

Triterpenoids from Aerial parts of *Pluchea lanceolata*

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Abstract:

Stigmasterol, -Sitosterol, Lupeol acetate, Taraxasteryl acetate, Taraxasterol were isolated from the aerial parts of *Pluchea lanceolata*. The structures were elucidated from spectroscopic data.

Introduction:

Pluchea lanceolata C.B. Clarke belongs to the family Compositae (syn. Asteraceae). *Pluchea lanceolata* is commonly known as Rasna. It is a shrub growing in Punjab, upper gangetic plain, Rajasthan and Gujrat[1]. This plant is used for the inflammatory conditions such as arthritis, bronchitis, psoriasis, osteoarthritis, cough and piles. It is a major ingredient of the famous anti-inflammatory ayurvedic decoction "Maharasnadi Qwath".

It is also used as antipyretic, analgesic, laxative and nervine tonic[2]. The decoction of plant is used to prevent the swellings of joints in arthritis, rheumatism and neurological diseases. Leaves are aperients and are used as substitute for Senna [2]. The roots are alexiteric, antipyretic, bitter, laxative and thermogenic and are used for allaying the pain caused by the sting of scorpions. It is highly effective for backaches, muscular strains and joint pains [2]. The plant extract is used as cooling agent in summers[2]. The genus *Pluchea* is rich in triterpenes, sterols[3,4,5,6], flavonoids, glycosides [7]and sesquiterpene[8].

We describe here in the isolation and structure elucidation of Stigmasterol, -Sitosterol, Lupeol acetate , Taraxasteryl acetate , Taraxasterol.

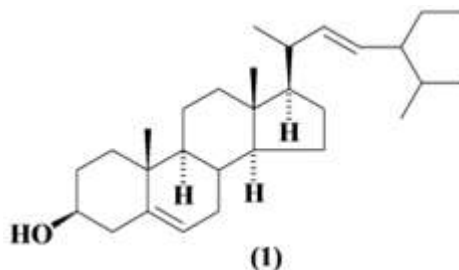
Keywords: *Pluchea lanceolata*; Compositae, triterpenoids, steroids.

Results And Discussion

Stigmasterol (1) was isolated as colourless shining flakes, m.p. 166-167° and displayed single spot on TLC-plate. It responded positive Liebermann-Burchard[9] and Noller tests for sterols. It also gave positive test for unsaturation.

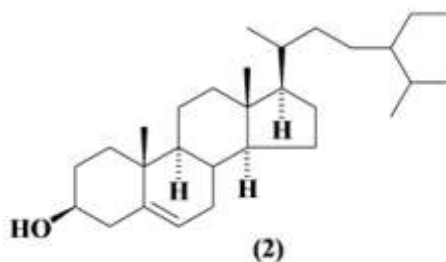
It has one hydroxyl group and two double bonds. The presence of hydroxyl group was ascertained by the appearance of a broad absorption band at 3400-3200 cm⁻¹ in IR-spectrum.

The ¹H NMR spectrum in CDCl₃ displayed a broad triplet at 5.34 for olefinic H-6 proton and a pair of double doublets at 5.04 and 5.12 for H-22 and H-23 olefinic protons respectively. Large coupling constants of order of 16 Hz in double doublets indicated their *trans* geometry. A multiplet centered at 3.52 was explainable to H-3 methine proton under oxygen function. A triplet at 0.81 corresponded to C-29 methyl protons, while a doublet at 0.91 (J = 7 Hz) and singlets at 0.79, 0.88 ppm were due to C-21, C-18 and C-19 methyl protons respectively. A doublet at 1.16 was observed for C-27 methyl protons.



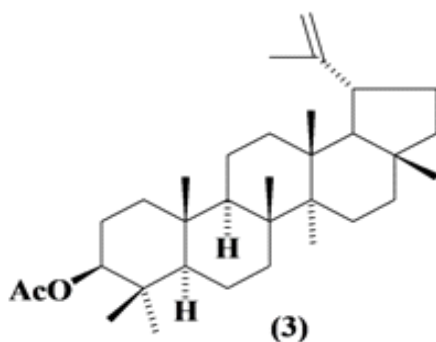
In mass spectrum, molecular ion peak $[M]^+$ was observed at m/z 412 corresponding to its molecular formula $C_{29}H_{48}O$. An intense peak at m/z 397 was due to the loss of methyl radical from 412. The other important peaks were observed at m/z 328, 302, etc.

-Sitosterol(2) was obtained as colourless needles, m.p. $136-37^\circ$ and responded positive Liebermann-Burchard and Noller tests for sterols[10]. From mass spectrum its molecular formula was ascertained as $C_{29}H_{50}O$. Presence of hydroxyl group (3450 cm^{-1}) was confirmed by its infra-red spectrum.



The ^1H NMR spectrum in CDCl_3 displayed the presence of an olefinic proton and hydroxymethine proton by the appearance of a broad triplet at 5.27 and a multiplet at 3.48 respectively. Rest of the protons were appeared in high field region (0.70-2.0 ppm). It formed an acetate, m.p. $127-28^\circ$ when it is refluxed with acetic anhydride and a drop of pyridine over water bath.

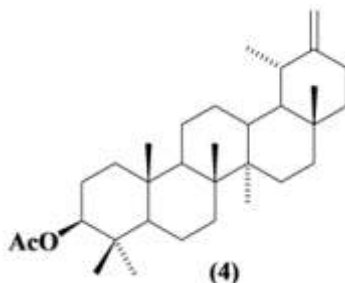
Lupeol acetate(3) was obtained as colourless crystals, m.p. $215-216^\circ$. It belongs to lupane series of triterpenoids. Its molecular formula $C_{32}H_{52}O_2$ was established from mass spectrum. Its IR spectrum clearly showed the presence of an acetoxy group by the appearance of a strong absorption band at 1725 cm^{-1} . An absorption at 1640 cm^{-1} showed the presence of olefinic double bond.



Its ^1H NMR spectrum in CDCl_3 displayed six sharp singlets in upfield region at 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03 for six tertiary methyl groups. A broad singlet at 1.69 showed the presence of methyl group attached to olefinic double bond. Vinylic protons were characterized by their broad singlets at 4.69 and 4.57. Proton under acetoxy function appeared in downfield region at 4.38 as a double doublet. H-19 proton of cyclopentane ring showed a multiplet centered at 2.38. A sharp singlet at 2.10 ppm confirmed the presence of an acetyl group.

A prominent parent ion peak appeared at m/z 468 corresponding to its molecular formula $\text{C}_{32}\text{H}_{52}\text{O}_2$ along with peaks at m/z 453 $[\text{M}-\text{Me}]^+$, 450 $[\text{M}-\text{H}_2\text{O}]^+$, 408 $[\text{M}-\text{AcOH}]^+$, 242, 240, 231 and 213 etc. in its mass spectrum.

Taraxasteryl acetate(4) was obtained as colourless crystals, m.p. 256-57°. Its molecular formula $\text{C}_{32}\text{H}_{52}\text{O}_2$ was established on the basis of spectral studies.



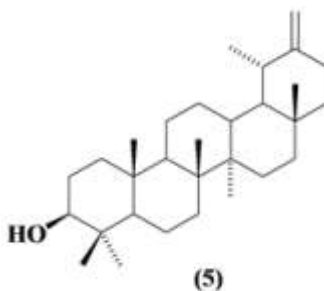
It was found to be an unsaturated compound as evidenced by its positive test with TNM. It gave pinkish violet colour changing to blue green with Liebermann-Burchard reagent, intense red colour with chlorosulphonic acid and positive Salkowski and Noller tests, indicating its triterpenoid nature.

In IR spectrum, an intense peak at 1725 cm^{-1} confirmed the presence of acetate moiety. The other absorption bands were at 3000-2800 (C-H stretch), 1470, 1460 (C-H bend) and 1400 cm^{-1} etc.

Its ^1H NMR spectrum in CDCl_3 revealed the presence of six singlets at 0.90, 0.89, 0.88, 0.87, 0.86, 0.85 and a doublet at 0.84 ($J=7\text{ Hz}$) corresponding to seven methyl groups in a pentacyclic triterpene. A multiplet at 4.60 indicated the presence of exomethylene protons. The appearance of a double doublet at 4.48 ($J=11, 7\text{ Hz}$) corresponded to H-3 proton. A characteristic acetoxy signal appeared at 2.04 ppm.

In the mass spectrum, molecular ion peak appeared at 468 $[\text{M}]^+$ confirmed its molecular composition. The other fragment ion peaks at m/z 453 and 408 were attributed to the loss of methyl radical and an acetic acid moiety from the molecular ion peak respectively.

Taraxasterol (-Lactuceryl)(5) was isolated as colourless crystals, m.p. 225-26°. Its molecular formula $\text{C}_{30}\text{H}_{50}\text{O}$ was established from its mass spectral studies.



It was found to be an unsaturated compound as evidenced by its positive test with TNM. It gave positive Salkowski[11] and Noller tests of triterpenes.

The important absorption bands in its infrared spectrum were observed[12] at 3470 (OH), 2980-2855 (C-H stretch), 1650 (C=C stretch), 1460, 1375 (gem dimethyl groups) and 1050 cm^{-1} (C-O stretch).

Six singlets at 0.88, 0.87, 0.86, 0.85, 0.84, 0.83 and a doublet at 0.82 ($J = 7\text{ Hz}$) were appeared due to the seven methyl groups in its ^1H NMR spectrum (Fig. 5) in CDCl_3 . A multiplet centered at 4.56 corresponded to exomethylene protons. A double doublet at 3.23 ($J = 11,7\text{ Hz}$) assigned to H-3 proton.

The molecular ion peak at m/z 426 [M^+] was observed in its mass spectrum corresponding to its molecular composition $\text{C}_{30}\text{H}_{50}\text{O}$. The other important fragment peaks were observed at m/z 411 [M-Me^+], 408 [$\text{M-H}_2\text{O}^+$], and 203.

Experimental

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus and are uncorrected. column chromatography (CC): silica gel (Merck 60-120 mesh). Prep.TLC: Merck silica gel 60 F_{254} precoated glass plates, UV spectra: Hitachi U-200 spectrophotometer, IR spectra: FT-IR Nicolet Magna 550 and Shimadzu QP-5000 spectrophotometer. ^1H and ^{13}C NMR spectra: JEOL AL-300 MHz and Bruker Avance DRX 500 FT NMR spectrometers, MS: JEOL JMS-SX 102A and JEOL D-300 spectrometers.

Plant material

The plant material were collected from the surroundings of Jaipur and identification was done with the help of Botany Department, University of Rajasthan, Jaipur India and a voucher specimen was deposited at RUBL Herbarium, Jaipur.

Extraction and Isolation

The air-dried aerial parts (5 kg) of *P. lanceolata* were extracted with petroleum-ether (60-80°) on water bath for 3x12 hours. The extract was concentrated in vacuo and resulting semi solid mass (15 g) was chromatographed over silica gel (Merck 60-120 mesh) to give seven fractions : fraction-1(Petroleum ether), fraction-2(Petroleum ether : chloroform,3:1), fraction-3(Petroleum ether : chloroform,1:1), fraction-4(Petroleum ether : chloroform,1:3), fraction-5 (Chloroform), fraction-6(Chloroform : acetone, 3:1), fraction-7 (Chloroform : acetone, 1:1).

Fractions 1 and 2 were abandoned as they were complex mixture of fatty material. Fraction 3 afforded as stigmasterol colourless needles, 100 mg, m.p. 166-67°. Fraction 4 gave -Sitosterol as colourless bright needles, 180 mg, m.p. 136-137° . . Fraction 5 afforded as Lupeol acetate colourless needles, 55 mg, m.p. 215-16°. Fraction 6 gave Taraxasteryl acetate as white crystals, 65 mg, m.p. 256-57°. Fraction 7 gave Taraxasterol as white crystals, 250 mg, m.p. 25-26°

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