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Polyhydroxyserratane triterpenoids from *Diphasiastrum complanatum*

Jian Yan [a,b], Ping Yi [a,b], Baohui Chen [a,b], Lu Lu [a,b], Zhongrong Li [a], Xianmin Zhang [a], Lin Zhou [a], Minghua Qiu [a,b,*]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, PR China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China*

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Abstract

Serratane triterpenoids were identified from *Diphasiastrum complanatum* (L.) Holub, including serratane-3α,14α,15α,20β,21β,24,29-heptol (**1**), 3α,20β,21β-trihydroxyserrat-14-en-24-oic acid (**2**), 3β,20β,21β-trihydroxyserrat-14-en-24-oic acid (**3**), 3α,20β,21β-trihydroxy-16-oxoserrat-14-en-24-oic acid (**4**), and 16-oxolyclanitin-29-yl E-4′-hydroxyl-3′-methoxycinnamate (**5**) on the basis of their spectroscopic data as well as nine known analogs.

Keywords: *Diphasiastrum complanatum*; Lycopodiaceae; Serratane triterpenoid; Polyhydroxylated derivatives

1. Introduction

Serratane triterpenoids were first isolated from *Lycopodium clavatum* L. growing in Japan by Inubushi Y. et al. in 1962. *Lycopodium* species contain lycopodium alkaloids (Ma and Gang, 2004) and serratane triterpenoids, some of which possess pharmacological activity (Liu et al., 1986a,b; Tanaka et al., 2003; Houghton et al., 2006). We have previously investigated *Lycopodium japonicum* (Yan et al., 2005a,b) and *Phlegmariurus squarrosus*. *Diphasiastrum complanatum* (L.) is distributed in Sichuang, Yunnan, Guizhou, Tibet Provinces and is used as a traditional Chinese herbal medicine for treatment of arthritic pain, quadriplegia, and contusion (Jiangsu, 1990). In our search for bioactive metabolites, we investigated the chemical constituents of *D. complanatum* (L.) Holub. We found five new polyhydroxy-derivatives: serratane-3α,14α,15α,20β,21β,24,29-heptol (**1**), 3α,20β,21β-trihydroxyserrat-14-en-24-oic acid (**2**), 3β,20β,21β-trihydroxyserrat-14-en-24-oic acid (**3**), 3α,20β,21β-trihydroxy-16-oxoserrat-14-en-24-oic acid (**4**), 16-oxolyclanitin-29-yl E-4′-hydroxyl-3′-methoxycinnamate (**5**), together with nine known compounds, lycoclavanol (**6**), serratenediol (**7**), 21-*epi*-serratenediol (**8**), lycoclaninol (**9**) (Haruo et al., 1988), wightianol B (**10**) (Tsuda and Tabata, 1980), lycernuic acid A (**11**) (Zhang et al., 2002), 16-oxolyclanitin-29-yl *p*-coumarate (**12**) (Cai and Pan, 1992), lycoclavanin (**13**) (Tsuda et al., 1975), and α-onocerin (**14**) (Cai et al., 1989; Pauli, 2000). In this paper, we wish to report the isolation and structure elucidation of five new compounds. Fig. 1

2. Results and discussion

The molecular formula of **1**, $C_{30}H_{52}O_7$, was established by negative ESI *m/z* 523 $[M-H]^-$, and was further confirmed by negative HRESIMS *m/z* 523.3621 (calcd. for $C_{30}H_{51}O_7$, 523.3634). Analysis of ^1H and ^{13}C NMR spectra indicated that **1** was a serratane derivative (Tables 1 and 2). In the ^1H and ^{13}C NMR spectra of **1**, the expected proton

* Corresponding author. Address: State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, PR China. Tel.: +86 871 5223327; fax: +86 871 5223255.
E-mail address: mhchiu@mail.kib.ac.cn (M.H. Qiu).

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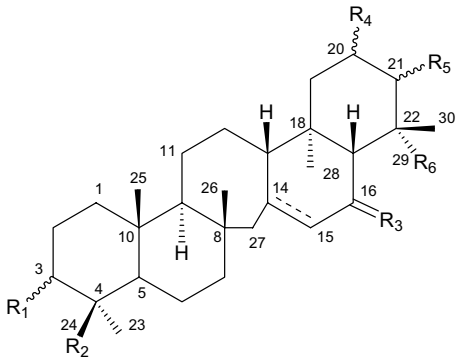

	R_1	R_2	R_3	R_4	R_5	R_6	
1	αOH	CH_2OH	H_2	βOH	βOH	CH_2OH	14α-OH 15α-OH
2	αOH	COOH	H_2	βOH	βOH	Me	$\Delta^{14,15}$
3	βOH	COOH	H_2	βOH	βOH	Me	$\Delta^{14,15}$
4	αOH	COOH	O	βOH	βOH	Me	$\Delta^{14,15}$
5	αOH	CH_2OH	O	βOH	βOH	-CH_2-O-(4′-hydroxyl-3′-methoxyl)-*E*-cinnamate	$\Delta^{14,15}$
6	αOH	CH_2OH	H_2	H	βOH	Me	$\Delta^{14,15}$
7	βOH	Me	H_2	H	αOH	Me	$\Delta^{14,15}$
8	βOH	Me	H_2	H	βOH	Me	$\Delta^{14,15}$
9	αOH	CH_2OH	H_2	βOH	βOH	Me	$\Delta^{14,15}$
10	βOH	CH_2OH	H_2	βOH	βOH	Me	$\Delta^{14,15}$
11	βOH	COOH	H_2	H	βOH	Me	$\Delta^{14,15}$
12	αOH	CH_2OH	O	βOH	βOH	-CH_2-O-*p* -coumatate	$\Delta^{14,15}$
13	αOH	CH_2OH	O	βOH	βOH	Me	$\Delta^{14,15}$

Fig. 1. Compounds 1–13 isolated from *D. complanatum* collected at Jinggu.

Table 1
^{13}C NMR spectroscopic data of **1**–**5** (pyridine-d_5, 100 MHz, δ in ppm)

Carbon	**1**	**2**	**3**	**4**	**5**[a]
1	34.2 (*t*)	34.8 (*t*)	39.7 (*t*)	34.8 (*t*)	34.1 (*t*)
2	26.7 (*t*)	27.9 (*t*)	29.5 (*t*)	27.9 (*t*)	26.7 (*t*)
3	70.2 (*d*)	70.6 (*d*)	78.3 (*d*)	70.5 (*d*)	69.9 (*d*)
4	44.2 (*s*)	48.6 (*s*)	49.4 (*s*)	48.6 (*s*)	44.2 (*s*)
5	50.3 (*d*)	49.6 (*d*)	56.6 (*d*)	49.4 (*d*)	50.1 (*d*)
6	19.6 (*t*)	21.7 (*t*)	21.3 (*t*)	20.2 (*t*)	19.5 (*t*)
7	45.3 (*t*)	45.8 (*t*)	45.7 (*t*)	45.9 (*t*)	45.8 (*t*)
8	38.2 (*s*)	37.6 (*s*)	37.7 (*s*)	38.1 (*s*)	38.2 (*s*)
9	59.6 (*d*)	62.6 (*d*)	62.6 (*d*)	62.2 (*d*)	62.6 (*d*)
10	38.7 (*s*)	39.4 (*s*)	39.7 (*s*)	39.3 (*s*)	38.6 (*s*)
11	25.8 (*t*)	25.8 (*t*)	25.8 (*t*)	25.4 (*t*)	26.7 (*t*)
12	26.7 (*t*)	27.6 (*t*)	27.7 (*t*)	26.8 (*t*)	25.4 (*t*)
13	59.9 (*d*)	57.6 (*d*)	57.7 (*d*)	59.1 (*d*)	59.3 (*d*)
14	77.9 (*s*)	139.0 (*s*)	138.8 (*s*)	163.7 (*s*)	164.6 (*s*)
15	76.8 (*d*)	122.8 (*d*)	122.9 (*d*)	129.3 (*d*)	128.9 (*d*)
16	28.2 (*t*)	24.4 (*t*)	24.4 (*t*)	200.9 (*s*)	200.5 (*s*)
17	46.5 (*d*)	41.3 (*d*)	43.2 (*d*)	58.9 (*d*)	59.2 (*d*)
18	40.2 (*t*)	37.8 (*t*)	37.7 (*t*)	45.5 (*t*)	45.8 (*t*)
19	43.1 (*t*)	43.3 (*t*)	41.3 (*t*)	41.3 (*t*)	40.9 (*t*)
20	66.5 (*d*)	66.6 (*d*)	66.4 (*d*)	65.9 (*d*)	65.7 (*d*)
21	74.3 (*d*)	79.6 (*d*)	79.5 (*d*)	80.4 (*d*)	74.3 (*d*)
22	45.1 (*s*)	38.9 (*s*)	38.8 (*s*)	38.8 (*s*)	43.5 (*s*)
23	23.4 (*q*)	25.5 (*q*)	24.8 (*q*)	25.5 (*q*)	23.6 (*q*)
24	65.8 (*t*)	180.7 (*s*)	180.7 (*s*)	180.5 (*s*)	65.7 (*t*)
25	17.2 (*q*)	14.2 (*q*)	14.3 (*q*)	14.1 (*q*)	16.6 (*q*)
26	23.3 (*q*)	20.0 (*q*)	19.7 (*q*)	19.8 (*q*)	20.0 (*q*)
27	54.7 (*t*)	56.9 (*t*)	56.6 (*t*)	56.1 (*t*)	55.9 (*t*)
28	18.1 (*q*)	14.7 (*q*)	14.7 (*q*)	16.1 (*q*)	16.7 (*q*)
29	65.8 (*t*)	21.7 (*q*)	21.3 (*q*)	21.7 (*q*)	65.7 (*t*)
30	23.6 (*q*)	28.7 (*q*)	28.7 (*q*)	29.0 (*q*)	23.7 (*q*)

[a] The ^{13}C NMR data for the ester moiety of **5**: δ 167.7 (*s*, C-9′), 115.3 (*d*, C-8′), 145.6 (*d*, C-7′), 126.5 (*s*, C-1′), 111.5 (*d*, C-2′), 149.0 (*s*, C-3′), 150.8 (*s*, C-4′), 116.9 (*d*, C-5′), 123.3 (*d*, C-6′), 55.9 (*q*, 3′-OCH_3).

and carbon signals for a typical serrate-14-ene double bond were absent and were replaced by two hydroxylated carbons [δ_C 77.9 (C-14) and 76.8 (CH, C-15); δ_H 3.65 *dd* (H-15)]. The α orientation of these two hydroxyl groups was assigned by comparing their carbon shifts with those of lycernuic acid C (Zhang et al., 2002). Two methyl signals of **1** [δ_C 23.4 (C-23) and 23.6 (C-30) were shifted upfield by 5–6 ppm relative to serratenediol (**7**) and 21-*epi*-serratenediol (**8**) indicating that two CH_2OH groups were located at C-24 and C-29. In the ROESY, strong correlations of H-24 with Me-25 and Me-28 with H-29 confirmed the positions of the CH_2OH groups. The broad resonances of H-3 and H-21 suggested that they were β and α positions, respectively. The hydroxyl group at C-20 caused a downfield shift of C-19 to 43.1 ppm. In the ROESY spectrum, the correlations of H-20 with H-29 and Me-28 showed that the C-20 hydroxyl group was β position. Thus the structure of diphasiastrol (**1**) is serratane-3α,14α,15α,20β,21β,24,29-heptol.

Compound (**2**) was assigned a molecular formula of $C_{30}H_{48}O_5$ as deduced from the negative HRFABMS (*m/z* 487.3403 $[M-H]^-$, calcd. for $C_{30}H_{47}O_5$, 487.3423), in conjunction with the proton and carbon NMR data. The ^{13}C NMR spectrum of **2** showed characteristic double bonds carbons at δ_C 139.0 (C-14) and 122.8 (C-15), 3 oxymethines at δ_C 70.6 (C-3), 79.6 (C-21), 66.6 (C-20), 6 methyls, 9 methylenes, 4 methines, 5 quaternary carbons, a carboxyl group. Analysis of the ^1H and ^{13}C NMR spectra indicated that **2** was related to 3α,21α-dihydroxyserrat-14-en-24-oic acid (Zhou et al., 2003) apart from ring E. The single peak at δ_H 3.82 (H-21) indicated that the C-21 OH was β orientation (Wang and Lou, 2005). The chemical shift differences of C-19 (+Δ11.1 ppm) and C-21 (+Δ4.0 ppm) relative to 3β,21β-dihydroxyserrat-14-en-24-oic acid indicated that there was a hydroxyl group attached to C-20. In the ROESY experiment, correlations of H-20α with H-29, and H-20α with H-21α confirmed that the C-20 OH was β orientated. On the basis of the above evidence, the chemical structure of **2** was assigned as 3α, 20β, 21β-trihydroxyserrat-14-en-24-oic acid.

Compound (**3**) had the same molecular formula as **2**, $C_{30}H_{48}O_5$, established by negative HRFABMS. After carefully analysing ^1H NMR and ^{13}C NMR spectra of **3** with those of **2**, we concluded that **3** differed from **2** only in the configuration of the hydroxyl group at C-3. The C-3 OH of **3** was assigned a β-orientation on the basis of its characteristic proton resonance at δ_H 3.40 (1H, *dd*, $J_1 = 11$ Hz, $J_2 = 4$ Hz, H-3α) (Fang et al., 1991). The significant chemical shift value differences of C1–C5 between

Table 2
^{1}H NMR spectroscopic data of **1**–**5** (pyridine-d_5, 400 MHz, J in Hz, δ in ppm)

H	1	2	3	4	5[a]
1	1.71 (*m*)/1.86 (*m*)	1.75 (*m*)/1.82 (*m*)	2.01 (*m*)/2.17 (*m*)	1.70 (*m*)/1.80 (*m*)	1.59 (*m*)/1.82 (*m*)
2	1.88 (*m*)/2.19 (*m*)	2.03 (*m*)/2.75 (*m*)	2.00 (*m*)/2.50 (*m*)	2.03 (*m*)/2.72 (*m*)	1.94 (*m*)/2.11 (*m*)
3	4.41 (*br.s*)	4.68 (*br s*)	3.40 (*dd*) 4.0, 11.0	4.69 (*br.s*)	4.40 (*br.s*)
5	1.90 (*m*)	2.01 (*m*)	1.02 (*m*)	2.05 (*m*)	1.88 (*m*)
6	1.59 (*m*)/1.80 (*m*)	1.98 (*m*)/2.44 (*m*)	1.98 (*m*)/2.44 (*m*)	1.93 (*m*)/2.42 (*m*)	1.60 (*m*)
7	1.52 (*m*)/1.60 (*m*)	1.30 (*m*)/1.50 (*m*)	1.30 (*m*)/1.50 (*m*)	1.29 (*t*) 12.0/1.47 (*m*)	1.26 (*m*)/1.37 (*m*)
9	1.39 (*m*)	1.05 (*m*)	1.05 (*m*)	1.05 (*m*)	1.04 (*m*)
11	1.70 (*m*)/1.78 (*m*)	1.73 (*m*)/1.86 (*m*)	1.73 (*m*)/1.86 (*m*)	1.72 (*m*)/1.82 (*m*)	1.08 (*m*)/2.00 (*m*)
12	1.05 (*m*)/2.05 (*m*)	1.10 (*m*)/2.01 (*m*)	1.05 (*m*)/2.02 (*m*)	1.07 (*m*)/2.02 (*m*)	1.08 (*m*)/1.83 (*m*)
13	1.75 (*m*)	2.08 (*m*)	2.08 (*m*)	2.54 (*m*)	2.58 (*d*), 9.2
15	3.65 (*dd*) 3.85, 9.50	5.45 (*br.s*)	5.45 (*br.s*)	5.94 (*s*)	5.95 (*s*)
16	2.18 (*m*)	1.99 (*m*)/2.11 (*m*)	1.99 (*m*)/2.11 (*m*)		
17	2.00 (*m*)	2.10 (*m*)	2.10 (*m*)	2.99 (*s*)	3.25 (*s*)
19	1.95 (*m*)/2.16 (*m*)	1.97 (*m*)/2.07 (*m*)	1.95 (*m*)/2.10 (*m*)	2.07 (*m*)/2.30 (*m*)	2.11 (*d*) 12.5/2.44 (*t*) 12.5
20	4.56 (*d*) 8.9	4.37 (*d*) 8.8	4.40 (*d*) 8.8	4.39 (*d*) 10.3	4.66 (*d*) 10.5
21	4.64 (*br.s*)	3.82 (*br.s*)	3.85 (*br.s*)	3.73 (*br.s*)	4.44 (*br.s*)
23	1.60 (*s*)	1.86 (*s*)	1.86 (*s*)	1.70 (*s*)	1.62 (*s*)
24	3.84 (*d*) 10.8/4.06 (*d*) 10.8				3.88 (*d*) 11.0/4.09 (*d*) 11.0
25	0.92 (*s*)	1.12 (*s*)	1.12 (*s*)	1.05 (*s*)	0.84 (*s*)
26	0.97 (*s*)	0.95 (*s*)	0.96 (*s*)	0.82 (*s*)	0.67 (*s*)
27	1.90 (*m*)	1.92 (*m*)/2.28 (*m*)	1.90 (*m*)/2.30 (*m*)	1.92 (*m*)/2.37 (*m*)	1.90 (*m*)/2.32 (*m*)
28	1.34 (*s*)	0.92 (*s*)	0.92 (*s*)	0.94 (*s*)	0.90 (*s*)
29	3.91 (*d*) 10.9/4.23 (*d*) 10.9	0.84 (*s*)	0.84 (*s*)	1.43 (*s*)	5.35 (*d*) 11.0/4.95 (*d*) 11.0
30	1.68 (*s*)	1.19 (*s*)	1.19 (*s*)	1.73 (*s*)	2.08 (*s*)

[a] The ^{1}H NMR data for the ester moiety of **5**: δ 6.64 (1H, *d*, $J = 15.0$, H-8′), 7.95 (1H, *d*, $J = 15.0$, H-7′), 7.35 (1H, *d*, $J = 1.8$, H-2′), 7.17 (1H, *d*, $J = 8.0$, H-5′), 7.28 (1H, *dd*, $J_1 = 8.0$, $J_2 = 1.8$, H-6′), 3.75 (3H, *s*, 3′-OCH_3).

3 and **2** further supported the β-orientation of the C-3 OH in **3** (Table 1). Thus the structure of **3** is 3β,20β,21β-trihydroxyserrat-14-en-24-oic acid.

Compound (**4**) had the molecular formula $C_{30}H_{46}O_6$, established by negative HRFABMS. The ^{13}C NMR spectrum exhibited 30 carbon signals, a ketone group at δ_C 200.9 (C-16), a carboxyl group δ_C 180.5 (C-24), a double bond δ_C 163.7 (C-14) and δ_C 129.3 (C-15), three oxymethines at δ_C 70.5 (C-3), δ_C 65.9 (C-20), δ_C 80.4 (C-21), six methyls, four methines, five quaternary carbons, eight methylenes. Comparison of **4** with **2** showed that they were much similar but differed in the numbers of methylene. There were 9 methylenes in **2**, and 8 methylenes and a ketone group in **4**, which suggested that one of the methylenes in **2** was oxidized to a ketone group. The position of the ketone group at C-16 was established by the HMBC spectrum and chemical shift changes of C-14 and C-15 (Table 1). Thus compound **4** is unambiguously determined as 3α,20β,21β-trihydroxy-16-oxoserrat-14-en-24-oic acid.

Compound (**5**) was deduced as $C_{40}H_{56}O_9$ from NMR spectra and confirmed by negative HRFABMS. The ^{1}H and ^{13}C NMR spectra of **5** were almost identical with those of 16-oxolyclanitin-29-yl *p*-coumarate (**12**) except for the presence of a 1,3,4-trisubstituted aromatic ring at δ_H 7.35 (1H, *d*, $J = 1.8$, H-2′), 7.17 (1H, *d*, $J = 8$ Hz, H-5′), 7.28 (1H, *dd*, $J_1 = 8$ Hz, $J_2 = 1.8$ Hz, H-6′) (Siddiqui et al., 1997; Zhu et al., 2002) in place of the *p*-coumaroyl moiety of **12**. The structure was also supported by ion fragments at *m/z* 503 $[M-H-C_{10}H_9O_3]^-$ (10), 193 $[C_{10}H_9O_4]^+$ (75) in the negative FABMS. In the HMBC spectrum, correlations between δ_H 5.35 (1H, *d*, $J = 11.0$ Hz, H-29), 4.95 (1H, *d*, $J = 11.0$ Hz, H-29) and δ_C 43.5 (C-22), 74.3 (C-21), and 167.7 (C-9′) indicated the attachment of the (4′-hydroxyl-3′-methoxyl)-*E*-cinnamate group at C-29. Moreover, HMBC correlations of δ_C 149.0 (C-3′) with 3.75 (3H, *s*, OCH_3-3′) suggested that the methoxyl group was at C-3′ (Siddiqui et al., 1997; Zhu et al., 2002). Hence, **5** was formulated as 16-oxolyclanitin-29-yl E-4′-hydroxyl-3′-methoxycinnamate (**5**) Fig. 2.

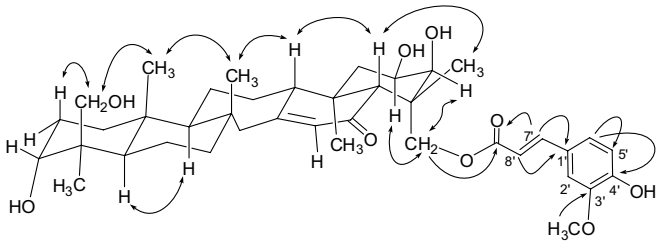

Fig. 2. The key ROESY (↔) and HMBC (→) correlations of **5**.

3. Conclusion

The structures of five new and nine known serratane triterpenoids from *D. complanatum* were determined by spectroscopic analysis and comparison with literature data. The heptol (**1**), diphasiastrol, is the most highly hydroxylated serratane derivative reported thus far.

4. Experimental

4.1. General experimental procedures

Silica gel (200–300 mesh, Qingdao Marine Chemical, China), Lichroprep RP-18 (40–63 μm, Merck, Darmstadt, German) and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.) were used for column chromatography (CC). Fractions were monitored by TLC, and spots were visualized by heating TLC sprayed with 10% H_2SO_4.

4.2. Plant material

D. complanatum (L.) Holub. was collected at Jinggu county, Yunnan province, located at 1110 m elevation. A voucher specimen (No. 20041022) has been deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

4.3. Extraction and isolation

The powdered material (8.0 kg) was exhaustively extracted with MeOH–H_2O (9:1, v/v, 30 L, 3 h, 3 h, 4 h) under reflux, and the methanol extract was combined and evaporated to dryness (750 g). The latter was dissolved in MeOH/H_2O (1:9, 3 L) and then partitioned with EtOAc (1.5 L× 4) to give an EtOAc-soluble fraction (250 g). Part of the EtOAc extract was absorbed on silica gel (300 g) and was fractionated by CC (1500 g) eluting with $CHCl_3$:MeOH (100:0, 80:1, 60:1, 40:1, 10:1, 5:1) to afford four fractions (*Fr.*): 1 (30 g), 2 (80 g), 3 (50 g), 4 (30 g).

*Fr.*1 was further submitted to CC (silica gel, $CHCl_3$:MeOH 100:1, 80:1) to give **14** (2 g) and **10** (100 mg). *Fr.* 2 (80 g) was subjected to CC (silica gel, $CHCl_3$:MeOH 40:1, 20:1) and gave two new fractions and **6** (the main constituent). *Fr.* 2.1 was further purified by repeated CC (silica gel, $CHCl_3$:MeOH 100:0, 50:1, 30:1) to yield **7** (50 mg) and **8** (20 mg). Repeated CC of *Fr.* 2.2 gave **4** (50 mg), **9** (30 mg) and **11** (15 mg). Similarly *Fr.* 3 (silica gel, $CHCl_3$:MeOH 40:1) gave **3** (10 mg) and **5** (15 mg) and a residual fraction which was purified by HPLC (MeOH:H_2O 60:40) to obtain **2** (100 mg). Repeated CC of *Fr.* 4 eluting with $CHCl_3$:MeOH (25:1, 20:1, 15:1) gave 2 sub-fractions and **13** (200 mg). *Fr.* 4.1, on CC as above with $CHCl_3$:MeOH (20:1) as eluant, yielded **12** (30 mg) and **1** (20 mg), which was purified by Sephadex LH-20.

4.3.1. Serratane-3α,14α,15α,20β,21β,24,29-heptol (*1*)

White powder; m.p > 350 °C; $[\alpha]_D^{23}$ −0.64 (MeOH; *c* 1.12); IR (KBr) ν_{max}:3396 (OH), 2935, 1457, 1389, 1250, 1161, 1038, 982, 672 cm^{-1}; for ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2; Negative ESIMS *m/z* (rel. int.): 523 (20), 339 (90), 325 (100), 311 (50); negative HRESIMS: 523.3621 $[M-H]^-$ (calcd. for $C_{30}H_{51}O_7$, 523.3634).

4.3.2. 3α, 20β, 21β-trihydroxyserrat-14-en-24-oic acid (*2*)

White powder; m.p. 271–273 °C;$[\alpha]_D^{23}$ −15.9 (MeOH; *c* 1.7); IR (KBr) ν_{max}:3477 (OH), 2969, 2873, 1690 (COOH), 1463, 1387, 1195, 946, 737 cm^{-1}; for ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS *m/z* (rel. int.): 487 $[M-H]^-$ (100); Negative HRFABMS *m/z* 487.3403 $[M-H]^-$ (29) (calcd. for $C_{30}H_{47}O_5$, 487.3423).

4.3.3. 3β, 20β, 21β-trihydroxyserrat-14-en-24-oic acid (*3*)

White powder; m.p. 258–260 °C; $[\alpha]_D^{23}$ −10.7 (MeOH; *c* 0.81); IR (KBr) ν_{max}: 3477 (OH), 2951, 2873, 1690 (COOH), 1463, 1387, 1195, 946, 737 cm^{-1}; for ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS *m/z* (rel. int.): 487 $[M-H]^-$ (100); Negative HRESIMS *m/z* 487.3408 $[M-H]^-$ (calcd. for C30H47O5, 487.3423).

4.3.4. 3α, 20β, 21β-trihydroxy-16-oxoserrat-14-en-24-oic acid (*4*)

White powder; m.p. 276–278 °C; $[\alpha]_D^{23}$ −22.1 (MeOH; *c* 1.12); IR (KBr) ν_{max}: 3444 (OH), 2968, 1710, 1652, 1387, 1231, 1190, 1033, 948, 880, 691 cm^{-1}; for ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS *m/z* (rel. int.): 501 $[M-H]^-$ (15); 339 (100), 325 (60); Negative HRESIMS *m/z* 501.3227 $[M-H]^-$ (24) (calcd. for $C_{30}H_{45}O_6$, 501.3216).

4.3.5. 16-oxolyclanitin-29-yl E-4′-hydroxyl-3′-methoxycinnamate (*5*)

White powder; m.p. 294–296 °C; $[\alpha]_D^{23}$ 3.22 (MeOH; *c* 0.77); IR (KBr) ν_{max}: 3440 (OH), 2967, 1710, 1652, 1631 1596, 1513, 1387, 1231, 1190, 1033, 948, 880, 849, 817, 691 cm^{-1}; for ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS *m/z* (rel. int.): 679 $[M-H]^-$(100), 589 (25), 339 (100), 193 (70); Negative HRFABMS *m/z* 679.3843 $[M-H]^-$ (calcd. for $C_{40}H_{55}O_9$, 679.3846).

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