Chapter 2

Long Chain Alkylated α-Methylene-γ-butyrolactone from *Artabotrys odoratissimus* Fruits
2.1. Introduction

*Solanum khasianum* Clarke (Solanaceae), a native plant of India, is a source of raw material for steroid drug industry [Fig. 2.1]. Its berries contain about 1.80-3.45% solasodine\(^1\). Major limiting factor of commercial cultivation [Fig. 2.2] of this plant is the leaf blight disease caused by *Alternaria tenuissima* Kunze Ex Pers.\(^2\) *A. tenuissima* is also an opportunistic causative pathogen of human allergy\(^3\). In continuation of our search for bioactive secondary metabolites from the flora of South Eastern sub-Himalayan region of biodiversity hotspot of Indo-Burma belt\(^4\)\(^-\)\(^7\), we have investigated *Artabotrys odoratissimus* R.Br Syn. *A uncinatus* (Lam) Merr, *A. hexapetalus* (family Anonaceae) and isolated an unusual long chain alkylated \(\alpha\)-methylene-\(\gamma\)-butyrolactone from the juice of its ripe fruits with good activity against *A. tenuissima*.

![Solasodine](image)

*Fig. 2.1. Solanum khasianum* (containing Solasodine) is a source of Steroid drugs
A. odoratissimus R.Br (family Anonaceae) is popularly known as chenichampa phool [Fig. 2.3(A)] in Assam\textsuperscript{8,9} for the typical fragrance of its mature flowers similar to that of ripe chenichampa banana [Fig. 2.3(B)] (*Musa champa* Hort. ex Hook. F., Family *Musaceae*) available in this region of India.

![Fig.2.2. Cultivation of Solanum khasianum](image)

![Fig.2.3. (A) Chenichampa phool and (B) Chenichampa banana](image)
A. odoratissimus is a scandent shrub10 [Fig. 2.4]. Its leaves are oblong, lanceolate, shortly acuminate, glabrous, shining, acute at the base. Its flowers are greenish yellow and are solitary or in pairs, 3-4 cm long. Pedicles are 2 cm long. Sepals are 6 mm long, cornate below, ovate, acute and tips are refluxed. Petals are lanceolate above saccate base, clothed with appressed silky hairs. Ripe carpels are obvoid and glabrous and yellow in colour. Flowers are not showy, but highly fragrant. Seeds are oblong and a little flattened, deeply grooved on one side. The plant is native to India, Srilanka and China. The flowers of A. odoratissimus is reported to be alexiteric, and is useful in vomiting, biliousness, diseases of blood and heart, itching, foul breadth, leucoderma, headache and diseases of bladder. A decoction of the leaves is given for cholera in some of the islands of Malaya Archipelago11.

Fig.2.4. Chenichampa phool shrub at NEIST Campus
The plant is also used in traditional Chinese medicine for the treatment of malaria and scrofula\textsuperscript{12-14}. The genus \textit{Artabotrys} is a rich source of diverse secondary metabolites. The isolated secondary metabolites of the genus include bisabolane\textsuperscript{15-17} and guaiane sesquiterpenes\textsuperscript{18}, steroids\textsuperscript{19}, aporphine\textsuperscript{20-25}, tetrahydroberberine\textsuperscript{26} and isoquinoline\textsuperscript{27} alkaloids, long chain hydrocarbons\textsuperscript{28}, flavonoid glycosides\textsuperscript{12-14} and β-methoxy-γ-methylene-α,β-unsaturated-γ-butyrolactones, artapetalins A–C, together with the unusual butyrolactones (+)-tulipalin B and (2R,3R)-(+)3-hydroxy-2-methylbutyrolactone\textsuperscript{29}. The pulp of ripe fruit of this plant has been used traditionally for topical fungal infection of domestic animals in some parts of Assam. Taking this cue, we investigated fruit of \textit{A. odoratissimus} and herein, we wish to report the isolation, characterization and antifungal property of a novel alkylated butyrolactone, 3-methylene-4-pentadecyldihydrofuran-2-one from the juice of ripe fruit [Fig. 2.5] of this plant.

\textbf{Fig.2.5.} Chenichampa phool fruit
2.1. Secondary Metabolite isolated from *Artabotrys species*

The plant is a rich source of diverse secondary metabolites. Phytochemical investigation of this plant leads to the isolation of a number of natural products which are described below.

Eloumi-Ropivia and co-workers\textsuperscript{20} analysed the bark of *A. lastourvillensis* and preliminary investigation showed the presence of alkaloid in the bark. Extraction of these alkaloids by conventional methods followed by spectroscopic analysis lead to the isolation of a new alkaloid lastourvilline (1) along with a few known alkaloids.

Cave and co-workers\textsuperscript{26} also studied the stem bark of *Artabotrys venustus* for the alkaloids present. Along with the previously known alkaloids which were identified by spectroscopic methods as well as by comparison with reference samples, one of the minor constituent was also characterized using spectroscopic methods and was found to be a new member of the rare class of catecholic isoquinoline alkaloid, (-) artavenustine (2)
In an attempt to establish the stereochemistry of yingzhaosu A and B which were previously isolated from the plant *Artabotrys unciatus*, Zhang et al.\textsuperscript{17} isolated another sesquiterpene peroxide named yingzhaosu C (3) and a sesquiterpenol named yingzhaosu D (4) along with yingzhaosu A and yingzhaosu B. The structures of the new bisabolene derivatives 3 and 4 were determined by spectroscopic analysis in combination with chemical degradation.

Bioassay guided phytochemical analysis of the stem and stem bark of *Artabotrys uncinatus* by Wu and co-workers\textsuperscript{21} afforded the isolation and characterization of two cytotoxic aporphines, liriodenine (5) and atherospermidine (6) in addition to the structural elucidation of an inactive aporphinoid, artacinatine (7) which represents the first example of a naturally occurring compound containing the
11-oxoaporphine skeleton. The structure of artacinatine (7) was confirmed from spectral data in conjunction with single-crystal X-ray analysis.

In an effort to explore anticancer active natural products with novel structural features Wijeratne and co-workers\textsuperscript{23} investigated the bioactive constituents in an extract derived from \textit{Artabotrys zeylanicus}. Bioassay guided fractionation resulted in the isolation of a novel N-methoxylated 4,5-dioxoaporphine alkaloid named artabotrine (8) along with a known alkaloid. The structure of the new alkaloid 8 was elucidated using spectral data and single crystal X-ray analysis. They were also subjected to yeast mutant bioassays and showed significant activities.

Phytochemical analysis of the dried and powdered stem bark of \textit{Artabotrys zeylanicus} by Wijeratne et al.\textsuperscript{24} lead to the isolation of a new 4, 5-dioxoaporphine alkaloid, 8-methoxyouregidione (9) along with a few known alkaloid which were
characterized by spectroscopic methods. They also screened the isolated compounds for their cytotoxicity and showed significant anticancer activity.

Fleischer and co-worker\textsuperscript{18} analysed a petroleum ether extract of the stem bark of \textit{Artabotrys stenopetalus} which yielded two novel sesquiterpenes, the guaiane pogostol O-methyl ether (10) and an isodaucane (11) named artabotrine. The compounds were identified by detailed analysis of their spectral data.

In the course of ethnopharmacological investigations of the genus \textit{Artabotrys hexapetalus}, Li and co-workers\textsuperscript{13} isolated two novel flavonol glycosides named arapetaloside A and B together with three known flavonoids. The structures of these novel flavonol derivatives were determined by detailed spectroscopic analysis.

In the search for novel bioactive compounds of Formosan annonaceous plants, Hsieh et al\textsuperscript{25} isolated two new aporphinoid alkaloids, artabonatine A (12) and B (13) along with five known alkaloids by systematic extraction and isolation from the fresh
unripe fruits of *Artabotrys uncinatus*. The structures of the new compounds were elucidated by spectroscopic analysis.

Chemical investigation of *Artabotrys odoratissimas* leaves yielded three novel compounds characterized as Pentadecyl-7-hydroxydodecanoate (14), pentadecyl-tritriacontanoate (15), and 4,5-epoxy-26-ol-dopentacontane (16) using IR, PMR, CMR and mass spectral data.

\[
\begin{align*}
\text{CH}_3(\text{CH}_2)_5 & \quad \text{CH} \quad (\text{CH}_2)_4 \quad \text{C} \quad \text{C} \quad (\text{CH}_2)_{14} \quad \text{CH}_3 \\
& \quad \text{O} \\
& \quad \text{HO} \\
& \quad \text{14}
\end{align*}
\]

\[
\begin{align*}
\text{H}_3\text{C} & \quad (\text{CH}_2)_{30} \quad \text{CH}_2 \quad \text{C} \quad \text{O} \quad (\text{CH}_2)_{14} \quad \text{CH}_3 \\
& \quad \text{O} \\
& \quad \text{15}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 & \quad (\text{CH}_2)_{25} \quad \text{CH} - (\text{CH}_2)_{20} \quad \text{CH} - \text{CH} - (\text{CH}_2)_{2} \quad \text{CH}_3 \\
& \quad \text{OH} \\
& \quad \text{16}
\end{align*}
\]

Phytochemical analysis of the dichloromethane extract of the aerial parts of *Artabotrys hexapetalus* afforded three β-Methoxy-γ-methylene-α,β-unsaturated-γ-
butyrolactones which are proposed to be derived from C\textsubscript{18} unsaturated fatty acid by a biosynthetic route similar to that proposed for the Annonaceous acetogenins. The structure of the unique β-Methoxy-γ-methylene-substituted,α,β-unsaturated-γ-butyrolactone ring of artapelins A-C(\textbf{17-19}) was determined by 2D-NMR spectroscopic analysis. Two unusual simple butyrolactones, (+) – tulipalin B (\textbf{20}) and (2R, 3R)-3-hydroxy-2-methylbutyrolactone (\textbf{21}) were also isolated from this species.

\[
\begin{align*}
\textbf{17} & \quad R=H \\
\textbf{18} & \quad R=OH \\
\textbf{19} & \quad R= \\
\textbf{20} & \quad R=\text{CH}_2 \\
\textbf{21} & \quad R=\text{β-CH}_3 
\end{align*}
\]

Artabotrys odoritissimus R.Br (Annonacea) is a shrub native to eastern Asia and is cultivated as ornamental tree widely in Bangladesh.\textsuperscript{30} The fruit are rerecorded as
containing fixed and volatile oils, glycosides, and resin; extracts are reported to exhibit hypotensive and spasmogenic effects.\textsuperscript{31} Decoctions of the leaves are used as remedy for cholera\textsuperscript{30} and have been found to exhibit antifertility effects in rats.\textsuperscript{32} Other species of Artabotrys are reported to contain aporphine alkaloids,\textsuperscript{33,20} cyanogenic glycosides\textsuperscript{34} and sesquiterpenes.\textsuperscript{34}

A new alkaloid, lastourvilline (22) along with six known compounds namely, suaveoline (23), glaucine (24), boldine (25), isoboldine (26), bracteoline (27) and liriotulipiferine (28) were isolated from \textit{Artabotrys lastourvillensis}.\textsuperscript{20}

\begin{table}
\centering
\begin{tabular}{ |l|c|c|c|c|c| } 
\hline
\textbf{Compounds} & \textbf{R\textsuperscript{1}} & \textbf{R\textsuperscript{2}} & \textbf{R\textsuperscript{3}} & \textbf{R\textsuperscript{4}} & \textbf{R\textsuperscript{5}} \\
\hline
Lastourvilline (22) & OH & OH & H & O-CH\textsubscript{3} & O-CH\textsubscript{3} \\
Suaveoline (23) & O-CH\textsubscript{3} & O-CH\textsubscript{3} & OH & OH & H \\
Glaucine (24) & O-CH\textsubscript{3} & O-CH\textsubscript{3} & H & O-CH\textsubscript{3} & O-CH\textsubscript{3} \\
Boldine (25) & OH & O-CH\textsubscript{3} & H & O-CH\textsubscript{3} & OH \\
Isoboldine (26) & O-CH\textsubscript{3} & OH & H & O-CH\textsubscript{3} & OH \\
Bracteoline (27) & O-CH\textsubscript{3} & OH & H & OH & O-CH\textsubscript{3} \\
Liriotulipiferine (28) & OH & O-CH\textsubscript{3} & H & OH & O-CH\textsubscript{3} \\
\hline
\end{tabular}
\end{table}
Extraction of the stem bark with petroleum ether followed by preparative TLC and then column chromatography gave 24-methylene-lanosta-7,9 (11) dien-3β-ol (29) in a yield of 0.003%.

Few triterpene derivatives have been reported from the Annonaceae, but the most common polycarpol (30) which is also lanosta-7,9(11)-dien is regarded as a useful chemotaxonomic marker.

Sichaem and co-workers analyzed the roots of *Artabotrys spinosus* and isolated a new dimeric aporphine (31) named artabotrysine along with five known compounds. All isolated compounds were assayed for their cytotoxicity and found to display significant activity.
A novel type of α,β-butenolide alkaloid, uncinine (32), two novel oxoaporphines, artabonatine C (33) and artabonatine D (34), a new oxazoloaporphine, artabonatine E (35), and a new 7,7′-bisdehydroaporphine, artabonatine F (36), along with 25 known alkaloids, were isolated from *Artabotrys uncinatus*.37

The following 25 known alkaloids were isolated from *Artabotrys uncinatus*,37 namely liriodenine, atherospermidine, *O*-methylmoschatoline, oxoasimilobine, (-)-anonaine, (-)-romerine, (-)-*N*-acetylnorstephalagine, (-)-norstephalagine, (-)-stephalagine, (-)-isopiline, (-)-*N*-methylisopiline, (-)-asimilobine, (-)-norushinsunine, artacinatine, (+)-isocorydine, (-)-nornuciferine, (+)-norisocorydine, (-)-*O*-methyl-*N*-

2.2. Result and Discussion

As part of our ongoing programme in the search for plant based bioactive molecules, we have investigated A. odoratissimus. The ethyl acetate extract of the fresh, ripe fruit juice of A. odoratissimus yielded one major compound. The compound was analyzed as C_{20}H_{36}O_{2} by elemental analysis and Electro Spray Ionization MS with [M+H]^+ at 309. In the $^{1}$H NMR spectrum recorded at 300 MHz in CDCl$_3$, two signals at $\delta$ 5.14 and 5.29 integrated to one proton each were assigned for an exomethylene group. A three-proton triplet signal at $\delta$ 0.89 was assigned to a chain end methyl group. Broad signal at $\delta$ 1.30 integrating to 18 protons indicates that the molecule contains 9 methylene groups in nearly identical environment. Two doublets of doublet at $\delta$ 4.14 and 4.29, each integrating to one proton were assigned to CH$_2$ group attached to an ester oxygen. In the IR spectrum, there was a strong band at 1755 cm$^{-1}$ indicating the presence of a $\gamma$-butyrolactone in the molecule. Based on these evidences, the structure of the molecule was assigned as 3-methylene-4-pentadecylidihydrofuran-2-one (37) [Fig. 2.6]. The structure of the molecule was further confirmed by its $^{13}$C NMR spectrum recorded at 75 MHz. The signal at $\delta$ 62.45 was assigned to the carbon under lactone oxygen. The signals at $\delta$ 128.45, 130.62 and 173.71 indicated the presence of the exomethylene group and the lactone carbonyl in the molecule. Other signals were assigned for the carbons of the pentadecyl side chain. The relative stereochemistry of H-4 of the compound (37) was determined as $\alpha$, on the basis of the
coupling constants of H-4 with H-5 ($J_{4,5α} = 6$ Hz and $J_{4,5β} = 4$ Hz) and was confirmed by nOe difference spectrum of it. In the nOe difference spectrum of compound (37), when the signal at $δ$ 4.14 ppm was irradiated, nOe was observed at $δ$ 2.31 and 4.29 ppm only.

![Diagram](image)

Fig. 2.6. Relative stereochemistry of 3-methylene-4-pentadecyldihydrofuran-2-one (37)

The compound (3-methylene-4-pentadecyldihydrofuran-2-one) (37) was found to have a good inhibitory effect against *Alternaria tenuissima* Kunze Ex Pers, isolated from *Solanum khasianum* Clarke. The test compound, 3-methylene-4-pentadecyldihydrofuran-2-one (37), showed 85.05 and 80.42 percent inhibition respectively at 250 and 200 µg/ml, while standard fungicide (Captan) exhibited 100 percent inhibition at 200 µg/ml against *A. tenuissima*, isolated from *S. khasianum*. The minimum inhibitory concentration (MIC) of 1 was found to be 300 µg/ml. This is the lowest concentration of the compound, which showed 100 percent visible inhibition of the growth of the test pathogen *A. tenuissima*. However, the IC$_{50}$ values of 1 and the standard (positive control) were found to be 51.37 and 35.52 µg/ml, respectively [Fig. 2.7]. This indicated that the compound possessed good antifungal activity. The untreated control sets of the experiments showed no inhibition of growth of the fungal pathogen (Table 2.1). The observed antifungal activity of the compound may be attributed to the $α$-methylene-$γ$-butyrolactone moiety present in the molecule.
Table 2.1. Antifungal activity of 3-methylene-4-pentadecyldihydrofuran-2-one (37) against *A. tenuissima*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition (%) at different concentrations in µg/ml (± Standard Deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
</tr>
<tr>
<td>3-methylene-4-pentadecyldihydrofuran-2-one (37)</td>
<td>100.00</td>
</tr>
<tr>
<td>Captan</td>
<td>100.00</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2.7. Inhibition (%) against *Alterneria tenuissima* for compound (37) and positive control
2.3. Biogenesis of alkylated $\alpha$-methylene-$\gamma$-butyrolactone (37)

Many secondary metabolites are now known to contain butyrolactone rings. However, to the best of our knowledge, this is the first report of 3-methylene-4-pentadecylidihydrofuran-2-one isolation from nature. Biogenesis of this unusual long chain alkylated $\alpha$-methylene-$\gamma$-butyrolactone (37), is baffling, formed probably from the fusion of one isoprenoid moiety (bold part) with long chain fatty acid moiety (Fig. 2.8). Meroisoprenoids are reported from lower organisms such as algae, bacteria, fungi, etc.\textsuperscript{39-43} The report of meroisoprenoid from a higher plants such as angiosperm and gymnosperm is very scanty\textsuperscript{44-47}.

However, Hutchinson and Leete\textsuperscript{48} has shown experimentally that $\alpha$-methylene-$\gamma$-butyrolactone, a cyclized aglycone of tulipaside A, was biosynthesized from the condensation of acetyl coenzyme A with pyruvate. Following the same pathway, we propose that the biogenesis of this unusual structural feature arose from the condensation of stearic acid (C17) with pyruvic acid (C3) forming pentadecyl citramalic acid (38) (Fig. 2.8). This was followed by unexceptional reduction and dehydration to form pentadecyl amethylene-$\gamma$-butyrolactone (37).
2.5. Experimental

2.5.1. General Experimental Procedures

UV-visible spectrum was recorded on Analyticjena UV-VIS specord200 operated with Aspect plus v1.7 software. Specific rotation of the compound was recorded on a Perkin Elmer polarimeter model 343 at sodium light of wavelength 589
nm. IR spectra were recorded on a Perkin Elmer System2000 FTIR spectrometer. $^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded on a Bruker AVANCE DPX 300 NMR spectrometer in CDCl$_3$ using TMS as the internal standard and mass spectra were recorded on Bruker Esquire 3000 system. Silica gel G was used for TLC. All solvents used were distilled prior to use.

2.5.2. Plant Material

About 300 g of the fresh ripe fruits of A. odoratissimus were collected in July, 2005 from two shrubs of the plant grown at NEIST, Jorhat as ornamental plants and a voucher specimen was deposited at NEIST Jorhat. The ripe fruits were washed properly with water to remove any contamination.

2.5.3. Extraction and Isolation

The washed fresh ripe fruits of A. odoratissimus were then crushed in a mixie to get gelly like material. It was then filtered and the fruit pulps were again shaked with distilled water. Then it was again filtered. The combined filtrate was then extracted with ethyl acetate. The dried ethyl acetate extract was then distilled under reduced pressure at below 50 °C. The crude extract was then subjected to column and thin layer chromatography to get 30 mg of pure 3-methylene-4-pentadecylidihydrofuran-2-one (37) as a gummy mass (Yield: 0.037 %) [Fig. 2.9].
**2.5.4. Spectral data 3-methylene-4-pentadecyldihydrofuran-2-one (37)**

Gum. \([\alpha]_{D}^{20} -0.4^\circ\) (c. 1.7, MeOH); UV \(\lambda_{\text{max}}^{\text{MeOH}}\) nm (log \(\varepsilon\)): 224.65 (3.1), IR \(\nu_{\text{max}}\) (thin film) cm\(^{-1}\): 3008, 2926, 2854, 1755, 1664, 1464, 1417, 1377, 1239, 1164, 1098 and 987. \(^1\)H NMR spectral data (300 MHz, TMS, CDCl\(_3\)): 0.87 (3H, t, \(J_{21,20}= 7\) Hz, H-21), 1.30 (18H, 9CH\(_2\), overlapping signals of H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19 and H-20), 1.60 (m, 6H, H-9, H-10 and H-11) 2.03 (m, 2H, H-8), 2.31 (2H, m, H-7), 2.78 (1H, m, H-4), 4.14 (1H, dd, \(J_{4\beta,5\alpha} = 6\) Hz and \(J_{5\alpha,5\beta} = 12.6\) Hz, H-5\(\alpha\), 4.29 (1H, dd, \(J_{4\beta,5\beta} = 4\) Hz and \(J_{5\alpha,5\beta} = 12.6\) Hz, H-5\(\beta\)), 5.25 br s (1H, H-6\(\alpha\)), 5.39 br s (1H, H-6\(\beta\)). \(^{13}\)C NMR spectral data (CDCl\(_3\)): \(\delta\) 14.5 (q, C-21), 23.26 (t, C-20), 26.02 (t, C-19), 26.0 (t, C-18), 27.60 (t, C-17), 29.53 (t, C-16), 29.60 (t, C-15), 119
29.74 (overlapping C-10, C-11, C-12, C-13, C-14), 29.94 (t, C-9), 30.00 (t, C-8), 30.08 (t, C-7), 30.12 (d, C-4), 62.45 (t, C-5), 128.45 (t, C-6), 130.62 (s, C-3), 173.71 (s, C-2). ESIMS m/z at 309 [M+H]^+. Elemental analysis: Found C 77.76, H 11.72. C_{20}H_{36}O_{2} requires C 77.87, H 11.76 %.

2.6. Bioassay of the 3-methylene-4-pentadecylidihydrofuran-2-one (37)

2.6.1. Micro-organism preparation

Stock culture of *Alternaria tenuissima* was used throughout the study. The fungus was isolated from *Solanum khasianum* and was maintained on Potato Dextrose (PDA) having standard formula consisting of infusion from potatoes 300 g dextrose 20 g and agar 15 g per L (Hi Media M096A). 41 g of standard PDA was suspended in 1000 ml distilled water, boiled to dissolve the medium completely, sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes, mixed well before dispensing. Fungus was grown for 72 hrs. on PDA at 28 °C. For use in experiment the fungus was grown separately on Potato dextrose broth (PDB) containing infusion from potato 200 g and dextrose 20 g (Hi Media, M 403). The broth culture was diluted to a concentration of 1x10^6 with sterile distilled water for preparation of spore suspension of the test fungus.

2.7.2. Test sample concentration

Stock solution of each sample was prepared initially dissolving in ethanol and then diluted in water to a concentration of 500 ppm. These varying concentrations of 300, 250, 200, 150, 100, and 50 ppm were prepared in water from the stock solution by standard broth dilution method. From each concentration of the sample 0.1 ml was
added to 10 ml of PDB Media containing spore, shaked well, kept in incubator at 28 ± 1 °C for growth and observed daily. Activity was judged by measuring the dry weight of the test fungus after one week. The experiments were conducted with three replications and data were statistically analyzed.

2.7.3. Minimum inhibitory concentration

Antifungal activity was expressed as % Inhibition = Sample weight (treated) / Control weight (untreated) x 100 (Lis-Balchin et al, 1996). Minimum Inhibitory Concentration (MIC) was considered as the highest dilution at which 100% inhibition of growth of the test fungus *A. tenuissima* was observed.
2.8. References


30. Chopra, R. N., Nayar, S. L., Chopra, I. C. *Glossary of Indian Medicinal Plants*, CSIR, New Delhi, India, **1956**.


