Serratene Triterpenoids from *Palhinhaea cernua* var. *sikkimensis*

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Seven new serratene triterpenoids, 3α,21β,29-trihydroxy-16-oxoserrat-14-en-24-oic acid (1), 3β,21β,29-trihydroxy-16-oxoserrat-14-en-24-oic acid (2), 3β,21β,29-trihydroxyserrat-14-en-24-oic acid 3β-(4-hydroxybenzoate) (3), 3β,21β,29-trihydroxyserrat-14-en-24-oic acid 3β-(4-hydroxy-3-methoxybenzoate) (4), 3β,14α,15α,-21β,29-pentahydroxyserrat-14-en-24-oic acid 3β-(4-hydroxybenzoate) (5), 3α,21β,24,29-tetrahydroxyserrat-14-en-16-one (6), 3α,21β,30-trihydroxyserrat-14-en-16-one (7) were isolated from *Palhinhaea cernua* var. *sikkimensis*, together with twelve known compounds (8—19). Their chemical structures were elucidated on the basis of spectroscopic evidence and comparison with literature values.

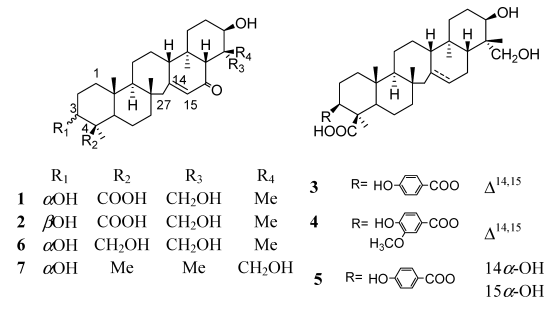
Key words serratene; triterpenoid; *Palhinhaea*

In our recent phytochemical research on some species of the family Lycopodiaceae, we reported some new serratene triterpenoids.[1,2] Serratene is a unique family of pentacyclic triterpenoids possessing seven to five tertiary methyl groups and a central seven-membered ring C. Some of the serratenes possessed important pharmacological activities, such as antitumor promotion: 3β-methoxyserrat-14-en-21β-ol, 13α,14α-epoxyserratan-3β,21β-diol, 13α,14α-epoxyserratan-3α,21β-diol[3] and inhibition *Candida albicans* secreted aspartic proteases: lycernuic acid C.[4] In continuation of our studies on other species of Lycopodiaceae, we investigated the traditional Chinese herb *Palhinhaea cernua* var. *sikkimensis*, which is used in the treatment of arthritic pain, quadriplegia, and contusion in folk medicine. Nineteen serratene-type triterpenoids have been obtained from the EtOH extract of the whole plant, including seven new compounds 3α,21β,29-trihydroxy-16-oxoserrat-14-en-24-oic acid (**1**), 3β,21β,29-trihydroxy-16-oxoserrat-14-en-24-oic acid (**2**), 3β,21β,29-trihydroxyserrat-14-en-24-oic acid 3β-(4-hydroxybenzoate) (**3**), 3β,21β,29-trihydroxyserrat-14-en-24-oic acid 3β-(4-hydroxy-3-methoxybenzoate) (**4**), 3β,14α,15α,21β,29-pentahydroxyserrat-14-en-24-oic acid 3β-(4-hydroxybenzoate) (**5**), 3α,21β,24,29-tetrahydroxyserrat-14-en-16-one (**6**), 3α,21β,30-trihydroxyserrat-14-en-16-one (**7**). In this paper, we report the isolation and structure elucidation of the seven new serratene-type triterpenoids.

Results and Discussion

The molecular formula $C_{30}H_{46}O_6$ for **1** was established by negative HR-FAB-MS *m*/*z* 501.3227 (Calcd $C_{30}H_{45}O_6$ for 501.3216). The ^{1}H- and ^{13}C-NMR spectra of **1** showed obvious signals for a ketone group (δ_C 202.2, C-16), a carbonyl group (δ_C 180.4, C-24), a double bond (δ_C 164.6, C-14; 128.9, C-15, and δ_H 5.94, H-15), two oxymethines (δ_C 70.5, C-3; 70.1, C-21, and δ_H 4.58, H-3; 4.69, H-21), one oxymethylene (δ_C 64.2, C-29, and δ_H 4.12, 4.78 (each 1H, d, 10.8)), five methyls, nine methylenes (including the most characteristic serratene signals at δ_C 54.9, C-27, δ_H 1.88, 2.34, H-27), four methines, and five quaternary carbons. Detailed analysis of ^{1}H- and ^{13}C-NMR spectra of **1**, and a comparison with the literature values of serratene[4—6] indicated that **1** was a serratene derivative. Positions of the ketone, carbonyl, and oxymethylene group were established by heteronuclear multiple-bond correlations (HMBC), and relative configuration of the carbonyl group at C-4, and the oxymethylene at C-22 were confirmed by nuclear Overhauser effect spectroscopy (NOESY) correlations (see Fig. 2). Both H-3 and H-21 were determined to have β- and α-orientation, as indicated by their characteristic ^{1}H-NMR signals at δ_H 4.58 (1H, s) and δ_H 4.69 (1H, s),[5] respectively. So, the chemical structure of **1** was established as 3α,21β,29-trihydroxy-16-oxoserrat-14-en-24-oic acid.

Compound **2** had the same molecular formula as **1**, $C_{30}H_{46}O_6$, established by negative HR-FAB-MS. Detailed analysis of their NMR spectra found that **2** differed from **1** only in the configuration of H-3. In compound **2**, the OH-3 was assigned a β-orientation, as indicated by the characteristic ^{1}H-NMR signal at δ_H 3.35 (1H, dd, J=4.3, 11.9 Hz).[5] The significant chemical shift differences at ring A between **2** and **1** further supported the β-orientation for OH-3 in **2**



	R_1	R_2	R_3	R_4
1	αOH	COOH	CH_2OH	Me
2	βOH	COOH	CH_2OH	Me
6	αOH	CH_2OH	CH_2OH	Me
7	αOH	Me	Me	CH_2OH

3 R= HO-⟨⟩-COO $\Delta^{14,15}$

4 R= HO-⟨⟩(H$_3$CO)-COO $\Delta^{14,15}$

5 R= HO-⟨⟩-COO 14α-OH, 15α-OH

Fig. 1. Structures of Newly Isolated Compounds **1**—**7** from *Palhinhaea cernua* var. *sikkimensis*

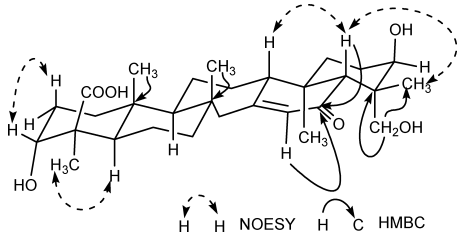


Fig. 2. Selected NOESY and HMBC Correlations of **1**

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Table 1. ^{13}C-NMR Spectroscopic Data of **1**—**7** (δ in ppm, in C_5D_5N)

C	1	2	3	4	5	6	7
1	34.7	39.0	38.9	38.9	39.1	34.7	34.7
2	27.8	29.1	26.7	26.7	33.9	26.7	26.5
3	70.5	78.1	79.9	81.3	76.8	69.9	69.6
4	48.5	49.4	49.4	49.4	49.4	44.2	37.7
5	49.4	56.7	57.5	57.5	56.6	50.2	49.9
6	21.0	21.1	21.2	21.2	21.5	19.7	19.7
7	45.6	45.4	45.8	45.8	44.1	45.8	44.7
8	38.2	37.9	37.3	37.3	37.9	38.3	38.1
9	62.6	62.1	62.6	62.6	59.7	62.8	62.5
10	39.3	38.9	39.1	39.1	38.8	38.6	39.0
11	25.9	25.4	25.7	25.7	25.7	25.4	25.2
12	26.9	30.0	27.7	27.7	26.7	26.0	26.8
13	59.2	59.1	56.9	56.9	58.7	59.4	58.8
14	164.6	164.1	138.9	138.9	78.2	162.9	162.9
15	128.9	128.9	123.1	123.1	76.8	128.4	128.4
16	202.2	202.0	24.7	24.7	28.2	200.7	200.7
17	60.3	60.3	43.5	43.5	47.1	60.3	58.3
18	45.6	44.7	36.4	36.4	39.0	44.8	44.8
19	32.1	32.0	32.0	32.0	34.0	32.1	31.3
20	25.9	25.9	25.2	25.2	26.5	26.8	25.9
21	70.1	70.0	69.7	69.8	70.1	70.0	75.2
22	43.9	43.8	43.5	43.5	44.3	43.9	41.8
23	25.5	24.7	24.5	24.5	24.5	23.6	29.0
24	180.4	180.7	177.0	177.0	180.7	65.9	23.3
25	14.1	14.3	14.1	14.1	14.7	16.6	15.2
26	19.8	19.7	19.8	19.8	23.2	20.0	19.9
27	56.3	55.9	56.9	56.9	56.6	56.3	55.9
28	15.9	15.9	14.7	14.7	17.0	15.9	16.5
29	64.2	64.1	65.8	65.8	65.8	64.3	22.1
30	23.2	23.1	23.0	23.0	23.6	23.1	68.9
1′	—	—	124.4	123.3	124.4	—	—
2′	—	—	132.6	113.7	132.6	—	—
3′	—	—	116.2	148.4	116.2	—	—
4′	—	—	163.6	153.2	163.6	—	—
5′	—	—	116.2	116.3	116.2	—	—
6′	—	—	132.6	124.9	132.6	—	—
7′	—	—	166.8	166.8	166.8	—	—
OCH_3	—	—	—	55.7	—	—	—

(see Table 1). The ^{1}H- and ^{13}C-NMR assignments were unambiguously made to use 2D NMR. Thus, the chemical structure of **2** was established to be 3β,21β,29-trihydroxy-16-oxoserrat-14-en-24-oic acid.

Compound **3** was assigned the molecular formula $C_{37}H_{52}O_7$ by negative HR-FAB-MS *m*/*z* 607.3639 (Calcd 607.3634 for $C_{37}H_{51}O_7$). The IR bands showed the presence of hydroxy (3567 cm^{-1}), ester carbonyl (1724 cm^{-1}), and aromatic (1620, 1517 cm^{-1}) groups. The ^{13}C-NMR and DEPT spectra displayed 37 carbon signals, indicating that serratene-type triterpenoid connected with an aromatic ring. Further analysis of ^{1}H-NMR spectra showed two sets of signals: one for 3β,21β,29-trihydroxyserrat-14-en-24-oic acid,[4] and the another for a 1,4-disubstituted aromatic ring [δ_H 7.12 and 8.42 (each 2H, d, J=8.5 Hz)]. The ion fragment peaks at *m*/*z* 607 $[M-H]^-$, 487 $[M-C_7H_5O_2]^+$ were observed in the negative FAB-MS. All the above evidence indicated 3β,21β,29-trihydroxyserrat-14-en-24-oic acid with a *p*-hydroxybenzoic group as an ester substituent. The ester unit was positioned at C-3 on the basis of HMQC and HMBC analysis (see Fig. 3). Therefore, the chemical structure of **3** was assigned as 3β,21β,29-trihydroxyserrat-14-en-24-oic acid 3β-(4-hydroxybenzoate).

The molecular formula $C_{38}H_{54}O_8$ for **4**, established by negative FAB-MS *m*/*z* 637, was 30 mass units higher than **3**. The ^{1}H- and ^{13}C-NMR signals of **4** were nearly superposed with those of **3**, expect for a 1,3,4-trisubstitute aromatic ring [δ_H 8.06 (1H, s), 8.13 (1H, d, J=8.2 Hz), and 7.16 (1H, overlap); δ_C 123.3 (s), 113.7 (d), 148.4 (s), 153.2 (s), 116.3 (d), 124.9 (d)] instead of 1,4-disubstitute aromatic ring in **3**. The structure was also supported by ion fragments at *m*/*z* 637 $[M-H]^-$, 487 $[M-C_8H_7O_3]^+$ in the negative FAB-MS. Additionally, HMBC correlation of δ_H 3.71 (s) with δ_C 148.4 (C-3′) indicated the methoxy group was attached at C-3′ of the aromatic ring. So, the structure of **4** was determined to be 3β,21β,29-trihydroxyserrat-14-en-24-oic acid 3β-(4-hydroxy-3-methoxybenzoate).

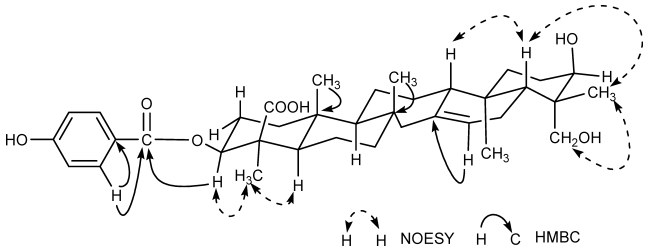

Fig. 3. Selected NOESY and HMBC Correlations of **3**

The molecular formula $C_{37}H_{54}O_9$ for **5**, established by negative FAB-MS, was 34 mass units (2×OH) higher than **3**, two hydroxy groups-attached carbons. Analysis of the ^{1}H- and ^{13}C-NMR spectra indicated that compound **5** was very similar to **3** (see Tables 1—3), apart from absence of a double bond. The typical serratene double bond was replaced by two hydroxylated carbons [δ_C 78.2 (C-14) and 76.8 (C-15); δ_H 3.62, dd, J=8.0 Hz (H-15)]. The α-orientation of these two hydroxy groups was assigned by comparing their carbon shifts with those of lycernuic acid C.[4] So, the structure of **5** was determined as 3β,14α,15α,21β,29-pentahydroxyserrat-14-en-24-oic acid 3β-(4-hydroxybenzoate).

Compound **6** had the molecular formula $C_{30}H_{48}O_5$, established by negative HR-FAB-MS. The IR absorptions disclosed hydroxy (3440 cm^{-1}) and unsaturated ketone (1660 cm^{-1}) groups. The ^{13}C-NMR spectrum exhibited 30 carbon signals, a ketone group at δ_C 200.7 (C-16), a double bond at δ_C 162.9 (C-14) and δ_C 128.4 (C-15), two oxymethines at δ_C 69.9 (C-3) and δ_C 70.0 (C-21), two oxymethylenes at δ_C 65.9 (C-24) and δ_C 64.3 (C-29), five methyls, four methines, five quaternary carbons, and nine methylenes. Comparison of **6** with 3α,21β,24,29-tetrahydroxyserrat-14-ene[6,7] showed that they were very similar, differing only in the number of methylenes, which suggested that one of the methylenes in **6** was oxidized to the ketone group. Position of the ketone group at C-16 was established by the HMBC spectrum (see Fig. 4), and was further confirmed by chemical shift changes of C-14 (+Δ 24.8), C-15 (+Δ 5.5), H-15 (+Δ 0.5), and H-17 (+Δ 1.12) (see Tables 1, 3) comparison with 3α,21β,24,29-tetrahydroxyserrat-14-ene. So, the structure of **6** was determined as 3α,21β,24,29-tetrahydroxyserrat-14-en-16-one.

The FAB-MS of compound **7** displayed a quasi-molecular ion peak at *m*/*z* 471 $[M-H]^-$, supporting a molecular formula of $C_{30}H_{48}O_4$. The ^{13}C-NMR and DEPT spectra of **7** showed signals for a ketone group at δ_C 200.7 (C-16), double bonds at δ_C 162.9 (C-14) and 128.4 (C-15), two oxymethines

at δ_C 69.6 (C-3) and 75.2 (C-21), a hydroxymethyl at δ_C 68.9 (C-30), six methyls, nine methylenes, four methines, and five quaternary carbons. The ^{1}H- and ^{13}C-NMR spectra of **7** were very similar to those of 3α,21β-dihydroxyserrat-14-en-16-one,[8] apart from the C-22 group. The hydroxymethyl at C-22 was established by HMBC correlations, and was shown to have a β-orientation by the presence of one key NOESY correlation between H-30 (δ_H 4.28, 4.58) and H-17 (δ_H 3.00). Meanwhile, the carbon signal of C-17 (δ_C 58.3) was shifted upfield 1.7 ppm in comparison with lycernuic ketone A[4] due to the γ-gauche effect from the additional hydroxymethyl group. Owing to molecular hydrogen bond effects from the

Table 2. ^{1}H-NMR Spectroscopic Data of **1**—**4** (δ in ppm, J in Hz, C_5D_5N)

H	1	2	3	4
1	1.60 (1H, m); 1.74 (1H, m)	0.99 (1H, m); 1.86 (1H, m)	1.11 (1H, m); 1.93 (1H, m)	1.11 (1H, m); 1.93 (1H, m)
2	1.93 (1H, m); 2.44 (1H, m)	1.95 (1H, m); 2.43 (1H, m)	1.99 (1H, m); 2.30 (1H, m)	1.99 (1H, m); 2.30 (1H, m)
3	4.58 (1H, s)	3.35 (1H, dd, 4.3, 11.9)	5.13 (1H, dd, 3.8, 11.8)	5.13 (1H, dd, 3.8, 11.8)
5	1.99 (1H, m)	0.98 (1H, m)	1.12 (1H, m)	1.12 (1H, m)
6	1.78 (2H, m)	1.82 (2H, m)	1.98 (1H, m); 2.32 (1H, m)	1.98 (1H, m); 2.32 (1H, m)
7	1.22 (1H, m); 1.42 (1H, m)	1.36 (1H, m); 1.40 (1H, m)	1.25 (1H, m); 1.40 (1H, m)	1.25 (1H, m); 1.40 (1H, m)
9	0.99 (1H, m)	0.81 (1H, m)	0.82 (1H, m)	0.82 (1H, m)
11	1.10 (1H, m); 1.85 (1H, m)	1.10 (1H, m); 1.85 (1H, m)	1.85 (1H, m); 2.10 (1H, m)	1.85 (1H, m); 2.10 (1H, m)
12	1.24 (1H, m); 1.30 (1H, m)	1.24 (1H, m); 1.30 (1H, m)	1.95 (1H, m); 2.01 (1H, m)	1.95 (1H, m); 2.01 (1H, m)
13	2.54 (1H, m)	2.54 (1H, m)	2.08 (1H, m)	2.08 (1H, m)
15	5.94 (1H, s)	5.96 (1H, s)	5.49 (1H, s)	5.49 (1H, s)
16	—	—	2.18 (2H, m)	2.18 (2H, m)
17	3.27(1H, s)	3.28 (1H, s)	2.35 (1H, s)	2.35 (1H, s)
19	1.61 (1H, m); 2.29 (1H, m)	1.61 (1H, m); 2.29 (1H, m)	1.70 (1H, m); 2.03 (1H, m)	1.70 (1H, m); 2.03 (1H, m)
20	1.93 (1H, m); 2.19 (1H, m)	1.93 (1H, m); 2.19 (1H, m)	1.15 (1H, m); 1.85 (1H, m)	1.15 (1H, m); 1.85 (1H, m)
21	4.69 (1H, s)	4.58 (1H, s)	4.58 (1H, s)	4.58 (1H, s)
23	1.73 (3H, s)	1.62 (3H, s)	1.57 (3H, s)	1.58 (3H, s)
25	0.81 (3H, s)	0.77 (3H, s)	1.12 (3H, s)	1.12 (3H, s)
26	1.09 (3H, s)	1.02 (3H, s)	0.88 (3H, s)	0.88 (3H, s)
27	1.88 (1H, m); 2.34(1H, m)	1.91 (1H, m); 2.36 (1H, m)	1.86 (1H, m); 2.36 (1H, m)	1.86 (1H, m); 2.36 (1H, m)
28	0.87 (3H, s)	0.91 (3H, s)	0.85 (3H, s)	0.85 (3H, s)
29	4.12, 4.78 (each 1H, d, 10.8)	4.13, 4.80 (each 1H, d, 10.8)	4.19, 3.96 (each 1H, d, 10.8)	4.19, 3.96 (each 1H, d, 10.8)
30	1.99 s	2.00 s	1.57 (3H, s)	1.56 (3H, s)
2′	—	—	7.12 (1H, d, 8.5)	8.06 (1H, s)
3′	—	—	8.42 (1H, d, 8.5)	—
5′	—	—	8.42 (1H, d, 8.5)	8.13 (1H, d, 8.2)
6′	—	—	7.12 (1H, d, 8.5)	7.16 (1H, overlap)
OCH_3	—	—	—	3.71 (3H, s)

Table 3. ^{1}H-NMR Spectroscopic Data of **5**—**7** (δ in ppm, J in Hz, C_5D_5N)

H	5	6	7
1	1.10 (1H, m); 1.90 (1H, m)	1.59 (1H, m); 1.81 (1H, m)	1.60 (1H, m); 1.74 (1H, m)
2	1.99 (1H, m); 2.43 (1H, m)	1.99 (1H, m); 2.28 (1H, m)	1.93 (1H, m); 2.02 (1H, m)
3	3.27 (1H, dd, 4, 12.0)	4.58 (br s)	4.16 (1H, s)
5	1.07 (1H, m)	1.84 (1H, m)	1.83 (1H, m)
6	1.92 (1H, m); 2.32 (1H, m)	1.68(1H, m); 1.65 (1H, m)	1.70 (2H, m)
7	1.49 (1H, m); 1.67 (1H, m)	1.32 (1H, m); 1.22 (1H, m)	1.18 (1H, m); 1.29 (1H, m)
9	1.27 (1H, m)	1.03 (1H, m)	1.00 (1H, m)
11	1.15 (1H, m); 1.75 (1H, m)	1.75 (1H, m); 1.23 (1H, m)	1.10 (1H, m); 1.85 (1H, m)
12	1.98 (1H, m); 2.05 (1H, m)	1.82 (1H, m); 1.27 (1H, m)	1.95 (1H, m); 2.05 (1H, m)
13	1.70 (1H, m)	2.52 (1H, m)	2.43 (1H, m)
15	3.62 (1H, t, 8.0)	5.94 (1H, s)	5.91 (1H, s)
16	2.20 (2H, m)	—	—
17	2.08 (1H, m)	3.28 (1H, s)	3.00 (1H, s)
19	1.65 (1H, m); 1.93 (1H, m)	1.63 (1H, m); 2.31 (1H, m)	1.63 (1H, m); 2.29 (1H, m)
20	1.98 (1H, m); 2.05 (1H, m)	1.87 (1H, m); 2.14 (1H, m)	1.99 (2H, m)
21	4.55 (1H, s)	4.44 (1H, br s)	3.57 (1H, s)
23	1.64 (3H, s)	1.59 (3H, s)	1.56 (3H, s)
24	—	4.08, 3.86 (each 1H, d, 10.7)	1.35 (3H, s)
25	1.04 (3H, s)	0.88 (3H, s)	0.85 (3H, s)
26	0.98 (3H, s)	0.72 (3H, s)	0.69 (3H, s)
27	1.78 (1H, m); 2.06 (1H, m)	2.31 (1H, m); 1.85 (1H, m)	1.83 (1H, m), 2.27 (1H, m)
28	1.36 (3H, s)	0.88 (3H, s)	0.81 (3H, s)
29	4.20, 3.95 (each 1H, d, 10.8)	4.80, 4.12 (each 1H, d, 10.7)	1.29 (3H, s)
30	1.56 (3H, s)	2.00 (3H, s)	4.28, 4.58 (each 1H, d, 11.2)
2′, 6′	7.12 (1H, d, 8.5)	—	—
3′, 5′	8.42 (1H, d, 8.5)	—	—

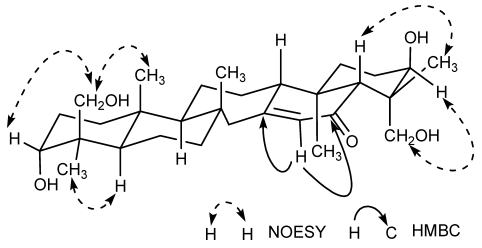

Fig. 4. Selected NOESY and HMBC Correlations of **6**

hydroxy group at C-21 and the hydroxymethyl group at C-22, the chemical shift C-21 (δ_C 75.2) was shifted downfield 4.9 ppm relative to that in lycernuic ketone A.[4] All evidence of chemical shift value change further confirmed that the hydroxymethyl was at C-30, rather than at C-29. A new natural product termed 3α,21β,30-trihydroxyserrat-14-en-16-one (**7**) was unambiguously assigned using HMQC, HMBC and ROESY correlations.

In addition, twelve known serratene triterpenoids were identified as inundosie-E (**8**),[9] 3α,21β,24-trihydroxyserrat-14-ene (**9**),[10] 3β,21β-dihydroxyserrat-14-ene (**10**),[11] 3β,21β,29-trihydroxyserrat-14-ene (**11**),[6] 3β,21β-dihydroxyserrat-14-en-24-oic acid (**12**),[4] lycernuic acid B (**13**),[4] 3β,21β,24,29-tetrahydroxyserrat-14-ene (**14**),[7] lycernuic acid C (**15**),[4] lycernuic acid D (**16**),[4] methyl 3α,21β,29-trihydroxy-16-oxoserrat-14-en-24-carboxylate (**17**) (lycernuic ketone B),[4] 3α,21β,24-trihydroxy-serrat-14-en-16-one (**18**),[4] and α-onocerin (**19**),[12] respectively, on the basis of comparison of their spectroscopic data with those in the literature.

Experimental

General Column chromatography (CC): Silica gel (200—300 mesh, Qingdao Marine Chemical, China); Lichroprep RP-18 (40—63 μm, Merck, Darmstadt, German); and Sephadex LH-20 (Pharmacia Fine Chemicals Co., Ltd.). All melting points: X-4 apparatus, uncorrected. Optical rotations: Horiba SEAP-300 spectropolarimeter. IR spectra: Shimadzu IR-450 instrument, in cm^{-1}, KBr pellets. FAB-MS and HR-FAB-MS: VG-AUTOSPEC-3000 spectrometer, in *m/z* (rel. int. in % of the base peak). NMR spectra: a Bruker AV-400 or DRX-500 instrument, chemical shifts (δ) in ppm, TMS as the internal standard, *J* in Hz; Fractions were monitored by TLC, and spots were visualized by heating TLC sprayed with 10% H_2SO_4.

Plant Material *Palhinhaea cernua* var. *sikkimensis* was collected in HuanJiang County, GuangXi Province in April, 2005. It was identified by Prof. Sugong Wu, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 200504012) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation The powdered material (5.0 kg) was exhaustively extracted with 90% EtOH under reflux, and concentrated *in vacuo* to give a crude extract. The alcoholic extract was dissolved in MeOH/H_2O (1 : 9, 2500 ml), and then partitioned with EtOAc (1500 ml×4) to give an EtOAc-soluble fraction (400 g). The EtOAc extract was absorbed on 400 g silica gel, and was chromatographed on CC (1600 g) eluting with $CHCl_3$: MeOH (100 : 0, 80 : 1, 60 : 1, 40 : 1, 10 : 1, 5 : 1) to afford four fractions (Fr.). Fr. 1 was not further researched. Fr. 2 was subjected to CC eluted by a gradient solvent system of increasing polarity of $CHCl_3$: acetone from 30 : 1 to 20 : 1 to get compound **6** (10 mg). Fr. 3 was chromatographed repeatedly on CC (silica gel, $CHCl_3$: MeOH 10 : 1) to yield compound **7**. Fr. 4 was repeatedly chromatographed using a combination of silica gel, RP_{18} column and Sephadex LH-20 to obtain compounds **1**—**5** as amorphous powder. During the process of separation of compounds **1**—**7**, twelve known compounds were also isolated as well.

3α,21β,29-Trihydroxy-16-oxoserrat-14-en-24-oic Acid (**1**): Colourless powder; mp 321—323 °C; $[\alpha]_D^{23}$ 7.0 (*c*=0.8, MeOH); IR (KBr) ν_{max}: 3410, 2945, 1560, 1457, 1250, 1160 cm^{-1}; Negative FAB-MS *m/z* 501 $[M-H]^-$; negative HR-FAB-MS *m/z* 501.3227 $[M-H]^-$ (Calcd $C_{30}H_{45}O_6$ for 501.3216); ^{13}C- and ^{1}H-NMR spectroscopic data see Tables 1 and 2.

3β,21β,29-Trihydroxy-16-oxoserrat-14-en-24-oic Acid (**2**): Colourless powder; mp 309—311 °C; $[\alpha]_D^{23}$ 10.0 (*c*=1.1, MeOH); IR (KBr) ν_{max}: 3405 (OH), 2950, 1570, 1450, 1250, 1168 cm^{-1}; negative FAB-MS *m/z* 501 $[M-H]^-$; negative HR-FAB-MS *m/z* 501.3227 $[M-H]^-$ (Calcd $C_{30}H_{45}O_6$ for 501.3216); ^{13}C- and ^{1}H-NMR spectroscopic data see Tables 1 and 2.

3β,21β,29-Trihydroxyserrat-14-en-24-oic Acid 3β-(4-Hydroxybenzoate) (**3**): Colourless powder; mp 335—337 °C; $[\alpha]_D^{23}$ 4.8 (*c*=1.2, MeOH); IR (KBr) ν_{max}: 3567 (OH), 2970, 1724, 1620, 1590, 1517, 1385, 1230, 1120, 1025, 948, 820, 690 cm^{-1}; negative FAB-MS *m/z* 607 $[M-H]^-$ (100), 487 (15); negative HR-FAB-MS *m/z* 607.3639 $[M-H]^-$ (5) (Calcd $C_{37}H_{51}O_7$ for 607.3634); ^{13}C- and ^{1}H-NMR spectroscopic data see Tables 1 and 2.

3β,21β,29-Trihydroxyserrat-14-en-24-oic Acid 3β-(4-Hydroxy-3-methoxybenzoate) (**4**): Colourless powder; mp 345—347 °C; $[\alpha]_D^{23}$ 4.0 (*c*=1.0, MeOH); IR (KBr) ν_{max}: 3430 (OH), 2965, 1720, 1652, 1631, 1590, 1515, 1385, 1230, 1120, 1025, 948, 820, 690 cm^{-1}; negative FAB-MS *m/z* 637 $[M-H]^-$ (100), 487 (10); negative HR-FAB-MS *m/z* 637.3729 $[M-H]^-$ (11) (Calcd $C_{38}H_{53}O_8$ for 637.3740); ^{13}C- and ^{1}H-NMR spectroscopic data see Tables 1 and 2.

3β,14α,15α,21β,29-Pentahydroxyserrat-14-en-24-oic Acid 3β-(4-Hydroxybenzoate) (**5**): Colourless powder; mp 330—332 °C; $[\alpha]_D^{23}$ 2.0 (*c*=0.77, MeOH); IR (KBr) ν_{max}: 3440 (OH), 2967, 1715, 1652, 1631, 1600, 1510, 1387, 1231, 1190, 1033, 948, 820, 690 cm^{-1}; negative FAB-MS *m/z* 641 $[M-H]^-$ (100); negative HR-FAB-MS *m/z* 641.3685 $[M-H]^-$ (Calcd $C_{37}H_{53}O_9$ for 641.3689); ^{13}C- and ^{1}H-NMR spectroscopic data see Tables 1 and 3.

3α,21β,24,29-Tetrahydroxyserrat-14-en-16-one (**6**): Colourless powder; mp 307—309 °C; $[\alpha]_D^{23}$ −24.0 (*c*=0.7, CH_3OH); IR (KBr) ν_{max} 3440, 2920, 1660, 1450, 1250, 1168, 1025 cm^{-1}; negative FAB-MS *m/z* 487 $[M-H]^-$ (6); negative HR-FAB-MS *m/z* 487.3440 $[M-H]^-$ (3.5) (Calcd $C_{30}H_{47}O_5$ for 487.3423); ^{1}H- and ^{13}C-NMR see Tables 1 and 3.

3α,21β,30-Trihydroxyserrat-14-en-16-one (**7**): Colourless powder; mp 245—247 °C; $[\alpha]_D^{23}$ −12.1 (*c*=0.91, CH_3OH); IR (KBr) ν_{max} 3420, 2930, 1580, 1445, 1250, 1168, 1030 cm^{-1}; negative FAB-MS *m/z* 471 $[M-H]^-$ (11); negative HR-FAB-MS *m/z* 471.3490 $[M-H]^-$ (2.5) (Calcd $C_{30}H_{47}O_4$ for 471.3474); ^{1}H- and ^{13}C-NMR see Tables 1 and 3.

Acknowledgements This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-G-038).

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