PART-I

Chemical constituents of

Pouzolzia indica
Section 1: A brief review of phytochemicals reported from different Pouzolzia species

The genus, *Pouzolzia* of family Urticaceae consists of about 30 species mostly distributed in tropical Asian and African countries; has 12 species in India [1]. The most common species found in India, Bangladesh, Sri Lanka, Burma and Thailand is *Pouzolzia indica* (L.) Gaud. syn. *P. zeylanica* Benn. Most of the species are herbs and used in traditional medicine in different countries. The whole plant of *P. indica* has been used in India against gonorrhoea, syphilis, wounds and snake poison [2,3], and in Vietnam against cough, sore throat, diuretic and galactogogue [3]. The leaves of the plant are also useful. In Malaysia, a poultice of leaves has been used against stomach ache and sores; in Indonesia, the poultice of leaves has been used against ulcers; in Java, the juice or decoction of leaves is used as a galactogogue and the Philippines, the leaves have been used against gangrene [3]. In China, the roots have been used against sores, abscesses, and swellings [3]. In Thailand, the whole plant has been used in the remedy of ailments in female infertility, cancer and inflammation and as emmenagogue and insecticide [4]. The tribes of Darjeeling hills, West Bengal, India have used the roots of *P. hirta* (Blume) in paste for treatment of bone fracture [5].

Phytochemical studies on different *Pouzolzia* species reported the isolation of a few phytochemicals, which are listed in Table 1.1
### Table 1.1: List of phytochemicals from different *Pouzolzia* species

<table>
<thead>
<tr>
<th>Str. No.</th>
<th>Name and structure of phytochemical</th>
<th>Plant source</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[A]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Friedelin</td>
<td><em>P. indica</em></td>
<td>[6]</td>
</tr>
<tr>
<td>2</td>
<td>28- hydroxy-3-fridelanone</td>
<td><em>P. indica</em></td>
<td>[6]</td>
</tr>
<tr>
<td>[B]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Lanceolone</td>
<td><em>P. indica</em></td>
<td>[7]</td>
</tr>
</tbody>
</table>
[C] Coumarins

4  7-Methoxy coumarin \(\text{P. indica} [6]\)

5  Scopoletin \(\text{P. indica} [6]\)

6  6, 7-Dimethyl coumarin \(\text{P. indica} [6]\)

[D] Norlignans

7  Pouzolignan A \(\text{P. occidentalis} [8]\)
Review on Different *Pouzolzia* Species

8  Pouzolignan B  *P. indica*  [8]

![Chemical structure of Pouzolignan B](image)

[E] Phenolics

9  Methyl caffeate  *P. indica*  [6]

![Chemical structure of Methyl caffeate](image)

[F] Steroid

10  β-Sitosterol glucoside  *P. indica*  [6]

![Chemical structure of β-Sitosterol glucoside](image)
Biological activities of crude extracts and isolated pure compounds from different *Pouzolzia* species.

Several biological / pharmacological activities of crude extracts and their pure isolates from different *Pouzolzia* species have been reported. These are discussed briefly.

a) *Antimicrobial activity*

The MeOH extract of the aerial parts of *Pouzolzia hirta* exhibited significant antimicrobial activity against bacteria, *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus* and mould, *Alternaria alternata* with MIC values of 5.0, 3.0, 0.5, 0.5 mg/disc, respectively. The MeOH extract of the aerial parts of *P. indica* also exhibited antimicrobial activity against *Bacillus cereus*, *B. pumilus*, *Staphylococcus aureus* and *Alternaria alternata* with MIC values of 6.0, 7.0, 6.0 and 1.0 mg / disc, respectively in disc diffusion assay [5].

The MeOH extracts of stem and roots of *Pouzolzia mixta* of South African origin exhibited significant antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus fecalis*, *Escherichia coli*, *Shigella*
flexneri and Serratia marcescens with MIC values in the range 3-6 mg / ml in micro dilution assay. Whereas methanolic leaves extract of the plant showed significant antibacterial activity against Aeromonas hydrophila, Proteus mirabilis, Klebsiella pneumonia, Salmonella cholerae-suis and Serratia marcescens with MIC value of 6 mg / ml in each strain in micro dilution method [9].

The EtOH extract of Pouzolzia zeylanica whole plant exhibited significant antibacterial activity against Bacillus subtilis, B. megaterium, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae and Salmonella typhi with inhibition zone diameter in the range of 11.5 – 35.75 mm at the concentration of 1000 µg / disc. The better activity was found against S. aureus and E. coli with inhibition zone diameter of 35.75 and 26.75 mm, respectively. Amoxicillin was used as standard drug [14].

Isoflavone, lanceolone (3) isolated from CHCl₃ extract of P. indica exhibited moderate antibacterial activity against Escherichia coli with MIC value of 32 µg / ml [7].

b) Anti-cancer activity

The methanolic extract of the aerial parts of Pouzolzia indica showed antiproliferative effect against acute promyelocytic leukemia cell lines, NB4 and HT93A with IC₅₀ values of 28.5 and 49.8 µg / ml, respectively in MTT assay [10].

The petroleum ether (PE), EtOAc and MeOH extracts of the leaves of Pouzolzia indica exhibited significant cytotoxic effects against leukaemia CCRF-CEM and multidrug-resistant CEM / ADR5000 subline cells with cell viability in the range 9.75 – 56.35 % at 10 µg / ml in XTT assay. The PE and EtOAc extracts of the leaves also showed cytotoxic effect against HeLa cells with IC₅₀ values of 214.27 ± 1.39 and 199.72 ± 2.07 µg / ml, respectively in MTT assay [11].
The isoflavone, lanceolone (3) isolated from CHCl₃ extract of *Pouzolzia indica* showed *in vivo* cytotoxic activity against brine shrimp nauplii with LC₅₀ value of 24.92 μg / ml [7].

c) *Antioxidant activity*

The petroleum ether (PE), EtOAc and MeOH extracts of the leaves of *Pouzolzia indica* exhibited antioxidant activities against DPPH with IC₅₀ values of 83.37, 67.23 and 0.60 μg / ml, respectively and against lipid peroxidation with IC₅₀ values of >100, >100 and 5.44 μg / ml, respectively [11].

The MeOH extracts of aerial parts of *Pouzolzia hirta* and *P. indica* exhibited antioxidant activities against DPPH scavenging, ABTS scavenging, hydroxyl radical scavenging, Fe (II)-chelating and lipid peroxidation with scavenging of 92 ± 2.1 and 26.8 ± 1.5 %; 64.8 ± 4.5 and ND (not determined) %; 29.1 ± 1.9 and ND %; 4.9 ± 0.3 and 4.9 ± 0.4 %; and 17.2 ± 2.1 and ND, Mm malondialdehyde / mg protein, respectively [5].

d) *Anti-inflammatory activity*

The MeOH extract of *Pouzolzia indica* leaves showed mild anti-inflammatory activity by inhibiting phorbol myristate acetate (PMA) – induced activation of NF- *kB* in HeLa cells with IC₅₀ value of 134.69 μg / ml, compared to standard reference, parthenolide of IC₅₀, 1.97 μg / ml. This extract also exhibited mild anti-inflammatory activity by inhibiting lipopolysaccharide (LPS) – induced release of pro – inflammatory mediators, IL-6 and PGE₂ in human monocytes with IC₅₀ values >50.0 μg / ml (for each), compared to standard drug, hydrocortisone of IC₅₀ of 0.32 and 0.89 μg / ml respectively[11].
e) **Anti-snake venom activity**

The EtOH and aqueous extracts of *Pouzolzia indica* aerial parts exhibited potent *in-vitro* and *in-vivo* anti snake venom activity against Russell viper venom. In *in-vitro* assay, both the extracts showed significant decrease in the activity of phospholipase A2 (PAL A2) in ADP-induced platelet aggregation in dose-dependent manner. At the concentration of 640 μg / ml, EtOH and aqueous extracts inhibited 36.58 and 31.58 % of PAL activity. In *in-vivo* model both EtOH and aqueous extracts of *P. indica* at the doses 250 and 500 mg / kg bw in mice and rats showed significant anti-snake venom activity by neutralising the haemorrhagic and necrotic activity of Russell viper venom. The Ethanolic extract at a dose of 500 mg / kg bw showed better anti venom activity with MHD (minimum haemorrhagic dose) value of 4.5 ± 0.131 mm and MND (minimum necrotizing dose) value of 2.23 ± 0.20 mm, which were comparable to that of standard reference, snake venom antiserum with MHD and MND values of 3.66 ± 0.171 and 1.266 ± 0.236 mm, respectively [12].

f) **FGFRI inhibitory activity**

Fibroblast growth factor receptor 1 (FGFR1) inhibitors are useful for the retention and / or growth of keratin fibers, such as growth of hair in the scalp, face, eyebrows etc. and are used in the formation of a variety of cosmetic and personal care products. The ethanolic extract of *Pouzolzia petandra* aerial parts at the concentration of 0.002% (w/v) exhibited inhibition of FGFR1 expression by 43.37 % in human dermal papilla cells, which was comparable to the standard drugs, L-4-thiazolylation (0.001 %) and cis-6-nonenol (0.002 %) having inhibition of FGFR1 expression of 59.13 and 28.25 %, respectively [13].
Section 2: A brief review of naturally occurring friedelanes reported during the last five years

Friedelanes are a group of pentacyclic triterpenoids mostly found in higher plants and rarely in secondary plants fungi. These metabolites are biogenetically derived from pentacyclic oleananes (13) by migration of methyl groups in D and A rings and due to this reason, these metabolites are also termed as D: A-friedo oleananes [15]. The first member of this group, friedelin (12) was reported from plant by Friedel in 1892 [16] and hence this group of metabolites are known as friedelanes. The structure elucidation of friedelin was completed by Corey and Ursprung in 1955 [17, 18] and its favoured conformation (12a) was determined by single-crystal X-ray diffraction analysis in 1991 [19, 20].
So far more than 600 friedelanes have been reported from plants of different families. On the basis of their structural features, they are subdivided into five major classes namely ‘normal’ friedelanes (Type I), seco friedelanes (Type II), nor-friedelanes (Type III), dimeric and trimeric friedelanes (Type IV), and rearranged friedelanes (Type V) as shown by general skeletal structures.
Friedelanes are commonly found in plants as free state or ester and glycosides. The published work in this area reported that they are mainly distributed in plant families such as Celastraceae, Hippocrateaceae, Euphorbiaceae, Flacourtiaceae and Guttiferae, the first two families bring the richest sources. Usually types I and II are found in aerial parts and types III-IV in roots and type V in both aerial parts and roots. To date, about 220 normal friedelanes, 40 nor friedelanes, 50 seco-friedelanes, 70 dimeric / trimeric friedelanes and 60 rearranged friedelanes have been reported from nature. Usually seco-friedelanes are found by cleavage of 2, 3-bond in A-ring and 20, 21-bond in E ring of friedelanes. Nor-friedelanes are found in nature by elimination of one methyl group either from C-5 or C-20 position. Dimeric and trimeric friedelanes are found by joining of monomeric units at C-3 and C-4 positions by ether bridges. Rearranged friedelanes are usually derived in nature by formation of spiro centre between C and D rings.

Several review articles on triterpenoids and its sub-class, friedelanes highlighting structures, distribution NMR data and bioactivities are available covering the reported data up to 2010 [1, 21-25]. The present investigator have reviewed the new friedelanes reported in the last five years (2009-2014) to highlight the diversity in their skeletal structures, distributions in different plant families and pharmacological activities. The list of new friedelanes reported in 2009-2014 is provided in Table 1.2.
Table 1.2. List of new friedelanes reported from plants during the period, 2009-2014

<table>
<thead>
<tr>
<th>Str. No.</th>
<th>Name and structure</th>
<th>Plant source(s)</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>[A]</td>
<td>‘Normal’ Friedelanes</td>
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<tr>
<td>19</td>
<td>Monospermol</td>
<td><em>Celastrus monospermus</em></td>
<td>[26]</td>
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<tr>
<td></td>
<td></td>
<td>(Celastraceae)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>28-Hydroxypolpunonic acid</td>
<td><em>Euonymus hederaceus</em></td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Celastraceae)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>16α-Hydroxy-1, 3-dioxofriedelane</td>
<td><em>Salacia elliptica</em></td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Hippocrateaceae)</td>
<td></td>
</tr>
</tbody>
</table>
22 7α, 30-Dihydroxyfriedelan-3-one \(\text{Salacia hainanensis}\) (Hippocrateaceae) [29]

\[\text{Structure Image}\]

23 3α-Hydroxyfriedelan-25-al \(\text{Drypetes inaequalis}\) (Euphorbiaceae) [30]

\[\text{Structure Image}\]

24 3β-Hydroxyfriedelan-25-al \(\text{Drypetes paxii}\) (Euphorbiaceae) [31]

\[\text{Structure Image}\]

25 12α-Hydroxyfriedelane-3, 15-dione \(\text{Drypetes paxii}\) (Euphorbiaceae) [31]

\[\text{Structure Image}\]
26 3β-Hydroxyfriedelane-7, 12, 22-trione  
*Drypetes laciniata*  
(Euphorbiaceae)  

27 1β-Hydroxy friedelin  
*Garcia parviflora*  
(Euphorbiaceae)  

28 3β-Hydroxy friedelan-23-oic acid  
*Garcia parviflora*  
(Euphorbiaceae)  

29 3β, 23-Epoxy-friedelan-28-oic acid  
*Calophyllum inophyllum*  
(Guttiferae)
30 3-Oxo-11α-hydroxyfriedelane  *Myrica rubra*  
(Myricaceae)  [35]

31 3-Oxo-12α,29-dihydroxy-friedelane  *Maytenus gonoclada*  
(Celastraceae)  [36]

32 7β-Hydroxy-3-oxo-friedelan-28-oic acid  *Celastrus vulcanicola*  
(Celastraceae)  [37]

33 7β-Hydroxy-3-oxo-friedelan-28-oic acid methyl ester  *Celastrus vulcanicola*  
(Celastraceae)  [37]
34 $7\beta, 29$-Dihydroxy-3-oxo-friedelane

35 $1\beta, 30$-Dihydroxy-3-oxo-friedelane

36 $1\beta$-Hydroxy-3-oxo-friedelan-30-oic acid

37 3-Oxo-friedelan-28,30-olide

*Maytenus jelskii* (Celastraceae)
38. 3β,24-Epoxy-2α,3α-dihydroxy friedelan-29-oic acid
   *Maytenus jelskii* [37]

39. 2α-Acetoxy-3β,24-epoxy-3α-hydroxy friedelan-29-oic acid methyl ester
   *Maytenus jelskii* [37]

40. 3α-Hydroxyfriedelan-28-oic acid
   *Maytenus jelskii* [37]

41. Ovalifolone A
   *Garcinia ovalifolia* (Guttiferae) [38]
42 Ovalifolone B \textit{Garcinia ovalifolia} (Guttiferae) \[38\]

43 3-O-Benzoylpluricostatic acid \textit{Trigonostemon xyphophyloides} (Euphorbiaceae) \[39\]

44 25-Hydroxyfriedelane-3, 21-dione \textit{Siphonodon celastrineus} (Celastraceae) \[40\]

45 25-Benzoyloxyfriedelane-3, 21-dione \textit{Siphonodon celastrineus} (Celastraceae) \[40\]
46 Maytensifolone  
*Maytenus distichophylla* (Celastraceae)  

47 3α-(E)-p-Coumaroyloxy-friedelan 7-one  
*Drypetes hoaensis* (Euphorbiaceae)  

48 3α-(E)-Caffeoyloxy friedelan-7-one  
*Drypetes hoaensis* (Euphorbiaceae)  

[B] Secofridelane  

49 1, 2-Dehydro-2, 3-secofridelan-3-oic acid  
*Garcia parviflora* (Euphorbiaceae)  

Natural Products Chemistry
[C] **Nor-friedelanes**

50 23-Norblepharodol  
*Maytenus retusa*  
(Celastraceae)  

51 21-Oxopristimerine  
*Maytenus retusa*  
(Celastraceae)  

52 Glaucalactone  
*Caloncoba glauca*  
(Flacourtiaceae)  

53 (16β)-16-Hydroxypristimerin  
*Maytenus salicifolia*  
(Celastraceae)
54 4β-Hydroxy-23-nor-friedel-3-one

\[ \text{Drypetes hainanensis} \]
(Euphorbiaceae) [46]

55 23, 24-Di-nor-1(10), 3(4), 5(6), 7(8)-octadehydro-3,4,6-trihydroxy-2-oxo friedelan-29-oic acid

\[ \text{Tripterygium wilfordii} \]
[46]

[D] Rearranged friedelanes

66 12-Nor-Dinor-1(10),3(4), 5(6), 7(8)-octadehydro-3-hydroxy 2,21-dioxo-29-oic-acid methyl ester

\[ \text{Celastrus hypoleucus} \]
(Celastraceae) [48]
Pharmacological activities

Several naturally occurring friedelanes exhibited different pharmacological activities. Some of these bio-activities are discussed briefly.

a. Anticancer / antitumor activity

Demethylzeylasteral (67) isolated from *Kokoona zeylanica* inhibited the tumor growth of human hepatoblastoma and B16-F10 melanoma cells in *in vivo* assays. It also inhibited the proliferation of vascular endothelial cells about 30 times more effectively than it does for the proliferation of human tumor cells [49].

3β, 23-Epoxyfriedelan-28-oic acid (29), canophyllic acid (68) and 3-oxofriedelan-28-oic acid (69) isolated from *Calophyllum inophyllum* showed significant antiproliferative effect against human leukemia HL-60 cells with IG₅₀ values of 10.66 ± 1.20, 4.64 ± 0.27 and 2.67 ± 0.49 μM, respectively in trypan blue exclusion assay [34].

1, 2-Dehydro-2,3-seco-friedelan-3-oic acid (49) and friedelin-3, 4-lactone (70) isolated from the leaves of *Garcia parviflora* showed moderate cytotoxicity against central nervous Glia U 251 cancer cells with IC₅₀ of 36.8 and 17.1 μM, respectively, when compared to the positive control adriamycin (IC₅₀, 0.3 μM) in SRB assay [33].

(16β)-16-Hydroxypristimerin (53) isolated from *Maytenus salicifolia* exhibited potent antiproliferative effect on human cancer HeLa, A-549 and HL-60 cells with IC₅₀ values of 2.2, 3.2 and 2.7 μM, respectively in MTT assay [45].

21β-Hydroxyfriedelan-3-one (71) isolated from the stem of *Siphonodon celastrineus* showed *in vitro* cytotoxicity against Hep G2 cells with IC₅₀ value of 10.2 μM in MTT assay [40].
3α-(E)-p-Coumaroyloxyfriedelan-7-one \((47)\) and 3α-(E)-caffeoxyloxyfriedelan-7-one \((48)\) isolated from the roots of *Drypetes hoaensis* showed strong cytotoxicity against Hep G2 cell line with IC\(_{50}\) values of 3.1 and 0.1 μM, respectively [42].

Friedelane-3, 7-dione \((72)\) isolated from *Drypetes hainanensis* leaves and stems showed moderate cytotoxicity against cancer BEL-7402, A-549 and HL-60 cells with growth inhibitory rate of 4.6, 21.1 and 43.1 %, respectively at the concentration of 10\(^{-5}\) M / L [46].

Celastrol \((73)\) isolated from *Maytenus aquifolium* and *Salacia campestris* showed potent anticancer activity against Erb B2 overexpressing breast cancer cells such as SK Br-3, BT-474, MCF-7, 21 MT-1 cells [50].

Celastrol \((73)\) also strongly inhibited the proliferation of androgen-independent prostate cancer cell lines, PC-3, DU145 and CL1, with IC\(_{50}\) values in the range of 1-2 μM. It suppressed the cell migration and invasion. It induced apoptosis of the cancer cells by increasing sub G-1 population, caspase activation and PARP cleavage. Furthermore, it increased cellular lκBα and various NF-κBw target genes in tumor tissues *in vivo* study in PC-3 xenograft nude mouse model [60].

Pristimerin \((74)\) isolated from *Maytenus chuchuhuasca*, *Maytenus laevis* and *Salacia cochinchinensis* induced apoptotic cell death in MDA-MB-231 breast cancer cells in a caspase-dependent manner, as well as in human acute myeloid leukemia cells [51, 52]. It also showed anticancer activity in Epidermal Growth Factor Receptor 2 (HER-2)- positive SKBR3 human breast cancer cells in dose-and time dependent manner with IC\(_{50}\) value of 2.4 μM after 24 h [53]. It also inhibited the cell growth of cancer A-594, MCF-7, Hep G2 and Hep G3 cells with IC\(_{50}\) values of 042-0.61 μM [51]. It inhibited the growth of breast cancer MDA-MB-435 and K-562 cells with IC\(_{50}\) values of 0.55 and 3.2 μM, respectively [54].
b. Antimicrobial activity

Triterpenes, 12α-hydroxyfriedelane-3,15-dione (25), 3β-hydroxyfriedelan-25-al (24), friedelin (75), friedelan-7-one (76) and friedelane-3, 15-dione (77) isolated from Drypetes paxii showed strong antibacterial activity against Gram positive bacterium, Staphylococcus aureus at the concentration of 200 µg / ml in DMSO [31].

3-Oxo-12α-hydroxyfriedelane (78) isolated from Maytenus gonoclada showed antifungal activity against Candida albicans in disk diffusion and micro dilution assay [36].

3β-Hydroxyfriedelane-7,12,22-trione (26) isolated from Drypetes laciniata showed antibacterial activity against Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi with MIC values of 256, 256 and 512 µg / ml, in XTT colorimetric assay as per method of Pettit et al. [55], modified by Kuete et al. [56, 32].

c. Antidiabetic activity

7β-Hydroxy-3-oxofriedelan-28-oic acid (33) and 7β,29-dihydroxy-3-oxofriedelane (34) isolated from Celastrus vulcanicola exhibited anti-diabetic activity by increasing insulin-mediated signaling in human hepatic cells, Huh-7 at the concentration of 100 µM [37].

7α, 30-Dihydroxyfriedelan-3-one (22) isolated from Salacia hainanensis roots showed in vitro α-glucosidase inhibitory activity with an IC₅₀ value of 0.87 µM [29].

d. Photosynthetic inhibitory activity

Epifriedelinol (79) and canophyllol (80) isolated from Celastrus vulcanicola exhibited significant herbicide activity by reducing the biomass production in the weed, Physalis ixocarpa in a concentration dependent manner, with IC₅₀ values of 82 and 124 µM, respectively. Epifriedelinol acts as an energy
transfer inhibitor, enhancing the light activated Mg\textsuperscript{2+} - ATPase, while canophyllol behaves as a Hill reaction inhibitor [57].

e. Anti-allergic activity

Celastrol (73) isolated from several Celastrus species showed potent anti-allergic activity by decreasing the secretion of β-hexosaminidase, release of histamine and expression of Th2 cytokines, calcium influx and cell adhesion in antigen stimulated RBL 2H3 cells [58].

i. Immunosuppressant activity

Correolide (81) isolated from Spachea correae exhibited immunosuppressant activity by blocking voltage-gated potassium channel with an IC\textsubscript{50} value of 86 nM. It also inhibited human T cells KV 1.3 proliferation with EC\textsubscript{50} value of 307 nM. T cell activation events, such as anti CD3-induced calcium elevation, IL-2 production and proliferation were inhibited by correolide in a dose-dependent manner. It may be noted that KV 1.3 is specifically expressed on human lymphocytes, where it controls membrane potential and calcium-influx [59].
Review on Friedelanes

R = O, R' = R'' = H

R = H, R' = O, R'' = H

R = O, R' = H, R'' = O

R = H

R = Me

R = O, R' = R'' = H₂

R = H₂, R' = O, R'' = H₂

R = O, R' = H₂, R'' = O
Section 3: A brief history, taxonomical description and classification of

*Pouzolzia indica*

Plate-1. Photographs of *Pouzolzia indica* (family: Urticaceae)
Pouzolzia indica Gaud, native of Indian subcontinent, is grown wild throughout in the state of Tripura and other North Eastern states of India [1] (Plate 1). It has few synonyms namely Pouzolzia indica var. alienta Wedd, Pouzolzia indica var. angustifolia (Wright) Wedd, Pouzolzia procumbens Wright, Pouzolzia zeylanica (L.) Benn and Parietaria zeylanica L. [1]. These plants are erect or prostrate, pubescent herbs with alternate, ovate to lanceolate, membranous leaves, flowers in small axillary androgynous clusters and white, ovoid achene.

The systematic position of Pouzolzia indica in botanical taxonomy as per reported in Bot. Voy. 563,1826 [61].

<table>
<thead>
<tr>
<th>Class</th>
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<tr>
<td>Sub-class</td>
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</tr>
<tr>
<td>Series</td>
<td>Curvembryae</td>
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<tr>
<td>Order</td>
<td>Amaranthales</td>
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<tr>
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<td>Urticaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Pouzolzia</td>
</tr>
<tr>
<td>Species</td>
<td>Pouzolzia indica (L.)</td>
</tr>
</tbody>
</table>
Section-4: Isolation and structure elucidation of pouzolzin

Isolation:

Pouzolzin (82) was isolated as amorphous powder from EtOAc soluble fraction of MeOH extract of *Pouzolzia indica* whole plants by silica gel column chromatography. It was homogeneous on TLC [Silica gel G, *R* sub f = 0.58 in PE-EtOAc, 5 : 1]

Structure elucidation:

a) Molecular formula:

The molecular formula of the compound was determined as C_{42}H_{72}O_{4} from its quasi-molecular ion at *m/z* 663.5322 [M + Na]+ (Calcd for C_{42}H_{72}O_{4}.Na: 663.5328) in its positive mode HR-FAB-MS and analysis of its $^{13}$C/DEPT-NMR data.

b) The IR spectrum:

The IR spectrum of the compound in KBr (Fig. 1.1) showed absorption bands for hydroxyl (3434 cm$^{-1}$), ester (1735 and 1077 cm$^{-1}$) and carbonyl (1715 cm$^{-1}$) functions.

c) The $^1$H-NMR spectrum:

The $^1$H-NMR spectrum of the compound in CDCl$_3$ (Fig. 1.2) (Table 1.3) showed proton signals for six tertiary methyls [δ$_H$ 0.82, 0.95, 0.99, 1.00, 1.04 and 1.18 (each, 3H, s)], one secondary methyl [δ$_H$ 0.88 (3H, d, *J* = 6.8 Hz), one methine proton [δ$_H$ 2.25 (1H, q, *J* = 6.8 Hz), one oxymethine proton [δ$_H$ 3.48 (1H, dd, *J* = 3.5 m and 4.0 Hz), and one methylene protons [δ$_H$ 2.38 (1H, ddd, *J* = 13.7, 5.2 and 2.5 Hz) and 2.30 (1H, ddd, *J* = 13.5, 6.5, 6.0 Hz) suggesting its hydroxy-friedelane structure [62-64]. The $^1$H-NMR spectrum also showed a primary methyl [δ$_H$ 0.86 (3H, t, *J* = 7.0 Hz)] and methylene
signals [δH 1.25 (18H, brs), 1.56 (2H, m) and 3.64 (2H, t, J = 7.0 Hz] indicating the presence of a long straight chain alkoxy group. The downfield chemical shifts of C-24, C-25 and C-26 methyl protons (δH 1.00, 0.82 and 1.18) in friedelane nucleus due to 1,3 diaxial interactions with the hydroxyl group suggested the location and orientation of hydroxyl group at C-7 position.

d) The 13C-NMR spectrum

The 13C-NMR spectrum of the compound in CDCl3 (Fig.1.3) (Table 1.3) recorded 39 carbon signals, which on DEPT experiments showed the signals for 8 methyl, 18 methylene, 5-methine and 8 quaternary carbons. The seven methyl carbon resonances at δC 6.8, 16.2, 17.9, 18.7, 20.3, 29.7 and 35.0 along with ester carbonyl resonance at δC 174.4, a carbinol methine carbon at δC 67.5 and a ketone carbonyl carbon at δC 213.9 suggested its hydroxy-3-oxo-friedelan acid ester structure [62-65]. The carbon resonances at δ 14.1(q), 22.7(t), 25.7(t), 32.1(t), 32.7(t), 29.4 (2C,t), 29.6 (2C,t), 29.9 (2C,t) and 61.5(t) indicated that an n-dodecyl (lauryl) group was present as an alkyl group in the compound [66]. The downfield carbon resonances at C-17 (δ 37.4), C-13 (δ 42.2), C-22 (δ 39.2) and C-19 (δ 35.3) compared to that of friedelin (Ia) suggested the attachment of ester group at C-17 position [65]. The downfield chemical shifts of C-24, C-25 and C-26-methyl carbons was possibly due to 1,3-diaxial interactions with the hydroxyl group at C-7 position.

e) The HMBC spectrum:

The HMBC spectrum of the compound showed the correlation of H-18 (δH 1.74) with C-17 (δC 37.4) confirming the attachment of ester group at C-17 (Fig.1.4).
f) **The NOESY spectrum:**

The NOESY spectrum of the compound showed correlation between H$_2$-6 ($\delta_H$ 1.68 and 1.39) and H-7 ($\delta_H$ 3.48) and between H-7 ($\delta_H$ 3.48) and H-8($\delta_H$ 1.91) supporting the assignment of hydroxyl group at C-7 position (Fig.1.4).

g) **The FAB-Mass spectrum:**

The FAB-MS of compound (Fig. 1.5) displayed mass ions at m/z 663 [M + Na]$^+$, 648 [M Na - Me]$^+$, 296, 278, 273, 221, 203 and 167 supporting its lauryl-hydroxy-oxo-friedelan-28-oate structure (Scheme 1.1).
h) The alkaline hydrolysis:

The compound on alkaline hydrolysis with 5% aqueous ethanolic KOH afforded lauryl alcohol (82b) and 7β-hydroxy-3-oxo-friedelan-28-oic acid (82c). Both the hydrolysis products 82b and 82c were identified by their IR and FAB-MS spectra as well as co-TLC of 82b with an authentic sample of lauryl alcohol.
i) **The IR spectrum of 82c:**

The IR spectrum of compound 82c in KBr (Fig. 1.6) showed absorption bands for hydroxyl (3434 cm\(^{-1}\)), carbonyl (1712 cm\(^{-1}\)) and carboxyl (1690 cm\(^{-1}\)) functions.

j) **The FAB-MS of 82c:**

The FAB-MS of compound 82c (Fig. 1.7) showed mass ions at \(m/z\) 495 [M + Na]\(^+\), 480 [MNa – Me]\(^+\), 289, 221, 203 and 95 supporting its 7-hydroxy-3-oxo-friedelan-28-oic acid skeletal structure (Scheme-1.2)

![Scheme 1.2. Plausible FAB-Mass Fragmentation of 82c](image-url)
k) Conclusion:

Considering all these evidence, the structure of the compound, pouzolzin was elucidated as 28-dodecyl-7β-hydroxy-3-oxo-friedelan-28-oate (82) (Fig. 1.8). It is a new natural product.

Figure 1.8. Structures of compounds 82, 82a-82c
### Table 1.3: NMR spectral data of compound 82 in CDCl₃ (δ in ppm)

(600 MHz for $^1$H and 150 MHz for $^{13}$C)

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<th>δ$_H$</th>
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<td>2.38 ddd (13.7, 5.2, 2.5) 2.30 ddd</td>
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<td>1.25 brs</td>
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<td>0.86 t (6.8)</td>
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<td>20</td>
<td>28.2 (C)</td>
<td>–</td>
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*a Assignment based on DEPT, HSQC and HMBC experiments  
*b $J$ in Hz in parentheses
Fig. 1.1. IR spectrum of punzolin (82) in KBr
Fig. 1.2. 1H-NMR spectrum of puzolin (82) in CDCl3.
Fig. 1.2b. $^1$H-NMR spectrum (expanded) of poutozolam (82) in CDC$_3$. 

1H-NMR in CDC$_3$. 

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Fig. 1.3a  $^{13}$C-NMR spectrum of pouzzalin (82) in CDCl$_3$
Fig. 1.3c. 1H-NMR spectrum (expanded) of porazolin (82) in CDCl₃

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Fig. 4: NOESY spectrum of ponzosin (82)
Fig. 1. a-b. NOESY spectrum of panolizou (82)
Fig. 1.5. FAB-MS spectrum of pozolzalin (82)
Fig. 1.6. IR spectrum of compound \textbf{82c} in KBr
Section 5: Isolation and structure elucidation of friedelin

Isolation:

Friedelin (82a) was isolated as colourless needles. mp 262ºC from CH₂Cl₂ soluble fraction of MeOH extract of *Pouzolzia indica* whole plants by silica gel column chromatography. It was homogeneous on TLC [Silica gel G, \( R_f : 0.50 \) in CH₂Cl₂-PE, 3 : 1].

Structure elucidation:

a) Molecular formula:

The molecular formula of the compound was determined as C₃₀H₅₀O from its mass ion at \( m/z \) 427.3938 [M + H]⁺ (Calcd for C₃₀H₅₁O : 427.3940) in HR-FAB-MS and analysis of its \(^{13}\)C-/DEPT NMR data.

b) The IR spectrum

The IR spectrum of the compound in KBr (Fig. 1.9) showed absorption bands for carbonyl (1717 cm\(^{-1}\)) function.

c) The \(^1\)H-NMR spectrum

\(^1\)H-NMR spectrum of the compound in CDCl₃ (Fig. 1.10) (Table 1.4) showed proton signals for seven tertiary methyls [\( \delta_H \) 0.73, 0.87, 0.95, 1.00, 1.01, 1.05 and 1.18 (each 3H, s)], one secondary methyl [\( \delta_H \) 0.88 (3H, d, \( J = 7.0 \) Hz), one downfield methine [\( \delta_H \) 2.25 (1H, m)], and one methylene [\( \delta_H \) 2.31 and 2.40 (each 1H, m)], characteristic of 3-oxo-friedelane [68].

d) The \(^{13}\)C-NMR spectrum:

The \(^{13}\)C-NMR spectrum of the compound in CDCl₃ (Fig. 1.11) (Table 1.4) showed 30 carbon signals, which on DEPT experiments revealed for 8-methyl, 11 methylene, 4 methine and 7 quaternary carbons. The carbonyl
carbon resonance at $\delta_c$ 213.2 and methyl carbon resonances at $\delta_c$ 6.8, 14.7, 17.9, 18.7, 30.5, 32.1 and 35.0 corroborated 3-oxofriedelane structure [65].

e) The FAB-MS:

The FAB-MS of the compound (Fig. 1.12) showed mass ions at $m/z$ 449 [M + Na]$^+$, 427 [M + H]$^+$, 425 [M – H]$^+$, 412 [MH – CH$_3$]$^+$, 220, 273, 205, 191 and 152. The formation of these mass ions could be rationalized by considering its 3-oxofriedelane or friedelin structure (Scheme 1.3)

Scheme 1.3. Plausible FAB-Mass Fragmentation of 82a.
f) Conclusion:

The spectral data of the compound were very similar to those reported for friedelin [67, 68]. On basis of these evidence, the structure of the compound was established as 3-oxofriedelane or friedelin (82a) (Fig. 1.13). It is a known natural product.

Fig. 1.13. Structure of compound 82a
Table 1.4. $^1$H- (600 MHz) and $^{13}$C-(150 MHz) NMR spectral data of compound 82a in CDCl$_3$ ($\delta$ in ppm)

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</tr>
<tr>
<td>4</td>
<td>58.2 (CH)</td>
<td>2.25 (m)</td>
</tr>
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<td>5</td>
<td>42.2 (C)</td>
<td>–</td>
</tr>
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Fig. 1.9. IR spectrum of friedelin (82a) in KBr.
Fig. 1.10 1H-NMR spectrum of finidolin (82a) in CDCl$_3$
Fig. 1.10a. ¹H-NMR spectrum (expanded) of friedelin (82a) in CDCl₃
Fig. 1.10b. \(^1\)H-NMR spectrum (expanded) of friedelin (82a) in CDCl\(_3\)
Fig. 1.11. \(^{13}\text{C}-\text{NMR}\) spectrum of friedelin (82a) in CDCl\(_3\).
Fig. 1 IDP. $^{13}$C-DEPT NMR spectrum of friedelcin (82a) in CDCl$_3$. 

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Fig. 1.2. FAB-MS of fiedulin (82a)

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Section 6: Isolation and structure elucidation of trichadonic acid

Isolation:

Trichadonic acid (83) was isolated as amorphous solid, mp. 249-251°C, from both CH₂Cl₂ and EtOAc soluble fractions of MeOH extract of *Pouzolzia indica* whole plant by repeated column chromatography over silica gel. It was homogeneous in TLC [Silica gel G, \( R_f = 0.45 \) in CH₂Cl₂-EtOAc, 6 : 1].

Structure elucidation:

a) Molecular formula:

The molecular formula of the compound was assigned as C₃₀H₄₈O₃ from its mass ion at m/z 457.3678 [M + H]⁺ (Calcd for C₃₀H₄₉O₃; 457.3682) in HR-FAB-MS and analysis of its ¹³C-NMR data.

b) The IR spectrum

The IR spectrum of the compound in KBr (Fig. 1.14) showed the absorption bands for carboxyl (3381 and 1685 cm⁻¹) and carbonyl (1710 cm⁻¹) functions.

c) The ¹H-NMR spectrum

The ¹H-NMR spectrum of the compound in CDCl₃ (Fig. 1.15) (Table 1.5) recorded signals for six tertiary methyls [\( \delta_H \) 0.73, 0.93, 0.95, 1.00, 1.18 and 1.23 (each 3H, s)], one secondary methyl [\( \delta_H \) 0.88 (3H, d, \( J = 6.6 \) Hz)], one downfield methine [\( \delta_H \) 2.24 (1H, q, \( J = 6.8 \) Hz)], and one methylene [\( \delta_H \) 2.38 (1H, dt) and 2.31(1H, m)] suggesting its 3-oxo-friedelane like structure [67].
d) **The $^{13}$C-NMR spectrum:**

The $^{13}$C-NMR spectrum of the compound in CDCl$_3$ (Fig. 1.16) (Table 1.5) showed 30 carbon signals, which on DEPT experiments revealed for 7-methyl, 11 methylene, 4 methine and 8 quaternary carbons. The carbon resonance at $\delta_C$ 213.0 was assigned to a carbonyl group of a cyclohexane system and carbon resonance at $\delta_C$ 181.3 was assigned for a carboxyl group. The methyl carbon resonance at $\delta_C$ 6.80 coupled with a secondary methyl protons at $\delta_H$ 0.88 suggested its 3-oxofriedelane structure [64].

e) **The FAB-MS spectrum:**

The FAB-MS of the compound (Fig. 1.17) displayed mass ions at $m/z$ 457 $[M + H]^+$, 442 $[MH – Me]^+$, 303, 235 and 191, which could be rationalized by its friedelane skeletal structure having a carboxyl group at C-13 / C-14 position. (Scheme 1.4)

![Scheme 1.4: Plausible FAB-Mass Fragmentation of 83](image-url)
f) Conclusion:

The spectral data of the compound were very similar to those reported for trichadonic acid [69]. Therefore, the structure of the compound was elucidated as 3-oxo-friedelan-27-oic acid or trichadonic acid (83) (Fig. 1.18). It is a known natural product but its isolation is reported first time from this plant. Earlier it was reported from *Trichademia zeylanica* [69], *Hydnocarpus octandra* [70, 71] and *Caloncoba glauca* [62] from family Flacourtiaceae.

![Fig. 1.18 Structure of compound 83](image)
Table 1.5. $^1$H- (600 MHz) and $^{13}$C-(150 MHz) NMR spectral data of compound 83 in CDCl$_3$ (δ in ppm)

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<td>18.2 (CH$_3$)</td>
<td>0.93 s</td>
</tr>
<tr>
<td>26</td>
<td>22.3 (CH$_3$)</td>
<td>1.18 s</td>
</tr>
<tr>
<td>27</td>
<td>181.3 (C)</td>
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</tr>
<tr>
<td>28</td>
<td>31.8 (CH$_3$)</td>
<td>1.23 s</td>
</tr>
<tr>
<td>29</td>
<td>30.5 (CH$_3$)</td>
<td>0.95 s</td>
</tr>
<tr>
<td>30</td>
<td>35.0 (CH$_3$)</td>
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Fig. 1.14. IR spectrum of trichadonic acid (83) in KBr
Fig. 1.15. 1H-NMR spectrum of tricholate acid (83) in CDCl₃.
Fig. 11.5a. 1H-NMR spectrum (expanded) triadaconic acid (8S) in CDCl₃.
Fig. 1.15b. 1H-NMR spectrum (expanded) of trihaloacid (63) in CDCl₃.
Fig. 1.17. FAB-MS of trichodonic acid (83)
Section 7: Isolation and structure elucidation of 1-hentriacontanyl palmitate

Isolation:

1-Hentriacontanyl palmitate (84) was isolated as an amorphous solid, mp 72-74°C from CH$_2$Cl$_2$ soluble fraction of MeOH extract of *Pouzolzia indica* whole plants by silica gel column chromatography. It was homogeneous in TLC [Silica gel G, $R_f = 0.62$ in PE-PhH, 4 : 1]

Structure elucidation:

a) Molecular formula:

The molecular formula of the compound was determined as C$_{47}$H$_{95}$O$_2$ from its mass ion at $m/z$ 691.7330 [M + H]$^+$ (Calcd for C$_{46}$H$_{95}$O$_2$: 691.7332) in its HR-FAB-MS and analysis of $^{13}$C-NMR data.

b) The IR spectrum:

The IR spectrum of the compound in KBr (Fig. 1.19) showed the absorption band for ester (1736 cm$^{-1}$ and 1175 cm$^{-1}$) function.

c) The $^1$H-NMR spectrum:

The $^1$H-NMR spectrum of the compound in CDCl$_3$ (Fig. 1.20) (Table 1.6) displayed signals for two primary methyls [$\delta_H$ 0.88 (6H, t, $J = 7.0$ Hz)] and methylenes [$\delta_H$ 1.25 (80H, br s), 1.56 (4H, m), 2.28 (2H, t, $J = 7.0$ Hz) and 4.05 (2H, t, $J = 7.0$ Hz)] suggesting its straight chain aliphatic ester structure [66].
d) The $^{13}$C-NMR spectrum:

The $^{13}$C-NMR spectrum of the compound in CDCl$_3$ (Fig. 1.21) (Table 1.6) recorded 15 carbon signals, which on DEPT experiments showed for 1 methyl, 13 methylene and 1 quaternary carbons. The methyl carbon resonance at $\delta_C$ 14.1 (2C), methylene carbon resonances at $\delta_C$ 22.7, 25.0, 25.9, 28.6, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 34.4 and 64.4 and a quaternary carbon resonance at $\delta_C$ 174.0 corroborated its straight chain ester structure [72].

e) The FAB-MS:

The FAB-Mass spectrum of the compound (Fig. 1.22) recorded mass ions at $m/z$ 691 [M + H]$^+$, 663 [MH - 28]$^+$, 635 [661 - 28]$^+$, 607 [635 - 28]$^+$, 451 [hentriacontanyl]$^+$, 435, 257 and 239. The formation of these mass ions could be rationalized by its hentriacontanyl palmitate structure (Scheme 1.4) [74].

f) The alkaline hydrolysis:

The alkaline hydrolysis of the compound with 2M aqueous methanolic KOH under inert atmosphere of nitrogen afforded palmitic acid (84a), C$_{16}$H$_{32}$O$_2$, (M$^+$ 256), mp 63$^\circ$C (lit mp 63$^\circ$C [74]) and 1-hentriacontanol (84b), C$_{31}$H$_{64}$O (M$^+$ 452), mp 87-88$^\circ$C (lit mp 87-87.5$^\circ$C [75]).
The $^1$H-NMR spectrum of 84a in CDCl$_3$ (Fig. 1.23) showed proton signals for one methyl \( [\delta_H 0.88 (3H, t, J = 7.0 \text{ Hz})] \), methylenes \( [\delta_H 1.26 (24H, \text{ br s}), 1.60 (2H, \text{ m}) \) and \( \delta_H 2.35 (2H, t, J = 7.0 \text{ Hz})] \) and carboxyl \( [\delta_H 11.84 (1H, \text{ br s})] \) suggesting its straight chain fatty acid structure [66].

**h) Conclusion:**

On the basis of the foregoing evidence, the structure of the compound was elucidated as 1-hentriacontanyl palmitate (84) (Fig. 1.24). It is a new natural product.

![Fig. 1.24. Structures of compounds 84, 84a and 84b.](image-url)
Table 1.6. $^1$H- (600 MHz) and $^{13}$C-(150 MHz) NMR spectral data of compound 84 in CDCl$_3$ ($\delta$, ppm, $J$/ Hz)

<table>
<thead>
<tr>
<th>C/H No</th>
<th>$\delta_C$ (DEPT)</th>
<th>$\delta_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>174.0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>34.4</td>
<td>2.28 (t, $J = 7.0$)</td>
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<tr>
<td>3</td>
<td>25.0-29.7</td>
<td>1.56 (m)</td>
</tr>
<tr>
<td>4-14</td>
<td>25.0-29.7</td>
<td>1.25 (br s)</td>
</tr>
<tr>
<td>15</td>
<td>22.7</td>
<td>1.25 (br s)</td>
</tr>
<tr>
<td>16</td>
<td>14.1</td>
<td>0.88 (t, $J = 7.0$)</td>
</tr>
<tr>
<td>1'</td>
<td>64.4</td>
<td>4.05 (t, $J = 7.0$)</td>
</tr>
<tr>
<td>2'</td>
<td>31.9</td>
<td>1.56 (m)</td>
</tr>
<tr>
<td>3'-29'</td>
<td>25.0-29.7</td>
<td>1.25 (br s)</td>
</tr>
<tr>
<td>30'</td>
<td>22.7</td>
<td>1.25 (br s)</td>
</tr>
<tr>
<td>31'</td>
<td>14.1</td>
<td>0.88 (t, $J = 7.0$)</td>
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</table>
Fig. 1.19. IR spectrum of 1-hentriacontyl palmitate (84) in KBr
Fig. 1.20. 1H-NMR spectrum of 1-beta-acetoxy-2-phenylisoquinoline (84) in CDCl₃
Fig. 1.21: 13C-NMR spectrum of 1-herbaacetyl palmitate (44) in CDCl₃.

TU 21 10 13C-NMR in CDCl₃

1740.3
Fig. 1.22. FAB-MS 1-heptacontyl palmitate
References


