $C_{31}H_{64}O$. TLC silica gel in $C_6H_6$, $R_f$ 0.26. IR $\gamma_{max}^{KBr}$ cm$^{-1}$: 3450, 2920, 2850, 1510, 1470, 720; EIMS (rel. abundances below 5% not given) *m/z* 453, 452 [M]$^+$ 424, 396, 368, 354, 353, 340, 339, 325, 312, 311, 297, 283, 269, 255, 241, 227, 213, 199, 185, 171, 157, 143, 129 (25), 115, 111 (18, [129−H$_2$O]$^+$), 101, 97 (23), 87 (15), 85 (25), 83 (27), 71 (45), 69 (41), 57 (90), 55 (65), 43 (100), 28 (99). Compound **1** (5 mg) was treated with pyridine (0.3 ml) and Ac$_2$O (0.3 ml) overnight at room temp. After work-up it afforded a thick residue; IR $\gamma_{max}^{neat}$ cm$^{-1}$: 2930, 2860, 1745, 1465, 1260 and 725.

The hexane–EtOAc (4:1) eluate on concn gave a crystalline compound, **2**, which was further purified by recrystallization from EtOAc–hexane (47 mg), mp 200–201°, EIMS *m/z*: 367 ([M−Cl]$^+$). $[\alpha]_D^{25°}$: +57.572 (pyridine; *c* 0.205), $^{13}$C NMR

(67.89 MHz, DMSO-$d_6$/TMS): 57.5, 67.7, 68.6, 73.8, 74.8, 127.9, 128.9, 129.3, 133.3, 165.7. The residue from the filtrate (1.1 g) on rechromatography (silica gel, 40 g) using hexane–EtOAc (9:1) gave 3 which was further purified by recrystallization from EtOAc–hexane (81 mg), mp 142–143°. $[\alpha]_D^{25°}$: −135.50 ($CHCl_3$; *c* 0.2). EIMS $[M]^+$ *m/z*: 372. $^{13}C$ NMR ($CDCl_3$/TMS): 149.5, 148.9, 135.3, 118.8, 111.5, 109.9, 88.3, 56.1, 51.1 and 14.0.

*Acknowledgement*—We thank the Sophisticated Instruments Facility, Indian Institute of Science, Bangalore for NMR analysis of compounds.

## REFERENCES

1. Kirtikar, K. R. and Basu, B. D. (1981) *Indian Medicinal Plants* Vol. III (Periodical Export Book Agency, Vivek Vihar, Delhi, India), p. 2135.
2. Kupchan, S. M., Hemingway, R. J., Coggon, P., Mcphail, A. T. and Suin, G. A. (1968) *J. Am. Chem. Soc.* 90, 2982.
3. Joshi, B. S., Gawad, D. H. and Fuhren, H. (1979) *Tetrahedron Letters* 2427.
4. Desai, S. J., Chaturvedi, R. N., Badheka, L. R. and Mulchandani, N. B. (1989) *Indian J. Chem.* 28, 775.
5. Stevenson, R. and Williams, J. R. (1977) *Tetrahedron* 33, 285.
6. Takaoka, D., Watanabe, K. and Hiroi, M. (1976) *Bull. Chem. Soc. Jpn* 49, 3564.
7. Stoianova-Ivanova, B. and Hadjieva, P. (1969) *Phytochemistry* 9, 1549.
8. Benon, J. H., Lester, G. R., Saunders, R. A. and Williams, A. E. (1961) *Trans. Faraday Soc.* 57, 1259.
9. Chakravarti, D. and Debnath, N. B. (1974) *J. Indian Chem. Soc.* 51, 260.
10. Holloway, P. J., Jeffree, C. E. and Baker, E. A. (1976) *Phytochemistry* 15, 1768.

# CONSTITUENTS OF *PIPER ATTENUATUM*

M.A. SUMATHYKUTTY, J. MADHUSUDANA RAO*
*Regional Research Laboratory (CSIR), Trivandrum 695 019, Kerala, India*

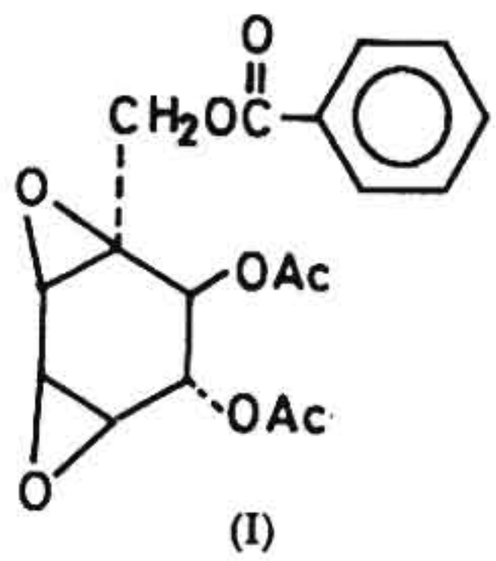
Received April 3, 1992 - Accepted July 6, 1992

Key words: *Piper attenuatum*; crotepoxide; lignans.

Plant. Berries and stem of *Piper attenuatum* Ham. (Piperaceaae), collected from local garden in January 1990 and identified by Tropical Botanic Garden and Research Institute, Palode, Trivandrum. A voucher specimen is available at TBGRI.

Uses in traditional medicine. It has an intense rubifacient effect and is used in poultices for headache and other pains.[1,2] The root is used as an excellent diuretic.[2]

Previously isolated constituents. Crotepoxide (I),[3,4] aristolactams[5] and dioxoaporphines[5] from aerial parts. Piperine, piperlongumine, N-isobutyl-deca-trans-2-trans-4-dienamide and guineensin from the roots.[6] Mono- and sesquiterpenes from the leaves, stem and berries.[7] 8-Hentriacontanol, (–)-galbelgin and pipoxide chlorohydrin from the leaves.[8]




(I)

New-isolated constituents. Berries (600 g): crotepoxide (1.93 g), pipoxide (147 mg), pipoxide chlorohydrin (290 mg), (–)-galbelgin (45 mg), and tetratriacontaneic acid (10 mg). Stem (250 g): pipoxide (590 mg) and β-sitosterol (20 mg).

Berries are good source of crotepoxide known to possess antitumor activity for Lewis lung carcinoma[9,10] and antifeedant activity.[11]

REFERENCES

1. "The Wealth of India. A Dictionary of Indian Raw Materials", Vol. VIII, CSIR, New Delhi, 1969, p 117. 2. Kirtikar K.R., Basu B.D., "Indian Medicinal Plants", Periodical Export Book Agency, Vivek Vihar, Delhi, India, Vol. III, 1981, p 2135. 3. Desai H.K., Gawad D.H., Govindachari T.R., Joshi B.S., Kamat V.N., *Indian J. Chem. 13B*, 97 (1975). 4. Joshi B.S., Gawad D.H., *Tetrahedron Lett.*, 2427 (1979). 5. Desai S.J., Chaturvedi R.N., Badheka L.P., Mulchandani B., *Indian J. Chem. 28B*, 775 (1989). 6. Dasgupta S., Ray A.B., *Indian J. Chem. 17B*, 538 (1979). 7. Sumathykutty M.A., Madhusudana Rao J., *Indian Perfumer 34*, 182 (1990). 8. Sumathykutty M.A., Madhusudana Rao J., *Phytochemistry 30*, 2075 (1991). 9. Kupchan S.M., Hemingway R.J., Coggon P., Mcphaiil A.T., Suin G.A., *J. Am. Chem. Soc. 90*, 2982 (1968). 10. Sumathykutty M.A., Madhusudana Rao J., *Indian Patent*, 1178/DEL/90 dated 27-11-1990. 11. Kubo I., Nakanishi K., *ACS Symposium Series No, 62*, 165 (1977).