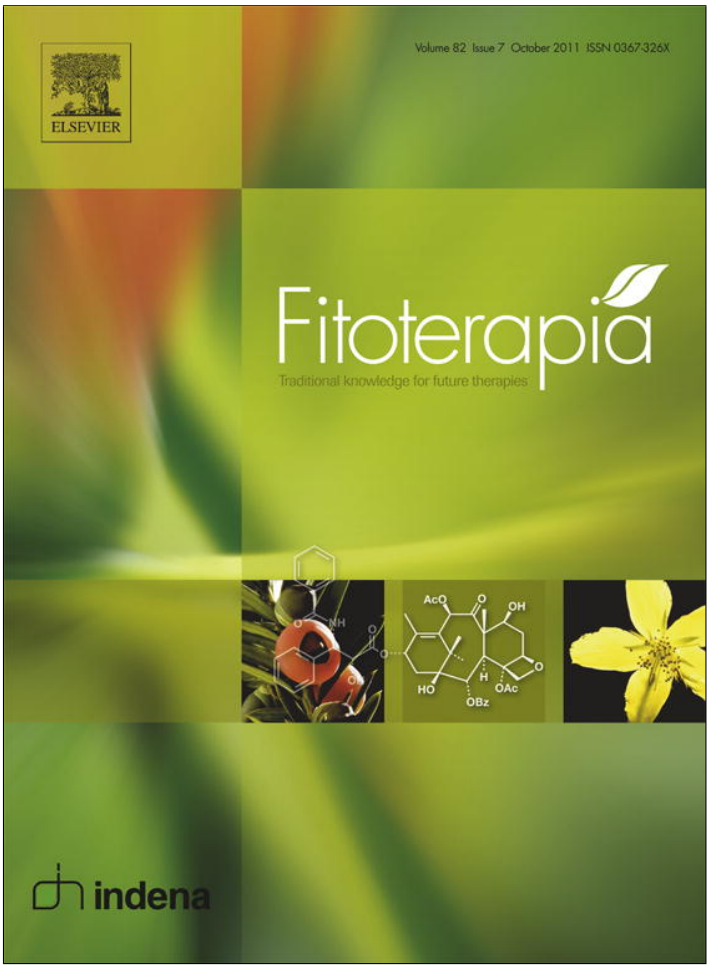

Fitoterapia 82 (2011) 1039–1043

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Minor pregnanes from *Caralluma adscendens* var. *gracilis* and *Caralluma pauciflora*

Kommidi Devendar Reddy [a], Belvotagi Venkatrao Adavi Rao [b], Gummadi Sridhar Babu [b], Bobbala Ravi Kumar [b], Alessandra Braca [c,*], Antonio Vassallo [d], Nunziatina De Tommasi [d], Ghanakota Venkateshwar Rao [a], Achanta Venkata Narasimha Appa Rao [b]

[a] Department of Chemistry, Kakatiya University, Warangal-506009, Andhra Pradesh, India
[b] University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506009, Andhra Pradesh, India
[c] Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy
[d] Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, via Ponte Don Melillo, 84084 Fisciano, Italy

ARTICLE INFO

Article history:
Received 22 April 2011
Accepted in revised form 24 June 2011
Available online 2 July 2011

Keywords:
Caralluma adscendens var. *gracilis*
Caralluma pauciflora
Asclepiadaceae
Pregnanes
Spectral analysis

ABSTRACT

Phytochemical investigation of *Caralluma adscendens* var. *gracilis* and *Caralluma pauciflora* (Asclepiadaceae) whole plant extracts allowed to isolate one pregnane glycoside and two pregnanes characterized as 12β,20-*O*-dibenzoyl-5α,6-dihydrosarcostin β-oleandropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-digitoxypyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside (**1**), 12β-*O*-benzoyl-3β,11α,14β,20*R*-pentahydroxy-pregn-5-ene (**2**), and 11α-*O*-benzoyl-3β,12β,14β,20*R*-pentahydroxy-pregn-5-ene (**3**), respectively. Their structural characterization was obtained on the basis of extensive NMR spectral studies. Three known pregnane glycosides along with lupeol and β-sitosterol were also isolated and characterized.

1. Introduction

Carallumas are succulents belonging to the Asclepiadaceae family. Many species of the genus such as *C. edulis*, *C. adscendens*, *C. indica*, *C. tuberculata*, and *C. umbellata* are edible and are eaten raw, cooked or pickled [1–6]. Some of the *Carallumas* have found medicinal uses. *C. edulis* is used to treat leprosy, diseases of blood, and as an anthelminthic [7]. Hot water extract of the stems of this plant is also a remedy for diabetes [8–10]. *C. umbellata* is claimed to be useful in treating stomach disorders and abdominal pains [11]. According to the folkloric practitioners of Africa, some of the *Carallumas*, are useful in treating rheumatism, diabetes, leprosy, and as antiseptics and disinfectants [12]. *C. negevensis* is used by Bedouins to treat chronic lung diseases, such as tuberculosis and cancer [13].

Caralluma pauciflora (Wright) N.E. Brown is a perennial herb growing wild in the states of Tamil Nadu and Karnataka, India. It resembles morphologically *C. indica*. It bears fewer (2–3) flowers (as umbels) than *C. indica* (8–10 flowers). It is rather difficult to distinguish between the two species except during flowering. *Caralluma adscendens* var. *gracilis* (Gravely et Mayaranathan) grows wild in Tamilnadu (Pudukottai, Salem and Ramnad districts) and Karnataka states of India. It grows to a height of 60 cm and has angled stems. It bears pink, hairy flowers in pairs all along the stem [6]. The genus *Caralluma* is a rich source of pregnane glycosides. In recent years, several pregnane glycosides with interesting biological activity have been reported from this genus [14–17].

As part of an ongoing investigation on Asclepiadaceae family, we have carried out the chemical study on the whole plants of *C. adscendens* var. *gracilis* and *C. pauciflora*, resulting in the isolation and structural characterization of two new pregnane esters and one new pregnane glycoside on the basis of extensive spectroscopic and spectrometric analyses, including 2D NMR and ESIMS spectra. Three known pregnane

* Corresponding author. Tel.: +39 050 2219688; fax: +39 050 2219660.
E-mail address: alessandra.braca@farm.unipi.it (A. Braca).

0367-326X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2011.06.011

glycosides along with lupeol and β-sitosterol were also isolated and characterized. This is the first report on the chemical investigations of these plants.

2. Experimental

2.1. General

Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (Time Proportional Phase Increment) used to achieve frequency discrimination in the ω_1 dimension. Standard pulse sequences and phase cycling were used for DQF-COSY, TOCSY, HSQC, and HMBC, experiments. NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software. HRESIMS were acquired in positive ion mode on a Q-TOF premier spectrometer equipped with a nanoelectrospray ion source (Waters-Milford, MA, USA). Column chromatographies were performed over silica gel (230–400 mesh). HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and Shimadzu injector on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, 10 μm Waters, flow rate 2.0 ml min^{-1}).

2.2. Plant material

C. pauciflora and *C. adscendens* var. *gracilis* were collected in January 2007 from Satyamangalam village of Pudukottai district, Tamil Nadu, India. The plants were identified by Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal, India. Voucher specimens of the plants were deposited at the herbarium of College of Pharmaceutical Sciences, Kakatiya University, Warangal, India (No. AVN-CP-1-07 and AVN-CG-1-07).

2.3. Extraction and isolation

The fresh whole plant (1 kg) of *C. gracilis* was chopped, crushed, and macerated in ethanol (3 L) at room temperature for 7 days. The extract was filtered and solvent was removed using rotary evaporator to give a dark greenish semi-solid residue. The alcoholic extract was dispersed in water (1 L) and fractionated successively with diethyl ether, butanone, and *n*-butanol. The diethyl ether fraction (6.5 g) was subjected to normal phase column chromatography. Elution of the column with benzene (500 ml) and concentration of the eluate gave a residue of 650 mg. Elution of the column with benzene–acetone (9:1) and concentration of the eluate gave a material which was not worked further. Elution of the column with benzene–acetone (8:2) and concentration of the eluate gave 550 mg of another residue. The benzene eluate (650 mg) contained mainly two compounds as revealed by TLC study and was subjected to silica gel column chromatography using benzene-*n*-hexane (1:1) as eluent, collecting fractions of 10 ml, to yield β-sitosterol (120 mg, 0.012%) and lupeol (80 mg, 0.008%). Benzene–acetone (8:2) eluate (550 mg) was subjected to silica gel column chromatography using *n*-hexane-acetone (8:2) as eluent. Fractions of 10 ml were collected and pooled basing on TLC study to give (5α,17*S*)-12-*O*-benzoyl-3β,8β,12β,14β-tetrahydroxypregnan-20-one-3-*O*-β-cymaropyranosyl-(1→4)-β-cymaropyranoside (45 mg, 0.0045%), together with a major fraction (220 mg). Part of this fraction (50 mg) was subjected to RP-HPLC using MeOH–H_2O (85:15) as eluent to yield compound **1** (t_R = 28 min, 10.5 mg, 0.0011%).

C. pauciflora (1 kg) was macerated in ethanol and the alcoholic extract was fractionated with different solvents as described for *C. adscendens* var. *gracilis*. The diethyl ether extract (15 g) was subjected to silica gel column chromatography with $CHCl_3$–MeOH (9:1) as eluent to give a material which was further subjected to silica gel column chromatography using benzene–acetone (7:3) as eluent and collecting fraction of 20 ml to obtain a major fraction (60 mg) which was submitted to RP-HPLC with MeOH–H_2O (55:45) to yield compounds **2** (t_R = 14 min, 5.5 mg, 0.00055%) and **3** (t_R = 15 min, 7.0 mg, 0.0007%). The *n*-butanol fraction (380 mg) was subjected to silica gel column chromatography using $CHCl_3$–MeOH–H_2O (4:1:1) as eluent and collecting fractions of 10 ml to yield a major fraction (50 mg) which was chromatographed using RP-HPLC with MeOH–H_2O (3:2) to yield carumbelloside III (t_R = 15 min, 5.0 mg, 0.0005%) and dihydrorusselioside B (t_R = 17 min, 4.5 mg, 0.00045%).

2.3.1. *12β,20-O-dibenzoyl-5α,6-dihydrosarcostin β-oleandropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-digitoxypyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside* (**1**)

Amorphous powder, $[\alpha]_D$: +60.6 (*c* 0.2, MeOH); ^1H and ^{13}C NMR (600 MHz, CD_3OD): see Tables 1 and 2; HRESIMS: *m/z* 1321.6722 $[M+Na]^+$ (calcd for $C_{69}H_{102}O_{23}$, 1321.6709); ESIMS: *m/z* 1321 $[M+Na]^+$, 1199 $[M+Na-122]^+$, 1077 $[M+Na-122-122]^+$, 933 $[M+Na-122-122-144]^+$, 789 $[M+Na-122-122-144-144]^+$, 515 $[M+Na-122-122-144-144-130-144]^+$, 729 $[144+144+130+144+144+Na]^+$, 585 $[144+130+144+144+Na]^+$, and 441 $[130+144+144+Na]^+$.

2.3.2. *12β-O-benzoyl-3β,11α,14β,20R-pentahydroxy-pregn-5-ene* (**2**)

Amorphous powder, $[\alpha]_D$: −11.5 (*c* 0.1, MeOH); ^1H and ^{13}C NMR (600 MHz, CD_3OD): see Table 1; HRESIMS: *m/z* 493.2560 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_6$, 493.2565); ESIMS: *m/z* 493 $[M+Na]^+$, 371 $[M+Na-122]^+$.

2.3.3. *11α-O-benzoyl-3β,12β,14β,20R-pentahydroxy-pregn-5-ene* (**3**)

Amorphous powder, $[\alpha]_D$: −10.8 (*c* 0.1, MeOH); ^1H and ^{13}C NMR (600 MHz, CD_3OD): see Table 1; HRESIMS: *m/z* 493.2558 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_6$, 493.2565); ESIMS: *m/z* 493 $[M+Na]^+$, 371 $[M+Na-122]^+$.

3. Results and discussion

Extracts from the whole plant of *Caralluma* ssp. were fractionated on silica gel and RP-HPLC to give pure compounds **1**–**3** (Fig. 1).

Compound **1** showed a quasi molecular ion peak at *m/z* 1321 $[M+Na]^+$ in the positive ESIMS spectrum. The

Table 1
^{1}H and ^{13}C NMR data for aglycon moieties of compounds **1**–**3** (CD_3OD, 600 MHz)[a].

	1		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	2.04 *br d* (11.0)	38.1	1.95 *br d* (11.0)	39.0	2.61 *br d* (11.0)	39.8
1b	1.03 *m*		1.28 *m*		1.22 *m*	
2a	2.18 *m*	33.2	1.75 *m*	31.8	1.77 *m*	31.8
2b	1.95 *m*		1.57 *m*		1.57 *m*	
3	3.63 *m*	78.0	3.42 *m*	72.1	3.37 *m*	71.5
4a	1.68[b]	34.7	2.30 *dd* (12.0, 9.0)	43.0	2.29 *dd* (12.0, 9.0)	43.0
4b	1.23 *m*		2.26 *dd* (12.0, 3.5)		2.26 *dd* (12.0, 3.5)	
5	1.14 *m*	45.6		140.0		140.0
6a	1.66[b]	25.0	5.50 *br t* (5.5)	122.4	5.52 *br t* (5.5)	122.4
6b	1.20 *m*					
7a	1.87 *m*	34.3	2.32 *m*	28.8	2.34 *m*	28.8
7b	1.43 *m*		1.96 *br d* (6.0)		1.98 *br d* (6.0)	
8		76.6	1.94 *br t* (6.0)	37.8	1.87 *br t* (6.0)	37.7
9	1.32 *dd* (9.0, 4.5)	47.0	1.45 *t* (10.0)	50.5	1.76 *br t* (10.0)	49.0
10		36.5		37.7		37.5
11a,b	1.97 *m*	24.5	4.00 *t* (11.0)	71.0	5.55 *t* (11.0)	75.1
12a	4.98 *dd* (12.0, 4.0)	75.6	4.96 *d* (11.5)	82.8	3.35 *d* (11.5)	78.4
12b						
13		57.7		49.5		50.8
14		88.5		85.3		85.2
15a	1.81 *m*	29.1	1.99[b]	33.4	1.99[b]	33.4
15b	1.46 *m*		1.69[b]		1.69[b]	
16a	1.74 *m*	38.3	1.99[b]	26.1	2.00[b]	26.1
16b	1.63 *m*		1.67[b]		1.68[b]	
17		89.4	1.94 *m*	53.4	2.19 *m*	53.7
18	1.71 *s*	11.3	1.40 *s*	11.8	1.20 *s*	10.3
19	0.97 *s*	12.5	1.23 *s*	18.9	1.24 *s*	18.9
20	4.84 *q* (6.2)	75.2	3.81 *m*	71.0	3.81 *m*	71.0
21	1.30 *d* (6.2)	15.0	1.12 *d* (6.5)	22.6	1.23 *d* (6.5)	22.4
Bz at C-11						
1						131.0
2 /6					8.10 *dd* (8.0, 2.0)	130.4
3 /5					7.55 *t* (8.0)	129.6
4					7.66 *t* (8.0)	133.4
COO						167.7
Bz at C-12						
1		132.0		131.0		
2 /6	7.63 *dd* (7.5, 1.5)	130.0	8.10 *dd* (8.0, 2.0)	130.5		
3 /5	7.10 *t* (7.5)	128.5	7.52 *t* (8.0)	129.0		
4	7.56 *t* (7.5)	133.0	7.63 *t* (8.0)	133.7		
COO		167.5		167.7		
Bz at C-20						
1		132.0				
2 /6	7.63 *dd* (7.5, 1.5)	130.2				
3 /5	7.34 *t* (7.5)	128.8				
4	7.44 *t* (7.5)	133.1				
COO		167.5				

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.
[b] Overlapped signal.

molecular formula was established to be $C_{69}H_{102}O_{23}$ by HRESIMS and ^{13}C NMR analyses. In the MS^2 spectrum, prominent fragments at *m/z* 1199 $[M+Na-122]^+$, 1077 $[M+Na-(122\times2)]^+$, 933 $[M+Na-(122\times2)-144]^+$, 789 $[M+Na-(122\times2)-(144\times2)]^+$, and 515 $[M+Na-(122\times2-(144\times2)-130]^+$ were observed, due to the consecutive losses of two benzoyl, two *O*-methyldideoxyhexose, and one dideoxyhexose residue, respectively. A fragment ion was detected at *m/z* 729 corresponding to the sodium-cationized etherified sugar chain of compound **1**, followed by fragment peaks at *m/z* 585 and 441, generated by elimination of one or two *O*-methyldideoxyhexose, respectively. From these fragments a pentasaccharide chain for **1** was deduced. Data from ^{13}C NMR spectra (Tables 1 and 2) confirmed a glycoside structure. The ^{1}H NMR of the aglycon portion (Table 1) showed signals for three methyl groups at δ 0.97 (3H, *s*), 1.71 (3H, *s*), 1.30 (3H, *d*, $J=6.2$ Hz), and three signals at δ 3.63 (1H, *m*), 4.84 (1H, *q*, $J=6.2$ Hz), and 4.98 (1H, *dd*, $J=12.0$, 4.0 Hz) corresponding to secondary oxygenated carbons. The ^{13}C NMR chemical shifts of all hydrogenated carbons were assigned unambiguously by the HSQC spectrum. The complete elucidation of the aglycon structure of **1** was achieved by HMBC experiments. The HMBC correlations between the proton signal at δ 0.97 (Me-19) and the carbon resonances at δ 36.5 (C-10), 38.1 (C-1), 45.6 (C-5), and 47.0 (C-9); the proton signal at δ 1.71 (Me-18) and the carbon

Table 2
^{1}H and ^{13}C NMR data for sugar moieties of compound **1** (CD_3OD, 600 MHz)[a].

	1	
	δ_H	δ_C
D-Cym I		
1	4.88 *dd* (9.5, 2.0)	96.5
2a	2.16 br *dd* (16.0, 3.0)	35.9
2b	1.55 br *dd* (16.0, 12.0)	
3	3.80 *dq* (3.0)	77.8
4	3.31 *dd* (9.5, 3.0)	83.3
5	3.86 *dq* (9.5, 6.0)	69.3
6	1.24 *d* (6.0)	18.1
–OMe	3.44 *s*	58.0
D-Cym II		
1	4.84 *dd* (9.5, 2.0)	100.2
2a	2.18 br *dd* (16.0, 3.0)	36.0
2b	1.65 br *dd* (16.0, 12.0)	
3	3.80 *dq* (3.0)	77.8
4	3.25 *dd* (9.5, 3.0)	83.1
5	3.85 *dq* (9.5, 6.0)	69.1
6	1.22 *d* (6.0)	18.0
–OMe	3.45 *s*	58.0
D-Digit		
1	4.87 *dd* (9.0, 2.0)	100.3
2a	2.01 br *dd* (16.0, 3.5)	36.1
2b	1.72 br *dd* (16.0, 12.0)	
3	4.24 *dq* (3.5)	67.8
4	3.22 *dd* (9.0, 3.5)	83.2
5	3.81 *dq* (9.0, 6.2)	69.0
6	1.17 *d* (6.2)	18.1
D-Cym III		
1	4.80 *dd* (9.5, 2.0)	100.0
2a	2.16 br *dd* (16.0, 3.0)	36.0
2b	1.63 br *dd* (16.0, 12.0)	
3	3.80 *dq* (3.0)	77.8
4	3.30 *dd* (9.5, 3.0)	83.3
5	3.85 *dq* (9.5, 6.0)	69.2
6	1.22 *d* (6.0)	18.0
–OMe	3.45 *s*	58.0
D-ole		
1	4.62 *dd* (8.5, 1.5)	102.6
2a	2.36 *m*	37.0
2b	1.40 *m*	
3	3.22 *m*	81.2
4	2.99 *t* (9.5)	76.2
5	3.27 *dq* (9.5, 6.0)	72.5
6	1.30 *d* (6.0)	17.9
–OMe	3.45 *s*	58.2

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.

resonances at δ 57.7 (C-13), 75.6 (C-12), 88.5 (C-14), and 89.4 (C-17); the proton signal at δ 1.97 (H_2-11) and the carbon resonances at δ 47.0 (C-9), 57.7 (C-13), 75.6 (C-12), and 76.6 (C-8); the proton signal at δ 4.84 (H-20) and the carbon resonance at δ 15.0 (C-21); the proton signal at δ 4.98 (H-12) and the carbon resonances at δ 11.3 (Me-18) and 89.4 (C-17) allowed us to deduce that the pregnane skeleton of **1** was 5,6-dihydrosarcostin [18]. In addition to the pregnane skeleton the ^{1}H and ^{13}C NMR spectra of compound **1** showed signals due to two benzoyl groups. The benzoyl moieties were located at C-12 and C-20 on the basis of chemical shifts of the respective carbons and protons. Moreover the ^{1}H and ^{13}C NMR spectra of **1** exhibited signals ascribable to the saccharidic portion (Table 2). In the ^{1}H NMR spectrum five anomeric proton signals (δ 4.62, 4.80, 4.84, 4.87, and 4.88) and five methyl doublets (δ 1.17, 1.22×2, 1.24, and 1.30) were observed. 1D-TOCSY experiments, together with the COSY spectrum led us to establish the proton sequence within these sugar fragments that were identified as three β-cymaropyranosyl, one β-digitoxypyranosyl, and one β-oleandropyranosyl unit. In the HSQC experiment glycosidation shifts were observed for C-4_{cymI}, C-4_{cymII}, C-4_{cymIII}, and C-4_{digit}. The position of sugar units was unambiguously defined by the HMBC experiment: β-cymaroseI unit was linked at C-3 as shown by the cross peak between δ 4.88 (H-1_{cymI}) and 78.0 (C-3); key correlations were observed between H-1_{cymII}–C-4_{cymI}, H-1_{digit}–C-4_{cymII}, and H-1_{ole}–C-4_{cymIII}. The β-linkages of the five sugar moieties were shown by the large coupling constants (J~8.5 Hz) of the anomeric proton signals as well as by the resonances of C-2, C-3, and C-5 characteristic of β-forms [19,20]. On the basis of these NMR evidences the structure of **1** was determined as 12β,20-*O*-dibenzoyl-5α,6-dihydrosarcostin β-oleandropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-digitoxypyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside.

Compound **2** had a molecular formula $C_{28}H_{38}O_6$ based on ESIMS (*m/z* 493 $[M+Na]^+$) and ^{13}C NMR data. Features characteristic for a pregnane unit [signals for three methyls, six methylenes, eight methines (including four oxymethines and one sp^2 carbon), and four quaternary carbons] were seen in the NMR spectra (Table 1). On the basis of 1D-TOCSY and COSY experiments three spin systems H-1–H-4, H-6–H-8, H-8–H-12, and H-15–H-20 were established for compound **2**. The presence of a benzoyl residue for **2** was deduced from the proton signals at δ 7.52 (2H, *t*, J = 8.0 Hz), 7.63 (1H, *t*, J = 8.0 Hz), and 8.10 (2H, *dd*, J = 8.0, 2.0 Hz), which correlated with the aromatic methine carbons at δ_C 129.0 (C-3, C-5), 133.7 (C-4), and 130.5 (C-2, C-6), in the ^{1}H and ^{13}C NMR spectra, respectively [21]. The assignments of all protonated carbons were accomplished by interpretation of the HSQC spectrum, while HMBC correlations revealed the substitution sites of the molecule, showing correlation peaks between δ 1.23 (Me-19) and 39.0 (C-1), 50.5 (C-9), and 140.0 (C-5); δ 1.40 (Me-18) and 53.4 (C-17), 82.8 (C-12), and 85.3 (C-14); δ 1.12 (Me-21) and 53.4 (C-17) and 71.0 (C-20); δ 4.96 (H-12) and 50.5 (C-9), 53.4 (C-17), and 85.3(C-14); δ 5.50 (H-6) and 37.7 (C-10), 43.0 (C-4), and 50.5 (C-9). The relative configurations of the hydroxylated carbons were assigned as 11α and 12β mainly on the basis of ^{1}H NMR coupling constants and by comparison with those reported for related compounds [22,23]. The location of benzoyl moiety at C-12 hydroxyl group was based on the chemical shift and coupling constant pattern of the proton at δ 4.96 (1H, *d*, J = 11.5 Hz) and its correlated carbon. Therefore, compound **2** was defined as 12β-*O*-benzoyl-3β,11α,14β,20*R*-pentahydroxy-pregn-5-ene.

Compound **3** ($C_{28}H_{38}O_6$) showed a quasimolecular ion peak at *m/z* 493 $[M+Na]^+$ and one significant fragment at *m/z* 371 $[M+Na-122]^+$ in the positive ESIMS. Comparison of NMR spectral data of **3** (Table 1) to those of **2** clearly suggested that **3** differed from **2** only in the location of the benzoyl moiety. The ester linkage was located at the C-11α hydroxyl group on the basis of the chemical shift and coupling constant of axial H-11α at δ 5.55 (1H, *t*, J = 11.0 Hz). Therefore, compound **3** was defined as 11α-*O*-benzoyl-3β,12β,14β,20*R*-pentahydroxy-pregn-5-ene.

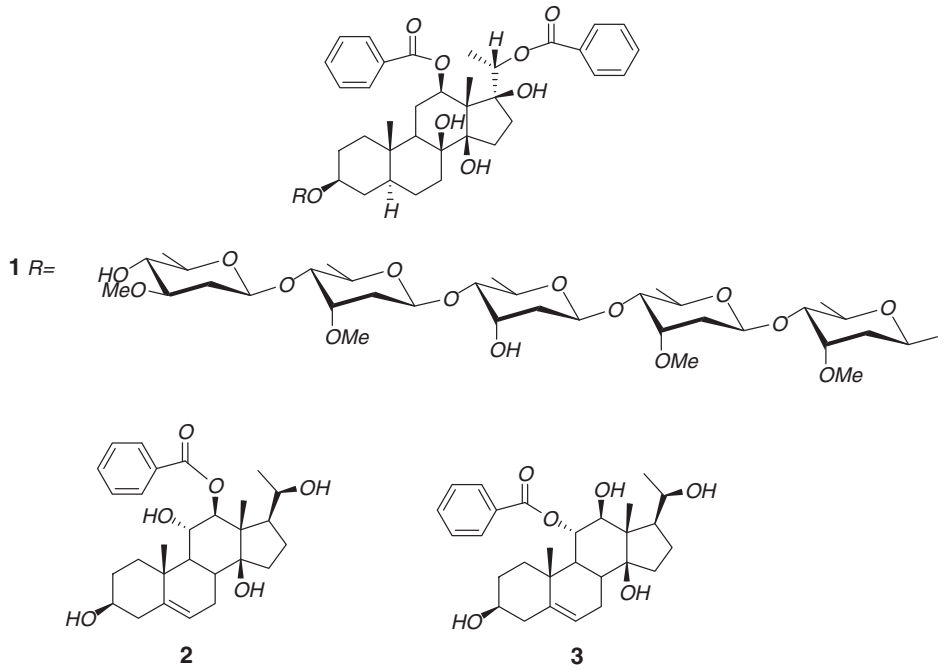

Fig. 1. Structures of compounds **1**–**3**.

Two known compounds, carumbelloside III and dihydrorusselioside B [13,24] were also isolated from *C. pauciflora* and β-sitosterol, lupeol, and a known pregnane glycoside were isolated from *C. adscendens* var. *gracilis*. The known pregnane glycoside was identified as (5α,17*S*)-12-*O*-benzoyl-3β,8β,12β,14β-tetrahydroxypregnan-20-one-3-*O*-β-cymaropyranosyl-(1→4)-β-cymaropyranoside isolated earlier from *C. fimbriata* [16]. The structures of all the above compounds were determined by spectral analysis and comparison of data with those reported in the literature.

Acknowledgements

Prof. Appa Rao wishes to thank AICTE (Grant No: 8023/BOR/RPS-150/2006-07).

References

[1] Bhandari MM. The flora of the Indian desert. 1 Ed. New Delhi. pp: Scientific Publishers; 1978. 220.
[2] Ahmad VU, Khan U, Rizwani GH. New pregnane glycosides from *Caralluma tuberculata*. J Nat Prod 1988;51:1092–7.
[3] Ahmed MM, Qureshi S, Al-Bekairi AM, Shah AH, Rao RM, Qazi NS. Anti-inflammatory activity of *C. tuberculata* alcoholic extract. Fitoterapia 1993;64:357–60.
[4] Ramachandran VS, Nair NC. Ethnobotanical observations on Irulars of Tamil Nadu (India). J Econ Tax Bot 1981;2:183–90.
[5] Singh NP. Flora of Eastern Karnataka, I Ed, Vol.1. New Delhi: Mittal Publications; 1988. p. 409–10.
[6] Gandhi R. *Carallumas* of the Indian Subcontinent, Indian Society of Cactii and Succulents, New Delhi; 1999.
[7] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi; 1956. p. 51.
[8] Akthar MS. Hypoglycemic activities of some indigenous medicinal plants traditionally used as antidiabetic drugs. J Pak Med Assoc 1992;42: 271–7.
[9] Leporatti ML, Lattanzi E. Traditional phytotherapy of coastal areas of Makran (Southern Pakistan). Fitoterapia 1994;65:158–61.
[10] Wadood A, Wadood N, Wahid Shah SA. Effects of *Acacia arabica* and *Caralluma edulis* on blood glucose levels of normal and alloxan-induced diabetic rabbits. J Pak Med Assoc 1989;39:208–12.
[11] Vedavathy S, Mridula V, Sudhakar A. Tribal medicine of chittor district of Andhra Pradesh, India. I Ed. Tirupati. pp: Herbal Folklore Research Centre; 1997. 46.
[12] Al-Yahya MAA, Abdel-Sattar E, Guittet E. Pregnane glycosides from *Caralluma rousseliana*. J Nat Prod 2000;63:1451–3.
[13] Braca A, Bader A, Morelli I, Scarpato R, Turchi G, Pizza C, et al. New pregnane glycosides from *Caralluma negevensis*. Tetrahedron 2002;58: 5837–48.
[14] Kunert O, Simic N, Ravinder E, Adavi Rao BV, Ravi Kumar B, Alex RM, et al. Steroidal glycosides from *Caralluma umbellata*. Phytochem Lett 2009;2:134–8.
[15] Abdel-Sattar E, Harraz FM, Abdullah Al-ansari SM, El-Mekkawy S, Ichino C, Kiyohara H, et al. Acylated pregnane glycosides from *Caralluma tuberculata* and their antiparasitic activity. Phytochemistry 2008;69: 2180–6.
[16] Kunert O, Gurunath Rao V, Sridhar Babu G, Sujatha P, Sivagamy M, Anuradha S, et al. Pregnane glycosides from *Caralluma adscendens* var. *fimbriata*. Chem Biodivers 2008;5:239–50.
[17] Suresh Babu K, Rama Subba Rao V, Vyasa Radhakrishnan S, Madhusudana Rao J, Siva Rambabu S. A new pregnane steroid from the stems of *Caralluma umbellata*. J Asian Nat Prod Res 2008;10:1013–6.
[18] Oyama M, Biya I, Tanaka T, Iinuma M. Five new steroidal glycosides from *Caralluma dalzielii*. Helv Chim Acta 2007;90:63–71.
[19] Nakagawa T, Hayashi K, Mitsuhashi H. Studies on the constituents of Asclepiadaceae plants LV. The structures of three new glycosides, glaucoside H, I, and J from the Chinese drug "Pa-ich'ien", Cyanachum glaucescens HAND-MAZZ. Chem Pharm Bull 1983;31:2244–53.
[20] Zhang ZX, Zhou J, Hayashi K, Mitsuhashi H. Studies on the constituents of Asclepiadaceae plants LVIII. The structures of five new glycosides, cynatratoside A-E, from the Chinese drug "Pai-Wei", Cyanachum atratum BUNGE. Chem Pharm Bull 1985;33:1507–14.
[21] De Leo M, De Tommasi N, Sanogo R, Autore G, Marzocco S, Pizza C, et al. New pregnane glycosides from *Caralluma dalzielii*. Steroids 2005;70: 573–85.
[22] Sahu NP, Panda N, Mondal NB, Banerjee S, Koibe K, Nikaido T. Polyoxypregnane glycosides from the flowers of *Dregea volubilis*. Phytochemistry 2002;61:383–8.
[23] Panda N, Mondal NB, Banerjee S, Sahu NP, Koibe K, Nikaido T, et al. Polyhydroxy pregnanes from *Dregea volubilis*. Tetrahedron 2003;59: 8399–403.
[24] Kunert O, Rao BVA, Babu GS, Padmavathi M, Kumar BR, Alex RM, et al. Appa Rao AVN. Novel steroidal glycosides from two indian *Caralluma* species, *C. stalagmifera* and *C. indica*. Helv Chim Acta 2006;89:201–9.