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Two New Triterpenoids from the Roots of *Ficus Racemosa* Linn.

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ABSTRACT

Ficus racemosa Linn. is a moderate-sized avenue tree found throughout India either wild or cultivated for its fruits eaten by villagers. It is popular in Indigenous system of medicine like Ayurveda, Siddha and Homeopathy. Two new triterpenoids of lanostene series were isolated from the roots of *Ficus racemosa* and their structure were determined as Lanost-20-en-3β-acetate (**1**) and Lanost-20-en-3β-ol (**2**) on the basis of various spectral and chemical studies.

Keywords: *Ficus racemosa* Linn. Triterpenoids, Lanost-20-en-3β-acetate (**1**), Lanost-20-en-3β-ol (**2**)

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INTRODUCTION

Ficus racemosa Linn. is a large spreading tree, belonging to family Moraceae. Its bark, milk sap and roots are used to wash wounds, in inflammation, diabetes and dysentery respectively. The ripe fruits are useful in blood diseases, biliousness, burning sensation, fatigue, urinary discharge, chronic bronchitis, thirst, leprosy, menorrhagia and nose bleeding[1-3]. The stem bark was found to exhibit hyperglycemic effect and the leaf extract showed hypotensive and cardiac depressant activity[4,5]. A survey of literature revealed the isolation of triterpenoids, steroids, coumarins and anthocyanins from the leaves, stem bark, heartwood, fruits and latex[6-19]. but the roots of *F. racemosa* Linn. have not been investigated so widely. Our phytochemical investigation of the roots of *F. racemosa* have resulted in the isolation of two new triterpenoids (Figure 1).

MATERIALS AND METHOD

General experimental procedure

Melting points were recorded in soft glass capillaries in an electrothermal melting point apparatus and are incorrect. All solvents used were of analytical grade. The column and thin layer chromatography were conducted on silica gel (60-120 mesh). Spots on TLC plates were visualized in UV light, by spraying with 2% ceric ammonium sulphate in 2N H_2SO_4. The infrared spectra were recorded as KBr pellets on Perkin-Elmer 557 model spectrometer and A400S, Shimadzu, FT-IR spectrometer. ^{1}H NMR spectra were recorded on and Bruker DRX 200 FT NMR and Jeol Al 500 MHz instruments using $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal reference. EIMS spectra were recorded on a Hitachi model RMU 6E and Jeol D-300 mass spectrometer.

Plant material

The plant material, roots of *F. racemosa* Linn., was collected from the road side of Ganesh Marg, Bapu Nagar, Jaipur and carefully identified in the Department of Botany, University of Rajasthan, Jaipur (Herbarium sheet No. RUBL 19764).

Extraction and isolation

The roots were air-dried, powdered and exhaustively extracted with ethanol (95%) on a steam bath for 8 hrs thrice. The extract was concentrated under reduced pressure when a dark brown semi-solid was obtained. The ethanolic extract was re-extracted with pet. ether, benzene and ethyl acetate successively whereby on concentration under reduced pressure brown pet. ether, yellowish-brown benzene and reddish-brown ethyl acetate fractions were obtained. Since all the fractions exhibited a similar TLC profile (benzene: ethyl acetate, 1:1), they were mixed together and The combined fractions were chromatographed over a column of silica gel. Elution was carried out with solvents of increasing polarity, viz., pet ether, benzene, ethyl acetate and methanol. The

fractions were collected with pet. ether: benzene (2:3) was repeatedly crystallized with ethyl acetate to afford compound **1** (145 mg). The fraction eluted with EA–MeOH (6:4) (1.3 g) was subjected to repeated column chromatography on silica gel EA-MeOH (1:1) leading to the isolation of compound **2** (170 mg).

Compound 1

White needle shaped solid. IR (KBr) ν_{max} : 1725 (C=O stretching), 1640 (C=C stretching) and 1245 cm^{-1} (-O-CO-CH_3, stretching). ^1H NMR (500 MHz, DMSO-d6) spectral data see Table 1. ESI-MS: m/z 470 [M^+], (calcd. for $C_{32}H_{54}O_2$, 470.41).

Table 1. ^1H (500 MHz) and 13C (125 MHz) NMR spectral data of compounds 1 and 2 (DMSO-d6, δ ppm, J, Hz).

S.N.	Protons	Compound 1	Compound 2
1	Vinylic proton	4.69 (d, 1H, 2.1)	4.60 (d, 1H, 2.3)
2	Vinylic proton	4.68 (d, 1H, 2.1)	4.58 (d, 1H, 2.3)
3	Acetoxy proton	2.04 (s, 3H)	
4	Geminal to acetoxy group	4.56 (m, 1H)	
5	Geminal to hydroxy group		3.45
6	Methyl groups	1.05-0.60 (7*CH_3)	1.05-0.60 (7*3CH_3)
7	Methylene and methine proton	1.62-1.14	1.62-1.14

Hydrolysis of lanost-20-en-3β-acetate

The compound (100 mg) was refluxed with 5% alc. potassium hydroxide (5 ml) for six hours. The solvent was then evaporated off, water added and the residual product was extracted with ether. The ether portion was washed with water, dried over anhydrous sodium sulphate and filtered. After removal of ether a white solid was obtained which was crystallized from MeOH-EA as white solid, m.p. 140-42 ^oC.

Compound 2

White solid. IR (KBr) ν_{max} : 3400 (O-H stretching), 1650 cm^{-1} (C=C stretching) and 1110 cm^{-1} (C-O stretching). ^1H NMR (500 MHz, DMSO-d6) spectral data see Table 1. ESI-MS: m/z 428 [M^+], (calcd. for $C_{30}H_{52}O_1$, 428.4).

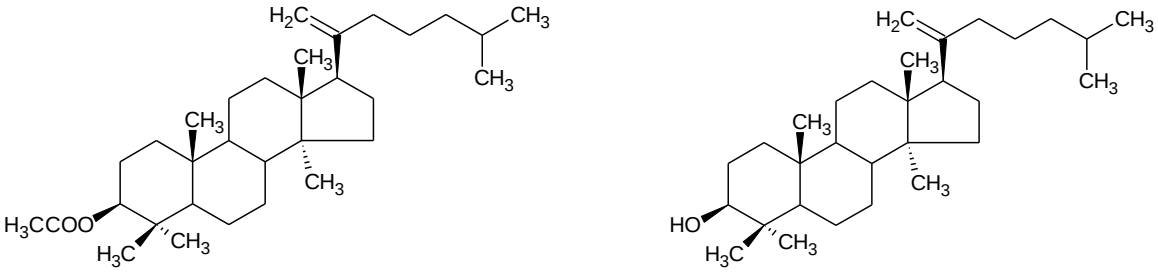
Acetate of Lanost-20-en-3β-ol

Compound (100 mg), acetic anhydride (5 ml) and pyridine (2-3 drops) were refluxed for 4 hrs and the resulting mixture was poured in ice-cold water. The solid was filtered washed well with cold water and crystallized Ethyl acetate as white needles shaped solid, m.p. 150-54 °C.

RESULTS AND DISCUSSION

Compound **1** was isolated as white needle shaped solid after crystallization with ethyl acetate m.p. 152-54^oC. In the mass spectrum, the molecular ion peak appeared at m/z 470 and peaks at m/z 455,

427 and 359 corresponded to the loss of CH_3, C_3H_7 and C_8H_{15} fragments respectively from the parent ion. The peak at m/z 410 [M^+– 60,CH_3COOH] indicated the presence of an acetyl group in the compound. The M^+– CH_3COOH ion (m/z 410) loses CH_3, C_3H_7 and C_8H_{15} fragments to give peaks at m/z 395, 367 and 299 respectively. The peaks at 359 [M^+ – C_8H_{15}] and 299 [M^+– CH_3COOH – C_8H_{15}] confirmed the unsaturated nature of the side chain while peaks at 410 [M^+ – C_5H_{10}] and 340 [M^+ – (CH_3COOH + C_5H_{10})] due to allylic cleavage were in conformity with the $\Delta^{20(21)}$double bond. In the ^{1}H NMR spectrum, a pair of doublets at δ 4.68 and δ 4.69 corresponded to the presence of vinylic protons. A singlet at δ 2.04 for the acetoxy proton and a multiplet centered at δ 4.56 for the proton geminal to acetoxy group were observed. Peaks in the region δ 0.60-1.05 were accountable for eight methyl group. The remaining protons appeared as a multiplet in the region δ 1.62-1.14. The hydrolysis of lanost-20-en-3β-acetate with 5% alcoholic potassium hydroxide yielded an alcohol, m.p. 140-42°C, identified as lanost-22-en-3β-ol in which all the spectral data were matched with the compound **2** . Based on these evidences, the compound **1** was identified as lanost-20-en-3β-acetate (Figure 1).



1 **2**

Figure 1. The chemical structures of compounds 1 and 2.

Compound **2** was obtained as white solid on crystallization with chloroform-methanol, m.p. 140-42 °C. In the ^{1}H NMR spectrum, a pair of doublets at δ 4.58 and δ 4.60 could be assigned to the vinylic protons. A multiplet centered at δ 3.45 appeared for the proton attached to the carbon with hydroxy group. Peaks in the region δ 0.60-1.05 were accountable for eight methyl group indicating its tetracyclic nature. The remaining protons appeared as a multiplet in the region δ 1.62-1.14. In the mass spectrum the molecular ion peak appeared at m/z 428 and peaks at m/z 413, 385 and 317 corresponded to the loss of CH_3, C_3H_7 and C_8H_{15} fragments respectively from the parent ion. The peaks at 317 [M^+ – C_8H_{15}] confirmed the unsaturated nature of the side chain. These physical and spectral data closely resembled those of the triterpenoid obtained on hydrolysis of compound **1**, hence it was identified as lanost-20-en-3β-ol (Figure 1). The structure of compound **2** was further confirmed by the acetate preparation which have all the physical and spectral data closely resembled to compound **1**.

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