# Dammarane and Ceanothane Triterpenes from *Zizyphus glabrata*

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From the leaves of *Zizyphus glabrata*, a new dammarane-type triterpene, pseudojujubogenin -3-O-$\beta$-D-glucopyranoside, along with the known ceanothane triterpenes, granulosic acid, ceanothic acid and daucosterol were isolated. The structures of the compounds were fully characterized by detailed NMR investigations including $^1$H and $^{13}$C NMR, HSQC, COSY, HMBC and NOESY experiments. In addition, the dammarane glycoside was tested for its potential to inhibit various bacteria and was found to possess significant bactericidal activity. The $^1$H, $^{13}$C and full 2D-NMR data on granulosic acid has also been presented. This is the first report on the chemical constituents of the leaves of *Z. glabrata*.

*Key words: Zizyphus glabrata Heyne* (syn: *Z. trinervia* Roxb), Rhamnaceae, Dammarane, Ceanothane Triterpenes, Antimicrobial Activity

## Introduction

*Zizyphus glabrata Heyne* (syn: *Z. trinervia* Roxb) is a small tree that grows up to 30 ft in height, having olive-brown wood and commonly found in the forests of Peninsular India and Bhutan [1, 2]. The leaves and aerial parts of the plant are traditionally used to treat inflammation, to relieve pain, convulsions and viral infections [3]. Plants belonging to the genus *Zizyphus* (Rhamnaceae) have been noted to produce a variety of characteristic secondary metabolites ranging from cyclopeptide alkaloids that possess antibacterial and antifungal activities [4], and the dammarane class of triterpenes that are reported as sweetness inhibitors [5 – 7]. The present work describes the isolation and characterization of a new dammarane-type triterpene, pseudojujubogenin-3-O-$\beta$-D-glucopyranoside along with the ceanothane triterpenes, granulosic acid, ceanothic acid and daucosterol.

## Results and Discussion

The leaves of *Z. glabrata* were extracted successively with hexane, chloroform and methanol that on concentration afforded three dark viscous semisolids. The methanolic residue was separated by silica gel column chromatography to furnish a new dammarane-type triterpene glycoside, together with the known ceanothane triterpenes, granulosic acid, ceanothic acid and daucosterol (**1 – 4**). Ceanothic acid and daucosterol were characterized by direct comparison of their physical and spectroscopic characteristics with those published in the literature (see experimental), while the two other isolated compounds were characterized by detailed NMR investigations including $^1$H and $^{13}$C NMR, HSQC, COSY, HMBC and NOESY experiments.

Compound **1** was the major isolate obtained in this investigation as pale green amorphous powder, m. p. 241 – 243 °C. It gave a positive for Liebermann-Burchard test for triterpenes and Molisch test for sugars. The IR spectrum indicated the presence of a tertiary hydroxyl at 3460 cm$^{-1}$ and the absence of a conjugated system in the molecule. The high resolution mass spectrum showed a molecular ion peak at *m/z* 650.85 [M]$^+$, supporting the molecular formula of $C_{36}H_{58}O_{10}$ for **1**, deduced from the mass spectrum in conjunction with the $^{13}$C NMR spectrum. The NMR spectrum (Table 1) exhibited signals for 36 carbons: nine methylene [two of them bearing oxygen atoms ($\delta = 66.2$ and 68.9)], seven methines [one oxymethine

Table 1. $^{1}$H, $^{13}$C NMR and COSY spectral data for the dammarane triterpene glycoside **1**.

| Position | $\delta$H | $\delta$C | COSY$^{\dagger}$ | Position | $\delta$H | $\delta$C | COSY$^{*}$ |
|---|---|---|---|---|---|---|---|
| 1 | a) 0.81 (m, 1H) | 39.0 | H-1b, H-11a | 16 | | 110.6 | |
| | b) 1.49 (m, 1H) | | H-1a, H-2b | | | | |
| 2 | a) 1.77 – 1.90 (m, 1H) | 26.9 | H-3$\alpha$ | 17$\alpha$ | 1.72 (m, 1H) | 53.9 | H-13$\beta$ |
| | b) 2.29 (m, 1H) | | H-1b | | | | |
| 3$\alpha$ | 3.38 (dd, 1H, 4.7, 11.5) | 89.0 | H-2a, H-2b | 18$\beta$ | 1.02 (s, 3H) | 19.0 | H-7 |
| 4 | | 37.6[a] | | 19$\beta$ | 1.02 (s, 3H) | 17.1 | H-7 |
| 5$\alpha$ | 0.70 (m,1H) | 56.3 | H-28$\alpha$ | 20 | | 69.1 | |
| 6 | 1.37 (m, 2H) | 18.5 | H-15b | 21 | 1.44 (s, 3H) | 29.8 | H-13$\beta$ |
| 7 | 1.54 (m,2H) | 36.2 | H-18, H-19 | 22 | 2.16 (m, 1H) | 46.9 | |
| 8 | | 37.4 | | 23 | 5.03 (m, 2H) | 68.9 | H-15a, H-24 |
| 9 | 0.81 (m, 1H) | 53.2 | | 24 | 5.42 (d, 1H, 8.0) | 127.2 | H-26, H-27 |
| 10 | | 37.4[a] | | 25 | | 135.4 | |
| 11 | a) 1.37 (m, 1H) | 21.8 | H-17$\alpha$ | 26 | 1.64 (s, 3H) | 25.9 | |
| | b) 1.49 (m, 1H) | | H-11a, H-17$\alpha$ | | | | |
| 12 | a) 1.77 – 1.90 (m, 1H) | 28.7 | | 27 | 1.72 (m, 3H) | 18.7 | H-23 |
| | b) 1.97 (m, 1H) | | H-11a | | | | |
| 13$\beta$ | 2.72 (m, 1H) | 38.6 | | 28$\alpha$ | 1.34 (m, 3H) | 28.4 | H-5$\alpha$ |
| 14 | | 53.5 | | 29$\beta$ | 0.74 (s, 3H) | 16.6 | H-1b |
| 15 | a) 1.77 – 1.90 (m, 1H) | 39.9 | H-13$\beta$ | 30 | 4.28 (d, 2H, 8.6) | 66.2 | |
| | b) 2.20 (d, 1H, 8.3) | | | | | | |
| $\beta$-D-Glucose | | | | | | | |
| G-1 | 4.97 (d, 1H, 7.7) | 107.2 | G-2 | G-4 | 4.20 (dd, 1H, 8.6, 9.1) | 72.2 | |
| G-2 | 4.07 (t, 1H, 8.7) | 76.0 | G-5, G-6b | G-5 | 4.25 (t, 1H, 8.6) | 79.0 | |
| G-3 | 4.02 (m, 1H) | 78.6 | G-4 | G-6 | a) 4.63 (dd, 1H, 2.4, 11.7) | 63.4 | G-6b |
| | | | | | b) 4.42 (dd, 1H, 5.5, 11.7) | | |

$^{*}$ Assignments were confirmed by 2D NMR experiments (HSQC, HMBC and 2D-NOESY); [a] signals are interchangeable. Coupling constants "J" in Hertz.

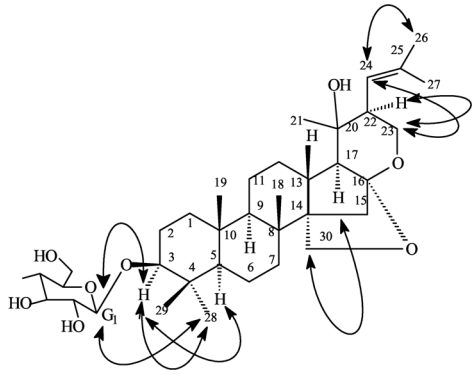



Fig. 1. Important NOESY interactions of **1**.

($\delta = 89.0$)], seven methyl carbons, an anomeric carbon $\delta = 107.2$ bound to $\delta = 4.97$ (1H, *d*, 7.7) according to the HSQC spectrum. Comparison of the NMR data for **1** (Table 1) with the COSY 45° spectrum, revealed the sugar (pyranose form) to be glucose. The coupling constant of the anomeric proton *i. e.* $\delta = 4.97$ (1H, *d*, 7.7) indicated $\beta$-configuration of glucopyranosyl moiety. A 1H double doublet at $\delta = 3.38$ ($J = 4.7$, 11.5 Hz) characteristic for H-3$\alpha$ having a sugar linked at C-3 was supported by $^{2}J$ HMBC correlations with the anomeric carbon 107.2 (G-1), and the geminal methyls [28.4 (C-28), 16.6 (C-29)] located at C-4. NOE correlation between H-3 of the genin and G-1 of the glucose confirmed the attachment of the sugar at position C-3 of the aglycone. These signals resembled a dammarane-type triterpene having a single sugar unit in the A ring at 3-O-$\beta$-position and a free tertiary hydroxyl group [8 – 11].

The spectrum also revealed an olefinic methine, $\delta =$ 5.42 ($\delta = 127.2$) along with signals typical to that of an isobutenyl side chain. The COSY 45° spectrum revealed that the methyls $\delta = 1.64$ and 1.72 and resonances at $\delta = 25.9$ and 18.7 ascribed to C-26 and C-27 were coupled to the unsaturated methine at $\delta =$ 5.42 and were assignable to H-24. The placement of the side chain at C-22 was accomplished through the HMBC experiment. The olefinic methine resonating at $\delta = 5.42$ (H-24) showed a $^{2}J$ correlation with the carbon $\delta = 46.9$ (C-22) and $^{3}J$ long-range couplings with the carbons $\delta = 68.9$ (C-23), 25.9 (C-26), 18.9 (C-27) supporting that the side chain was located at C-22. Of the three oxygen functions in **1**, one was assigned to a tertiary hydroxyl group $\delta = 69.1$ (C-20) while the two other oxygen's were directly involved in ethers of

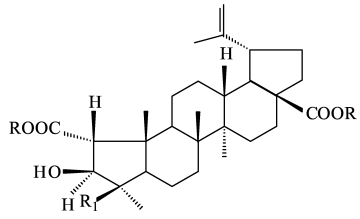
Table 2. Key HMBC correlations observed for the dammarane triterpene glycoside **1**.

| Protons | $^2J$ | $^3J$ |
|---|---|---|
| H-1 | 26.9 (C-2) | |
| H-2 | 89.0 (C-3) | |
| H-3 | 107.2 (G-1), 28.4 (C-28), 16.6 (C-29) | |
| H-5 | | 89.0 (C-3) |
| H-12 | 38.6 (C-13) | 53.9 (C-17) |
| H-13 | 53.5 (C-14) | 69.1(C-20) |
| H-18 | 36.2 (C-7), 53.5 (C-14) | 37.6 (C-10) |
| H-19 | 56.3 (C-5) | 38.6 (C-4), 21.8 (C-11) |
| H-21 | 46.9 (C-22) | |
| H-22 | 53.9 (C-17), 69.1 (C-20) | |
| H-23 | 110.6 (C-16) | 127.2 (C-24) |
| H-24 | 46.9 (C-22) | 68.9 (C-23), 25.9 (C-26), 18.9 (C-27) |
| H-26/27 | 127.2 (C-24) | |
| H-28 | 89.0 (C-3), 56.3 (C-5) | 16.6 (C-29) |
| H-30 | 53.5 (C-14) | 38.6 (C-13) |
| G-1 | 89.0 (C-3) | 78.6 (G-3) |
| G-3 | 78.6 (G-4) | 107.2 (G-1), 79.0 (G-5) |
| G-4 | 79.0 (G-5) | 63.4 (G-6) |

a ketal group $\delta = 5.03$ and 4.28 (*d*, $J = 8.6$ Hz, H-30) with resonances $\delta = 68.9$ and 66.2 assignable to $sp^3$ carbons C-23 and C-30 respectively. The relative stereochemistry at C-3/5/28 and C-18/19 were confirmed by means of the NOESY spectrum. The H-3$\alpha$ proton showed strong NOE interactions with $H_3$-28 resonance and H-5 methine suggesting that they were $\alpha$-oriented and the H-24 olefinic methine showed two interactions with the angular methyls $H_3$-18 and $H_3$-19 establishing $\beta$-orientation of the methyls.

Some key HMBC correlations (Table 2) observed were between the methyls ($\delta = 1.34$ and 0.74) that exhibited $^3J$ couplings between themselves indicating their geminal nature and $^2J$ couplings with the oxymethine C-3 and the methine C-5, while the methyl at $\delta =$ 1.44 showed $^2J$ correlation with the methine at C-22. The angular methyl, $H_3$-18 ($\delta = 1.02$) showed $^2J$ correlation to the methylene at C-7 and the quaternary carbon at C-14 and $^3J$ couplings with the quaternary carbon C-10. On the basis of the above spectral data, compound **1** was identified as pseudojujubogenin-3-O-$\beta$-D-glucopyranoside, a new natural product. $^1$H and $^{13}$C NMR resonances were assigned using COSY, HMBC and NOESY spectra and are presented in Tables 1 and 2 and on structure **1**.

Compound **3** was obtained as colourless flakes, m. p. 237 – 239 °C. The HR-EI mass spectrum showed a molecular ion peak at *m/z* 503.69 $[M]^+$ that corresponded to the molecular formula $C_{30}H_{46}O_6$. The $^{13}$C NMR (Table 3) displayed 30 carbon resonances,




R =H, $R_1$=Me., **2**
R =Me, $R_1$=$CH_2OH$., **3a**

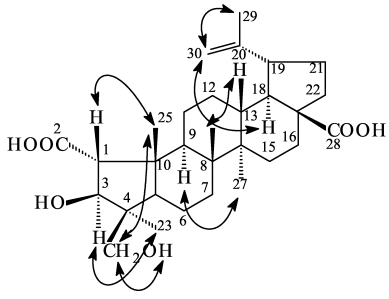



Fig. 2. Important NOESY interactions of **3**.

while the HSQC experiment confirmed that 22 out of the 30 carbons were directly attached to protons. The *J* modulated $^{13}$C experiment revealed the presence of five methyls, ten methylenes, seven methines and eight quaternary carbons that included resonances for two carboxylic functions $\delta = 178.0$ and 179.2. The $^1$H NMR (Table 3) showed five tertiary methyl singlets, a hydroxymethyl group $\delta = 3.70$, 4.64 (*d*, $J =$ 9.0 Hz), an oxymethine $\delta = 4.96$ and a saturated methine $\delta = 3.26$ and a series of multiplets from $\delta = 1.49$ to 1.59. These features revealed the backbone of **3** as a ceanothic acid derivative. This presumption was further confirmed when the $^1$H and $^{13}$C NMR chemical shifts of the ceanothane triterpenes **2** and **3** were found to be almost superimposable [12, 13]. However, there were minor changes for signals in the $^1$H and $^{13}$C NMR of the A and B rings of the ceanothane skeleton which were also observed in the COSY and the HMBC experiments. The remaining assignments of the $^1$H resonances in **3** were made by comparison with those published for **3a** in the literature. The $^{13}$C assignments were based on the HMBC experiments using $^2J$ and $^3J$ correlations while the $^1J$ C–H interactions observed in the HSQC spectrum allowed unambiguous assignments of the hydroxymethyl and methylene protons in **3**. The relative stereochemistry at H-1/3/9/13 and $H_3$-23/25/26/27 and the carboxylic groups at H-2/28 were finally determined by 2D NOESY experiments as shown on **3**. Accordingly, the structure of **3** was deter-

Table 3. $^{1}$H, $^{13}$C NMR, HMBC, COSY and NOESY spectral data for the ceanothane triterpene **3**.

| H/C | $^{\delta}$H | $^{\delta}$C | HMBC | COSY | NOESY |
|---|---|---|---|---|---|
| 1$\beta$ | 3.26 (s, 1H) | 66.8 | C-2, C-3, C-5, C-10, C-25 | | H-25$\beta$ |
| 2-COOH | | 178.0 | | | |
| 3$\alpha$ | 4.96 (s, 1H) | 86.2 | C-2, C-10, C-23 | | |
| 4 | | 48.9 | | | |
| 5 | 2.31 (m, 1H) | 57.6 | C-4, C-6 | | |
| 6a/b | 1.49 (m, 2H) | 18.8 | C-5, C-10 | | |
| 7 | a) 1.49 (m, 1H) | | | | |
| | b) 1.41 (m, 1H) | 35.5 | C-4, C-5, C-24 | | |
| 8 | | 42.4 | | | |
| 9 | 2.18 (m, 1H) | 45.7 | C-6, C-8, C-10, C-11, C-25 | | H-1$\beta$, H-27$\alpha$ |
| 10 | | 50.4 | | | |
| 11 | a) 1.59 (m, 1H) | 24.7 | C-13 | | |
| | b) 2.10 (m, 1H ) | | | H-25$\beta$ | |
| 12 | a) 1.98 (m, 1H) | 26.6 | C-14 | H-26$\beta$ | |
| | b) 1.33 (m, 1H) | | | | |
| 13$\beta$ | 2.78 (m, 1H ) | 39.5 | C-8, C-18 | | |
| 14 | | 43.9 | | | |
| 15 | a) 1.22 (m, 1H) | 30.9 | | | |
| | b) 1.90 (m, 1H) | | | | |
| 16 | a) 1.49 (m, 1H) | 33.3 | C-8, C-17, C-18, C-20, C-28 | H-21, H-29 | H-16b |
| | b) 2.60 (m, 1H) | | | | |
| 17 | | 57.0 | | | |
| 18 | 1.69 (m, 1H) | 50.1 | | | H-30 |
| 19$\alpha$ | 3.51 (m, 1H) | 48.0 | C-30 | H-21, H-22, H-29 | |
| 20 | | 151.6 | | | |
| 21 | a) 2.23 (m, 1H) | 31.7 | C-18, C-28 | H-21b, H-22 | H-19, H-21b |
| | b) 1.49 (m, 1H) | | | | H-21a |
| 22 | a) 2.23 (m, 1H) | 37.9 | C-28 | | |
| | b) 1.55 (m, 1H) | | | | |
| 23$\alpha$ | 1.81 (s, 3H) | 26.1 | C-3, C-17, C-24 | | H-3$\alpha$, 24-OH |
| 24$\beta$ | 4.64 (d, 2H, 9.0) | 67.0 | C-23 | | 24-OH |
| 24-OH | 3.70 (d, 1H, 9.0) | | C-4, C-23 | H-24 | |
| 25$\beta$ | 1.47 (s, 3H) | 19.4 | C-9 | | H-24$\beta$ |
| 26$\beta$ | 1.13 (s, 3H) | 17.4 | C-7, C-8 | | H-13$\beta$ |
| 27$\alpha$ | 1.06 (s, 3H) | 15.4 | C-13, C-14, C-15 | | H-6, H-7 |
| 28-COOH | | 179.2 | | | |
| 29 | 1.67 (s, 3H) | 20.1 | C-19, C-20, C-20, C-30 | | H-18, H-30a |
| 30 | a) 4.68 (d, 1H, 13.6) | 110.1 | C-12, C-19, C-20, C-27 | H-29, H-30b | H-19 |
| | b) 4.87 (s, 1H) | | C-19, C-29 | | |

mined as 24-Hydroxyceanothic acid, known as granulosic acid. Although dimethyl granulosate **3a**, has been previously reported from the heartwood of *Colubrina granulosa* [12] and also from *Paliurus ramosissimus* [13], the present study constitutes the first isolation of **3** as a pure natural product and is reported as a rare ceanothane triterpene. In addition to its $^{1}$H NMR data, $^{13}$C and full 2D-NMR spectral data has been presented for the first time (see Table 3 and structure **3**).

Ceanothic acid **2** and daucosterol **4** were characterized by analysis of NMR spectra and comparison with the published data [12, 14]. The dammarane-type triterpene glycoside is the major compound in *Z. glabrata*. Jujubogenin glycosides, jujuboside A, C and lotoside I, II have been reported from *Z. lotus* [15] however, this is the first report of a pseudojujubogenin glycoside isolated from the genus, *Zizyphus*. The ceanothane triterpene, 3-O-protocatechuoylceanothic acid has been reported from *Z. jujuba* [16]. The isolation of the dammarane-type glycoside from a plant of the *Zizyphus* genus is not surprising, but it is remarkable to note that *Z. glabrata* produces both dammarane and the ceanothane class of terpenoids. Compound **1** was tested for its potential to inhibit various bacteria by established methods [17]. It inhibited the growth of *Bacillus pumilus*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* with MICs being 51.2, 102.2, 12.8, 25.6 $\mu$g/ml, respectively. The dammarane and

ceanothane terpenoids have been reported to possess potent anti-inflammatory activity [18], the biological activities of the three compounds are of interest and are presently taken up for investigation.

## Experimental Section

*General experimental procedures*

Melting points were measured on a Cipla I-28 digital melting point apparatus and are reported uncorrected. The IR spectra were recorded on a Buck Scientific 500 infrared spectrophotometer. Silica gel (Acme, 60 – 120 mesh) for column chromatography and silica gel (Acme) was used for preparative thin layer chromatography. Spots on chromatogram were detected under UV light and by spraying with 5% $H_2SO_4$ in methanol. The NMR experiments were performed on a Bruker AVANCE DRX-500 spectrometer operating at 500.13 MHz and 125.77 MHz, respectively. Mass spectra were obtained using an Agilent 1100 series LC/MSD in APCI or API-ES mode.

*Plant material*

The leaves of *Z. glabrata* (1.2 kg) were collected at the Khailasa hills, India, in April 2002. The sample was authenticated by Dr. M. Venkaiah, Taxonomist, Botany Department, Andhra University, Visakhapatnam. A voucher specimen (SG/ZGL/03/345) has been deposited at the Herbarium, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam India (Herbarium Code = SKU).

*Extraction and isolation*

Powdered plant material (900 g) was extracted in a Soxhlet apparatus, successively with hexane, $CHC1_3$ and MeOH and the extracts were concentrated using a rotary evaporator at a maximum temperature of 45 °C. The dark viscous green residue 11 g from the methanol extract was separated over silica gel eluting with different mixtures of petroleum ether-chloroform and chloroform-methanol to give 25 fractions. Fraction 12 – 18 were combined, purified by repeated preparative TLC that recrystallized from methanol to give **2** (43.0 mg) and **3** (38.0 mg). Fraction 20 was further purified by repeated small column chromatography and recrystallized with chloroform-methanol to give **1** (64.0 mg). Fraction 23 from the original column was crystallized using methanol to give **4** (14.0 mg).

*Pseudojujubogenin-3-O-β-D-glucopyranoside* (**1**): Pale green amorphous powder, m. p. 241 – 243 °C. – IR (KBr): $\nu$ = 3200, 3640 (OH), 1465, 1285, 1078, 1012 $cm^{-1}$. – $^1$H NMR (500.13 MHz, $d_5$-pyridine, $^{13}$C NMR (125.77 MHz, $d_5$-pyridine), COSY, HMBC and NOESY see Tables 1 and 2 and structure **1**. -HR-EIMS: *m/z* (%) = 650 (18) ($M^+$). -$C_{36}H_{58}O_{10}$ (650.85): calcd. C 66.43, H 8.98, O 24.58; found C 66.32, H 8.94, O 24.54.

*Ceanothic acid* (**2**): Colourless needles from $Me_2CO$-methanol, m. p. 356 – 357 °C. Lit. m. p. 333 – 335 °C dec. -$[\alpha]_D^{24} = -51.5°$ (*c*, 1.01 in $CHCl_3$). IR and MS in agreement with the published data [12]. $^1$H NMR (500.13 MHz, $d_5$-pyridine) $\delta$ = 1.09, 1.17, 1.29, 1.41, 1.44, 1.68 (6 × s, 18H, CH*Me*) 1.71, (m, 1H, 18-H), 3.22 (s, IH, 1-H), 4.84 (s, 1H, 3-H), 2.23 (d, 1H, $J$ = 2.8 Hz, 5/9-H), 1.45, 1.54 (m, 2H, 6/7-H), 1.60, (m, 1H, 11-H), 2.11 (d, 1H, $J$ = 11.4 Hz, 11-H), 1.34, (m, 1H, 12-H), 1.98 (d, 1H, $J$ = 10.5 Hz, 12-H), 2.79 (dd, 1H, $J$ = 2.8, 8.5 Hz, 13-H), 1.25, (m, 1H, 15-H), 1.92 (dd, 1H, $J$ = 2.8, 10.5Hz, 15-H), 1.50, 2.61 (d, 2H, $J$ = 11.4 Hz, 16=H), 3.51 (d, 1H, $J$ = 2.3 Hz, 19-H), 1.50, 2.23 (m, 1H, 21-H), 1.50, 2.23 (m, 1H, 22-H), 4.68, (s, 1H, 30-H ($CH_2$=C), 4.87 (d, 1H, $J$ = 10.0 Hz, 30-H ($CH_2$=C). $^{13}$C NMR ($d_5$-pyridine) $\delta$ = 20.7 (24-$CH_2$), 15.5, 17.4, 19.2, 20.0, 31.9 (all CH*Me*), 67.4 (C-1), 85.1 (C-3), 44.2 (C-4), 57.4 (C-5), 19.5 (C-6), 35.1 (C-7), 42.5 (C-8), 45.5 (C-9), 50.0 (C-10), 24.6 (C-11), 26.6 (C-12), 39.5 (C-13), 43.9 (C-14), 30.9 (C-15), 33.3 (C-16), 57.0 (C-17), 50.1 (C-18), 48.0 (C-19), 31.7 (C-21), 38.0 (C-22), 110.1 (30-$CH_2$), 178.4 (2-COOH), 179.3 (28-COOH). NOESY correlations: H-1↔H-3, H-19↔$H_2$-30, H-5↔H-3, $H_3$-29↔$H_2$-30a/b, $H_3$-23↔H-3, $H_3$-24↔H-3, H-13↔$H_3$-26, H-13↔$H_2$-12 and $H_3$-29↔H-19.

*Granulosic acid* (**3**): Colourless flakes from methanol, m. p. 237 – 239 °C. -$[\alpha]_D^{24} = -51.5°$ (*c*, 1.01 in $CHCl_3$). $^1$H NMR (500.13 MHz, $d_5$-pyridine), $^{13}$C NMR (125.77 MHz, $d_5$-pyridine), COSY, HMBC and NOESY, see Table 3 and structure **3**. – HR-EIMS: *m/z* (%) = 502 (20) ($M^+$). – $C_{30}H_{46}O_6$ (502.32): calcd. C 71.68, H 9.22, O 19.10; found C 71.64, H 9.16, O 19.06.

*Daucosterol* (**4**): White powder, m. p. 279 – 281 °C. Lit. m. p. 287 – 289 °C. IR (KBr): $\nu$ $cm^{-1}$, – $^1$H NMR (500.13 MHz, $d_5$-pyridine), $^{13}$C NMR (125.77 MHz, $d_5$-pyridine) data was in agreement with the literature [14].

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