

This article was downloaded by:

On: 22 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713454007>

### New sterol esters from the flowers of *Punica granatum* Linn.

Priyanka Bagri<sup>a</sup>; Mohammed Ali<sup>a</sup>; Shahnaz Sultana<sup>a</sup>; Vidhu Aeri<sup>a</sup>

<sup>a</sup> Phytochemistry Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India

**To cite this Article** Bagri, Priyanka , Ali, Mohammed , Sultana, Shahnaz and Aeri, Vidhu(2009) 'New sterol esters from the flowers of *Punica granatum* Linn.', Journal of Asian Natural Products Research, 11: 8, 710 – 715

**To link to this Article:** DOI: 10.1080/10286020903004291

**URL:** <http://dx.doi.org/10.1080/10286020903004291>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## New sterol esters from the flowers of *Punica granatum* Linn.

Priyanka Bagri, Mohammed Ali\*, Shahnaz Sultana and Vidhu Aeri

Phytochemistry Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi 110062, India

(Received 27 December 2008; final version received 29 March 2009)

Two new  $\beta$ -sitosterol esters have been isolated from the flowers of *Punica granatum* Linn. (Punicaceae) along with the known compounds *n*-tricosane (**3**), *n*-heptacosanyl *n*-hexanoate (**4**), olean-5,12-dien-3 $\beta$ -ol-28-oic acid (**5**), and olean-12-en-3 $\beta$ -ol-28-oic acid (**6**). The structures of the new phytosterols have been elucidated as stigmast-5-en-3 $\beta$ -ol-3 $\beta$ -dodecanoate ( $\beta$ -sitosterol laurate, **1**) and stigmast-5-en-3 $\beta$ -ol-3 $\beta$ -tetradeconoate ( $\beta$ -sitosterol myristate, **2**) on the basis of spectral data and chemical analyses.

**Keywords:** *Punica granatum*; Punicaceae; steroidal esters;  $\beta$ -sitosterol laurate;  $\beta$ -sitosterol myristate

### 1. Introduction

*Punica granatum* Linn. (Punicaceae), commonly called as pomegranate, is an ancient fruit with an illustrious medical history [1]. It is a shrub or a small tree and considered to be a native of Iran and Afghanistan. It is also found growing wild in the warm valleys and outer hills of the Himalayas, and is cultivated throughout India [2]. In Ayurvedic medicine, the pomegranate is considered 'a pharmacy unto itself'. The bark and root possess anthelmintic and vermifuge properties, and the peels are a powerful astringent and used to cure diarrhoea and oral aphthae [3,4]. The powdered flower buds are ingested to relieve bronchitis, diarrhoea, and dysentery; as a styptic to the gums and to allay biliousness, ulcers, sore throat, and sore eyes [5]. In Unani medicine, pomegranate flowers serve as a remedy for diabetes mellitus [6]. The earlier studies of *P. granatum* flowers have shown

the presence of sterols, phenolic compounds, and pentacyclic triterpenes [7,8].

The present paper describes the isolation and characterization of two unknown steroidal esters from the flowers of *P. granatum* of the unexplored North Indian Delhi region along with the four known compounds *n*-tricosane (**3**), *n*-heptacosanyl *n*-hexanoate (**4**), olean-5,12-dien-3 $\beta$ -ol-28-oic acid (**5**), and olean-12-en-3 $\beta$ -ol-28-oic acid (**6**).

### 2. Results and discussion

Compound **1**, named  $\beta$ -sitosterol laurate, was obtained from petroleum ether–chloroform (1:1) eluants as a colorless crystalline mass. It responded positively to steroidal tests. Its IR spectrum showed characteristic absorption bands for the ester group (1736  $\text{cm}^{-1}$ ), unsaturation (1629  $\text{cm}^{-1}$ ), and long aliphatic chain (724  $\text{cm}^{-1}$ ). On the basis of MS, HR-MS, and  $^{13}\text{C}$  NMR spectra, its molecular weight was established at  $m/z$  596 consistent

\*Corresponding author. Email: maliphyto@gmail.com

with the molecular formula of a steroid esterified with a C<sub>12</sub> fatty acid, C<sub>41</sub>H<sub>72</sub>O<sub>2</sub>. Its molecular formula indicated six double bond equivalents. Four of them were adjusted in the tetracyclic carbon framework of the steroidal nucleus, one in the vinylic linkage and the remaining one in the ester group. The prominent ion peaks generated at *m/z* 581 [M–Me]<sup>+</sup>, 553 [M–CHMe<sub>2</sub>]<sup>+</sup>, 413 [M–CO(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>]<sup>+</sup>, 183 [CO(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>]<sup>+</sup>, 397 [413–Me]<sup>+</sup>, 396 [M–CH<sub>3</sub>(CH<sub>2</sub>)<sub>10</sub>COOH]<sup>+</sup>, 272 [413–C<sub>10</sub>H<sub>21</sub>, side chain]<sup>+</sup>, 270 [272–2H]<sup>+</sup>, 255 [270–Me]<sup>+</sup>, 240 [255–Me]<sup>+</sup>, 213 [255–ring D fission]<sup>+</sup>, and 198 [213–Me]<sup>+</sup> suggested that a C<sub>12</sub> acyl moiety was esterified with the hydroxyl group of the sterol containing a C<sub>10</sub> saturated side chain. The <sup>1</sup>H NMR spectrum of **1** displayed a one-proton broad multiplet at δ 5.27 ascribed to the vinylic H-6. A one-proton broad multiplet at δ 4.16 with a half width of 16.5 Hz was attributed to an α-oriented C-3 carbinol proton. Two three-proton broad signals at δ 0.67 and 1.04 were assigned to C-18 and C-19 tertiary methyl protons, respectively. Four doublets at δ 0.93 (*J* = 6.3 Hz), δ 0.87 (*J* = 6.6 Hz), δ 0.80 (*J* = 6.0 Hz), and δ 0.83 (*J* = 3.6 Hz), all integrating for three protons each, were due to C-21, C-26, and C-27 secondary and C-29 primary methyl protons, respectively. The presence of all methyl signals in the range of δ 1.04–0.67 suggested the location of these functionalities on the saturated carbons. Two one-proton doublets at δ 2.50 (*J* = 5.2 Hz) and δ 2.48 (*J* = 5.2 Hz) were accounted to the C-2' methylene protons adjacent to the ester group. A one-proton triplet at δ 0.85 (*J* = 6.1 Hz) was due to H-12' terminal primary methyl protons. The remaining methylene and methine protons resonated between δ 2.39 and 1.17. The <sup>13</sup>C NMR spectrum of **3** showed important signals for the ester carbon at δ 173.2 (C-1'), the vinylic carbon at δ 141.1 (C-5) and δ 119.8 (C-6), the carbinol carbon at δ 69.7 (C-3), the primary methyl carbon at δ 13.8 (C-12'), and the remaining methyl, methylene, and methine carbons between δ

55.4 and 11.3. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data were compared with β-sitosterol [9], lawsaritol [10], and other related steroids [11]. Alkaline hydrolysis of **1** yielded β-sitosterol and lauric acid. The <sup>1</sup>H and <sup>13</sup>C HETCOR spectrum of **1** showed correlations of C-6 with H<sub>2</sub>-4 and H<sub>2</sub>-7; C-5 with H-3, H<sub>2</sub>-4, and H-6; and C-1' with H-3 and H<sub>2</sub>-2'. On the basis of the spectral data analysis and chemical reactions, the structure of **1** has been established as stigmast-5-en-3β-ol-3β-dodecanoate. It is an unreported sterol ester (Figure 1).

Compound **2**, named β-sitosterol myristate, was obtained from petroleum ether–chloroform (1:3) eluants as a colorless crystalline mass. Its IR spectrum showed characteristic absorption bands for the ester group (1737 cm<sup>-1</sup>), unsaturation (1640 cm<sup>-1</sup>), and long aliphatic chain (727 cm<sup>-1</sup>). On the basis of FAB-MS, HR-MS, and <sup>13</sup>C NMR spectra, its molecular weight was established at *m/z* 624 consistent with the molecular formula of a sterol ester, C<sub>43</sub>H<sub>76</sub>O<sub>2</sub>. Its molecular formula indicated six double bond equivalents; four of them were adjusted in the tetracyclic skeleton of the steroid and one each in the vinylic linkage and ester group. The MS of **2** exhibited prominent ion fragments generated at *m/z* 609 [M–Me]<sup>+</sup>, 413 [M–CO(CH<sub>2</sub>)<sub>12</sub>CH<sub>3</sub>]<sup>+</sup>, 211 [CH<sub>3</sub>(CH<sub>2</sub>)<sub>12</sub>CO]<sup>+</sup>, 396 [M–CH<sub>3</sub>(CH<sub>2</sub>)<sub>12</sub>COOH]<sup>+</sup>,

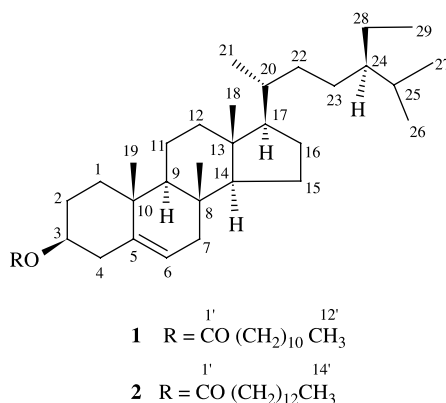


Figure 1. Structures of compounds **1** and **2**.

228 [CH<sub>3</sub>(CH<sub>2</sub>)<sub>12</sub>COOH]<sup>+</sup>, 398 [413-Me]<sup>+</sup>, 272 [413-C<sub>10</sub>H<sub>21</sub>, side chain]<sup>+</sup>, 270 [272-2H]<sup>+</sup>, 255 [270-Me]<sup>+</sup>, 240 [255-Me]<sup>+</sup>, 213 [255-ring D fission]<sup>+</sup>, and 198 [213-Me]<sup>+</sup> suggested that a sterol was esterified with a C<sub>14</sub> fatty acid. The ion fragments that arose at *m/z* 108 [C<sub>6,7</sub>-C<sub>9,10</sub> fission]<sup>+</sup>, 122 [C<sub>7,8</sub>-C<sub>9,10</sub> fission]<sup>+</sup>, 164 [C<sub>8,14</sub>-C<sub>9,11</sub> fission]<sup>+</sup>, 178 [C<sub>8,14</sub>-C<sub>11,12</sub> fission]<sup>+</sup>, and 192 [C<sub>8,14</sub>-C<sub>12,13</sub> fission]<sup>+</sup> supported the existence of the vinylic linkage in ring B at C<sub>5</sub> and the saturated nature of ring C. The <sup>1</sup>H NMR spectrum of **2** displayed a one-proton multiplet at δ 5.26 ascribed to the vinylic H-6. A one-proton broad multiplet at δ 4.01 with a half width of 16.5 Hz was attributed to an α-oriented C-3 carbinol proton. Two three-proton broad signals at δ 0.67 and 1.04 were assigned to C-18 and C-19 tertiary methyl protons, respectively. Four doublets at δ 0.93 (*J* = 6.2 Hz), δ 0.87 (*J* = 6.4 Hz), δ 0.80 (*J* = 6.1 Hz), and δ 0.82 (*J* = 5.6 Hz), all integrating for three protons each, were due to C-21, C-26, and C-27 secondary and C-29 primary methyl protons, respectively. The presence of all methyl signals in the range of δ 1.04–0.67 suggested the location of these functionalities on the saturated carbons. Two one-proton doublets at δ 2.50 (*J* = 5.2 Hz) and 2.48 (*J* = 5.2 Hz) were accounted to C-2' methylene protons adjacent to the ester group. A one-proton triplet at δ 0.84 (*J* = 6.1 Hz) was due to H-14' terminal methyl protons. The remaining methine and methylene protons appeared in the range of δ 2.27–1.17. The <sup>13</sup>C NMR spectrum of **2** showed important signals for the ester carbon at δ 173.1 (C-1'), the vinylic carbon at δ 141.1 (C-5) and δ 119.9 (C-6), the carbinol carbon at δ 69.8 (C-3), the primary methyl carbon at δ 14.5 (C-14'), and the remaining methyl, methylene, and methine carbons between δ 55.4 and 11.4. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data of the steroidal nucleus of **2** were compared with β-sitosterol [9], lawsaritol

[10], and other related steroids [11]. Alkaline hydrolysis of **2** afforded β-sitosterol and myristic acid. The <sup>1</sup>H and <sup>13</sup>C HETCOR spectrum of **2** showed correlations of C-5 with H-3, H<sub>2</sub>-4, and H-6; and C-1' with H-3 and H<sub>2</sub>-2'. On the basis of the foregoing discussion, the structure of **2** has been elucidated as stigmat-5-en-3β-ol-3β-tetradecanoate. It is an unknown sterol ester (Figure 1).

Compounds **3**, **4**, **5**, and **6** are the known phytoconstituents and their structures have been established as *n*-tricosane, *n*-heptacosanyl *n*-hexanoate [12], olean-5,12-dien-3β-ol-28-oic acid, and olean-12-en-3β-ol-28-oic acid [13], respectively.

### 3. Experimental

#### 3.1 General experimental procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. The IR spectra were recorded on KBr pellets using a Jasco FT/IR-5000 instrument. The UV spectra were scanned in methanol on a Lambda Bio 20 spectrophotometer. The <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (75 MHz) spectra were recorded on an Advance DRY 400, Bruker Spectrospin in CDCl<sub>3</sub>. The MS were measured in the FAB ionization mode with a JEOL-JMS-DX 303. Silica gel (60–120 mesh; Qualigens, Mumbai, India) was used for column chromatography. Silica gel (Qualigens) was used for analytical TLC. Spots were visualized by exposure to iodine vapors, UV radiation, and by spraying reagents.

#### 3.2 Plant material

The flowers of *P. granatum* were purchased from the Khari-Baoli market of Delhi and identified by Dr M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen (No. PRL/JH/05/21) has been deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

### 3.3 Extraction and isolation

The flowers (800 g) dried at 45°C in an electric oven were coarsely powdered and exhaustively extracted with methanol in a Soxhlet apparatus for 72 h. The combined extracts were concentrated and dried on a steam bath under reduced pressure to obtain 420 g of a dark brown mass. It was dissolved in 250 ml methanol and adsorbed on silica gel (60–120 mesh) for the preparation of slurry. The slurry was dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, petroleum ether–chloroform (9:1, 3:1, 1:1, 1:3 v/v), chloroform, chloroform–methanol (99:1, 98:2, 95:5, 9:1, 3:1, 1:1, 1:3 v/v), and methanol, successively, in the order of increasing polarity.

#### 3.3.1 $\beta$ -Sitosterol laurate (1)

Elution of the column with a petroleum ether–chloroform (1:1) mixture yielded colorless crystals of **1**, recrystallized from methanol, 756 mg (0.094% yield);  $R_f$ : 0.68 (toluene:ethyl acetate:formic acid, 10:10:3); mp 108–109°C; UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 211 (5.8) nm; IR  $\nu_{\max}$  (KBr): 2920, 2852, 1736, 1629, 1463, 1463, 1373, 1225, 1173, 724  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) spectral data: Table 1; + ve FAB-MS  $m/z$  (rel. int.): 596  $[\text{M}]^+$  ( $\text{C}_{41}\text{H}_{72}\text{O}_2$ ) (6.2), 581 (19.3), 553 (52.3), 413 (37.8), 397 (100), 396 (41.3), 383 (20.1), 381 (16.2), 272 (10.2), 270 (10.1), 255 (23.6), 240 (13.2), 213 (26.5), 198 (21.3), 192 (11.6), 183 (16.2), 178 (32.8), 164 (23.7), 159 (83.6), 145 (84.2), 133 (65.3), 122 (31.9), 108 (69.3), 107 (73.8), 95 (97.3), 91 (70.2); HR-MS: 597.5610  $[\text{M}+\text{H}]^+$  (calcd for  $\text{C}_{41}\text{H}_{73}\text{O}_2$ , 597.5598).

**3.3.1.1 Alkaline hydrolysis of 1.** Compound **1** (60 mg) was dissolved in aqueous ethanol (5 ml) and then 1 N NaOH solution was added. It was heated on a steam bath for 30 min. The solution was dried under reduced pressure and the residue was

dissolved in  $\text{CHCl}_3$  (5 ml). It was washed with  $\text{H}_2\text{O}$  ( $3 \times 5$  ml), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and crystallized to obtain  $\beta$ -sitosterol, mp 135–137°C, EI-MS  $m/z$  414  $[\text{C}_{29}\text{H}_{50}\text{O}]^+$ ; Co-TLC comparable. The residue after separating the chloroform-soluble portion was dissolved in water (5 ml) and acidified to Congo red with dil. HCl. It was extracted with  $\text{CHCl}_3$  ( $3 \times 5$  ml). The  $\text{CHCl}_3$  extract was dried under reduced pressure to obtain lauric acid, mp 42–44°C (co-TLC comparable).

#### 3.3.2 $\beta$ -Sitosterol myristate (2)

Elution of the column with a petroleum ether–chloroform (1:3) mixture gave colorless crystals of **2**, recrystallized from methanol, 137 mg (0.017% yield);  $R_f$ : 0.47 (petroleum ether:chloroform:ethyl acetate, 1:1.5:0.5); mp 116–117°C; UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 209 (5.2), 229 (3.1) nm; IR  $\nu_{\max}$  (KBr): 2927, 2857, 1737, 1640, 1461, 1375, 1244, 1181, 1106, 1049, 960, 727  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) spectral data: Table 1; + ve FAB-MS  $m/z$  (rel. int.): 624  $[\text{M}]^+$  ( $\text{C}_{43}\text{H}_{76}\text{O}_2$ ) (5.3), 609 (8.3), 413 (37.2), 398 (16.2), 396 (12.6), 272 (11.3), 270 (11.5), 255 (21.6), 240 (6.2), 228 (13.2), 213 (38.1), 211 (15.6), 198 (43.2), 192 (17.9), 178 (33.1), 173 (65.8), 164 (23.6), 159 (90.3), 145 (87.6), 133 (73.2), 192 (7.1), 178 (9.2), 164 (12.5), 122 (3.1), 108 (12.6), 95 (100); HR-MS: 625.5932  $[\text{M}+\text{H}]^+$  (calcd for  $\text{C}_{43}\text{H}_{77}\text{O}_2$ , 625.5951).

**3.3.2.1 Alkaline hydrolysis of 2.** Compound **2** (60 mg) was dissolved in aqueous ethanol (5 ml) and then 1 N NaOH solution was added. It was heated on a steam bath for 30 min. The solution was dried under reduced pressure and the residue was dissolved in  $\text{CHCl}_3$  (5 ml). It was washed with  $\text{H}_2\text{O}$  ( $3 \times 5$  ml), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and crystallized to obtain  $\beta$ -sitosterol, mp 135–137°C, EI-MS  $m/z$  414  $[\text{C}_{29}\text{H}_{50}\text{O}]^+$ ; co-TLC comparable. The residue after separating the

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of  $\beta$ -sitosterol laurate (1) and  $\beta$ -sitosterol myristate (2).

Position	1		2	
	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR
	$\alpha, \beta$		$\alpha, \beta$	
1	1.35 m, 2.39 m	36.7	1.35 m, 2.27 m	36.7
2	1.90 m, 1.79 m	31.1	1.95 m, 1.78 m	31.1
3	4.16 m ( $w_{1/2} = 16.5$ )	69.7	4.01 m ( $w_{1/2} = 16.5$ )	69.8
4	1.78 m, 1.73 m	41.9	1.78 m, 1.71 m	41.9
5	—	141.1	—	141.1
6	5.27 m	119.8	5.26 m	119.9
7	2.14 dd (7.2, 4.8), 2.16 m	31.1	2.14 dd (7.2, 4.8), 2.18 m	31.3
8	1.17 m	31.1	1.19 m	31.1
9	1.49 m	49.5	1.47 m	49.5
10	—	38.1	—	38.9
11	2.06 m, 1.43 m	21.1	2.03 m, 1.47 m	23.5
12	1.19 m, 1.90 m	39.8	1.21 m, 1.95 m	39.8
13	—	41.9	—	41.9
14	1.52 m	55.9	1.52 m	55.9
15	1.17 m, 1.68 m	24.2	1.17 m, 1.70 m	24.1
16	1.68 m, 1.49 m	28.7	1.70 m, 1.47 m	28.9
17	1.43 m	55.4	1.43 m	55.4
18	0.67 brs	11.3	0.67 brs	11.4
19	1.04 brs	19.2	1.04 brs	19.3
20	2.06 m	36.7	2.03 m	36.7
21	0.93 d (6.3)	18.3	0.93 d (6.2)	18.4
22	1.52 m, 1.19 m	33.3	1.52 m, 1.19 m	33.2
23	1.26 brs, 1.79 m	25.7	1.27 brs, 1.78 m	25.7
24	1.68 m	27.3	1.69 m	27.8
25	1.52 m	45.1	1.50 m	45.1
26	0.87 d (6.6)	20.3	0.87 d (6.4)	20.4
27	0.80 d (6.0)	18.7	0.80 d (6.1)	22.5
28	1.52 m, 1.66 m	23.5	1.54 m, 1.63 m	23.9
29	0.83 d (3.6)	11.3	0.82 d (5.6)	11.6
1'	—	173.1	—	173.1
2'	2.50 d (5.2), 2.48 d (5.2)	40.1	2.50 d (5.2), 2.48 (5.2)	40.1
3'	2.39, 2.16 m	28.5	2.27 m, 2.25 m	28.6
4'	2.14 m, 2.06 m	28.5	2.14 m, 2.03 m	28.6
5'	1.26 brs, 1.29 brs	28.5	1.25 brs, 1.25 brs	28.6
6'	1.26 brs, 1.29 brs	28.5	1.25 brs, 1.25 brs	36.7
7'	1.26 brs, 1.29 brs	28.5	1.25 brs, 1.25 brs	31.1
8'	1.26 brs, 1.29 brs	28.5	1.25 brs, 1.25 brs	69.8
9'	1.26 brs, 1.29 brs	28.5	1.25 brs, 1.25 brs	41.9
10'	1.26 brs, 1.29 brs	28.4	1.25 brs, 1.25 brs	141.1
11'	1.26 brs, 1.29 brs	28.4	1.25 brs, 1.25 brs	119.88
12'	0.85 t (6.1)	13.8	1.25 brs, 1.25 brs	31.2
13'	—	—	1.25 brs, 1.25 brs	31.1
14'	—	—	0.84 t (6.1)	14.5

Coupling constants in Hz are provided in parentheses.

chloroform-soluble portion was dissolved in water (5 ml) and acidified to Congo red with dil. HCl. It was extracted with  $\text{CHCl}_3$

(3  $\times$  5 ml). The  $\text{CHCl}_3$  extract was dried under reduced pressure to obtain myristic acid, mp 57–58°C (co-TLC comparable).

### 3.3.3 *n*-Tricosane (**3**)

Elution of the column with petroleum ether furnished a colorless amorphous powder of **3**, recrystallized from acetone, 1.47 g (0.183% yield);  $R_f$ : 0.77 (petroleum ether); mp 46–47°C; IR  $\nu_{\max}$  (KBr): 2921, 2852, 1463, 1375, 724  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  1.39 (16H, brs,  $8 \times \text{CH}_2$ ),  $\delta$  1.32 (26H, brs,  $13 \times \text{CH}_2$ ), 0.99 (3H, brs, Me-1), 0.92 (3H, brs, Me-23); + ve FAB-MS  $m/z$  (rel. int.): 324  $[\text{M}]^+$  ( $\text{C}_{23}\text{H}_{48}$ ).

### 3.3.4 *n*-Heptacosanyl *n*-hexanoate (**4**)

Elution of the column with a petroleum ether–chloroform (3:1) mixture afforded colorless crystals of **4** [12], 252 mg (0.031% yield);  $R_f$ : 0.66 (petroleum ether:chloroform, 4:1); mp 52–53°C; IR  $\nu_{\max}$  (KBr): 1736  $\text{cm}^{-1}$ ; + ve FAB-MS  $m/z$  (rel. int.): 494  $[\text{M}]^+$  ( $\text{C}_{33}\text{H}_{66}\text{O}_2$ ) (10.5), 395 (57.8), 99 (11.6).

### 3.3.5 *Olean-5,12-dien-3 $\beta$ -ol-28-oic acid* (**5**)

Elution of the column with a chloroform–methanol (99:1) mixture yielded pale yellow crystals of **5** [13], 599 mg (0.074% yield);  $R_f$ : 0.49 (chloroform); mp 274–276°C; IR  $\nu_{\max}$  (KBr): 3423, 1691, 1635  $\text{cm}^{-1}$ ; + ve FAB-MS  $m/z$  (rel. int.): 454  $[\text{M}]^+$  ( $\text{C}_{30}\text{H}_{46}\text{O}_3$ ) (49.6).

### 3.3.6 *Olean-12-en-3 $\beta$ -ol-28-oic acid* (**6**)

Elution of the column with a chloroform–methanol (97:3) mixture afforded colorless crystals of **6** [13], 698 mg (0.087% yield);  $R_f$ : 0.63 (chloroform); mp 308–310°C; IR  $\nu_{\max}$  (KBr): 3424, 1690,

1645  $\text{cm}^{-1}$ ; + ve FAB-MS  $m/z$  (rel. int.): 456  $[\text{M}]^+$  ( $\text{C}_{30}\text{H}_{48}\text{O}_3$ ) (24.8).

## Acknowledgement

The authors are thankful to the Head, Regional Sophisticated Center, Central Drug Research Institute, Lucknow for recording the spectral data of the compounds.

## References

- [1] Anonymous, *The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products* (Publication and Information Directorate (CSIR), New Delhi, 2003), Vol. V, p. 425.
- [2] N.D. Prajapati, S.S. Purohit, A.K. Sharma, and T. Kumar, *A Handbook of Medicinal Plants* (Agrobios India, Jodhpur, 2003), p. 432.
- [3] S.A.H. Naqvi, M.S.Y. Khan, and S.B. Vohora, *Fitoterapia* **62**, 221 (1991).
- [4] K.R. Kirtikar and B.D. Basu, *Indian Medicinal Plants* (Sri Satguru Publications, New Delhi, 2000), Vol. IV, p. 1508.
- [5] A.K. Nadkarni, *Nadkarni's Indian Materia Medica* (Popular Prakashan, Bombay, 1976), Vol. 1, p. 1031.
- [6] M.I. Jurjani, *Zakheera-Khwazaram-Shahi* (Munshi Nawal Kishore, Lucknow, 1878) p. 145 [Urdu translation].
- [7] E.P. Lansky and R.A. Newman, *J. Ethnopharmacol.* **109**, 177 (2007).
- [8] T.H.W. Huang, Q.L. Yang, M. Harada, G.Q. Li, J. Yamahara, B.D. Roufogalis, and Y.H. Li, *J. Cardiovasc. Pharmacol.* **46**, 856 (2005c).
- [9] M.S. Greca, P. Monaco, and L. Previtera, *J. Nat. Prod.* **53**, 1430 (1990).
- [10] S. Gupta, M. Ali, M.S. Alam, M.S. Niwa, and T. Sakai, *Phytochemistry* **31**, 2558 (1992).
- [11] M. Ali, *Techniques in Terpenoids Identification* (Birla Publication, New Delhi, 2001), p. 407.
- [12] S. Sultana, M. Ali, S.H. Ansari, and P. Bagri, *J. Saudi Chem. Soc.* **11**, 527 (2007).
- [13] M. Maillard, C.O. Adewunmi, and K. Hostettman, *Phytochemistry* **31**, 1321 (1992).