CHAPTER- 2

2.2 CHROMATOGRAPHIC SEPARATION AND ISOLATION OF COMPOUND FROM THE PLANT *CHISOCHETON PANICULATUS*

2.1 PLANTS OF MELIACEAE FAMILY

The plants of Meliaceae family are widely distributed in the North-Eastern Region of India. Some species of it are popularly known as Neem[1-4], Bandardima, Poma, Gandheli poma etc. These plants are used in folk medicine as antifeedant[5-6] and antifungal agents. Photochemical investigation of Meliaceae family has revealed the occurrence of limonoids[7-14] (a tetranortriterpenoid), most of them showing antifungal activity. Among the family Meliaceae, the species *Chisocheton paniculatus* is the most popular, which powdered fruits show marked antifungal[15] activities. More recently limonoids have shown anticarcinogenic and antitumourogenic activities.

2.2 INTRODUCTION TO THE PLANT *CHISOCHETON PANICULATUS* HIERN

See part-I, secion-1.1 and page-12-13

2.3 SAMPLE COLLECTION

The plant material particularly the fruits have been collected from different parts of Assam during April-may, 2003 as well as 2004. The identification of the plant species are made from the Herbarium maintained at Botany Department, Gauhati University, Assam. All the samples are well classified and documented with proper labeling after drying in the shed.

2.4 CHEMICAL CONSTITUENTS

The present investigation deals with the isolation, structure elucidation and study of crystal structure of known Paniculatin[16] (6α-acetoxy azadirone) (MK 001) from the plant material of *Chisocheton paniculatus* Hiern.

6.5 EXTRACTION OF FRUITS OF *CHISOCHETON PANICULATUS*

6.5.1 EXTRACTION AND ISOLATION OF PANICULATIN (MK 001)

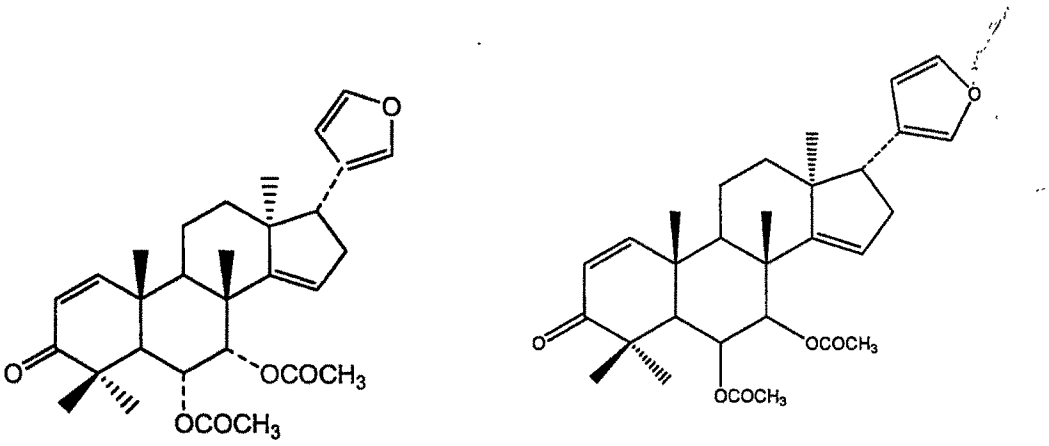
Collected leaves and fruits of *Chisocheton paniculatus* are first shed dried and powdered. Powdered leaves and fruits are extracted with petroleum ether (60^0-80^0C) in a soxhlet apparatus (Figure 2.3).However, the collected leaves have not responded as our expectation in these studies. Therefore leaving aside the studies of the leaves for future précised works, the studies of collected fruits are undertaken for the present works. The extract on concentration under reduced pressure gave a dark material (F_1). These materials (F_1) are washed several times by petroleum ether followed by ethanol. This amount is then column chromatographed in silica gel, using solvents of increasing polarity.

After careful chromatographic separation one compound is isolated from the Petroleum ether fraction and purified by TLC. On repeated crystallization (Toluene: MeOH :: 8:2) a pure crystalline white solid is obtained. The compound gave a purple spot on TLC plate, when sprayed with vanillin/H_2SO_4 followed by heating at 100°C. After doing different chemical tests, the compound is confirmed to be an unsaturated triterpene. The pure compound is identified and confirmed as Paniculatin[16] (6α-acetoxy azadirone, m.p. 192°C) (MK 001) by comparing the values of M.P., IR, ^{1}HNMR and M.S data with the reported[17-18] values (Figure 2.9,2.10,2.11) and table 2.1 as well as by comparing with authentic samples. It has been found that the compound MK 001 exists

as α- as well as β-isomer. The crystal structure of β-isomer (6β-acetoxy azadirone, MK 002) is already known[19]. From the literature survey, it has been found that no work on crystal structure of α-isomer has been undertaken so far by any group of scientists. So, we report here the molecular and crystal structure of its α-isomer in this thesis. The chemical structures of the compound α-isomer and β -isomer are shown in figure 2.1.

Table 2.1: Physical and Spectral data of the compound MK-001

Physical and Spectral Data	MK-001
Molecular formula	$C_{30}H_{38}O_6$
Molecular weight (gm)	494
Melting point (°C)	191.9
^{1}H NMR (δppm)	1.13 (s, 5-CH_3), 2.16 (6H, s, 2-Ac), 6.39 (1H, d, H-1) 6.80 (1H, d, H-2), 5.42 (1H, H-6), 5.47 (1H, H-7), 7.51 and 7.53 (1H, m, furan α-H) 6.62 (1H, m, furan β-H)
IR υ_{max} (KBr)cm^{-1}	1742(C=O), 1673(C=O),1373,734(β-substituted),furan,1245(C-O-C)
Mass Spectra	m/Z 494 $[M]^+$



MK 001 (α-Isomer) **MK 002 (β -Isomer)**

Figure 2.1: Structures of 6α-acetoxy azadirone (I) isomers

Figure 2.2: Soxhlet apparatus

Figure 2. 3: Column chromatography.

Figure 2.4: Thin Layer Chromatography Plate, applying sample.

Figure 2.5: Developing a TLC plate in solvent chamber.

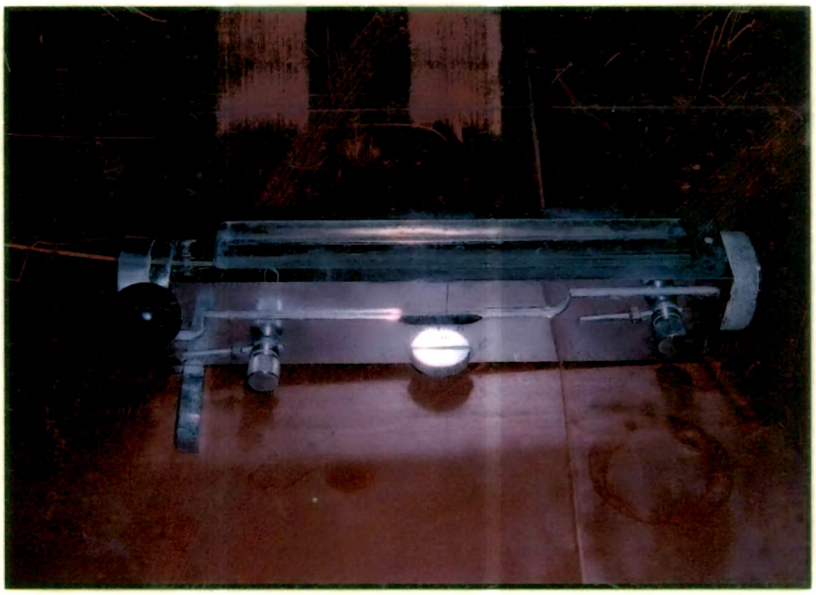


Figure 2.6: TLC Spreader / Applicator

Figure 2.7: Preparation of TLC Plates

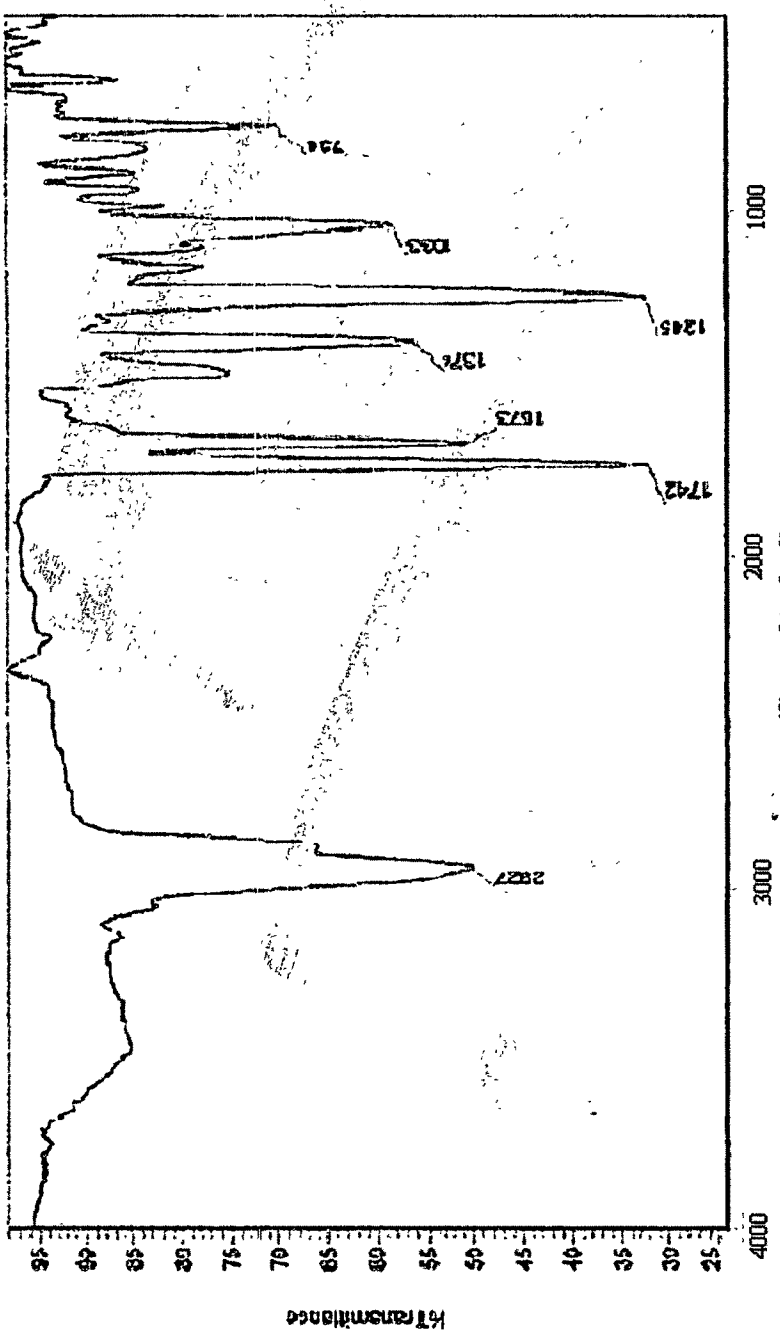


Figure 2.8: IR Spectra of MK-001

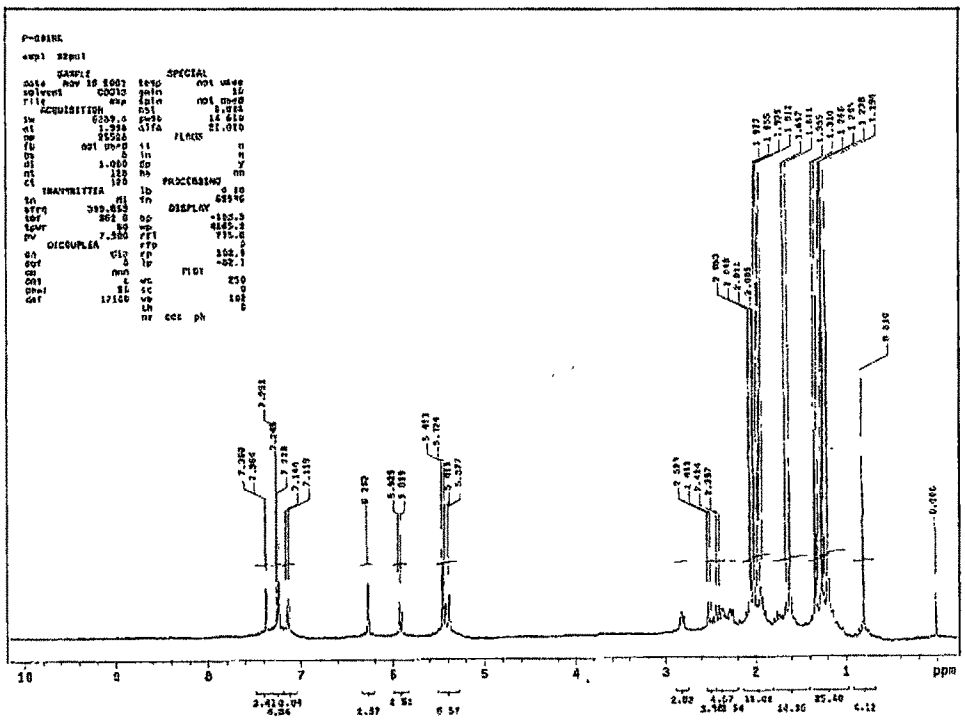


Figure 2.9: ^{1}H NMR Spectra of MK-001

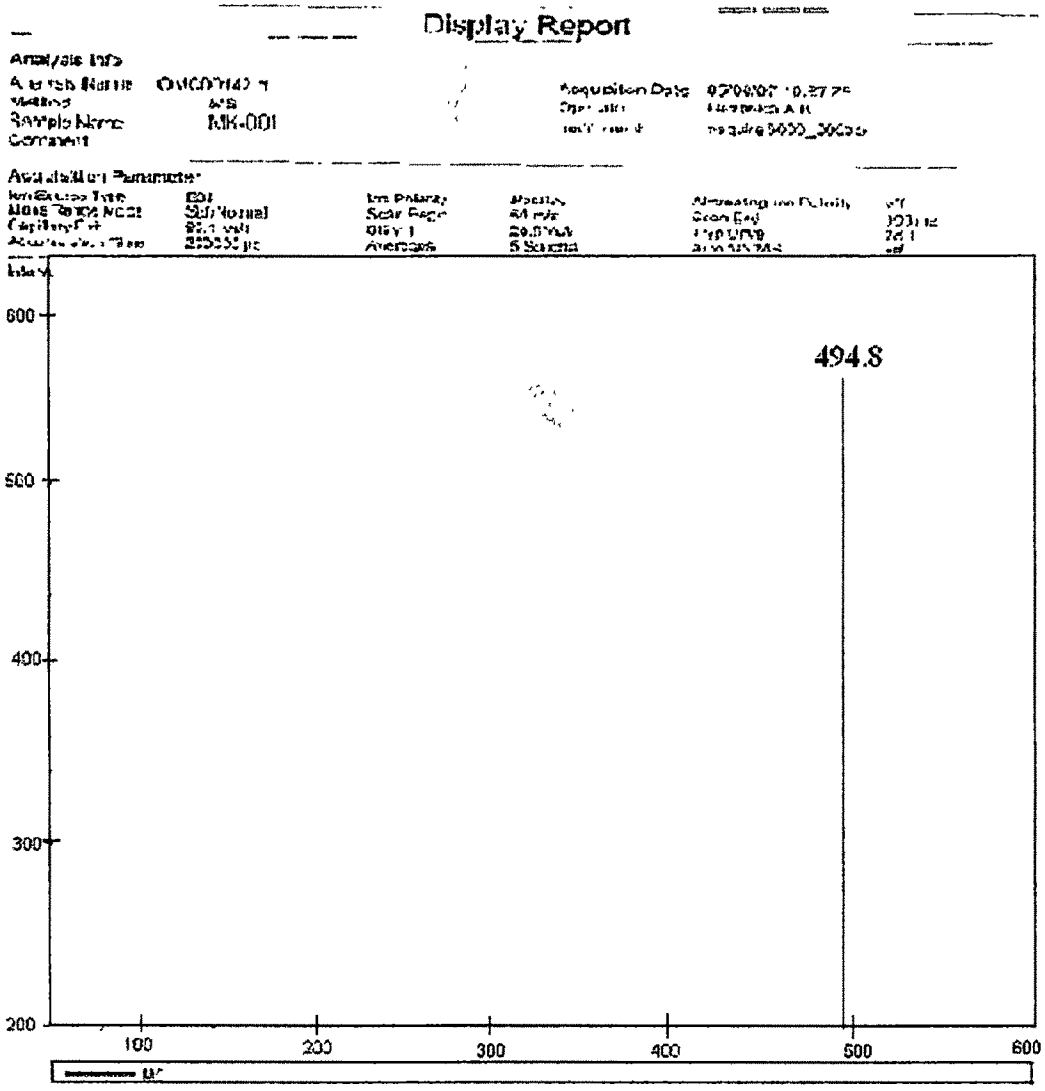


Figure 2.10: Mass spectra of MK 001

2.6. RESULT & DISCUSSION

From the chromatographic separation method of the fruits *Chisocheton peniculatus* (Bandordima) one compound is isolated from the Ptroleum ether fraction and purified by TLC.On repeated crystallization (Toluene: MeOH:: 8:2) a pure crystalline white solid is obtained. The pure compound is identified and confirmed as Paniculatin (6α-acetoxy azadirone, melting point = 192°C) (MK 001). It has been found that the compound MK 001 exists as α- as well as β-isomer. The crystal structure of β-isomer (6β-acetoxy azadirone, MK 002) is already established. From the literature survey, it has been found that no work on crystal structure of α-isomer has been undertaken so far by any group of scientists. So, we report here the molecular and crystal structure of its α-isomer in this thesis. The chemical structure of the compound (6α-acetoxy azadirone, MK-001) and β-isomer (6β-acetoxy azadirone, MK 002) are shown in figure: 2.2.

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