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Bisdesmosidic pregnane glycosides from *Caralluma lasiantha*☆

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Abstract

From the whole plant of *Caralluma lasiantha*, two new bisdesmosidic C-21 steroidal (pregnane) glycosides, named as lasianthoside-A and -B, were isolated and their structures elucidated solely based on extensive 2D-NMR and MS/MS spectral analysis as caralasigenin 3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-digitalopyranoside-20-*O*-α-L-rhamnosyl(1 → 6)-β-D-glucopyranoside and caralumagenin 3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-digitalopyranoside-20-*O*-α-L-rhamnosyl(1 → 6)-β-D-glucopyranoside. In addition, a known flavonoid glycoside, luteolin neohesperidoside, was also isolated.

Keywords: Caralluma lasiantha; Asclepiadaceae; Bisdesmosidic pregnane steroidal glycosides; Lasianthoside-A; Lasianthoside-B; Luteolin neohesperidoside; Caralasigenin; Caralumagenin; Structure elucidation; NMR assignments; Two-dimensional NMR techniques

1. Introduction

Plants of the Asclepiadaceae family are reported to be rich in pregnane glycosides (Christiane, Klaus & Eberhard, 1993). In recent years, the pregnanes and their glycosides have been shown to possess antitumor and anti-cancer activities (Luo et al., 1993). Earlier, we reported the isolation of five new steroidal glycosides from *Caralluma umbellata* Haw. (syn. *Boucerosia umbellata* W. & A.) (Asclepiadaceae) (Lin, Lin, Gil, Cordell, Ramesh, Reddy et al., 1994; Qiu, Lin, Cordell, Ramesh, Ravi Kumar, Radhakishan et al., 1997). *Caralluma lasiantha N. E. Br.* (syn. *Boucerosia lasiantha* Wt.), with a sour taste, is a succulent, perennial herb growing wild in Tirupathi and surrounding places of Andhra Pradesh, India. In a continuation of work on plants of the genus *Caralluma*, we report the isolation, structure characterization and NMR assignments of two new steroidal glycosides lasianthoside-A (**1**) and B (**2**) from the whole plant of *C. lasiantha* by means of 1D- and 2D-NMR techniques.

2. Results and discussion

Lasianthoside-A (**1**), being a steroidal glycoside as evident from a positive Liebermann–Burchard reaction, exhibited a molecular formula $C_{46}H_{74}O_{20}$ (MW = 946) based on its ^{13}C-NMR (DEPT) and mass spectra, in which a sodiated molecular ion $[M + Na]^+$ at *m/z* 969 and a quasimolecular ion $[M-H]^+$ at *m/z* 947 were detected in the positive FAB and DCI mass spectra, respectively. A quasimolecular ion $[M-H]^-$ observed at *m/z* 945 in the negative electrospray (ES) mass spectrum provided further supporting evidence.

Earlier methods for the structure elucidation of complex steroidal glycosides are quite tedious and involve substantial chemical derivatization and/or degradation work. Usually, this is carried out by permethylation

☆ Part VIII in the series 'Studies on the C-21 steroids from Asclepiadaceous plants', For part VII see Luo, Lin, Cordell, Xue & Johnson, 1993.

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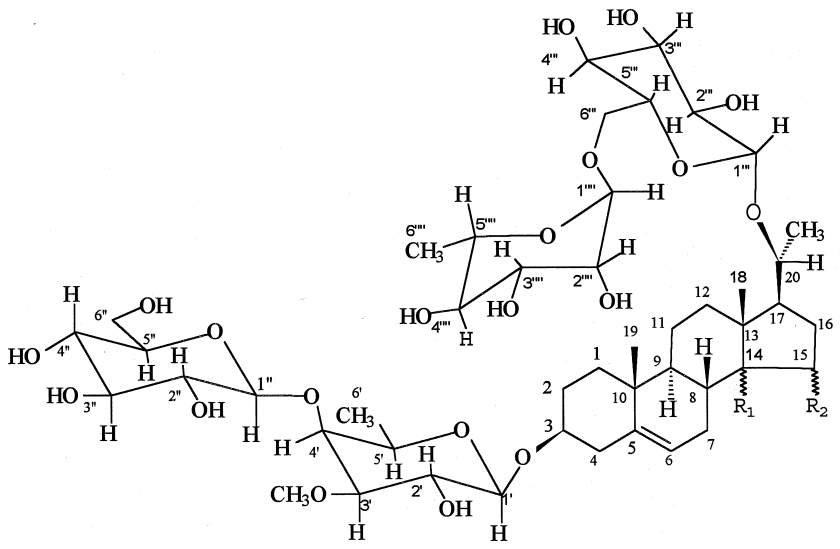
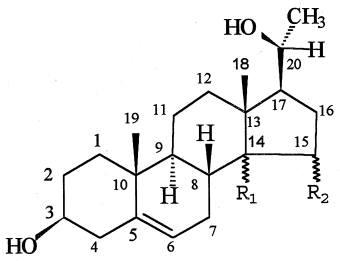


Lisianthoside A (**1**) $R_1 R_2$: $\Delta^{14\text{-}15}$

Lisianthoside B (**2**) R_1 = β-OH; R_2 = H



Caralasigenin (**3**) $R_1 R_2$: $\Delta^{14\text{-}15}$

Caralumagenin (**4**) R_1 = β-OH; R_2 = H

using Hakomori's method (Hakomori, 1964), followed by identification of the methylated monosaccharides. These studies, although providing proof of the structure, consume large quantities of sample that in most cases is not available. In this respect, as shown in our previous work (Qiu et al., 1997), NMR techniques, particularly ^{1}H-detected 2D-NMR spectroscopy offers a convenient and non-destructive method for studying the structures of glycosides without relying on prior information or using chemical shift theory. The techniques provide the direct through-bond-connectivity of one bond (HMQC) and multiple-bond (two or three) bonds (HMBC), with stereochemical characterization based on the coupling constants and spatial proximity information from DQF-COSY (TOCSY) and ROESY. These are a function of dihedral angles and arise from the dipole–dipole relaxation depending on the r^{-6} relationship of given protons.

The ^{1}H-NMR spectrum of **1** displayed four anomeric protons, one appears as a singlet at δ 5.43, the others are all doublet signals resonating at δ 4.73, 4.84 and 5.11, respectively, with coupling constants around 8.0 Hz, diagnostic of the axial orientations for all three sugar moieties (Agrawal, 1992). The ^{13}C DEPT experiments of **1** revealed a total of 46 carbon signals, of which six corresponded to methyl carbons, nine to methylene carbons, twenty-seven to methine carbons, and four to quaternary carbons, corresponding to a pregnane steroid glycoside carrying four hexoses, among which, one is a 6-deoxy-3-*O*-methyl-hexose, as usually found in pregnane glycosides (Qiu et al., 1997). The ^{13}C NMR signals of **1** due to the glycone portion were very similar to those of carumbelloside III (Qiu et al., 1997), except for a set of additional signals corresponding to an α-L-rhamnopyranosyl unit (Yoshikawa, 1989) by taking the glycosidation shifts at the methylene carbon into account. This suggested that **1** contained an analogous glycone portion with a terminal α-L-rhamnopyranosyl attached to a methylene carbon of the glucopyranosyl unit, linked either to C-20 or to a β-D-digitalopyranosyl unit. This is in good agreement with the negative ES-MS fragmentation pattern of **1**, which showed two significant fragmentation ions at *m/z* 799 $[M-H-146]^-$ and 637 $[M-H-146-162]^-$, rather than the quasimolecular ion *m/z* 945 $[M-H]^-$, and thus confirmed the presence of the terminal 6-, or 2-deoxy hexose.

The aglycone of **1** could be established as a previously unknown pregnane steroid, designated as caralasigenin (**3**), based on ^{13}C NMR comparison with the data of caralumagenin (**4**) (Qiu et al., 1997) and interpreting the correlation information from DQF-COSY, HOHAHA, ROESY and HMBC spectra. Aglycone **3** possesses two, non-conjugated double bonds as evident from the absence of UV absorption and the ^{1}H– and ^{13}C-NMR spectra, in which two olefinic protons were detected as broad singlets at δ 5.37 and 5.10, and four olefinic carbon signals were observed at δ 140.61, 121.78, 155.81 and 117.88, respectively. Caralasigenin (**3**) possesses the molecular formula $C_{21}H_{32}O_2$ (MW = 316) as deduced from the subtraction of the glycone units from that of **1**, and was observed to be closely related to caralumagenin (**4**) (Qiu et al., 1997) by comparison of their ^{13}C spectral data. The primary

difference between them was that the tertiary hydroxyl group of **4** was replaced by a double bond located at C-14 and C-15 in **3**. In support of this structure, the HMBC experiment showed conclusive three- and two-bond correlations between H-15 at δ 5.10 and the carbon signals δ 155.81 (C-14), 36.09 (C-16), 47.72 (C-13) and 59.04 (C-17).

Accordingly, the NMR spectral data of **3** were completely assigned (Table 1) by following the same methodology as described for caralumagenin in carabelloside-III (Qiu et al., 1997). The absolute configuration at C-20 of **3** could be similarly assigned to 20(*R*) based on the ROE correlations (Fig. 1) observed for the anomeric protons H-1‴ with H-20 and H-1‴ with H_3-21 (Qiu et al., 1997).

The NMR spectral signals of the β-D-digitalopyranosyl unit in **1** were well-resolved and the assignments could be achieved by cross-inspection of the correlation information from DQF-COSY, TOCSY, HMQC, HMBC and ROESY experiments utilizing the anomeric proton H-1′ at δ 4.73 as starting point, which showed strong correlation contours with H-3 (δ 3.78) and C-3 (δ 78.32) in the ROESY and HMBC spectra, respectively. In contrast, the assignment of the two β-D-glucopyranosyls were relatively difficult due to the barrier of the substantial signal overlapping, and thus only the anomeric protons H-1″ and H-1‴ and a few recognizable signals could be assigned unambiguously. As envisioned, the key for the structure of **1** rests on the location of the terminal α-L-rhamnopyranosyl unit.

Starting from the well-separated anomeric proton resonance H-1‴ at δ 4.84, which showed correlation ROE contours with H_3-21 and H-20, and a ^{13}C–^{1}H long-range contour with C-20 in ROESY and HMBC spectra, ring protons within the same residue could be assigned from cross peaks in the DQF-COSY and TOCSY contour maps as H-2‴ (δ 3.91), H-3‴ (δ 4.16), H-4‴ (δ 4.05), H-5‴ (δ 3.89), and, particularly a pair of non-equivalent methylene protons (H-6‴s) at δ 4.06 and 4.65. Thus, their directly coupled ^{13}C resonances were assigned by delineating the direct ^{13}C–^{1}H correlation from the HMQC experiment.

With the unequivocal assignments of H_2-6‴, C-6‴ and C-5‴ in hand, the attachment of the terminal rhamnose was ascribed to C-6‴, since an indicative ROE correlation contour was observed between H-1⁗/H-6‴ in the ROESY experiment. This conclusion was further supported by the observation of the glycosidation effects at C-6‴ (+6.5 ppm) and C-5‴ (–4.2 ppm) by comparison with those carbons of the terminal glucose. Interestingly, a medium ROE correlation was also observed between H-1⁗/H_3-18, which can only be accountable with the attachment of the rhamnose to the upper portion of the molecule. There is also a strong ^{13}C–^{1}H long-range correlation observed between H-1⁗ and the nearly overlapping ^{13}C signals resonating at around δ 69–70 ppm. Because this could arise from either C-6‴ or C-5⁗ from the point of view of HMBC, no clear assignment could be made without

Table 1
^{13}C NMR spectral data of Lasianthoside A (**1**) and B (**2**)*

	1	**2**
Aglycone		
1	37.52 (t)	37.57 (t)
2	30.35 (t)	30.19 (t)
3	78.32 (d)	78.25 (d)
4	39.36 (t)	39.11 (t)
5	140.61 (s)	139.56 (s)
6	121.78 (d)	122.72 (d)
7	30.35 (t)	27.81 (t)
8	31.38 (d)	37.57 (d)
9	50.71 (d)	46.72 (d)
10	37.48 (s)	37.57 (s)
11	22.02 (t)	20.95 (t)
12	41.76 (t)	41.27 (t)
13	47.72 (s)	47.57 (s)
14	155.81 (s)	85.25 (s)
15	117.88 (d)	25.41 (t)
16	36.09 (t)	32.70 (t)
17	59.04 (d)	56.17 (d)
18	16.93 (q)	17.23 (q)
19	19.34 (q)	19.39 (q)
20	75.26 (d)	76.18 (d)
21	19.41 (q)	18.29 (q)
Digitalose		
1′	103.10 (d)	102.69 (d)
2′	71.48 (d)	71.40 (d)
3′	85.56 (d)	85.45 (d)
4′	76.88 (d)	76.77 (d)
5′	70.61 (d)	70.48 (d)
6′	17.88 (q)	17.73 (q)
–OCH_3	59.04 (q)	58.93 (q)
Glucose		
1″	105.37 (d)	105.31 (d)
2″	76.29 (d)	76.32 (d)
3″	78.32 (d)	78.14 (d)
4″	71.96 (d)	71.85 (d)
5″	78.74 (d)	78.64 (d)
6″	63.13 (t)	63.06 (t)
Glucose		
1‴	101.28 (d)	100.83 (d)
2‴	76.14 (d)	75.25 (d)
3‴	78.74 (d)	78.57 (d)
4‴	72.37 (d)	71.86 (d)
5‴	74.50 (d)	75.25 (d)
6‴	69.62 (t)	67.85 (t)
Rhamnose		
1⁗	102.87 (d)	102.39 (d)
2⁗	72.46 (d)	72.22 (d)
3⁗	72.82 (d)	72.52 (d)
4⁗	74.06 (d)	74.06 (d)
5⁗	69.84 (d)	69.47 (d)
6⁗	18.83 (q)	18.74 (q)

* ppm from internal standard TMS in pyridine-d_5; multiplicity by DEPT experiments in parentheses, s, quaternary; d, methine; t, methylene and q, methyl carbons.

ambiguity. Instead, a strong ^{13}C–^{1}H long-range correlation contour was unprecedently observed between one of the well-isolated H-6‴s at δ 4.65 and the anomeric carbon resonance C-1‴′ at δ 102.87, providing further supporting evidence for the attachment of the α-L-rhamnosyl unit.

Therefore, based on the previous combination of information, the structure of **1** was established as caralasigenin 3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-digitalopyranoside-20-*O*-α-L-rhamnosyl(1 → 6)-β-D-glucopyranoside. It could be regarded as biogenetically derived from its direct precursor, lasianthoside-B (**2**), through dehydration.

Lasianthoside-B (**2**) was assigned the molecular formula $C_{46}H_{76}O_{21}$ (MW = 964) based on its ^{13}C-NMR (DEPT) and mass spectra, which displayed a protonated molecular ion $[M + H]^+$ and the adduct ions $[M + Na]^+$ at *m/z* 965 and *m/z* 987 in the positive electrospray (ES) and FAB mass spectra, respectively. As expected, a quasi-molecular ion was detected at *m/z* 963 $[M-H]^-$ in the negative ion mode ES mass spectrum.

From the DEPT spectra of **2**, the presence of six methyl, ten methylene, twenty-six methine and four quaternary carbons were evident. Four anomeric proton signals were observed at δ 4.79, 4.93, 5.15 and 5.48 in the ^{1}H NMR spectrum, and also four anomeric carbon resonances at δ 102.69, 100.83, 105.31 and 102.39, respectively, from the ^{13}C and HMQC spectra. A closer inspection of its ^{13}C spectrum revealed a pregnane tetraglycoside bearing the same glycone portions as **1**, since all of the ^{13}C signals due to the sugar moieties were nearly identical with those of **1**. Moreover, the aglycone of **2** was readily determined as caralumagenin (**4**) based on the comparable ^{13}C data with literature values (Qiu et al., 1997) with a slight deviation for some of the carbons in rings C and D due to the introduction of a terminal α-L-rhamnosyl unit with respect to carumbelloside III. Thus, the structure of **2** was deduced as caralumagenin 3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-digitalopyranoside-20-*O*-α-L-rhamnosyl(1 → 6)-β-D-glucopyranoside. This conclusion was in agreement with the large number of through-bond connectivities observed in the DQF-COSY, TOCSY and HMBC spectra.

Theoretically, because the HMBC experiment is a ^{1}H-detected multiple-bond heteroatomic correlation, it is a very sensitive method for establishing glycosidic linkages. This method, in addition to the intra-residue multiple bond correlations, is valuable for confirming ^{13}C and ^{1}H assignments, provides inter-residue multiple bond correlations between either the anomeric carbon and the aglycone (or adjacently linked monosaccharide) or the anomeric proton and the aglycone carbon (or adjacently linked monosaccharide), and thus serves to determine the interglycosidic linkage. However, the intensities of the cross peaks depend on many factors, such as the coupling constants involved,

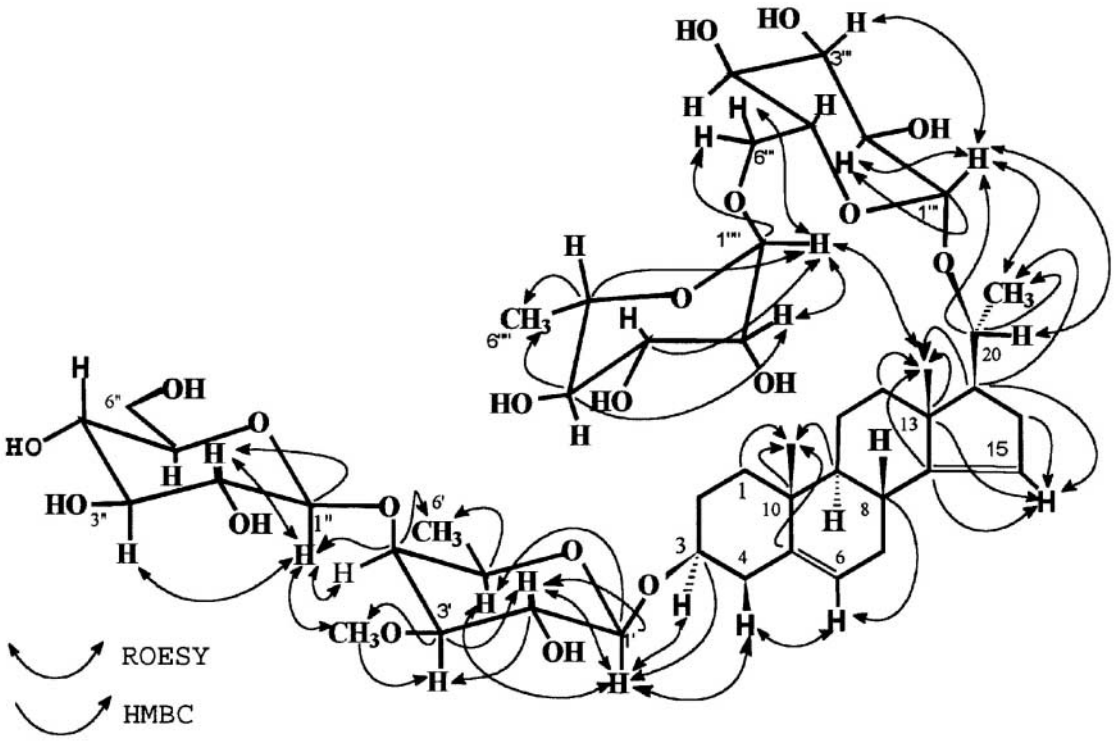


Fig. 1.

i.e. cross peaks are generally more detectable between resonance for which there are relatively large two- and three-bond ^{13}C–^{1}H coupling constants (Morat, Taravel & Vignon, 1988), and also on the multiplicity of the proton resonances, namely, a broad multiplicity of the proton signal leads to poor cross peaks (Byrd, Egan, Summers & Bax, 1987). In other words, the intensity of the HMBC correlations of intra- and inter-residue correlations depends on the configuration and the relative stereochemistry of the sugar units. Therefore, the HMBC correlation profile and the relative intensity of cross peaks involved in anomeric protons are characteristic of given monosaccharides, and can be employed for the structure elucidation of oligosaccharides. As for **1** and **2**, the only observed HMBC cross peaks involved in the anomeric protons of β-D-digitalopyranosyl and two β-D-glucopyranosyl units were those between the glycosidic linkage, consistent with the observation by Abeygunawardana and Bush (1990). These result from the fact that all of the couplings of the anomeric proton to the β-D-pyranosyl ring carbons are small. In the α-L-rhamnosyl unit, some 'abnormality', possibly conformationally linked, was observed in the HMBC correlations involved for the anomeric proton H-1‴, in which the intraresidue cross peaks to C-3‴ and to C-5‴ predominated and no correlation across the glycosidic linkage was observed.

It is interesting to note that this represents the first case for the absence of HMBC correlation of an anomeric proton across a glycosidic linkage, although it may be specific to an α-L-rhamnopyranosyl linkage, since it has been believed that while the three-bond ^{13}C–^{1}H coupling constant across the glycosidic linkage does depend on the conformation, only the intensity and not its existence is in question (Abeygunawardana & Bush, 1990). On the other hand, a ^{13}C–^{1}H correlation between H-6‴ and C-1‴ was observed across the glycosidic linkage.

The coupling maps of the β-D-digitalopyranosyl units in compounds **1**, **2** and carumbelloside III (Qiu et al., 1997) are in excellent agreement. As expected for the monosaccharide residue with a β-D-galacto configuration (Morat, Taravel & Vignon, 1988), the anomeric proton H-1′ showed cross peaks to H-2′, H-3′, and H-4′ in a TOCSY experiment, but the connectivity of H-1′ to H-5′ and further to H-6′ was not observed owing to the small scalar coupling between H-4′ and H-5′ for a sugar having the β-D-galacto configuration. Nevertheless, the assignment of H-5′ could be rigorously established by an intraresidue ROE to the anomeric proton H-1′.

The anomeric proton of the α-L-rhamnosyl H-1‴ showed no scalar coupling with H-2‴ in the DQF-COSY spectrum, which is only visible at low contour level in the TOCSY spectrum. Although connectivity beyond H-2‴ was not seen in the cross section taken through the anomeric resonance in the TOCSY spectrum, connectivity up to the same H-2‴ resonance from the well-resolved methyl proton resonance H-6‴ at δ 1.62 ppm was clearly traced in the TOCSY spectrum.

A known flavonoid glycoside luteolin neohesperidoside [luteolin-4′-*O*-(α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranoside] was also isolated from the EtOAc and *n*-BuOH extracts of the title plant, whose structure was determined by physical and spectral comparison with those of an authentic compound (Ahmad, 1988). It is interesting to point out that such a compound was also isolated from *C. attenuata* and *C. umbellata* (unpublished results). Furthermore, it was reported by Ahmad *et al.* that luteolin neohesperidoside was also isolated from *C. tuberculata* (Ahmad, 1988). Thus, this compound appears to be a common constituent of plants of the *Caralluma* genus.

3. Experimental

3.1. General

Mps: uncorr. IR spectra were recorded in a KBr pellet on a MIDAC FT-IR interferometer. The optical rotations were measured with a Perkin–Elmer 241 polarimeter. The ^{1}H, ^{13}C-NMR, DEPT, HMQC, HMBC, DQF-COSY, HOHAHA and ROESY spectra were recorded with a Bruker Avance DPX-300 and DRX-500 instruments, using Bruker standard programs in C_5D_5N solution. FAB-MS was recorded by the direct-inlet method on a VG ZAB-HS mass spectrometer using glycerol as matrix. Electrospray MS/MS was performed on a Macro mass Quattro II electrospray triple quadrupole mass spectrometer.

3.2. Plant material

The plant material of *C. lasiantha* was collected in Tirupathi, Andhra Pradesh, India, and identified by Dr. V. S. Raju, Department of Botany, Kakatiya University, Warangal, India. A voucher sample was deposited in the herbarium of the University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India.

3.3. Extraction and isolation

The fresh whole plant (5 kg) of *C. lasiantha* was chopped and crushed and extracted with EtOH (15 l) at rt for 7 days. The extract was filtered and the solvent was removed under vacuum. To the concentrate, water (500 ml) was added, and extd successively with toluene, ether, EtOAc and *n*-BuOH. After evaporation of the solvent, the *n*-BuOH ext was subjected to vac-

uum liquid chromatography on Silica gel cc to yield compounds **1** and **2**, respectively.

3.4. Isolation of lasianthoside-A (*1*) and B (*2*)

The *n*-BuOH extract (3.6 g) was dissolved in MeOH and adsorbed onto silica gel (10 g). This was subjected to vacuum liquid chromatography (silica gel 10–40 μ, 500 g) using EtOAc–MeOH–H_2O (75:15:10, v/v) as the eluent collecting fractions of 20 ml each. Fractions 35–53 contained lasianthoside-A (**1**) (CL-1) which was further separated by re-chromatography using the solvent system $CHCl_3$–MeOH–H_2O (70:20:2) as eluent. Fractions 68–78 contained lasianthoside-B (**2**) (Cl-2) which was further purified by charcoalisation.

Lasianthoside-A (**1**) (170 mg, yield 0.0034%). amorphous powder, hygroscopic; mp 159–60°C; $[\alpha]_D^{19}$ -4.5° (MeOH, *c* 0.40). IR ν_{max} (cm^{-1}): 3600–3200 (OH), 980, 920, 902, 856. FAB-MS *m/z* (positive): 969 $[M + Na]^+$, 807 $[M + Na - 162]^+$. DCI-MS *m/z* (positive): 947 $[M + H]^+$, 911 $[M + H\text{-}2\times H_2O]^+$, 765 $[911–146]^+$, 639 $[M + H - 146–162]^+$, 477 $[M + H - 146–162–162]^+$, 461 $[M + H - 146–162–162 - O]^+$. ES-MS *m/z* (negative): 945 $[M - H]^-$, 799 $[M - H - 146]^-$, 783 $[M - H - 146 - O]^-$, 637 $[M - H - 146–162]^-$. ^{1}H NMR (ppm from internal standard pyridine-d_5) δ: 1.04 (1H, m, H-1α), 1.72 (1H, m, H-1β), 2.03 (1H, m, H-2α), 1.47 (1H, m, H-2β), 3.78 (1H, m, H-3α), 2.62 (br.d, J = 11.6 Hz, H-4α), 2.31 (1H, t, J = 11.6 Hz, H-4β), 5.37 (1H, br.s, H-6), 1.67 (1H, m, H-7α), 1.95 (1H, m, H-7β), 2.07 (1H, m, H-8β), 0.98 (1H, m, H-9α), 2.10 (1H, m, H-11α), 1.98 (1H, m, H-11β), 1.26 (1H, m, H-12α), 2.50 (1H, br.d, J = 12.9 Hz, H-12β), 5.10 (1H, br.s, H-15), 1.99 (1H, m, H-16α), 2.07 (1H, m, H-16β), 1.98 (1H, m, H-17α), 1.39 (3H, s, H_3-18), 0.85 (3H, s, H_3-19), 4.22 (1H, m, H-20), 1.18 (3H, d, J = 5.7 Hz, H_3-21), 4.73 (1H, d, J = 7.6 Hz, H-1′), 4.39 (1H, t, J = 9.3 Hz, H-2′), 3.55 (1H, dd, J = 9.7, 2.8 Hz, H-3′), 4.07 (1H, m, H-4′), 3.69 (1H, q, J = 6.3 Hz, H-5′), 1.52 (3H, d, J = 6.3 Hz, $H_3$6′), 3.65 (3H, s, OCH_3 of digitalose), 5.11 (1H, d, J = 7.9 Hz, H-1″), 3.92 (1H, m, H-2″), 4.18 (1H, m, H-3″), 4.10 (1H, m, H-4″), 4.06 (1H, m, H-5″), 4.30 (1H, m, H_A-6″), 4.48 (1H, dd, J = 2.5, 8.4 Hz, H_B-6″), 4.84 (1H, d, J = 7.6 Hz, H-1‴), 3.91 (1H, m, H-2‴), 4.16 (1H, m, H-3‴), 4.05 (1H, m, H-4‴), 3.89 (1H, m, H-5‴), 4.06 (1H, br.d, J = 9.0 Hz, H_A-6‴), 4.65 (1H, br.d, J = 9.0 Hz, H_B-6‴), 5.43 (1H, br.s, H-1⁗), 4.55 (1H, t, J = 3.2 Hz, H-2⁗), 4.34 (1H, m, H-3⁗), 4.40 (1H, m, H-4⁗), 4.30 (1H, m, H-5⁗), 1.57 (3H, d, J = 5.8 Hz, H_3-6⁗). ^{13}C-NMR data are shown in Table 1.

Lasianthoside-B (**2**) (207 mg, yield 0.0041%). amorphous powder, hygroscopic; mp 109–113°C; $[\alpha]_D^{19}$ −32.9° (MeOH, c 0.50). IR ν_{max} (cm^{-1}): 3450, 1700, 1600, 1450, 1280. FAB-MS *m/z* (positive): 987 $[M + Na]^+$, 825 $[M + Na\text{-}162]^+$; *m/z* (negative): 963 $[M - H]^-$, 817 $[M - H - 146]^-$. ES-MS (positive): 965 $[M + H]^+]^-$. ^{1}H NMR (ppm from internal standard pyridine-d_5) δ: 1.03 (1H, m, H-1α), 1.77 (1H, m, H-1β), 2.12 (1H, m, H-2α), 1.66 (1H, m, H-2β), 3.86 (1H, m, H-3α), 2.64 (dd, J = 11.6, 4.5 Hz, H-4α), 2.31 (1H, t, J = 11.6 Hz, H-4β), 5.34 (1H, d, J = 4.5 Hz, H-6), 2.02 (1H, m, H-7α), 2.51 (1H, m, H-7β), 1.96 (1H, m, H-8β), 1.17 (1H, m, H-9α), 2.08 (1H, m, H-11α), 1.80 (1H, m, H-11β), 1.52 (1H, m, H-12α), 1.37 (1H, m, H-12β), 1.77 (1H, m, H-15), 1.76 (1H, m, H-16α), 1.88 (1H, m, H-16β), 2.00 (1H, m, H-17α), 1.55 (3H, s, H_3-18), 0.82 (3H, s, H_3-19), 4.52 (1H, q, J = 6.0 Hz, H-20), 1.33 (3H, d, J = 6.0 Hz, H_3-21), 4.79 (1H, d, J = 7.7 Hz, H-1′), 4.42 (1H, dd, J = 9.6, 7.6 Hz, H-2′), 3.61 (1H, dd, J = 9.6, 3.2 Hz, H-3′), 4.37 (1H, m, H-4′), 3.77 (1H, q, J = 6.5 Hz, H-5′), 1.58 (3H, d, J = 6.5 Hz, $H_3$6′), 3.71 (3H, s, OCH_3 of digitalose), 5.15 (1H, d, J = 7.7 Hz, H-1″), 3.98 (1H, m, H-2″), 4.21 (1H, m, H-3″), 4.20 (1H, m, H-4″), 3.98 (1H, m, H-5″), 4.31 (1H, m, H_A-6″), 4.58 (1H, m, H_B-6″), 4.93 (1H, d, J = 7.7 Hz, H-1‴), 3.90 (1H, m, H-2‴), 4.18 (1H, m, H-3‴), 4.00 (1H, m, H-4‴), 3.95 (1H, m, H-5‴), 4.15 (1H, dd, J = 10.4, 5.0 Hz, H_A-6‴), 4.60 (1H, m, H_B-6‴), 5.48 (1H, br.s, H-1⁗), 4.59 (1H, t, J = 3.2 Hz, H-2⁗), 4.28 (1H, m, H-3⁗), 4.42 (1H, m, H-4⁗), 4.32 (1H, m, H-5⁗), 1.62 (3H, d, J = 6.0 Hz, H-6⁗). ^{13}C-NMR data are shown in Table 1.

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References

Abeygunawardana, C., & Bush, C. A. (1990). *Biochemistry, 29,* 234–248.

Agrawal, P. (1992). *Phytochemistry, 31,* 3307.

Ahmad, V. U. (1988). *J. Nat. Prod., 51,* 1092.

Byrd, R. A., Egan, W., Summers, M. F., & Bax, A. (1987). *Carbohydr. Res., 166,* 47.

Christiane, S., Klaus, R., & Eberhard, B. (1993). *Liebigs Ann. Chem., 10,* 1057.

Hakomori, S. (1964). *J. Biochem., 55,* 205.

Lin, L.-J., Lin, L.-Z., Gil, R. R., Cordell, G. A., Ramesh, M., Reddy, B., & Rao, A. V. N. A. (1994). *Phytochemistry, 35,* 1549.

Luo, S. Q., Lin, L. Z., Cordell, G. A., Xue, L., & Johnson, M. E. (1993). *Phytochemistry, 34,* 1615.

Morat, C., Taravel, F. R., & Vignon, M. R. (1988). *Magn. Reson. Chem., 26,* 264.

Qiu, S. X., Lin, L. Z., Cordell, G. A., Ramesh, M., Ravi Kumar, B., Radhakishan, M., Krishna Mohan, G., Reddy, B. M., Nageshwar Rao, Y., Srinivas, B., Sunil Thomas, N., & Rao, A. V. N. A. (1997). *Phytochemistry, 46,* 333.

Yoshikawa, K., Arihara, S., Wang, J. D., Narui, T., & Okuyama, T. (1989). *Chem. Pharm. Bull., 39,* 1185.