CHAPTER II
AILANTHUS ALTISSIMA (MILL.) SWINGLE SYN.
A. GLANDULOSA DESF (SIMARUBACEAE)

Ailanthus Desf is a genus of tall deciduous trees. About 5 species are reported from India, a few species are observed to show anti-leukemic activity (The Wealth of India, 1985).

DESCRIPTION AND DISTRIBUTION

The large deciduous “Tree of Heaven”, A. altissima, is native to China and Japan and found in the hills of northern India up to an altitude of 2,400 m. The species is often cultivated as an ornamental avenue tree. It is reported to be a good food for silkworms. It can be grown on sandy soils, being a quick growing tree, it is suitable for shelter belts.

Leaves are up to 0.9 m long, pubescent, alternate, pinnate, leaflets alternate or subopposite, numerous, flowers in terminal or axillary, branched panicles, small, pedicels bracteate. Calyx short, 5-fid, lobes equal imbricate. Petals 5, spreading, in duplicate valvate. Disk 10 lobed. Stamens 10 in the male, 2-3 in the hermaphrodite and 0 in the female flowers, inserted at the base of the disk, filaments very short or filiform, without scales. Ovary 2-5 partite (rudimentary in the male flowers), ovule 1 each cell, semianatropous, styles connate. Samaras (fruits) 1-5, large, purplish yellow, twisted at the top, membranous, linear-oblong, veined, one-seeded in the middle. Seeds 0.6 x 0.25 cm, compressed, testa membranous, albumen scanty, adhering to the testa, cotyledons flat, foliaceous, suborbicular (Kirtikar and Basu, 1991).

VERNACULAR NAMES

Chinese: Ch 'on ch' un, Ch'un Shu, Fen Yen Tsao, Teheou Tch'o'ven.
German: Goetherbaum, Indochina, Bachbi, Hu, Bach-bi, Vu bachbi.
CHEMICAL CONSTITUENTS

*A. altissima* contains a number of bitter principles, flavonoids, benzoquinone, indole alkaloids, β-sitosterol, malanthin, triacontane and hexatriacontane (The Wealth of India, 1985). Three bitters, namely 2,6-dimethoxyquinone, ailanthone and an unidentified compound have been reported (Casinovi *et al.*, 1964). Ailanthone has also been reported by some other workers (Gaudemer *et al.*, 1967, Naora *et al.*, 1982, Casinovi *et al.*, 1965) and along with chaparrinone from the seeds (Polonsky and Fourrey, 1964). The structure of shinjudilactones, isolated from root bark (Varga *et al.*, 1981, Ishibashi *et al.*, 1981, 1983) was confirmed by X-ray crystallography. Shinjulactone A was isolated and characterized and the configuration of hydroxyl group at C-12 of ailanthone revised to α-orientation by X-ray analysis (Naora *et al.*, 1983). Shinjulactones B and C were isolated from stem bark and their structures established (Furono *et al.*, 1981, 1984, Ishibashi *et al.*, 1982, 1983b, 1984a, 1985). 2-Dihydroxyailanthone (Casinovi *et al.*, 1983) and 13,18-dehydroglaucarubolone (Moron *et al.*, 1966; Kupchan and Lacadie, 1975) were isolated from root bark and it was characterized as shinjulactone A (Casinovi *et al.*, 1983). Shinjulactones D, E (Ishibashi *et al.*, 1983a, 1983c, 1984b, 1984d, Furono *et al.*, 1984, Niimi *et al.*, 1987), shinjulactones F, G, H, I, J, K (Ishibashi *et al.*, 1984c, 1984d, Takahashi *et al.*, 1984, Niimi *et al.*, 1987), shinjulactone L (Ishibashi *et al.*, 1985) and shinjulactones M and N (Niimi *et al.*, 1986, 1987) were also isolated and characterized. Amarolide 11-acetate, amarolide (Casinovi *et al.*, 1965; Stocklin *et al.*, 1970), 13,18-dehydroexcelsin, shinjuglucosides A, B, C and D (Yoshimura *et al.*, 1984), chaparrolide (Mitchell *et al.*, 1971), glaucarubinone (Gaudemer and Polonsky, 1965), 13,18-dehydroglaucarubionone (Polonsky *et al.*, 1987), shinjuglucosides E and F (Niimi *et al.*, 1987), and some other quassinoids (Jaziri, 1990) have also been reported from *A. altissima*. Quassine and neoquassine were isolated from the bark and characterized (Chiarlo and Pinca, 1965). Two new quassinoids, ailantinols A and B, and related compounds were isolated from the stem bark of *Ailanthus altissima*, and their
structures were elucidated (Kubota et al., 1996).

The following β-carboline indole alkaloids have been isolated and characterized: canthin-6-one, 1-methoxycanthin-6-one and methyl 4-methoxy-β-carboline 1-carboxylate from root bark (Varga et al., 1980; Szendrei et al., 1977; Varga et al., 1980), 1-(2-hydroxyethyl)-4-methoxy-β-carboline, 1-(1,2 dihydroxyethyl)-4-methoxy-β-carboline and 1-methoxycanthin-6-one 3N-oxide (Ohmoto et al., 1981), Canthin-6-one, its 3-oxide and 1-methoxycanthin-6-one from the wood (Ohmoto et al., 1976), 1-hydroxycanthin-6-one and 1-(1,2-dihydroxyethyl)-4-methoxy-β-carboline from root bark (Varga et al., 1981). Canthin-6-one, 1-methoxycanthin-6-one, 4-methoxy-1-vinyl-β-carboline, 1-(2-hydroxy-1-methoxy)-ethyl-4-methoxy-β-carboline, 5-hydroxymethyl-canthin-6-one along with β-carboline-1-propionic acid, 1-carbamyl-β-carboline and 1-carbomethoxy-β-carboline have been isolated from leaves (Ohmoto et al., 1984); 1-hydroxycanthin-6-one has been isolated from the wood (Khan and Shamsuddin, 1981) of A. giraldii Dode, synonym of A. altissima (Hegnauer, 1973; Nooteboom, 1962). The roots of this plant have also been reported to contain 4-methoxy-1-methyl-3[3,3-dimethylallyl]-quinol-2-one (Behlmann and Rao, 1969).

Canthine-6-one and 1-methoxycanthin-6-one from plant cell cultures (Anderson et al., 1983) and 1-methoxycarbonyl-4, 8-dimethoxy-β-carboline from leaves culture (Souleles and Kokkalou, 1989) have also been reported. In addition to these, fatty acids from the bark, isoquercetin, rhamnosyl-1,3-quercetin, apigenin, its 7-O-glucoside, luteolin-7-O-glucoside, quercetin-3-O-galactoside, kempferol, quercetin, and 2,6-dimethoxybenzoquinone (Inamota et al., 1961; Casinovi and Grandolini, 1963) were also isolated.

The seeds are a rich source of a fatty oil. A sample of decorticated seeds from USA (1,000 seed wt. 9 g) yielded 28% protein and 56% fatty oil. The oil is bitter in taste and has the following characteristics: nD, 1.4677, saponification value 188 and iodine value 133. The fatty oil from USSR sample (yield 22%, iodine value 121.4) had the following composition: oleic 80, linoleic 5-6, linolenic traces, palmitic and stearic acids, 12: and phytosterol, 2%. The
Shinjulactone L

Shinjulactone M

Shinjulactone N

Ailanthone

Shinjuglycoside E

Shinjuglycoside F
Shinjudilactone

I

\[ R = \text{CH}_2\text{CH}_2\text{OH} \]

\[ R = \text{CH(OH)}\text{CH}_2\text{OH} \]

II

III

Excelsin

\[ R = \alpha\text{-Me}, H \]

13(18)-Dehydroexcelsin

\[ R = \text{CH}_2 \]
1 - Methoxycarbonyl-4,8-dimethoxy-β-carbol ine

1 - Acetyl-4-methoxy-β-carbol ine (R = COCH₃)
1 - (2'-Hydroxyethyl)-4-methoxy-β-carbol ine (R = CH₂CH₂OH)
1 - (1',2'-Dihydroxyethyl)-4-methoxy-β-carbol ine (R = CH(OH)CH₂OH)

Canthin-6-one (R=H)
1 - Methoxycanthin-6-one (R=OCH₃)
1 - Hydroxycanthin-6-one (R = OH)

4 - Methoxy-1-methyl-3-(3,3-dimethylallyl) -quinol - 2-one

Canthin-6-One 3-oxide
bitter taste of the crude oil can be removed by refining. Besides the quassinoids the seeds contain dimethoxybenzoquinone (Wealth of India, 1985).

**BIOLOGICAL ACTIVITY AND MEDICINAL USES**

The bark is astringent, antispasmodic, anthelmintic and parasiticidal. In powdered form it is narcotic with a strong nauseating odour. It exercises a powerful depressing influence on the nervous system similar to that of tobacco. The fresh bark is useful in diarrhoea and dysentery. The root bark is recommended for heart ailments, epilepsy and asthma (Kirtikar and Basu, 1991). The fruits are used as an emmenagogue and for ophthalmic diseases. The leaves are astringent and used in the preparation of lotions prescribed in seborrhoea and scabies. The flowers are reported to produce dermatitis (Wealth of India, 1985).
EXPERIMENTAL METHODS

GENERAL

- All the plant materials collected from different places were identified by Dr. M.P. Sharma, Senior Lecturer, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi-110062.
- Silica gel used for analytical and column chromatography was obtained from Qualigens Fine Chemicals, Mumbai.
- Iodine vapours, ceric ammonium sulphate, ultraviolet lamp and 1% vanillin in sulphuric acid were used for visualisation of TLC spots.
- All chemicals and other solvents were mostly of AR. grade; anhydrous sodium sulphate was used for drying organic solvents.
- All melting points were determined in centigrade scale in one-end open capillary on Perfit melting point apparatus and are uncorrected.
- UV spectra were recorded in methanol on Shimadzu, Backman DU-64 and Hitachi 150-20 spectrophotometers, $\lambda_{\text{max}}$ values are in nm.
- IR spectra were taken on Perkin-Elmer 1710 and 580B, Hitachi 260-30 or Shimadzu 480 spectrophotometers using KBr pellets or nujol mull, $\gamma_{\text{max}}$ values are in cm$^{-1}$.
- $^1$H NMR spectra were screened on Jeol 60 MHz, Jeol FT-90 MHz, or JNM Fx-100 MHz in CDCl$_3$ (unless otherwise stated) using tetramethylsilane (TMS) as the internal standard. Chemical shifts are expressed in $\delta$ ppm with respect to the internal TMS and reported from downfield to upfield region. The coupling contents ($J$ values) are expressed in Hertz (Hz). Notations used for spin-coupling pattern throughout the manuscript are designated as: $s =$ singlet, $d =$ doublet, $dd =$ double doublet, $ddd =$ doublet of double doublets, $t =$ triplet, $q =$ quartet, $m =$ multiplet, $brs =$ unresolved broad singlet, $w_{1/2} =$ half-width.
- Mass spectra were scanned by effecting electron impact ionization at 70-eV on a Jeol D-300 (El/Cl) or Jeol JMS-DX 303 GC-mass spectrometer equipped with direct inlet probe system. The $m/z$ values of the more intense peaks are mentioned and the figures in bracket attached to each $m/z$ values indicate relative intensities with respect to the base peak.
Analytical GLC of volatile oils was carried out on a Varian 3300 gas chromatograph filled with silicone DB-1 column (30 x 0.25 mm i.d.); carrier gas N₂, flow rate 1.5 ml/min, split mode, isothermal temperature programmed 80-225 °C at 4 °C/min. Injection temperature 280°, detector used FID, temperature 300 °C, injection volume for all samples 0.1 μl.

GC-MS analyses of volatile oils were carried out on a Hewlett-Packard 5890 gas chromatographed filled with a fused silica SE-30 capillary column (12 m x 0.25 mm i.d.) and FID; carrier gas He, flow rate 1.5 ml/min, temperature programmed 70-250 °C, injection temperature 250 °C, mass selective detector HP 5971 A, 70 eV.

¹³C FT-NMR spectra were determined on a Jeol FT 22.5 MHz in CDCl₃, with TMS as internal standard in 5 mm spinning tube at 27 °C.

Optical Rotations were measured on a PA-1R polarimeter in sodium light.

Silica gel (60-120 mesh) was used for column chromatography. The lower end of a clean dry column was plugged with adsorbent cotton, over which a piece of filter paper was placed. The column was then half filled with petroleum ether. Silica gel was added in small portion and allowed to settle down gently until the necessary length of column was attained. All the air bubbles were allowed to escape by running the column blank thrice with the solvent. The dried Silica gel slurry of the extract was packed in the column and then eluted successively in order of increasing polarity with different solvents. The development and elution of the column was carried out with successive series of solvents in various combination, viz. petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform, chloroform - methanol (49:1, 19:1, 9:1, 3:1 and 1:1, v/v) and methanol. The completion of elution of the components(s) was confirmed via evaporating a small fraction of the eluant.

The fractions collected were subjected to thin layer chromatography to check homogeneity of various functions. Chromatographically identical fractions were combined and concentrated.

Preparation of the plates: Silica gel-G (100 g) was mixed with distilled water (400 ml) by trituration in a glass pastel and mortar to form a fine
thin cream. The slurry was poured on clean glass plates and spread uniformly by means of an applicator to achieve 0.25 mm thickness. The plates were allowed to dry in air at room temperature. The dried plates were activated by heating kept in an electric oven at 100°C for twenty minutes and were stored in a dessicator.

Equilibration of the chromatographic chamber: About 1 cm height of the solvent was taken in a clean dry chamber, after the walls of the chamber were lined with a strip of filter paper impregnated with the solvent system. The chamber was closed and allowed to saturate with vapours of solvent.

Application of spots: The base line was marked at about 1.5 cm above from the lower edge. The dissolved fractions were spotted on the plates with the fine capillary tubes and then allowed to dry in air. The spotted places were kept in the chromatographic chambers containing the solvent mixture. The chambers were covered with greased glass plates. The solvent system was allowed to ascend up to 2-3 cms below the upper edge of Silica gel-G layer. The plates were taken out, solvent fronts marked and dried.

Solvent system for TLC plates:

i) Benzene

ii) Benzene:Petroleum ether (1:1 v/v)

iii) Petroleum ether (60-80°C)

iv) Petroleum ether:Chloroform (4:1, 1:1, 1:3)

v) Chloroform

vi) Chloroform:Methanol (9:1, 4:1, 7:3, 1:1)

After drying the plates were viewed in ultra light chamber to look for any activation. Ceric ammonium citrate solution (in concentrated sulphuric acid) was used as spray reagent for detection of spots. This spray reagent was then carefully sprayed on the plates which were then heated in an oven at 110 °C for 10-15 minutes. The distance travelled by spots and their colour were noted down and their Rf values calculated.

Yield of the isolated compound has been calculated on the basis of dried material.
EXPERIMENTAL

PHYTOCHEMICAL INVESTIGATION OF SEEDS OF
AILANTHUS ALTISSIMA MILL. (SWINGLE)

Plant material: Seeds (900 g) of A. altissima were collected from the forests of Solan (H.P.).

Extraction: The dried and powdered seeds were extracted with CHCl₃ in a Soxhlet apparatus. The extract was concentrated under reduced pressure to get a dark brown viscous semi-solid mass.

Isolation of chemical constituents: The concentrated extract was dissolved in minimum amount of MeOH and adsorbed on silica gel to form a slurry. The slurry was air-dried and subjected to Si-gel column chromatography prepared in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in order of increasing polarity to isolate the following compounds:

Ailanthusterol A (AA-1):

Elution of the column with petroleum ether (fraction 1-7) furnished colourless amorphous powder of AA-1, recrystallized from CHCl₃-MeOH (1:1), 0.78 g (0.08% yield), m.p. 271 - 272°C, \([\alpha]_D^{30} = + 1.47 \ (C\ 0.6, \ AcOH)\).

\[\text{IR } \gamma_{\text{max}} (\text{KBr}): 3425, 2910, 1630, 1598, 1460, 1380, 1365, 1160, 1020 \ \text{cm}^{-1}\]

\[\text{\textsuperscript{1}H NMR (60 MHz, DMSO-d}_6): \alpha 5.30 (1H, m, H-6), 4.77 (2H, bm, H₂-21), 3.50 (1H, brm, w 1/2 = 9.5 Hz, H-3B), 0.98 (3H, brs, Me-19), 0.90 (3H, d, J = 6.0 Hz, Me-29), 0.85 (3H, d, J = 6.0 Hz, Me-26), 0.70 (3H, d, J = 6.0 Hz, Me-27), 0.63 (3H, brs, Me-18)\]

\[\text{EIMS } m/z \ (\text{rel. int.}): 412[M]^+ (C_{28}H_{48}O) \ (1.1), 394 (7.4), 379 (2.4), 273 (2.3), 255 (4.1), 213 (5.3), 201 (1.1), 192 (4.1), 174 (2.1), 164 (3.1), 160 (3.9), 152 (4.2), 146 (5.9), 144 (7.0), 135 (5.3), 132 (4.1), 124 (5.3), 120 (5.1), 118 (3.9), 108 (5.9), 106 (6.6), 95 (8.3), 83 (5.1), 81 (11.0), 72 (6.5), 69 (8.0), 57 (10.4)\].
Acetylation of AA-1: Compound AA-1 (15 mg) was treated with Ac₂O (3 ml) and pyridine (1 ml) at room temperature overnight. Water (10 ml) was added and the reaction mixture extracted with CHCl₃. The CHCl₃-layer was washed with water, dried over Na₂SO₄ and evaporated to obtain monoacetyl product (AA-1a), m.p. 146-147°, IR γ max: 1725 cm⁻¹.

Oxidation of AA-1: Compound AA-1 (15 ml) was dissolved in Me₆CO (10 ml) and freshly prepared Jones reagent added dropwise till the persistent of brown colour at 4°C. The reaction mixture was left at 25° for 2 hours, water (10 ml) added and worked up as usual to obtain 3-oxo derivative (AA-1b), m.p. 133-134°, IR γ max: 1710 cm⁻¹.

Ailanthusterol B (AA-2):
Elution of the column with petroleum ether (fraction 8-12) gave colourless beads of AA-2, recrystallized from CHCl₃-MeOH (1:1), m.p. 127-128°, [α]D³⁰ = -7.5 (C 1.37, CHCl₃).

UV λ max: 212 nm (log ε 5.7).
IR γ max (KBr): 3430, 2920, 2840, 1590, 1455, 1390, 1360, 1310, 1045 cm⁻¹.
¹H NMR (400 MHz, CDCl₃): δ 5.36 (1H, d, J = 5.5 Hz, H-6), 3.52 (1H, br m, w 1/2 = 16.50 Hz, H-3α), 1.20 (3H, brs, Me-21), 1.00 (3H, brs, Me-19), 0.92 (3H, t, J = 6.5 Hz, Me-29), 0.86 (3H, d, J = 6.5 Hz, Me-26), 0.80 (3H, d, J = 6.5 Hz, Me-27), 0.68 (3H, brs, Me-18).

EIMS m/z (rel. int.): 430 [M⁺]+(C₂₉H₄₅O₂)(N.O.), 412 (6.8), 394 (7.1), 379 (6.1), 326 (5.3), 301 (5.8), 273 (3.2), 271 (3.6), 255 (7.8), 231 (2.4), 213 (8.6), 198 (4.3), 160 (8.1), 157 (8.9), 174 (1.5), 144 (12.3), 134 (7.3), 124 (2.4), 106 (14.9), 94 (14.5), 83 (10.1), 81 (12.5), 72 (9.7), 57 (16.0), 55 (21.6), 43 (27.0).

Acetylation of AA-2: Compound AA-2 (25 mg) was acetylated with Ac₂O (5 ml) and pyridine (1 ml) at room temperature for 24 hours. Water (20 ml) was added and the reaction mixture extracted with CHCl₃ (3 x 10 ml). The organic phase was washed with H₂O, dried over Na₂SO₄ and evaporated to get monoacetyl product (AA-2a), m.p. 114-115°, IR γ max: 3400, 1725 cm⁻¹.
Jones oxidation of AA-2: Compound AA-2 (10 mg) was treated with a mixture of conc. $\text{H}_2\text{SO}_4$ (2 ml) and $\text{CrO}_3$ in acetone (5 ml) at 0° for 2 hours. Water (10 ml) was added and the reaction mixture extracted with CHCl$_3$ (3 x 10 ml). The organic phase was washed with H$_2$O (3 x 10 ml), dried over Na$_2$SO$_4$ and evaporated to get 3-oxo derivative (AA-2b), m.p. 91-93°, IR $\nu_{\text{max}}$: 3400, 1705 cm$^{-1}$. 
DISCUSSION

COMPOUND AA-1

Compound AA-1, namely ailanthusterol A, was obtained as colourless amorphous powder from petroleum ether eluants. It gave positive Liebermann-Burchard test and showed characteristic IR absorption bands for hydroxyl groups (3425 cm\(^{-1}\)) and unsaturation (1630, 1598 cm\(^{-1}\)). Its mass spectrum showed a molecular ion peak at \(m/z\) 412 corresponding to a steroidal molecular formula, \(C_{29}H_{48}O\). The important ion peaks observed at \(m/z\) 394 \([M-H_2O]^+\), 379 \([394-\text{Me}]^+\), 273 \([M-C_{10}H_{19}\text{SC}]^+\), 255 \([273-H_2O]^+\) and 213 \([255\text{-ring D}]^+\) suggested that the compound possessed a \(C_{10}\)-unsaturated side chain and a hydroxy group and olefinic linkage in the steroidal carbon framework. The ion fragments at \(m/z\) 72 \([C_{1,10}-C_{4,5} \text{ fission}]^+\), 201 \([M-71-\text{SC}]^+\), 106 \([124(C_{6,7}-C_{8,10} \text{ fission})-H_2O]^+\), 120 \([138(C_{7,8}-C_{9,10})-H_2O]^+\), 135\([M-138-\text{SC}]^+\), 83 \([C_{2,3}-C_{5,10}-C_{7,8} \text{ fission}]^+\) and 69 \([83-\text{CH}_3]^+\) supported the presence of the hydroxyl group in ring A, placed at C-3 on biogenetic analogy, and the olefinic linkage at C-5. The saturated nature of ring C was inferred from the ion peaks appearing at \(m/z\) 146 \([164(C_{6,14}-C_{9,11})-H_2O]^+\), 160 \([178(C_{6,14}-C_{9,14})-H_2O]^+\), 174 \([192(C_{6,14}-C_{12,13})-H_2O]^+\), 108 \([M-164-\text{SC}]^+\), 95\([M-178-\text{SC}]^+\) and 81 \([M-192-\text{SC}]^+\) (Scheme AA-I).

The \(^1\)H NMR spectrum of AA-1 displayed one-proton multiplet at \(\delta\) 5.30 assigned to H-6. A two-proton multiplet at \(\delta\) 4.77 was due to C-21 methylene groups. A broad multiplet at \(\delta\) 3.50 (\(\text{w} \ 1/2 = 9.5 \text{ Hz}\)), integrating for one proton, was ascribed to C-3 \(\beta\)-proton. Two three-proton each signals at \(\delta\) 0.96 and 0.63 were attributed correspondingly to C-19 and C-18 methyl protons. Three doublets at \(\delta\) 0.90, 0.85 and 0.70, integrated for three protons and with coupling interaction of 6.0 Hz each, were accounted to C-29 primary and C-27 and C-26 secondary methyls, respectively. The appearance of all the methyls in the range \(\delta\) 0.96-0.63 attested their location on the saturated carbons. Acetylation of AA-1 yielded a monoacetyl product AA-1a. Treatment of AA-1 with Jones reagent produced a 3-oxo compound (AA-1b), which responded to Zimmermann's test positively, thus confirming the presence of hydroxyl group at C-3 (Barton and Mayo, 1954).
Scheme AA - I: Mass Fragmentation Pattern of Ailanthusterol A (AA - 1)
On the basis of these findings the structure of ailanthusterol A (AA-1) has been elucidated as stigmas-5, 20(21)-diene-3β-ol. This is a new natural sterol containing C-21 unsaturated methylene group.

COMPOUND AA-2:
Compound AA-2, namely ailanthusterol B, was obtained as colourless beads from petroleum ether eluants. It showed positive Liebermann-Burchard test and characteristic IR absorption bands for hydroxyl group (3430 cm⁻¹), unsaturation (1590 cm⁻¹) and gem-dimethyl/isopropyl group (1390, 1360, 1310 cm⁻¹). The electron impact mass spectrum showed the presence of diagnostically important peaks at m/z 412 [M-H₂O], 394 [M-2 x H₂O], 157 [C₁₀H₁₈O, side chain, SC]⁺, 273 [M-SC], 255 [273-H₂O]⁺, 231 [273-ring D]⁺, 213 [255-ring D]⁺ and 198 [213-Me]⁺ (Scheme AA-II). These fragments suggested (Knight, 1967; Gupta et al., 1992; 1994) that the sterol possessed a saturated C₁₀-side chain with an ethyl group, placed on C-24 on the basis of biogenetic consideration, and with a hydroxyl group and an unsaturated stigmastane carbon framework of the steroidal nucleus with a hydroxyl group. In addition to these, peaks at m/z 72 [C₁₀-C₁₂ fusion]⁺, 124 (ring B cleavage), 55 [72-H₂O]⁺, 106 [124-H₂O]⁺, 120 [134-H₂O]⁺, 146 [M-266 (ring C cleavage)-H₂O]⁺, 160 [M-252 (ring C cleavage)-H₂O]⁺, 174 [M-238 (ring C cleavage)-H₂O]⁺ and 83 [C₂-C₆-C₅-C₇,₈ fusion]⁺ suggested the location of another hydroxyl group in ring A which was placed at C-3 on biogenetic ground, and the presence of trisubstituted double bond at C-5. The ¹H NMR spectrum of AA-2 exhibited a one-proton downfield doublet at δ 5.36 (J = 5.5 Hz) assigned to H-6. A one-proton broad triplet at δ 3.52 with half-width of 16.5 Hz is associated with C-3 axial proton. A three-proton broad signal at δ 1.20 was accounted to C-21 methyl group attached to a C-20 tertiary carbon containing a hydroxyl group. Two tertiary methyl signals at δ 1.00 (Me-19) and 0.68 (Me-18), one three-proton triplet for primary methyl at δ 0.92 (J = 6.5 Hz, Me-29) and two three-proton each doublets at δ 0.86 (J = 6.5 Hz, Me-26) and 0.80 (J = 6.5 Hz, Me-27) were in perfect agreement with the stigmaster-5-ene diol skeleton. The remaining methylene and methine protons resonated in between δ 2.80-1.08. Appearance of all the methyl signals in the region δ 1.20-0.68 supported the existence of these groups on the saturated carbons. Acetylation of AA-2 with acetic anhydride-pyridine afforded
Scheme: AA-II Mass Fragmentation Pattern of Ailanthusterol B (AA - 2)

AA - 1 : R = α - OH, H  
AA - 1a : R = α - OAc, H  
AA - 1b : R = O  

AA - 2 : R = β - OH, H  
AA - 2a : R = β - OAc, H  
AA - 2b : R = O
a monoacetyl product (AA-2). Jones oxidation of AA-2 formed a 3-oxo derivative which gave positive Zimmermann's test for 3-keto sterols, thus supporting 3-hydroxyl group in the compound (Barton and Mayo, 1954). On the basis of these chemical and spectral evidences the structure of ailanthusterol B (AA-2) has been established as stigmast-5-ene-3β,21β-diol. This is also a new sterol derivative containing one hydroxyl group in the carbocyclic framework and another in the side chain.
Mass Spectrum of Ailanthusterol B (AA-2)


34. Moron, J., Rondest, J. and Polonsky, J., "Biosynthesis of bitter constituents of simarubiacal", Experientia, 2, 511-516.


