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Pentacyclic triterpenes from *Chuquiraga ulicina*

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Abstract

Four taraxastane triterpenes, 3β-acetoxy-6β-hydroxytaraxasta-20-ene, 6β-hydroxytaraxasta-20-en-3-one, 6β-hydroxytaraxasta-20-ene 3β-palmitate and 3β,6β-dihydroxytaraxasta-20-ene were isolated from the dichloromethane-methanol extract of *Chuquiraga ulicina* ssp. *ulicina* together with the known triterpenes lupeol, lupenyl acetate, lupenone, friedelinol, 3β-acetoxy-30-nor-lupan-20-one, and 30-nor-lupan-3β-ol-20-one.

Keywords: *Chuquiraga ulicina*; Asteraceae; Mutisieae; Pentacyclic triterpenes; Taraxastanes; Lupanes

1. Introduction

The western edge of the Atacama desert in northern Chile is characterized by a coastal range of varying altitudes which blocks the inland movement of clouds from the Pacific ocean. This range traps the moisture in valleys within 10–15 km from the coast, thus creating unique fog-zones referred to locally as *lomas* ('small hills'). Of the 1200 species native to the Atacama desert, the majority are concentrated within the *lomas* which represent floristic islands surrounded by hyperarid areas where virtually no plants exist. Near Paposo, a small mining village, the significant height of the coastal range allows for the formation of a pronounced fog-zone that provides an optimal environment for the establishment of a diverse and unique flora (Dillon, 1989).

One of the representative species in the area of Paposo is *Chuquiraga ulicina* (H. et A.) H. et A. ssp. *ulicina* (Asteraceae, Mutisieae), a dense shrub known locally as 'araña de la ballena' and 'hierba de la yesca' (Muñoz Pizarro, 1966). *Chuquiraga* is a New World genus with 25 species distributed in the Andes Mountains and Patagonia of Chile and Argentina (Mabberly, 1987). *C. ulicina*, distributed in Chile from Coquimbo to Antofagasta, is used locally as a remedy for hyperglycemia and as fuelwood (Ezcurra, 1986).

C. ulicina presents xerophytic anatomical foliar adaptations including an abaxial thickened cuticle and an adaxial layer of filamentous trichomes that surround the stomata (Flagg and Gomez, personal observation).

This study is part of continuing phytochemical and biological investigations of medicinal plants from arid environments in Chile and Argentina. We report here on the isolation and structure elucidation of four new and six known triterpenoids from *C. ulicina* ssp. *ulicina* and on the presence of some of these compounds in eight related species from Chile and Argentina.

2. Results and discussion

The CH_2Cl_2–CH_3OH (1:1) extract of *C. ulicina* was subjected to normal phase silica gel column chromatography and HPLC. Fractionation yielded four new taraxastane triterpenes, 3β-acetoxy-6β-hydroxytaraxasta-20-ene (**1**), 6β-hydroxy-taraxasta-20-en-3-one (**2**),

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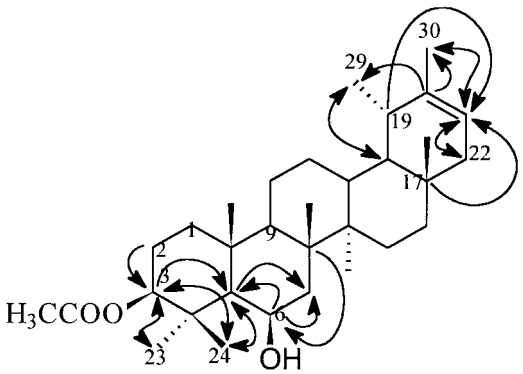


Fig. 1. Selected HMBC correlations of compound **1**.

6β-hydroxytaraxasta-20-ene 3β-palmitate (**3**) and 3β,6β-dihydroxytaraxasta-20-ene (**4**), along with six known triterpenes lupeol (**5**), lupenyl acetate (**6**), lupenone (**7**), friedelinol (**8**), 3β-acetoxy-30-nor-lupan-20-one (**9**) and 30-nor-lupan-3β-ol-20-one (**10**). The identities of compounds **5–10** were established by comparison of their spectral data with those reported in the literature (Ho, Chang & Chang, 1995; Hui, Li & Lee, 1977; Thompson & Bowers, 1968; Wenkert, Baddeley, Burfitt & Moreno, 1978).

Compound **1** was isolated as a white amorphous powder. High resolution EI-MS showed the $[M]^+$ at m/z = 484.3921 corresponding to the molecular formula $C_{32}H_{52}O_3$ (calc. 484.3916). The IR spectrum indicated the presence of a hydroxy group (3510 cm^{-1}) and an ester functionality (1716 cm^{-1}). On the basis of its spectroscopic data, compound **1** was assigned to be a taraxastane-type triterpene (Della Loggia et al., 1994; Reynolds, McLean & Poplawski, 1986). The ^1H NMR (pyridine-d_5) spectrum showed signals for nine methyl groups, six of which were positioned at quaternary carbons as singlets at δ 0.79, 0.98, 1.10, 1.52, 1.59 and 1.62. One methyl appeared as a doublet at δ 1.04 and one olefinic methyl occurred as a singlet at δ 1.71. An acetoxy methyl was observed as a singlet at δ 2.08, corresponding to the carbon at δ 21.2 in the HMQC experiment, with the acetoxy carbonyl occurring at δ 170.7. The signal at δ 4.73 (1H, m) corresponded to a proton geminal to the acetoxy group and correlated to the carbon at δ 81.2 (C-3). The multiplet at δ 4.72 that correlated to the carbon signal at δ 67.6 (C-6) was assigned to a proton geminal to a hydroxy group. A broad doublet, appearing at δ 5.35, corresponded to the only olefinic proton present and correlated to the carbon at δ 119.4 (C-21). The C-20 olefinic carbon was quaternary and appeared at δ 140.0.

HMQC and HMBC spectra were utilized to assign the positions of the carbons and protons in **1** (see Fig. 1). The double bond was established to be located between C-20 and C-21 due to HMBC correlations from H-22, H-29, and H-30 to C-20, correlations from H-30 and H-22 to C-21, and correlations from H-21 to C-17, C-19, C-22 and C-30. The acetoxy group was assigned to be at the C-3 position based on the HMBC correlations observed from H-3 to the carbonyl carbon, C-2, C-4, C-23, and C-24. In addition, we observed that H-23 showed a correlation to C-3. The hydroxy group was assigned to the C-6 position based on an HMBC correlation from H-6 to C-8 and a correlation from H-7 to C-6. The stereochemistry, of both the acetoxy (at C-3) and hydroxy (at C-6) groups in **1**, was assigned to be beta due to the coupling constants noted in the ^1H NMR ($CDCl_3$) [δ_H 4.50 (1H, ddd, $3 \times J \cong 2.5$ Hz, H–6), 4.41 (1H, dd, J = 4.8 Hz, J = 10.8 Hz, H–3)]. This coupling pattern is similar to that of other 6β-OH triterpenes (Aquino, De Simone, Vincieri & Pizza, 1990) in contrast to 6α-OH triterpenes, such as missourin that shows a doublet of a triplet (J = 11.4, 7.2 Hz) pattern (Wong, Oshima, Pezzuto, Fong & Farnsworth, 1986). The structure of compound **1** was established to be 3β-acetoxy-6β-hydroxytaraxasta-20-ene.

Compound **2** was isolated as a white amorphous powder. High resolution EI-MS showed the $[M]^+$ at m/z = 440.3669 corresponding to the molecular formula $C_{30}H_{48}O_2$ (calc. 440.3654). The IR spectrum indicated the presence of a hydroxy group (3440 $cm^{-1)}$ and a carbonyl group (1706 cm^{-1}, 1692 cm^{-1}). The ^1H and ^{13}C spectral data for compound **2** were similar to those of **1** (see Table 1) with the exception that signals for the acetoxy group were missing and a signal for a ketone group appeared at δ = 213.2. The only other difference in the ^{13}C NMR spectral data between **1** and **2** occurred for carbons C-1–C-6, C-23 and C-24. Carbons 1–6 were all shifted downfield in comparison with the same positions in **1**, with the most marked differences occurring for C-2, C-3, and C-4, indicating that the ketone group in **2** has replaced the acetoxy group in **1** at position C-3. This position was confirmed by HMBC correlations observed from H-1 and H-2 to C-3. The hydroxy group at C-6 was also corroborated by HMBC correlations from H-6 to C-8 and C-10 and from H-5 to C-6. On the basis of its spectroscopic data and comparison with those of compound **1**, compound **2** was assigned to be 6β-hydroxytaraxasta-20-en-3-one.

Compound **3** was isolated as a white amorphous powder. High resolution EI-MS showed $[M]^+$ at m/z = 680.6127 corresponding to the molecular formula $C_{46}H_{80}O_3$ (calc. 680.6107). The IR spectrum indicated the presence of a hydroxy group (3480 cm^{-1}) and an ester functionality (1708 cm^{-1}, 1728 cm^{-1}). The ^1H and ^{13}C spectral data for compound **3** were identical to that of **1** (see Table 1), with the only difference being that **3** showed an additional group of methylene signals from δ 29.2 to 29.7. This cluster of methylenes is typical for a fatty acid chain. In order to identify

Table 1
^{13}C NMR spectral data for compounds **1–4**; ^{1}H and ^{13}C NMR and HMBC correlations for compounds **1** and **2**

	1 (pyridine-d_5)			**2** (pyridine-d_5)			**3** (pyridine-d_5)	**4** ($CDCl_3$)
position[a]	δ_C	δ_H	hmbc	δ_C	δ_H	hmbc	δ_C	δ_C
1a	40.7t	0.99	25,26	42.2t	1.11	2a	40.6t	40.7t
1b		1.17			1.16			
2a	24.5t	1.81	3	34.8t	2.37*dt*(4,12)	1	24.6t	27.5t
2b		1.92			2.96*dt*(6,14)			
3	81.2d	4.73*m*	5,23,24	213.2s		1,2a,2b,23,24	81.0d	79.1d
4	39.1s		3,5,23,24	49.4s		5,23,24	39.2s	39.6s
5	56.0d	0.88	7b,23,24,25	56.8d	1.26	23,24	56.0d	55.5d
6	67.6d	4.72*m*	5,7b	68.5d	4.67b*s*	5	67.6d	69.0d
7a	42.3t	1.64		42.5t	1.65	5	42.3t	42.0t
7b		1.87			1.85			
8	40.7s		6,15b,27	40.7s		6,26,27	40.6s	40.7s
9	51.3d	1.43	7b,25,26	51.0d	1.46	25	51.2d	51.1d
10	37.0s		5,6,9,25	37.2s		1,2b,5,6,25	37.0s	36.6s
11a	22.0t	1.43	9	22.2t	1.44	9	22.0t	21.7t
11b		1.61			1.64			
12a	28.1t	1.28		28.0t	1.34		28.1t	27.7t
12b		1.65			1.70			
13	38.6d	1.73	18,27	38.6d	1.74	18,27	38.6d	38.4d
14	42.7s		7b,12a,16a,16b,26,27	42.8s		26,27	42.7s	42.5s
15a	27.4t	1.02	27	27.4t	1.01	27	27.4t	27.1t
15b		1.87			1.86			
16a	37.1t	1.19	15b,28	37.0t	1.13	28	37.1t	36.6t
16b		1.33			1.30			
17	34.6s		18,21,28	34.6s		21,22,28	34.6s	34.4s
18	49.1d	1.11	12a,16a,16b,28,29	49.1d	1.10	22,28,29	49.1d	48.8d
19	36.5d	1.61	18,21,29,30	36.5d	1.64	21	36.5d	36.3d
20	140.0s		22,29,30	139.9s		22,29,30	140.0s	139.8s
21	119.4d	5.35*d*(6)	22,30	119.4d	5.36*d*(6)	22,30	119.4d	118.9d
22a,b	42.5t	1.57	18,21,28	42.5t	1.58	21,28	42.5t	42.2t
23	27.8q	1.10*s*	3,24	25.2q	1.36*s*	5,24	27.9q	27.6q
24	18.6q	1.59*s*	3,5,23	23.9q	1.68*s*	5,23	18.7q	16.9q
25	18.1q	1.52*s*	9	17.2q	1.67*s*	5	18.0q	17.8q
26	17.1q	1.62*s*	9	17.2q	1.65*s*	7a	17.1q	17.2q
27	15.2q	0.98*s*	15b	15.1q	0.94*s*	15b	15.2q	15.0q
28	18.0q	0.79*s*	16b,18	18.0q	0.80*s*	18	17.9q	17.7q
29	22.9q	1.04*d*(6)	18	22.8q	1.03*d*(6)	18	22.8q	22.5q
30	21.9q	1.71*s*	21	21.8q	1.70*s*	21	21.8q	21.6q
OO$\underline{C}$Me	170.7s		3					
OO$\underline{C}$$\underline{Me}$	21.2q	2.08*s*						
OO$\underline{C}$$(CH_2)_{14}CH_3$							170.5s	
OO$\underline{C}$$(\underline{CH_2})_{14}CH_3$							32.1t	
							30.0-29.4t	
							25.6t	
							23.0t	
$OOC(CH_2)_{14}\underline{CH_3}$							14.3q	

[a] a,b indicates the relative configuration of protons.

this fatty acid, compound **3** was saponified and palmitic acid was identified in the acid fraction by comparison of its EI-MS with a computer reference database (NBS/Wiley). The structure of **3** was established to be 6β-hydroxytaraxasta-20-ene 3β-palmitate (**3**).

Compound **4** was isolated as a white amorphous powder. High resolution EI-MS showed the $[M]^+$ at m/z = 442.3825 corresponding to the molecular formula $C_{30}H_{50}O_2$ (calc. 442.3811). The IR spectrum indicated the presence of a hydroxy group (3450 cm^{-1}). The ^{1}H and ^{13}C spectral data for compound **4** were similar to those of **1** (see Table 1), with the major difference being the absence of the acetoxy signals and the appearance of a carbon signal at δ_C ($CDCl_3$) 79.1 that correlated to a proton signal at δ_H ($CDCl_3$) 3.15 [1H, t, J = 8.0 Hz, H–3]. These data indicated the presence of a second hydroxy substituent in **4** when compared with **1**. The only other differences occurred in

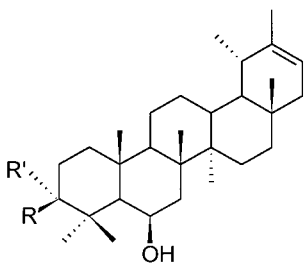
Table 2
Distribution of compounds **1**, **2**, **3** and **5** in various *Chuquiraga* spp. (presence suggested by LC-MS +; not present −)

C. ulicina (H. et A.) H. et A. ssp. *ulicina* (Chile)	**1**	**2**	**3**	**5**
C. oppositifolia D. Don (Chile)	−	−	+	+
C. acicularis D. Don (Chile)	+	−	+	+
C. kuschelii Acev. (Chile)	−	−	+	+
C. erinacea Don. (Argentina)	+	−	+	+
C. aurea Skottsberg (Argentina)	+	−	+	+
C. rosulata Gaspar (Argentina)	+	−	+	+
C. avellanedae (Lorentz) (Argentina)	+	−	+	+
C. straminea Sandwith (Argentina)	+	+	+	+

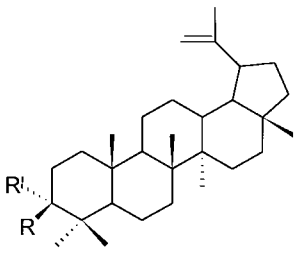
the ^{13}C NMR spectral data for C-2–C-6 and C-24 indicating that the hydroxy group had replaced the acetoxy group in **1** at the C-3 position. On the basis of its spectroscopic data and comparison with those of compound **1**, compound **4** was assigned to be 3β,6β-dihydroxytaraxasta-20-ene.

To our knowledge, this is the first time that taraxastane-type triterpenes have shown the presence of a functional group at C-6. In a routine screen, compounds **1**–**4** were tested for in vitro antimicrobial activities against methicillin-sensitive (MSSA) and (MRSA) -resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium* (VREF), *E. coli*, *E. coli imp* (a mutant strain with increased permeability to large molecular weight compounds) and *Candida albicans* by the agar diffusion method but showed no activity.

Isocratic normal-phase LC-MS of hexane-soluble fractions of the dichloromethane–methanol (1:1) extracts from *C. ulicina* and eight other related species from Chile and Argentina was performed as a comparative study within the genus. The results for each species were compared for the presence of compounds **1**, **2**, **3** and **5** and the distribution within the various species is shown in Table 2. It is interesting to note that compound **3** is present in all of the species investigated and it could serve as a possible chemotaxonomic marker for this genus.



1 R = OAc, R' = H
2 R, R' = O
3 R = $OOC(CH_2)_{14}CH_3$, R' = H
4 R = OH, R' = H



5 R = OH; R' = H
6 R = OAc; R' = H
7 R, R' = O

3. Experimental

3.1. General

Mps: uncorr. IR: determined on a Buck Scientific 500 IR spectrometer. Optical rotations were determined on a Jasco P1020 polarimeter. LC-MS was performed on a Finnegan TSQ 7000 in APCI positive mode with an Alltech adsorbosil (5 μm) 4.6 × 250 mm column, hexane–EtOAc 9:1 as an isocratic eluent at a flow rate of 1.5 ml/min. Under these conditions, the retention times and ions observed for cmpds **1**, **2**, **3** and **5** were, respectively, 4.2 min. ($[M–HOAc+H]^+$ at m/z 425 and $[M–HOAc–H_2O+H]^+$ at m/z 407); 5.4 min. ($[M–H_2O+H]^+$ at m/z 423); 2.3 min. ($[M-H_2O+H]^+$ at m/z 663); and 7.1 min. ($[M–H_2O+H]^+$ at m/z 409). NMR spectra were recorded in pyridine-d_5 and $CDCl_3$, on a Varian Unity 300 at 300 MHz (^1H) and 75 MHz (^{13}C) with residual pyridine ($\delta_H = 7.19$) and ($\delta_C = 135.5$) and $CHCl_3$ ($\delta_H = 7.24$) and ($\delta_C = 77.0$) as references. HMBC spectra were acquired with 1/2 $J = 0.05$ s. High resolution EI-MS were recorded on a JEOL HX 110A with a resolution of 5,000. Low resolution EI spectra were obtained with a Hewlett Packard 5988A (70 eV). Compound **3** was saponified by heating in 20% ethanolic KOH for 1 h, neutralization with acetic acid and extraction with EtOAc. Palmitic acid was isolated but compound **4** was not detectable in the neutral fraction. Visualization of compounds on silica gel TLC was carried out by spraying with an anisaldehyde sulfuric acid spray reagent (Krebs, Heusser & Wimmer, 1969) followed by heating.

3.2. Plant material

In Chile: *C. ulicina* (H. et A.) H. et A. ssp. *ulicina* was collected in April 1997 (25°00′ S, 70°26′ W, Paposo, Departamento Taltal, Region II), collection number 0826; *C. oppositifolia* D. Don was collected in January 1993 (33°15′ S, 70°20′ W, Farellones, Departamento Santiago, Region Metropolitana), collection number PUC 0; *C. acicularis* D. Don was collected in November 1993 (30°03′ S, 71°30′ W, El Chape, Departamento La Serena, Region IV), collection number 0189; and *C. kuschelii* Acev. was collected in December 1995 (18°15′ S, 69°10′ W, Laderas Lago Chungara, Departamento Arica, Region I), collection number 0705. All voucher specimens have been deposited in the Herbarium of the Pontificia Universidad Católica de Chile, Santiago, Chile. In Argentina: *C. erinacea* Don. was collected in February 1994 (38°28′ S, 68°33′ W, Añelo, Departamento Añelo, Provincia Neuquen) collection number RHF4291; *C. aurea* Skottsberg was collected in April 1994 (41°53′ S, 68°28′ W, Maquinchao, Departamento 25 de Mayo,

Provincia Rio Negro) collection number RHF4368; *C. rosulata* Gaspar was collected in February 1994 (38°30′ S, 69°02′ W, Añelo, Departamento Añelo, Provincia Neuquen) collection number RHF4295; *C. avellanedae* (Lorentz) was collected in Feb. 1994 (38°04′ S, 70°07′ W, Loncopué, Departamento Loncopué, Provincia Neuquen) collection number RHF4325; and *C. straminea* Sandwith was collected in March 1994 (41°19′ S, 69°40′ W, Jacobacci, Departamento 25 de Mayo, Provincia Rio Negro) collection number RHF4373. All voucher specimens have been deposited at the herbarium of the Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Buenos Aires, Argentina. Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between The University of Arizona and each of the collaborating institutions in Chile and Argentina in this study.

3.3. Extraction and isolation

The air-dried, ground aerial parts of *C. ulicina* (1.5 kg) were extracted three times in 4 L CH_2Cl_2–MeOH (1:1), followed by MeOH (4 L) overnight. Initial fractionation was done on silica gel 60, 50–200 μm Macherey Nagel, Germany and all subsequent fractionations on silica gel 60, 40–63 μm Lagand Chemical Co., Elmhurst, NY. The comb. CH_2Cl_2–MeOH extracts (68.6 g) were subjected to CC on silica gel. The column was eluted with a hexane–EtOAc gradient (0–100% EtOAc), followed by a CH_2Cl_2–MeOH gradient (0–100% MeOH). The column was subsequently washed with MeOH–H_2O (9:1) and MeOH–H_2O–HOAc, yielding 24 frs. Known compound **5**, **6**, **7**, and **8** were isolated from original frs. 9, 2, 5 and 7, respectively, which were eluted with hexane–EtOAc (49:1). **9** was isolated from original fr. 13 (hexane–EtOAc 19:1 eluate) and **10** from original fr. 15 (hexane–EtOAc 9:1 eluate). Fr. 11 (0.81 g, hexane–EtOAc 19:1 eluate) was subjected to CC on silica gel (hexane–EtOAc, 93:7) to give crude **1**. Final purification of **1** (54.0 mg) was performed by washing the fr. with hexane. Fr. 12 (1.72 g, hexane–EtOAc 19:1 eluate) was also subjected to CC on silica gel (hexane–EtOAc, 9:1) to give **2** (274.1 mg) as a pure compound. Fr. 7 (0.70 g, hexane–EtOAc, 49:1 eluate) was chromatographed over silica gel and eluted with hexane–EtOAc (19:1) to produce 10 sub-frs. Comb. sub-frs. 7.1–7.3 (527.0 mg) underwent CC on silica gel (hexane–EtOAc, 97:3) to yield **3** (38.1 mg). Fr. 13 was subjected to CC on silica gel (1.23 g, hexane–EtOAc, 1:4 eluate) to yield Fr. 14 (2.14 g, hexane–EtOAc, 9:1 eluate) was subjected to CC on silica gel (hexane–EtOAc, 17:3) to furnish 17 sub-frs. Comb. sub-frs. 14.11 and 14.12 (170.0 mg) were chromatographed over silica gel (hexane–EtOAc, 7:3) to produce 8 sub-frs. Sub-fr. 14.11.3 was subjected to HPLC [Alltech adsorbosil (5 μm) 4.6 × 250 mm column; flow rate 1 ml/min, hexane–EtOAc 43:7] to yield **4** with a retention time of 19.7 min.

3.4. Antimicrobial testing

Media used were Difco nutrient agar (pH 6.8) for *S. aureus*, LB (Luria-Bertani) agar for *E. faecium* and *E. coli* and YM agar for *C. albicans*. Assay plates (12″ × 12″ Sumilon) were prepared by pouring 125 ml volume of agar medium (tempered at 50°C) inoculated with an overnight broth culture of the test organisms (adjusted to approximately 10^6 cells per ml). Ten μl volume of antibiotic solution diluted in DMSO were spotted onto the agar surface and the plates were incubated at 37°C for 18 h. Zones of inhibition were measured using a hand-held digital caliper.

3.5. 3β-Acetoxy-6β-hydroxytaraxasta-20-ene (*1*)

Amorphous powder, $[\alpha]_D^{25} = +25.7$ ($CHCl_3$; *c* 0.3). Mp. dec. above 256°. HR-EIMS: Calcd for $C_{32}H_{52}O_3$ (M^+), 484.3916; Found: 484.3921. EI-MS *m/z* (rel. int.): 484 (5), 424 (6), 406 (2), 301 (1), 272 (2), 257 (3), 229 (6), 205 (81), 187 (88), 175 (20), 161 (18), 151 (26), 133 (39), 123 (100), 107 (62), 81(54), 69 (39), 55 (38), 43 (77). IR ν_{max} cm^{-1}: 3510, 2940, 1716, 1264, 920, 868. ^1H NMR (pyridine-d_5): See Table 1. ^1H NMR ($CDCl_3$) δ: 5.23 (1H, bd, J = 6.6 Hz, H-21), 4.50 (1H, bddd, 3 × $J \cong$ 2.5 Hz, H-6), 4.41 (1H, dd, J = 4.8 Hz, J = 10.8 Hz, H-3), 2.03 (3H, s, H-OAC), 1.61 (3H, s, H-30), 1.33, 1.23, 1.20 (3 × 3H, s, H-24, H-25, H-26), 0.96 (3H, d, J = 6.6 Hz, H-29), 0.91 (3H, s, H-23), 0.89 (3H, s, H-27), 0.71 (3H, s, H-28). ^{13}C NMR: see Table 1.

3.6. 6β-Hydroxytaraxasta-20-en-3-one (*2*)

Amorphous powder, $[\alpha]_D^{25} = +7.5$ ($CHCl_3$; *c* 0.5). Mp. 197–201°. HR-EIMS: Calcd for $C_{30}H_{48}O_2$ (M^+), 440.3654; Found: 440.3669. EI-MS *m/z* (rel. int.): 440 (9), 422 (1), 407 (2), 358 (1), 229 (6), 221 (13), 203 (62), 189 (59), 175 (42), 161 (25), 147 (27), 135 (47), 121 (89), 107 (79), 95 (100), 81 (60), 69 (60), 55 (78), 43 (86). IR ν_{max} cm^{-1}: 3440, 2940, 1706, 1692, 1458, 1448, 1376. ^1H NMR (pyridine-d_5) δ: see Table 1. ^{13}C NMR: see Table 1.

3.7. 6β-Hydroxytaraxasta-20-ene 3β-palmitate (*3*)

Amorphous powder, $[\alpha]_D^{25} = +22.4$ ($CHCl_3$; *c* 0.8). Mp. 55–58°. HR-EIMS: Calcd for $C_{46}H_{80}O_3$ (M^+), 680.6107; Found: 680.6127. EI-MS *m/z* (rel. int.): 680 (0.5), 424 (9), 406 (6), 391 (5), 257(4), 229 (9), 217 (9), 205 (100), 187 (96), 153 (28), 135 (37), 123 (84), 109

(58), 95(57), 81 (37), 57 (37), 43 (31). IR ν_{max} cm^{-1}: 3480, 2930, 2850, 1728, 1708, 1454, 1374. ^1H NMR (pyridine-d_5) δ: 5.68 (1H, bd, J = 3.0 Hz, OH), 5.35 (1H, d, J = 6.6 Hz, H-21), 4.82 (1H, dd, J = 4.7 Hz, J = 11.3 Hz, H-3), 4.76 (1H, bs, H-6), 2.47 (2H, t, J = 7.4 Hz, CH_2COO), 1.70 (3H, s, H-30), 1.67, 1.63 (2 × 3H, s, H-24, H-26), 1.54 (3H, s, H-25), 1.18 (3H, s, H-23), 1.03 (3H, d, J = 6.3 Hz, H-29), 0.98 (3H, s, H-27), 0.85 (3H, t, J = 6.0 Hz, $CH_3(CH_2)_x$), 0.79 (3H, s, H-28). ^{13}C NMR: see Table 1.

3.8. 3β,6β-Dihydroxytaraxasta-20-ene (*4*)

Amorphous powder, $[\alpha]_D^{25} = +13.4$ ($CHCl_3$; c 0.2). Mp. 205–209°. HR-EIMS: Calcd for $C_{30}H_{50}O_2$ (M^+), 442.3811; Found: 442.3825. EI-MS m/z (rel. int.): 442 (4), 424 (2), 229 (6), 205 (32), 187 (68), 133 (29), 123 (100), 107 (59), 95 (66), 81 (71), 67 (50), 55 (54), 43 (58). IR ν_{max} cm^{-1}: 3450, 2940, 2880. ^1H NMR ($CDCl_3$) δ: 5.26 (1H, d, J = 7.2 Hz, H-21), 4.54 (1H, ddd, 3 × $J \cong$ 2.5 Hz, H-6), 3.15 (1H, t, J = 8.0 Hz, H-3), 1.64 (3H, s, H-30), 1.36, 1.23, 1.16 (3 × 3H, s, H-24, H-25, H-26), 1.06 (3H, s, H-23), 0.99 (3H, d, J = 6.3 Hz, H-29), 0.93 (3H, s, H-27), 0.75 (3H, s, H-28). ^{13}C NMR: see Table 1.

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