CHAPTER IV

DISCUSSION

In the investigation of *Aglaia pirifera* Hance, the stem bark powders were exhaustively extracted with methanol by maceration method. The concentrated methanolic extract was then partitioned with pentane in a continuous liquid-liquid extractor. This procedure removed most of chlorophyll, pigments, sterols, triterpenoids, terpenes, lipid materials and some other non polar compounds from the aqueous methanolic extract.

The separation process of individual substances was based on the use of adsorption chromatographic method.. The column chromatographic procedure followed the technique called short column chromatography. This technique was devised and pioneered by Rigby and Hunt (90) in 1967. It provides reliable preparative separations of mixtures. Moreover, separations are carried out more rapidly and with less solvent than conventional techniques. It is essential that the appropriate solvent system be employed, and that the column be packed uniformly. Examination of a mixture to be separated by analytical tlc in several mixed solvent systems enabled selection of an eluting solvent mixture yielding the best separation of the components. Ideally, these components should appear as spots at about hR_f values of 30-40 on the plate. Moving these spots around

with various solvent combinations of differing polarities were carried out to ensure that each spot was a single compound. Having selected the best solvent system for tlc, the polarity for the column eluent can then be chosen. Since a compound on a column runs somewhat faster than on a plate, the concentration of the more polar component in the column eluent was usually decreased to about 50% of that found to be suitable for analytical tlc. The best system for analytical tlc was found to be benzene/ethyl acetate (8:2),the eluent mixture used for the column separation was benzene/ethyl acetate (9:1). The main advantage of using this method of chromatography is that the columns are short, resulting in rapid separations, efficient solvent utilization, and excellent material recovery.

From this study, a colorless crystalline Ap was isolated from the column. It was subsequently identified as a known lignan compound called grandisin. The high resolution mass spectral study of Ap showed molecular ion at *m/z* 432.2139 and the base peak at *m/z* 208. The other important peaks appeared at *m/z* 224, 236 and 196. The ms fragmentation pattern of Ap was shown in Fig. 2.

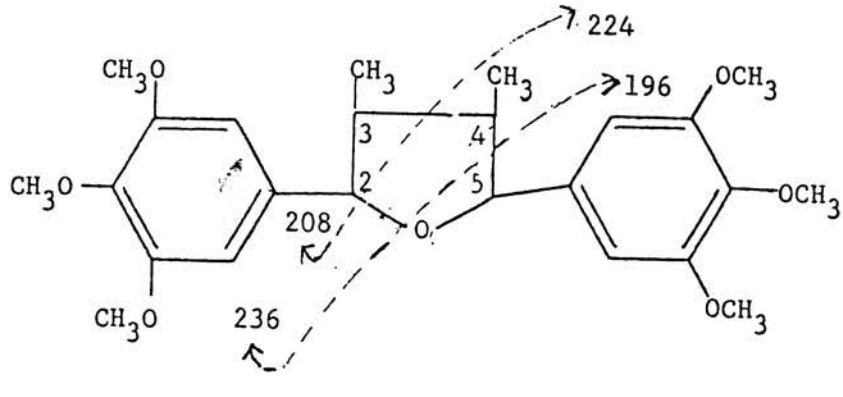


Fig. 2 ms Fragmentations of Ap

The ir spectrum of Ap (Fig. 9, Page 77) showed phenyl group at 1600 cm^{-1} and at 1470 cm^{-1} and the ether group at 1130 cm^{-1} and at 1000 cm^{-1}.

The ^{1}H nmr spectrum of Ap showed signal corresponding to two pyrogallol trimethyl ether moieties with six methoxyl groups, the corresponding signal appeared at δ 3.89 (12H) and δ 3.84 (6H) respectively. A four proton singlet at δ 6.63 indicated four magnetically equivalent aromatic protons. The symmetry of the rest of the spectrum indicated a lignan of tetrahydrofuran type which was confirmed by the ms fragmentation pattern (Fig. 2, Page 48). The proton chemical shifts could be assigned to the molecule by comparison with reported values of nmr spectrum of grandisin as shown in Fig. 3.

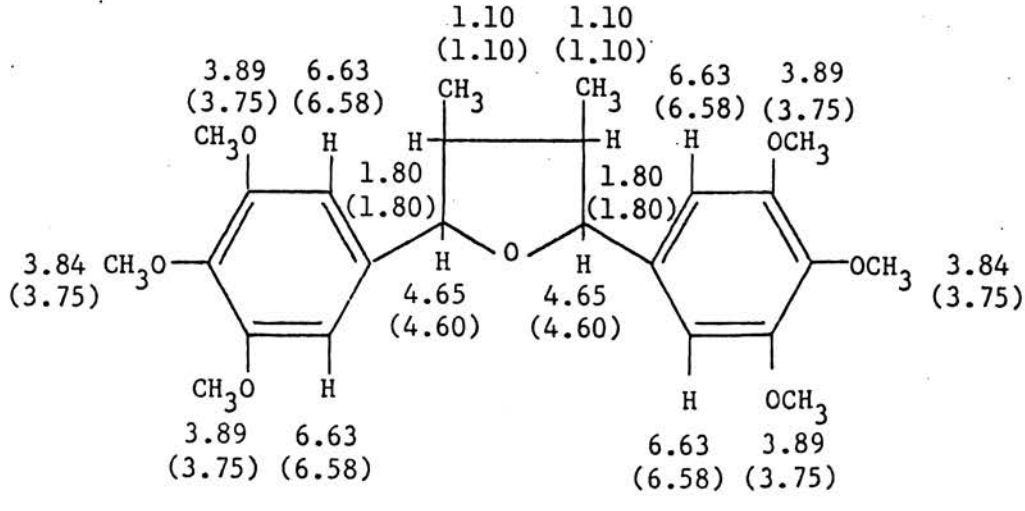


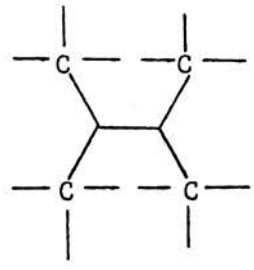
Fig. 3 ^{1}H nmr Assignment of Ap

(Shift values in parentheses are for report values of grandisin)(91).

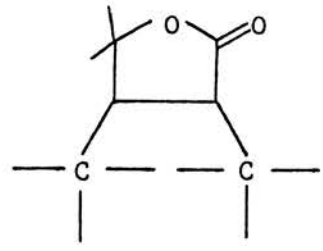
Lignans are dimers of phenylpropanoid (C_6-C_3) units linked by the central carbons of their side chains. Naturally occurring dimers that exhibit linkages other than this C_8-C_8'' type linkages are known as neolignans and are more limited in number and phylogenetic distribution.

According to the way in which oxygen is incorporated into the skeleton, four structural groups of linear lignans can be recognized : lignans or derivative of butane (LXXIV); linanolide, or derivative of butanolide (LXXV); monoepoxylignans, or derivative of tetrahydrofuran (LXXVI) and bis-epoxylignans (LXXVII).

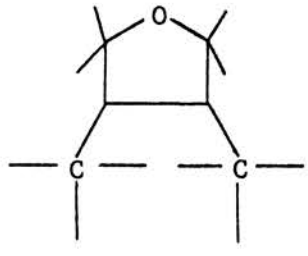
Further cyclization resulting from the introduction of a C-7/C-6'' linkage allows the existence of a large class of compounds collectively known as cyclolignans. These occur either as tetrahydronapthalene (LXXVIII) or napthalene (LXXIX) derivatives.



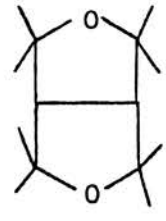
(LXXIV)



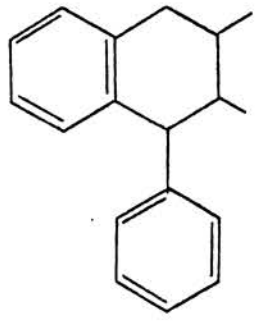
(LXXV)



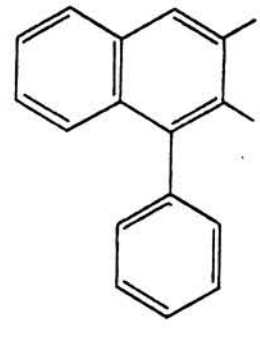
(LXXVI)



(LXXVII)



(LXXVIII)



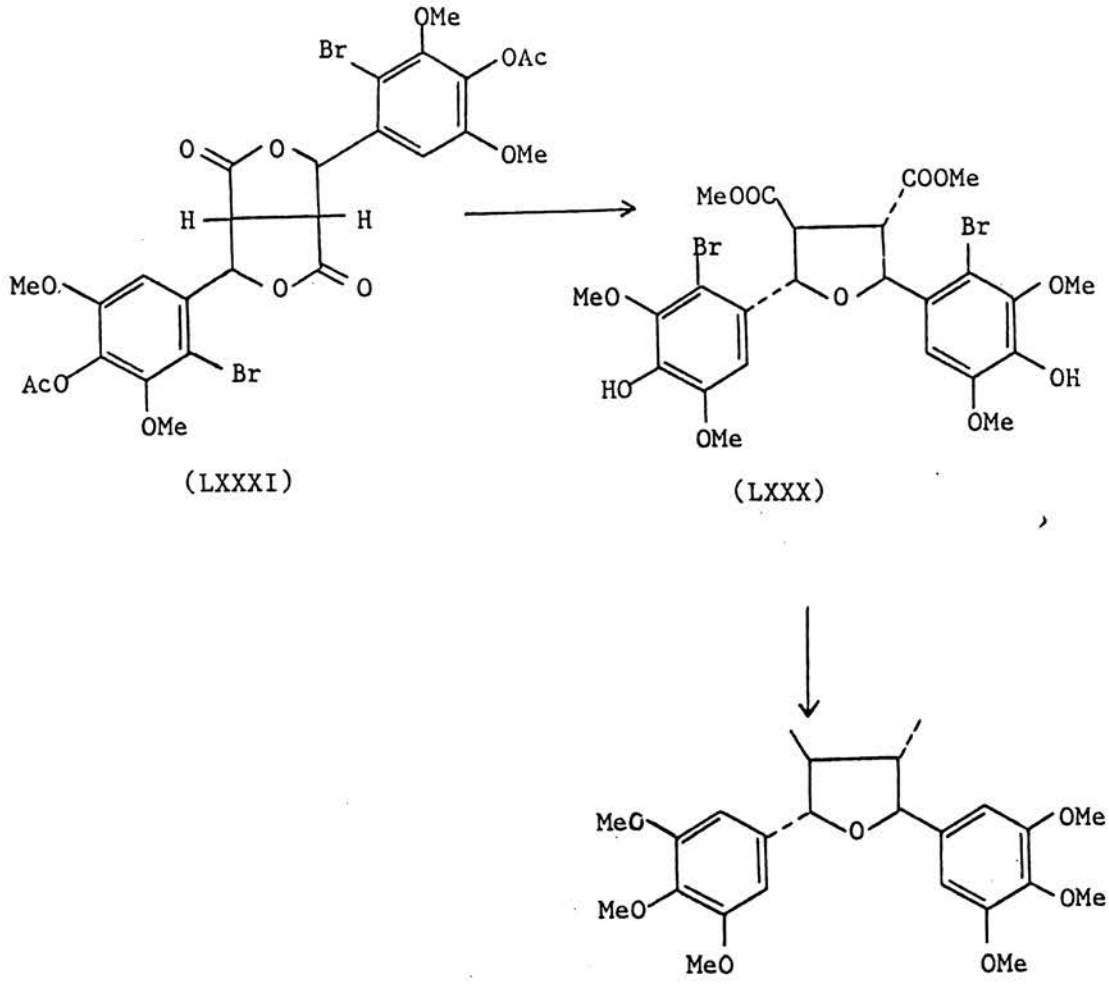
(LXXIX)

Lignans have been isolated from all parts of plants, including, roots (92), heartwood (93), bark (94), leaves (95), flowers (96) and seeds (97).

From the literature survey, it was found that grandisin was first isolated from *Litsea grandis* (Wall.) Hook.f. in 1974 by Holloway and Scheinmann (91).

In the same year Ashrafova and Bazhenova (98) studied the effect of grandisin on animals during long term use by given grandisin to rat (sc), at 10 mg/kg/day for 3 weeks, produced no micro or macroscopic changes in the central nervous system or internal organs. However, at 50 mg/kg/day for 3 weeks, grandisin produced perivascular and pericellular edema in the brain, pyknosis of nuclei of some nerve cells, dystrophic changes in the heart, fatty accumulation in the liver and vacuolar dystrophy and necrosis in the kidney.

In 1977 Stevenson and Williams (99) synthesized grandisin by routine reduction of the tetrahydrofuran dicarboxylic esters (LXXX), obtained by mild acid treatment of the readily available diaryl dilactones (LXXXI).



(LXXXI)

(LXXX)

In 1979 Biftu *et al.* (100) synthesized deoxyschizandrin by developing from 3,4,5-trimethoxypropiophenone. In the path way of synthesis, grandisin was an intermediate.

In 1983 Takeya, *et al.* (101) synthesized grandisin by oxidation of (E)-and (Z)-1-(3,4,5-trimethoxyphenyl)-1-propenes with the new reagent systems, CrO_3-HBF_4-MeCN and CrO_3-$HClO_4$-MeCN.

In 1984 Hikono *et al.* (102) studied the pharmacological activity of deoxyschizandrin and found that it showed anti-hepatotoxic activity in carbon tetrachloride-induced cytotoxicity assay.

The result of this investigation showed that the main constituent of *Aglaia pirifera* stem bark is neither a sterol nor a triterpenoid as it were proposed. It turned out to be a lignan compound which gave false reaction with Liebermann-Burchard's test.