NEW LANOSTENE-TYPE TRITERPENES FROM THE OLEO-GUM RESIN OF COMMIPHORA MYRRHA (NEES) ENGL.

MOHD. SHUAIB*a,b, MOHD. ALI*c, KAMRAN J. NAQUVIa

• Phytochemistry Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110 062, India. *Kalka Institute for Research & Advanced Studies, Meerut-250006, Uttar Pradesh.
Email: mailphyto@gmail.com

Received: 23 Mar 2014 Revised and Accepted: 25 Apr 2014

INTRODUCTION
Commiphora myrrha (Nees) Engl. (Burseraceae) is a small tree which grows in small sandy and rocky regions of Somalia, Sudan, Ethiopia, Kenya and Saudi Arabia. The schizogenous cavities of the stem and branches of this tree produce a scented oleo-resin which is known as myrrh. It is imported into India since long time and used in perfumery as food additive, fragrance, incense, antiseptic, astringent, stimulant, stomachic, tonic and for embalming. It is an ingredient of toothpastes, mouthwashes and dentifrices. Myrrh tincture is useful in menstrual disorders and chlorosis. In China, it is prescribed to treat wounds, inflammation and menstrual pain due to blood stagnation [1].

Cadinenes, calamenes, triaccont-1-ene [2], commiphoric acids, furanosesquiterpenoids [3-10], eudesmol and triterpenoids [11] and volatile oil [12] have been reported from the oleo-resin of C. myrrha. This paper describes the isolation of three new tetracyclic lanostene-type triterpenoids along with three known aliphatic esters from the oleo-resin obtained from the Khari Baoli market of Delhi.

MATERIALS AND METHODS
Instrumentation and techniques
Melting points were determined on a Perkin melting apparatus (Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra red red spectra were recorded on Bio-Rad PTIR 5000 (FTS 135, Kawlooin, Hong Kong) spectrophotometer using KBr pellets; values are given in cm
1
.1H and 13C NMR spectra were screened on Avance DRX 400, Bruker spectrometer 400 and 100 MHz instruments (Karlsruhe, Germany) using TMS as an internal standard.

Mass spectra were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was ran on silica gel G (Qualigen). Spots were visualised by exposing to iodine vapours, UV radiation, and spraying with ceric sulphate.

Plant material
The crude oleo-gum resin of C. myrrha was procured from the local market of the Khari Baoli, Delhi. The sample was authenticated by Dr. H.B. Singh, Taxonomist, NISCAIR, CSIR, New Delhi. A voucher specimen of the sample (No. N/R/C/06-07/803/120) was deposited in the NISCAIR, RHM Division, Dr. K.S. Krishnan Marg (Near Pusa Gate), New Delhi.

Extraction and isolation
The air dried oleo-gum resin (2.5 kg) was coarsely powdered and extracted with methanol at room temperature for one week. The extract was filtered and concentrated under reduced pressure to get 185 g (yielded 7.4 %) of dark brown mass. The concentrated extract of the oleo-resin was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) to form slurry. The slurry was air dried and filled up in a silica gel column (1.6 m × 16 mm × 2 mm) loaded in petroleum ether and then eluted successively with different solvents in increasing order of polarity in various combinations, such as petroleum ether, petrol ether- chloroform (9:1, 3:1, 1:1, 1:3), chloroform, chloroform-methanol (19:9:0.1, 99:1, 97:3, 19:1, 93:7, 9:1, 1:7, 0.1, 3:1, 3:2, 2:3) and methanol. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same Rf values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of C. myrrha oleo-gum resin:

n-Heptadecanyl capriate (1)

Elution of the column with petroleum ether produced a light coloured sticky mass of 1, recrystallized from methanol-acetone (9:1), 460 mg (0.0184 % yield); Rf: 0.73 (petroleum ether- chloroform; 9: 1); m.p.: 76-78°C; +ve ion FAB MS m/z (rel. int.): 410 [M]+ (C27H45O3) 171 (75.7) 155 (61.3).

n-Dodecanyl myristate (2)

Further elution of the column with petroleum ether gave a colourless amorphous powder of 2, recrystallized from acetone-methanol (1:1), 1.41 g (0.0564 % yield); Rf: 0.77 (chloroform-
Elution of the column with petroleum ether-chloroform (1:1) yielded yellow solid mass of 3, recrystallized from petroleum ether-acetone (1:1), 1.1 g (0.044% yield). Rf: 0.81 (petroleum ether-chloroform; 1:1); m.p. 70-71°C; UV \( \lambda_{\text{max}}(\text{MeOH}) \): 223, 242.66 (log \( \epsilon \) 4.3, 3.7); IR \( \nu \) (KBr): 15.72 (1H, m, H-11), 5.94 (1H, m, H-12), 5.75 (1H, d, \( J=7.5 \) Hz, H-24), 3.23 (1H, dd, \( J=7.5, 7.5 \) Hz, H-6), 2.87, 2.85 (2H, each, H-23, H-23), 2.40 (1H, d, \( J=4.5 \) Hz, H-24), 1.32 (2H, m, H-21), 1.19 (3H, Me-30), 0.91 (3H, Me-31) \( \delta \) ppm (CDCl\(_3\), ppm): 6.94.18 \( \delta \) ppm (CDCl\(_3\)).

**RESULTS AND DISCUSSION**

Compounds 1, 2 and 4 are fatty acid esters characterized as \( \alpha \)-heptadecanoyl caprate, \( \alpha \)-dodecanoyl myristate and \( \alpha \)-hexatriacontanol, respectively (Figure 1).

Compound 3, designated as myrrhalanosteryl acetate, was obtained as a yellow solid mass from petroleum ether-chloroform (9:1) eluents. Its IR spectrum displayed characteristic absorption bands for ester group (1741 cm\(^{-1}\)) and unsaturation (1640 cm\(^{-1}\)). On the basis of \( \psi \) FAB mass and \( \psi \) C NMR spectra, the molecular ion peak of 3 was determined at \( m/z \) 462 consistent with the molecular formula of triterpene ester C\(_{36}H_{40}O_2\). The fragment formed at \( m/z \) 194 [C\(_{36}H_{36}O_2\), fission], 204 [C\(_{36}H_{36}O_2\), fission] indicated the location of acyl group in ring A and the existence of a vinylic linkage at C-5. The ion peaks arising at \( m/z \) 215, 247 [C\(_{36}H_{36}O_2\), fission], and 261 [C\(_{36}H_{36}O_2\), fission] suggested the presence of other vinylic linkage in ring B at C-11. The ion peaks produced at \( m/z \) 107 [C\(_{28}H_{44}O_2\), side chain], 419 [M-\( \alpha \)-Ac]; 402 [M-\( \alpha \)-AcOH]; 355 [M-\( \alpha \)-side chain], 314 [355-\( \alpha \)-AcOH] and 295 [355-\( \alpha \)-AcOH] indicated that the \( \alpha \)-side chain contained three vinylic linkages. The \( \psi \) NMR spectrum of 3 displayed two one-proton multiplets at \( \delta \) 5.49 and 5.73 assigned to vinyl H-6 and H-11 protons, respectively. Four one-proton broad singlets at \( \delta \) 5.24, 4.87 and at \( \delta \) 4.70, 4.77 were ascribed correspondingly to methyl H-21 and H-26 protons. One-proton triplet doublet at \( \delta \) 5.21 (J=15.6, 5.5 Hz) and a one-proton doublet at \( \delta \) 5.27 (J=15.6 Hz) were assigned to \( \psi \)-CH=O and C-25 methyl protons, respectively. A one-proton doublet-doublet at \( \delta \) 6.13 (J=7.5, 5.7 Hz) was ascribed to \( \psi \)-oriented oxygenated H-3 methyl proton. The remaining methylene protons resonated between \( \delta \) 2.35-1.45. Two three-proton broad singlets at \( \delta \) 1.66 and 2.02 were ascribed to methyl protons Me-27 attached to olefinic carbon and acetyl protons, respectively. Five-proton broad singlets at \( \delta \) 0.91, 1.23, 1.30, 1.09 and 1.19 were associated correspondingly with C-18, C-19, C-28, C-29, and C-30 tertiary methyl protons. The \( \psi \) C NMR spectrum of 3 exhibited signals for ester carbon at \( \delta \) 170.57 (Ac) and vinylic carbons between 149.89-109.78, carbonil carbon at \( \delta \) 84.88 (C-3) and methyl carbons from \( \delta \) 26.72-14.08. The \( \psi \) NMR spectrum of 3 was compared with the related lanosterol type triterpenoids [13-15]. On the basis of the above discussion, the \( \alpha \)-side chain was characterized as lanost-5,9(11),20(21)-23,25-pentaene-3\( \beta \)-ol acetate (Figure 1). It is a lanostane-type triterpene isolated from a natural source for the first time.

Compound 5, designated as myrrhalanostanol, was obtained as a light brown mass from petroleum ether-chloroform (1:1) eluents. Its IR spectrum displayed absorption bands for hydroxyl group (3440 cm\(^{-1}\)), lactone group (1742 cm\(^{-1}\)) and unsaturation (1645 cm\(^{-1}\)). On the basis of mass and \( \psi \) C NMR spectra, the molecular ion peak of 5 was established at \( m/z \) 542 consistent with the molecular formula of a triterpenoid, C\(_{36}H_{40}O_2\). The fragment ion peaks at \( m/z \) 150 [C\(_{28}H_{28}O\), fission], 164 [C\(_{28}H_{28}O\), fission], 206 [C\(_{28}H_{28}O\), fission] and 220 [C\(_{28}H_{28}O\), fission] supported saturated nature of ring C, the presence of a vinylic linkage at C-5 and hydroxyl group in ring D. The \( \psi \) NMR spectrum of 5 showed three one-proton downfield double-doublets at \( \delta \) 5.60 (J=7.5, 5.6 Hz), 5.52 (J=7.5, 4.9 Hz) and 5.37 (J=7.3, 10.7 Hz) assigned correspondingly to cis-oriented H-23, H-22, and H-26 vinylic protons, respectively. A two-proton broad signal at \( \delta \) 4.84 was ascribed to H-26 methyl protons. A doublet and a double doublet, one proton each, at \( \delta \) 6.59 (J=5.6 Hz) and \( \delta \) 3.25 (J=8.7, 4.5 Hz) were attributed to oxygenated methine H-24\( \beta \) and carbinol H-3\( \alpha \) protons, respectively. Six three-
proton broad singlets at δ 1.67, 1.16, 1.12, 1.09, 1.01 and 0.96 were accounted correspondingly to C-26 methyl protons to a vinylic carbon and to tertiary C-19, C-29, C-28, C-30 and C-18 methyl protons. The 13C NMR spectrum of 5 exhibited 30 carbon signals. The important signals appeared for vinylic carbons at δ 142.33 (C-5), 122.76 (C-6), 137.39 (C-22), 137.01 (C-23), 147.48 (C-25) and 107.81 (C-26), lactone carbon at δ 172.81 (C-21) and oxygenated methine carbons at δ 76.56 (C-3) and 86.02 (C-24). The 1H NMR and 13C NMR spectral data of 5 were compared with the lanoster type triterpenoids [13-15]. On the basis of above discussion the structure of the 5 was elucidated as lanost-5, (Z)-22,25(27)-trien-3β-olide-26-oic acid (Figure 1). It is a new lanostane-type triterpenoid isolated from a natural source.

Compound 6, designated as myrrhalanostenoic acid was obtained as a colourless solid mass from chloroform eluants. It responded positively to tests for triterpenoids and produced effervescences with sodium bicarbonate solution. Its IR spectrum exhibited important absorption bands for hydroxyl group (3425 cm⁻¹), carboxyl group (3218, 1680 cm⁻¹), unsaturation (1645 cm⁻¹) and lactone ring (1740 cm⁻¹). Its molecular weight was established at m/z 486 on the basis of +ve FAB mass and 13C NMR spectra consistent with the molecular formula of a triterpenic acid, C₃₀H₄₀O₃. The ion peaks arising at m/z 192, 294 [C₁₈H₂₂O₃], 306, 280 [C₁₉H₂₄O₃], 320, 266, 312 [C₂₀H₂₆O₃] indicated the presence of olefinic linkage in ring B at C-5, and the hydroxyl group in ring A which was placed at C-3 on the biogenetic considerations. The diagnostic ion peaks arising at m/z 192, 294 [C₁₈H₂₂O₃], 306, 280 [C₁₉H₂₄O₃], 320, 266, 312 [C₂₀H₂₆O₃] suggested the saturated nature of ring C. The other fragment ion peaks at m/z 171 [CH₃OH], side chain (SG) + 300 [M-side chain-Me]- and 297 [M-side chain-H₂O] supported the presence of C₉-side chain containing a lactone moiety and carboxylic group and saturated ring D. The 1H NMR spectrum of 6 exhibited a one-proton downfield signal at δ 5.03 attributed to vinylic H-6 proton. A one-proton broad multiplet at δ 4.82 with half-width value (Δν₀.₅=9.2 Hz) was ascribed to oxygenated methine H-24 proton placed in β-orientation. Another one-proton doublet at δ 3.34 (J=3.6, 4.5 Hz) was assigned to β-oriented H-3 carbonyl proton. Five three-proton broad singlets at δ 1.12, 1.08, 1.03, 0.98, and 0.77 were attributed to C-19, C-28, C-29, C-30 and C-18 tertiary methyl protons, respectively, all attached to the saturated carbons. A three-proton doublet at δ 0.85 (J=6.5 Hz) was assigned to C-27 secondary methyl protons. The 13C NMR spectrum of 6 showed 30 carbon signals and the important signal were observed for δ-lactone at δ 173.08 (C-21) and 82.65 (C-24), carboxyl carbon at 181.57 (C-26), vinylic carbons at δ 141.35 (C-5) and 121.48 (C-6), carbonyl carbon at 80.27 (C-3) and methyl carbons between δ 28.29-14.89. The 1H NMR and 13C NMR values of 6 were compared with lanoster-type triterpenoids [13-15]. On the basis of the above evidences the structure of 6 has been established as lanost-5-en-3β-olide (Figure 1). It is a new lanostane-type triterpenoid isolated from a natural source.

CH₃(CH₂)₁₀CO - OCH₂(CH₂)₁₅CH₃

CH₃(CH₂)₁₂CO - OCH₂(CH₂)₁₀CH₃

CH₃(CH₂)₁₀CO - OCH₂(CH₂)₁₅CH₃

Fig. 1: Structures of compound 1-6.

REFERENCES


