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Alru-1, A new steroid from *Ailanthus Excelsa* Roxb. (Mahanimba)

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Abstract
A new steroid, designated Alru-1, isolated from the hexane extract of *Ailanthus excelsa* Roxb, has been characterised from spectroscopic analysis and chemical transformation as stigmasta-4, 22-dien-3*β*-ol.

Keywords: Alru-1, stigmasta-4, 22-dien-3□-ol, steroids, *Ailanthus excelsa*

Introduction
As part of our programme of analysis, isolation and characterisation of active principles of Indian medicinal plants[1], we took up the reinvestigation of *Ailanthus excelsa* Roxb.
Ailanthus excelsa Roxb. (*Simaroubaceae*), commonly known as Mahanimba (Sanskrit), Alru (Hindi) and tree of heaven, is a large deciduous tree found in India and Sri Lanka. It is well-reputed in the Indian system of medicine – Ayurveda. Its leaves are used to treat asthma, bronchitis, dyspepsia; its bark has antipyretic, antispasmodic, antiasthmatic, astringent and anthelmintic properties [2, 3]. *A. excelsa* is reported to be rich source of quassinoids – a class of highly oxygenated terpenoids, alkaloids and steroids [3]. An earlier publication of Chatterjee, Mandal and others reported the isolation and characterisation of a new steroid, designated AE-23, as stigmasta-4, 22-dien-3-one (I) [4]. We are reinvestigating the hexane extract of the stem bark of *A. excelsa*.

Materials and Methods
Plant material
Stem bark of *Ailanthus excelsa* was purchased from the local market at Kolkata from reputed suppliers of traditional drugs. The material was identified at the Botany Department, NRIADD; a voucher specimen AE/Stem Bark has been preserved.

General
Samples of compounds isolated and prepared were routinely dried *in vacuo* over anhydrous $CaCl_2$. IR spectra were recorded with a Perkin-Elmer RX-9 spectrophotometer. 300 MHz ^{1}H-NMR and 75.5 ^{13}C-NMR spectra were recorded in $CDCl_3$ solution with a Bruker Avance 300 spectrometer. Mass spectrum was recorded with a JEOL JMS600 Mass spectrometer. Silica gel (Qualigens 60-120) was used for column chromatography, Precoated aluminium plate with silica gel 60 F254, 0.2 mm thickness, Merck, was used for TLC. Spots on TLC were visualised by iodine vapour and also by spraying with 20% aqueous sulphuric acid followed by heating. Silica gel G (Merck) was used for PTLC.

Extraction and isolation of compounds
Stem bark of *A. excelsa* (1 kg) was dried and coarsely powdered. This was extracted for 24 h. in Soxhlet apparatus with hexane (5 lit.). The hexane extract was concentrated in a rotary evaporator. The concentrated extract, a brown gummy mass (8 g), was chromatographed over silica gel. The 10% EtOAc in hexane eluate furnished a mixture of compounds, which was rechromatographed over silica gel, and further resolved by PTLC over silica gel G (20% EtOAc in hexane as developing solvent). The compounds obtained were AE-23 (stigmasta-4, 22-dien-3-one), m.p. 97 ^{0}C (yield 200 mg), *β*-sitosterol, m.p. 135^{0}C (yield 350 mg) and the new compound Alru-1, (yield 100 mg). Alru-1 on crystallisation from hexane-acetone (1:1) provided white shining crystals, m.p. 124-125^{0}C, R_f 0.54 (hexane-EtOAc 1:1 as developing solvent).

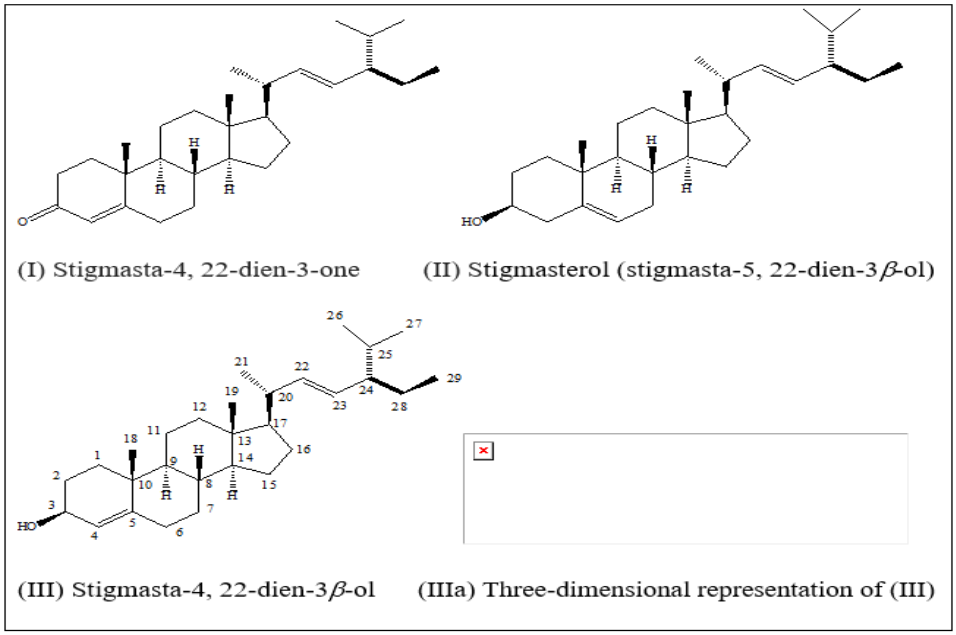
PCC Oxidation of Arlu-1

Arlu-1 (40 mg) was taken in dry methylene chloride (10 ml). Pyridinium chlorochromate (80 mg), anhydrous sodium acetate (40 mg) and Celite 545 (200 mg) were added and the solution stirred magnetically for 1 hr. The reaction mixture was filtered though a Celite bed, washed twice with 3x2 ml of methylene chloride. The combined extracts were washed with 2% sodium hydrogen carbonate solution (10 ml), then with water (10 ml), and dried over anhydrous sodium sulphate. Removal of solvent yielded a white solid (34 mg) which was identical in spectroscopical properties (IR, ^{1}H-NMR, ^{13}C-NMR) and co-TLC with stigmasta-4, 22-dien-3-one.

Results and Discussions

The present work constitutes the reinvestigation of the stem bark of *A. excelsa*. Extraction of the stem-bark with hexane, and chromatography of the extract over silica-gel followed furnished (I), *β*-sitosterol and a new compound, designated Alru-1 in the 10% EtOAc in hexane eluates. These fractions were further subjected to PTLC to obtain the pure compounds. Alru-1 was purified by crystallisation from hexane-acetone (1:1) as white crystals, m.p. 124-125^{0}C. Arlu-1 showed a positive Liebermann-Burchardt test giving the green colour characteristic of steroids. It exhibited IR bands (KBr disc) at 3429 (hydroxyl), 1646 (alkenyl, =CH), 1458, 1374, 1057 (C-O) and 966 (trisubstituted double bond) cm^{-1}. Structure elucidation was achieved by detailed spectroscopical studies, including detailed 300 MHz ^{1}H-NMR and 75.5 MHz ^{13}C- NMR studies, and confirmed by chemical transformation. Its 70ev mass spectrum showed a molecular ion peak at m/z 412 (14% relative intensity), corresponding to the molecular formula, $C_{29}H_{48}O$. Other peaks appeared at 397 (M-Me, 5%), 394 (M-H_2O, 18%), 273 (M-$C_{10}H_{19}$, 12%, indicative of a $C_{10}H_{19}$ side-chain), 271 (273 – 2H, 23%), 256 (271-Me, 65%), 147 (33%), 145 (38%), 69 (63%) and 55 (100%, base peak).

Its ^{1}H-NMR spectrum ($CDCl_3$) showed the presence of three olefinic protons. Two olefinic protons appeared as close-coupled double doublets a δ 5.01 (J 15.3, 8.4 Hz) and δ 5.13 (J 15.3, 8.4 Hz). The magnitude of the coupling constant (J 15.3 Hz) established the presence of a *trans*-double bond in the unit -CH-CH=CH-CH- in the compound. The third olefinic proton (H-4) appeared at δ 5.34 (broadened doublet) overlapped with the hydroxyl proton. H-3 appeared as a multiplet centred at δ 3.71 (signal width ~ 27 Hz). The other protons – methyl, methylene and methine linked to sp^3 carbons – appeared between δ 0.66-2.32. ^{13}C-NMR spectral studies (fully decoupled, and DEPT-90^{0}, DEPT-135^{0} experiments multiplicities to determine multiplicities) revealed the presence of six methyls, three olefinic methines, one sp^2 quaternary, nine sp^3 methylene groups, eight sp^3 methines and two sp^3 quaternary carbons. Comparison of the 300 MHz ^{1}H-NMR and 75.5 MHz ^{13}C-NMR spectra with those of stigmasta-4,22-dien-3-one (I) and stigmasterol (stigmasta-5,22-dien-3*β*-ol; II) revealed general similarities with some specific differences [4, 5]. This allowed the formulation stigmasta-4, 22-dien-3*β*-ol (III) to be made for Alru-1. Complete ^{13}C-NMR assignments are as follows – C-1 δ 33.9; X–2 δ 35.6; X–3 δ 71.7; X–4 δ 121.4; X–5 δ 140.7; X –6 & X–7 δ 32.9 & δ 32.0; X–8 δ 35.9; X–9 δ 53.7; X–10 δ 3 8.5; X–11 δ 20.9; X–12 δ 39.5; X–13 δ 42.2; X–14 & X–17 δ 55.9 & δ 55.7; X–15 δ 24.1; X–16 δ 28.7; X–18 & X–29 δ 1 2.1 & δ 12.3; X–19 δ 17.3; X–20 δ 40.4; X–21& X–26 δ 21.4 & δ 21.1; X–22 δ 138.2; X–23 δ 129.2; X–24 δ 51.2; X–25 δ 31.8; X–27 δ 19.0; X–28 δ 25.3.



The stereochemistry at C-3 was settled as follows. The multiplet at H-3 (signal width ~ 27 Hz) had couplings to C-2 methylene, hydroxyl and H-4. D_2O-exchange removed the hydroxyl and sharpened the signal at δ 5.34 (H-4) to a doublet (J 2.4 Hz), and simplified the H-3 multiplet to ddd pattern. Calculation of torsion angles involving H-3 were done by computing the three dimensional representation of the molecule with the MM2 programme [6]. The molecular geometry of the (III) was optimised by MM2 calculations carried out on a personal computer. The three dimensional representation of Alru-1 is given in (IIIa). Some computed selected parameters are given regarding rings A and B: selected bond lengths (in Angstrom): C(1)-C(2) 1.522, C(2)-C(3) 1.514, C(3)-O 1.422, C(3)-C(4) 1.496, C(4)-C(5) 1.336, C(5)-C(6) 1.498, C(5)-C(10) 1.497; selected bond angles (in degrees): C(3)-C(4)-C(5) 122.0^{0}, C(4)-C(5)-C(6) 121.4^{0}, C(4)-C(5)-C(10) 121.4^{0}, C(6)-C(5)-C(10) 117.2^{0}, C(1)-C(10)-C(5) 109.5^{0}. The torsion angles were also calculated. The

magnitude of the coupling constants indicated an □-axial orientation for H-3 - 7.1 Hz (H-2 equatorial, torsion angle 51.2^0), 11.9 Hz (H-2 axial, 178.3^0) and 2.4 Hz (H-4, - 80.1^0). Final confirmation of structure (III) came from PCC (pyridinium chlorochromate) oxidation of Alru-1 to stigmasta-4, 22-dien-3-one (I). The oxidised product was identical with an authentic sample of stigmasta-4, 22-dien-3-one, isolated earlier from this plant.

Conclusion

Re-investigation of the well-known Indian medicinal plant *Ailanthus excelsa* Roxb. (Mahanimba) yielded a new sterol, designated Alru-1. Spectroscopical investigations revealed its structure to be stigmasta-4, 22-dien-3β-ol.

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