Serratene-Type Triterpenoids from *Huperzia serrata*

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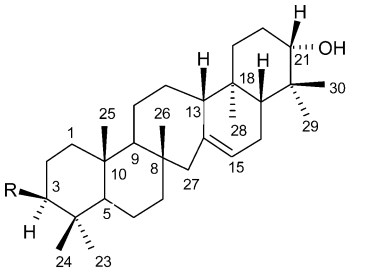
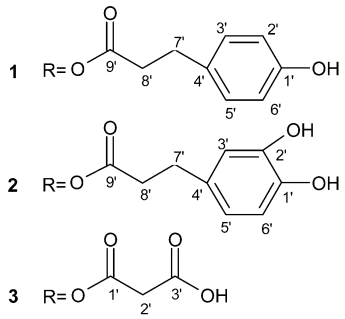
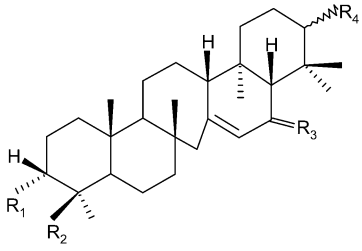
Seven new and 15 known serratene-type triterpenoids were isolated from the whole plant of *Huperzia serrata*. The structures of these new triterpenoids were elucidated as 21α-hydroxyserrat-14-en-3*β*-yl *p*-dihydrocoumarate (**1**), 21α-hydroxyserrat-14-en-3*β*-yl dihydrocaffeate (**2**), 21α-hydroxyserrat-14-en-3*β*-yl propanedioic acid monoester (**3**), 3α,21α-dihydroxyserrat-14-en-24-oic acid (**4**), 16-oxo-3α,21*β*-dihydroxyserrat-14-en-24-al (**5**), 16-oxo-3α,21*β*-dihydroxyserrat-14-en-24-oic acid (**6**), and 16-oxo-21*β*-hydroxyserrat-14-en-3α-yl acetate (**7**), respectively, by means of spectroscopic analysis.

Huperzia serrata (Thunb.) Trev. (Huperziaceae, also named *Lycopodium serratum*)[1] is one of the most commonly encountered traditional Chinese herbal medicines for the treatment of contusions, strains, swelling, and schizophrenia.[2] The discovery of huperzine A, a potent acetylcholinesterase (AChE) inhibitor,[3,4] prompted us to reinvestigate the chemical constituents of this plant (25 kg of dry whole plants). Recently, we have reported some new *Lycopodium* alkaloids obtained from the basic fraction of *H. serrata*.[5] Further work on the nonalkaloidal fraction has led to the isolation of seven new serratene-type triterpenoids, 21α-hydroxyserrat-14-en-3*β*-yl *p*-dihydrocoumarate (**1**), 21α-hydroxyserrat-14-en-3*β*-yl dihydrocaffeate (**2**), 21α-hydroxyserrat-14-en-3*β*-yl propanedioic acid monoester (**3**), 3α,21α-dihydroxyserrat-14-en-24-oic acid (**4**), 16-oxo-3α,21*β*-dihydroxyserrat-14-en-24-al (**5**), 16-oxo-3α,21*β*-dihydroxyserrat-14-en-24-oic acid (**6**), and 16-oxo-21*β*-hydroxyserrat-14-en-3α-yl acetate (**7**), along with 15 known serratenes. In this paper, we describe the isolation and structure elucidation of compounds **1**−**7**.







4	R_1=OH	R_2=COOH	R_3=H_2	R_4=αOH
5	R_1=OH	R_2=CHO	R_3=O	R_4=βOH
6	R_1=OH	R_2=COOH	R_3=O	R_4=βOH
7	R_1=OAc	R_2=Me	R_3=O	R_4=βOH
8	R_1=OH	R_2=Me	R_3=O	R_4=βOH

Results and Discussion

Serratenes are a group of naturally occurring pentacyclic triterpenoids, which possess seven tertiary methyls and a seven-membered ring C (instead of eight methyls and a six-membered ring C in common pentacyclic triterpenoids), usually with a double bond between C-14 and C-15 and oxygen functionalities at both C-3 and C-21.[6]

Compound **1** was assigned the molecular formula $C_{39}H_{58}O_4$ by HREIMS (found M^+ *m*/*z* 590.4312, calcd 590.4335). The IR bands showed the presence of hydroxyl (3572 cm^{-1}), ester carbonyl (1724 cm^{-1}), and aromatic (1618 and 1517 cm^{-1}) groups. Its ^1H NMR spectrum (Table 1) displayed signals for seven tertiary methyls, two axial protons of an oxygenated methine [*δ* 3.50 (dd, *J* = 9.1, 6.4 Hz, H-21*β*) and 4.70 (dd, *J* = 11.5, 4.7 Hz, H-3α)], an olefinic proton [*δ* 5.47 (br s, H-15)], two methylenes with an A_2B_2 pattern [*δ* 3.06 and 2.78 (each 2H, t, *J* = 7.5 Hz)], and a 1,4-disubstituted aromatic ring [*δ* 7.15 and 7.28 (each 2H, d, *J* = 8.4 Hz)]. The ^{13}C NMR and DEPT spectra displayed 39 carbon signals including an ester carbonyl, three sp^2 quaternary carbons, five sp^2 methines, two oxygenated methines, five quaternary carbons, four methines, 12 methylenes, and seven methyls. The ion fragment peaks at *m*/*z* 425.3791 $[C_{30}H_{49}O]^+$, 165.0552 $[C_9H_9O_3]^+$, and 149.0593 $[C_9H_9O_2]^+$ were also observed in the HREIMS. All the above evidence indicated a serratene-type triterpenoid with a *p*-dihydrocoumarate group as ester substituent.[7]

With the exception of the *p*-dihydrocoumarate group signals, the remaining 30 carbon signals were similar to those of 21α-hydroxyserrat-14-en-3*β*-ol,[8] suggesting **1** to have the same triterpene moiety. The ester unit was positioned at C-3 on the basis of HMQC and HMBC

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**7** (δ, J in Hz)

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[b]
1	0.94, 1.68 m	0.94, 1.68 m	0.93, 1.69 m	1.88, 1.94 m	1.15, 1.56 m	1.79, 1.93 m	1.12, 1.47 m
2	1.64, 1.90 m	1.64, 1.90 m	1.75, 1.92 m	2.14, 2.85 m	1.89, 2.04 m	2.12, 2.82 m	1.58, 1.86 m
3	4.70, dd (11.5, 4.7)	4.70, dd (11.5, 4.7)	4.84, dd (11.8, 4.5)	4.80 br s	4.47 br s	4.80 br s	4.56, t (2.6)
5	0.80 m	0.80 m	0.82 m	2.21 m	1.98 m	2.18 m	1.26 m
6	1.38, 1.40 m	1.38, 1.40 m	1.38, 1.40 m	2.11, 2.45 m	1.67, 1.74 m	2.08, 2.52 m	1.38, 1.51 m
7	1.16, 1.39 m	1.16, 1.39 m	1.17, 1.37 m	1.62, 1.62 m	1.20, 1.41 m	1.39, 1.55 m	1.26, 1.42 m
9	0.74 m	0.74 m	0.74 m	1.14 m	0.97 m	1.15 m	0.98 m
11	1.13, 1.74 m	1.12, 1.74 m	1.09, 1.74 m	1.23, 1.85 m	1.08, 1.83 m	1.20, 1.91 m	1.19, 1.83 m
12	1.38, 1.90 m	1.38, 1.90 m	1.10, 1.89 m	1.21, 2.20 m	1.09, 2.13 m	1.20, 2.18 m	1.20, 2.04 m
13	1.83 m	1.83 m	1.82 m	1.93 m	2.46 m	2.59 m	2.31 m
15	5.47 br s	5.47 br s	5.46 br s	5.54 br s	5.94 s	6.04 s	5.65 s
16	2.01, 2.15 m	2.01, 2.15 m	2.03, 2.15 m	2.08, 2.22 m			
17	1.38 m	1.38 m	1.37 m	1.45 m	3.02 s	3.11 s	2.48 s
19	2.01, 2.15 m	2.01, 2.15 m	1.17, 1.87 m	1.23, 1.94 m	1.56, 2.24 m	1.66, 2.33 m	1.48, 1.72 m
20	1.64, 1.75 m	1.64, 1.75 m	1.75, 1.84 m	1.23, 2.01 m	1.86, 2.00 m	1.97, 2.11 m	1.56, 1.82 m
21	3.50, dd (9.1, 6.4)	3.52, dd (9.3, 6.2)	3.51, dd (9.8, 5.6)	3.58, dd (9.6, 5.8)	3.58 br s	3.67 br s	3.29, t (2.9)
23	0.88 s	0.86 s	1.04 s	1.85 s	1.29 s	1.83 s	0.78 s
24	0.92 s	0.89 s	0.95 s		9.99 s		0.82 s
25	0.82 s	0.78 s	0.79 s	1.25 s	0.74 s	1.18 s	0.77 s
26	0.92 s	0.90 s	0.90 s	1.12 s	0.72 s	0.92 s	0.81 s
27	1.85, 2.31 d (14.3)	1.85, 2.31 d (14.3)	1.85, 2.31 d (14.3)	1.97, 2.40 d (14.3)	1.88, 2.34 d (14.5)	1.83, 2.49 d (14.9)	1.87, 2.43 d (14.9)
28	0.78 s	0.78 s	0.79 s	0.86 s	0.87 s	0.98 s	0.73s
29	1.08 s	1.10 s	1.09 s	1.18 s	1.36 s	1.45 s	1.06 s
30	1.19 s	1.19 s	1.18 s	1.26 s	1.69 s	1.78 s	1.09 s
2′	7.15 d (8.4)		3.83 s				2.03 s ($COCH_3$)
3′	7.28 d (8.4)	7.28 s					
5′	7.28 d (8.4)	7.23 d (8.1)					
6′	7.15 d (8.4)	6.85 br d (8.1)					
7′	3.06 t (7.5)	3.08 t (7.5)					
8′	2.78 t (7.5)	2.80 t (7.5)					

[a] In C_5D_5N. [b] In $CDCl_3$.

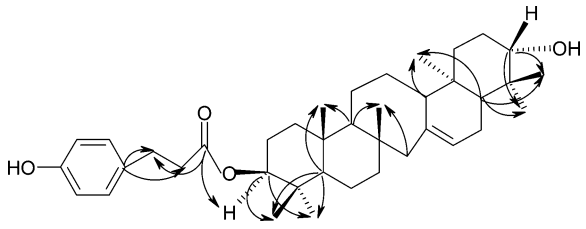

Figure 1. Key HMBC (C to H) correlations of **1**.

analysis. As shown in Figure 1, evident correlations were observed between C-27 (δ_C 56.4) and Me-26 (δ_H 0.92),[8,9] C-9 (δ_C 62.6) and Me-25 (δ_H 0.82) and Me-26, C-5 (δ_C 55.8) and Me-25 and Me-24 (δ_H 0.92) and Me-23 (δ_H 0.88), C-3 (δ_C 80.7) and Me-24 and Me-23, and C-9′ (δ_C 172.3) and H-3α (δ_H 4.70). Therefore, **1** was assigned structurally as 21α-hydroxyserrat-14-en-3β-yl *p*-dihydrocoumarate.

The molecular formula for compound **2**, $C_{39}H_{58}O_5$, was determined by HREIMS. The ^{1}H and ^{13}C NMR signals of **2** (Tables 1 and 2) were nearly superposable with those of **1**, except for a 1,3,4-trisubstituted aromatic ring [δ_H 7.28 (s), 7.23 (d, J = 8.1 Hz), and 6.85 (br d, J = 8.1 Hz); δ_C 145.4 (s), 147.1 (s), 116.7 (d), 132.3 (s), 119.5 (d), and 116.3 (d)] instead of the 1,4-disubstituted aromatic ring in **1**, suggesting a dihydrocaffeic unit rather than a *p*-dihydrocoumaric group as the acid moiety in **2**. This structure was also supported by ion fragments at *m*/*z* 425.3790 [M − $C_9H_9O_4]^+$, 181.0503 $[C_9H_9O_4]^+$, and 165.0551 $[C_9H_9O_3]^+$ in the HREIMS. Hence, **2** was formulated as 21α-hydroxyserrat-14-en-3β-yl dihydrocaffeate.[10]

The molecular formula of **3** was deduced as $C_{33}H_{52}O_5$ from the combination of ESIMS (*m*/*z* 1256 $[2M]^+$) and ^{1}H and ^{13}C NMR and DEPT spectra. The similarities of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) disclosed the skeleton to be the same as that of **1** and **2**. The other signals were attributed to a propanedioic acid group [δ_C 167.8, 169.9, 43.2; δ_H 3.83 (2H, s); IR (ν_{max}) 1745 and 1700 cm^{-1}]. The HMBC correlations observed between C-3 (δ 81.6) and Me-23 (δ_H 0.88) and Me-24 (δ_H 0.92) and between C-1′ (δ 167.8) and H-3α (δ 4.84, dd, J = 11.8, 4.5 Hz) demonstrated the propanedioic acid group to be attached to C-3. Thus,

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**7**

carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[b]
1	38.4	38.1	38.4	34.8	33.6	34.8	33.8
2	28.7	28.4	28.7	27.9	26.8	28.0	24.5
3	80.7	80.3	81.6	70.6	68.3	70.6	78.1
4	38.3	37.9	38.4	48.6	53.3	48.6	36.7
5	55.8	55.5	55.8	49.7	49.5	49.5	50.4
6	19.1	18.8	19.0	21.2	18.6	21.1	18.5
7	45.3	45.0	45.2	45.8	45.0	45.6	44.7
8	37.4	37.1	37.4	37.6	38.9	38.3	38.2
9	62.6	62.3	62.6	62.6	61.4	62.4	62.4
10	38.2	37.9	38.2	39.4	37.9	39.4	38.0
11	25.5	25.2	25.5	25.8	25.2	25.5	24.9
12	27.5	27.3	27.5	27.7	27.1	27.0	26.4
13	57.6	57.3	57.6	57.7	58.9	59.1	58.5
14	138.6	138.4	138.6	138.9	163.4	163.8	163.5
15	122.9	122.6	122.9	122.8	129.1	129.1	128.6
16	24.7	24.4	24.7	24.7	201.3	201.4	201.2
17	50.1	49.8	50.1	50.1	59.5	59.6	58.7
18	36.5	36.2	36.5	36.5	44.7	44.8	44.3
19	37.6	37.3	37.6	37.6	32.0	32.1	31.4
20	24.3	24.0	24.2	28.7	25.9	26.0	22.9
21	78.3	78.0	78.3	78.3	75.8	76.0	76.7
22	39.5	39.3	39.5	39.5	37.6	37.7	36.8
23	28.2	27.9	28.2	25.5	20.6	25.6	27.9
24	16.9	16.6	16.8	180.7	205.7	180.6	21.7
25	16.0	15.7	15.9	14.2	14.6	14.2	14.8
26	20.1	19.8	20.1	20.0	19.8	19.9	20.0
27	56.4	56.1	56.4	56.9	55.9	56.3	55.8
28	13.8	13.6	13.8	13.8	15.3	15.3	15.6
29	15.5	15.2	15.5	15.5	22.2	22.3	21.4
30	28.3	28.0	28.3	28.3	29.0	29.1	28.0
1′ (C=O)	157.5	145.4	167.8				170.3
2′ (CH_3)	116.4	147.1	43.2				21.3
3′	130.0	116.7	169.9				
4′	131.6	132.3					
5′	130.0	119.5					
6′	116.4	116.3					
7′	30.8	30.8					
8′	36.9	36.7					
9′	172.3	172.6					

[a] In C_5D_5N. [b] In $CDCl_3$.

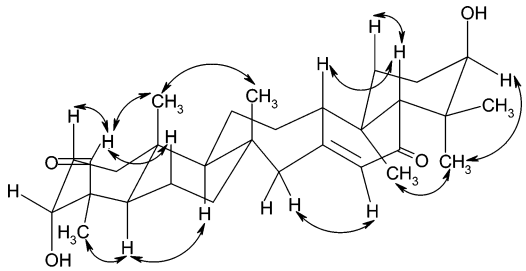

Figure 2. Significant NOESY correlations for **5**.

the structure of **3** was determined as 21α-hydroxyserrat-14-en-3*β*-yl propanedioic acid monoester.

Compound **4** was assigned a molecular formula of $C_{30}H_{48}O_4$, as deduced from the HREIMS. Its IR spectrum showed the presence of hydroxyl (3365 cm^{-1}) and carboxyl (1699 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra displayed 30 carbon signals [6 × CH_3, 10 × CH_2, 2 × OCH, 4 × CH (sp^3), 1 × CH (sp^2), 1 × C (sp^2), 5 × C (sp^3), and 1 × COOH], indicating a serratene-type triterpenoid with one methyl group being oxidized to a carboxyl group. Two oxygenated methine protons at δ_H 4.80 (br s, H-3) and 3.58 (dd, J = 9.6, 5.8 Hz, H-21) demonstrated both hydroxyl groups (OH-3 and OH-21) to have an α orientation. In the HREIMS, **4** exhibited characteristic serratene triterpenoid ion fragment peaks due to the cleavage of the C ring (Scheme S1; Supporting Information).[11−14] The presence of peaks at m/z 237.1492 $[C_{14}H_{21}O_3]^+$ and m/z 219.1381 $[C_{14}H_{19}O_2]^+$ suggested a carboxyl group was attached to the A and B ring moiety of **4**. The carboxyl group could be further assigned at C-24 by means of ^{13}C NMR data comparison of **4** with **3**, which was confirmed by the NOESY correlations of Me-23 (δ_H 1.85) with H-5 (α, δ 2.21). Thus, **4** was determined as 3α,21α-dihydroxyserrat-14-en-24-oic acid.

Compound **5**, $C_{30}H_{46}O_4$, was obtained as colorless prisms. The IR absorptions disclosed hydroxyl (3438 cm^{-1}), aldehyde (1716 cm^{-1}), and α,*β*-unsaturated ketone (1660 cm^{-1}) groups. The olefinic protons at δ 5.94 (H-15) and H-17 at δ 3.02 (s) were shifted downfield compared to those of **1** at δ 5.47 and 1.38, respectively, suggesting the ketone group (δ 201.3) at C-16 was conjugated with a C-14 (C-15) double bond. On comparing the ^1H and ^{13}C NMR spectra with those of 3α,21*β*-dihydroxyserrat-14-en- 16-one (**8**) (see Experimental Section),[15] the important differences were that in **5** an aldehyde group (δ_C 205.7; δ_H 9.99) replaced Me-24 (δ_C 22.6 and δ_H 0.88) in **8**, and some chemical shifts varied [$\Delta\delta_{(5-8)}$: C-3, −7.6; C-23, −8.7; C-4, +14.7], caused by the *γ*-gauche and deshielding effects from the CHO-24,[16] suggesting **5** to be 16-oxo-3α,21*β*-dihydroxyserrat-14-en-24-al. The above structure was further validated by NOESY correlations (Figure 2) of H-24 (δ 9.99) with Me-25 (δ_H 0.74) and H-2*β* (δ 1.75).

The HREIMS suggested the molecular formula of **6** to be $C_{30}H_{46}O_5$, one more oxygen atom than that of **5**. The IR and ^1H and ^{13}C NMR signal patterns of **6** were similar to those of **5**, except for the presence of a carboxyl group (δ_C 180.6) instead of an aldehyde group. The whole structure of **6** was determined unambiguously as 16-oxo-3α,21*β*-dihydroxyserrat-14-en-24-oic acid by carefully analyzing the HMQC, HMBC, NOESY, and HREIMS data.

Compound **7** was obtained as fine needles. The molecular formula, $C_{32}H_{50}O_4$, was deduced from HREIMS. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) exhibited an additional acetyl group (δ_C 170.3 and 21.3; δ_H 2.03, 3H, s) besides several signals similar to those of **8**, indicating **7** to be an acetylated derivative of **8**. The presence of a fragment peak at m/z 358.2537 $[C_{23}H_{34}O_3]^+$ in the HREIMS due to retro-Diels−Alder cleavage of the D ring demonstrated the acetyl group to be connected to C-3, which was also confirmed by the HMBC correlations [OAc (δ_C 170.3)/H-3*β* (δ 4.56), C-3 (δ 78.1)/Me-23 (δ_H 0.78) and Me-24 (δ_H 0.82)]. Thus, the structure of **7** was determined as 16-oxo-21*β*-hydroxyserrat-14-en-3α-yl acetate.

In addition, 15 known triterpenoids were identified as 21α-hydroxyserrat-14-en-3*β*-ol,[8] 21*β*-hydroxyserrat-14-en-3α-ol,[8] 3α,21*β*-dihydroxyserrat-14-en-16-one (**8**),[15] 21α-hydroxyserrat-14-en-3*β*-yl acetate,[15] 3*β*-hydroxyserrat-14-en-21-one,[14] 21*β*-hydroxyserrat-14-en-3*β*-yl acetate,[15] 21*β*-hydroxyserrat-14-en-3*β*-ol,[7] 3*β*-hydroxyserrat-14-en-21α-yl acetate,[15] 3*β*,21*β*-dihydroxyserrat-14-en-16-one,[17] 3*β*,21α-dihydroxyserrat-14-en-24-ol,[10] 3*β*,21*β*-dihydroxyserrat-14-en-24-ol,[18] 3α,21*β*-dihydroxyserrat-14-en-24-ol,[9] 3α,21*β*,24-trihydroxyserrat-14-en-16-one,[17] 3*β*,21*β*-dihydroxyserrat-14-en-29-ol,[10] and 3α,21*β*-dihydroxyserrat-14-ene-24,29-diol,[19] respectively, on the basis of the comparison of their spectroscopic data with those in the literature. Among them, 21*β*-hydroxyserrat-14-en-3α-ol, 3*β*,21*β*-dihydroxyserrat-14-en-16-one, 3*β*,21*β*-dihydroxyserrat-14-en-24-ol, 3α,21*β*-dihydroxyserrat-14-en-24-ol, 3α,21*β*,24-trihydroxyserrat-14-en-16-one, 3*β*,21*β*-dihydroxyserrat-14-en-29-ol, and 3α,21*β*-dihydroxyserrat-14-ene-24,29-diol were isolated from this plant for the first time, and the ^{13}C NMR data of **8** have not appeared in the literature previously.

Experimental Section

General Experimental Procedures. All melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 MC polarimeter in $CHCl_3$ or C_5D_5N. The IR spectra were recorded on a Nicolet Magna 750 FTIR (KBr) spectrophotometer. EIMS, HREIMS, and ESIMS data were obtained with a MAT-95 mass spectrometer. All NMR spectra were recorded on a Bruker AM-400 instrument. Silica gel (100−200, 200−300, 400 mesh) and precoated plates of silica gel (HSGF254) (Qingdao Haiyang Chemical Group Co., Qingdao, People's Republic of China) were used for column chromatography and for TLC, respectively.

Plant Material. Fresh whole plants of *Huperzia serrata* were collected in Yukang Natural Protection District of Xianju County, Zhejiang Province, People's Republic of China, in August 1999, and identified by Dr. X. Q. Ma. A voucher specimen was deposited in the Herbarium of Shanghai Institute of Materia Medica.

Extraction and Isolation. The air-dried whole plants (25 kg) of *H. serrata* were powdered and extracted with 1% aqueous tartaric acid at room temperature. After filtering the acid-soluble fraction, the residue was extracted with 95% ethanol (30 L × 3) and then concentrated under reduced pressure to give a nonalkaloid extract (530 g). This was partitioned successively with solvents of increasing polarity (each 10 L × 3) to yield petroleum ether (35 g), chloroform (120 g), acetone (75 g), and ethanol (240 g) fractions. The petroleum ether fraction was subjected to silica gel column chromatography with a petroleum ether−$CHCl_3$ gradient system (40:1 to 8:1) to afford fractions A-1−A-6. Fraction A-4 was purified by silica gel column chromatography using petroleum ether−Me_2CO (10:1) as eluent to give 21*β*-hydroxyserrat-14-en-3*β*-yl acetate (34 mg) and 21*β*-hydroxyserrat-14-en-3α-ol (68 mg). 3*β*-Hydroxyserrat-14-en-21α-yl acetate (240 mg) and 3*β*-hydroxyserrat-14-en-21-one (82 mg) were obtained from fraction A-6 by chromatography on silica gel eluted with petroleum ether−CH_2Cl_2 (8:1).

The $CHCl_3$ fraction was chromatographed over a silica gel column with $CHCl_3$−Me_2CO mixtures of increasing polarity (40:1, 30:1, 20:1, 10:1, and 5:1) and then $CHCl_3$−CH_3OH (10:1, 8:1, 6:1, 5:1, and 4:1) to furnish fractions B-1−B-10. Fraction

B-1 was purified by silica gel column chromatography with $CHCl_3$−Me_2CO (40: 1) to provide 21α-hydroxyserrat-14-en-3*β*-yl acetate (15 mg) and **7** (23 mg). 21α-Hydroxyserrat-14-en-3*β*-ol (430 mg) and 21*β*-hydroxyserrat-14-en-3*β*-ol (1.2 g) were obtained from fraction B-2 after purification on a silica gel column with $CHCl_3$−Me_2CO (20:1). Fraction B-4 was further separated into fractions B-4.1−4.4 on a silica gel column with $CHCl_3$−CH_3OH (15:1) as eluent. Fraction B-4.2 provided **8** (36 mg) and 3*β*,21*β*-dihydroxyserrat-14-en-16-one (21 mg) after purification on a silica gel column eluted with $CHCl_3$−CH_3OH (12:1); fraction B-4.4 was dissolved with $CHCl_3$−CH_3OH (5:1) and yielded crystalline needles of **1** (23 mg). Fraction B-6 was separated into fractions B-6.1−6.2 on a silica gel column with $CHCl_3$−CH_3OH (10:1 and 8:1). Compound **5** (14 mg) was obtained as prisms from fraction B-6.1, and compound **2** (17 mg) was obtained from fraction B-6.2 by chromatography on a silica gel column with $CHCl_3$−CH_3OH (8:1). 3*β*,21α-Dihydroxyserrat-14-en-24-ol (43 mg) and 3*β*,21*β*-dihydroxyserrat-14-en-24-ol (8 mg) were isolated from fraction B-7 by chromatography on silica gel eluted with $CHCl_3$−CH_3OH (5:1). Fraction B-8 was subjected to silica gel column chromatography with a $CHCl_3$−CH_3OH gradient system (10:1 to 5:1) to furnish fractions B-8.1−8.4. Fractions B-8.2 and B-8.4 gave **4** (9 mg) and 3α,21*β*-dihydroxyserrat-14-en-24-ol (25 mg) after further purification on a silica gel column with $CHCl_3$−CH_3OH (8:1 and 6:1), respectively. Compound **6** (18 mg) and 3*β*,21*β*-dihydroxyserrat-14-en-29-ol (62 mg) were derived from fraction B-9 by repeated chromatography on a silica gel column eluted with $CHCl_3$−CH_3OH (4:1). Fraction B-10 was chromatographed over a silica gel column by $CHCl_3$−CH_3OH (2:1) to afford **3** (26 mg).

The Me_2CO fraction afforded fractions C-1−C-10 through silica gel column chromatography with a $CHCl_3$−CH_3OH gradient system (10:1 to 1:1). 3α,21*β*,24-Trihydroxyserrat-14-en-16-one (43 mg) and 3α,21*β*-dihydroxyserrat-14-ene-24,29-diol (54 mg) were obtained from fractions C-4 and C-5, respectively, by repeated column chromatography on silica gel with $CHCl_3$−CH_3OH (3:1).

21α-Hydroxyserrat-14-en-3*β*-yl *p*-dihydrocoumarate (1): colorless needles ($CHCl_3$−CH_3OH); mp 296−298 °C; $[\alpha]^{20}_D$ −20.5° (*c* 0.47, C_5D_5N); IR (KBr) ν_{max} 3572, 3292, 1724, 1618, 1517, 1452, 1355, 1238, 825 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 590 $[M]^+$ (20), 572 $[M - H_2O]^+$ (5), 558 $[M - H_2O - Me]^+$ (3), 425 $[M - C_9H_9O_3]^+$ (100), 407 (59), 287 (11), 269 (15), 189 (98), 187 (21), 165 (5), 149 (4), 123 (26); HREIMS *m*/*z* 590.4312 $[M]^+$ (calcd for $C_{39}H_{58}O_4$, 590.4335).

21α-Hydroxyserrat-14-en-3*β*-yl dihydrocaffeate (2): white powder ($CHCl_3$−CH_3OH); mp 282−286 °C; $[\alpha]^{20}_D$ −11° (*c* 0.12, C_5D_5N); IR (KBr) ν_{max} 3354 (OH), 1726 (COO−), 1618 and 1529 (benzene ring), 1450, 1367, 1284, 808 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC (400 MHz, C_5D_5N) [C-1, Me-25], [C-3, Me-23, Me-24], [C-4, Me-23, Me-24, H-3α], [C-5, Me-23, Me-24, Me-25], [C-8, Me-26], [C-9, Me-26, Me-25], [C-10, Me-25], [C-13, Me-28], [C-17, Me-28, Me-29, Me-30], [C-18, Me-28, H-17*β*], [C-19, Me-28], [C-21, Me-29, Me-30], [C-22, Me-29, Me-30, H-21*β*], [C-27, Me-26], [C-1′, H-3′, H-5′, H-6′], [C-2′, H-6′], [C-4′, H-6′, H-7′, H-8′], [C-7′, H-3′, H-5′, H-8′], [C-9′, H-3α, H-7′, H-8′]; EIMS *m*/*z* 606 $[M]^+$ (9), 588 $[M - H_2O]^+$ (7), 573 $[M - H_2O - Me]^+$ (5), 425 $[M - C_9H_9O_4]^+$ (75), 407 (39), 287 (8), 269 (9), 189 (50), 187 (22), 181 (8), 165 (22), 123 (100); HREIMS *m*/*z* 606.4300 $[M]^+$ (calcd for $C_{39}H_{58}O_5$, 606.4316).

21α-Hydroxyserrat-14-en-3*β*-yl propanedioic acid monoester (3): white powder ($CHCl_3$−CH_3OH); mp 306−310 °C; $[\alpha]^{20}_D$ −19° (*c* 0.23, C_5D_5N); IR (KBr) ν_{max} 3411, 1745, 1700, 1442, 1384, 1234, 825 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC (400 MHz, C_5D_5N) [C-1, Me-25], [C-3, Me-23, Me-24], [C-4, Me-23, Me-24, H-3α], [C-5, Me-23, Me-24, Me-25], [C-8, Me-26], [C-9, Me-26, Me-25], [C-10, Me-25], [C-13, Me-28], [C-17, Me-28, Me-29, Me-30], [C-18, Me-28, H-17*β*], [C-19, Me-28], [C-21, Me-29, Me-30], [C-22, Me-29, Me-30, H-21*β*], [C-27, Me-26], [C-1′, H-3α, H-2′], [C-3′, H-2′]; EIMS *m*/*z* 484 $[M - CO_2]^+$ (53), 469 $[M - CO_2 - Me]^+$ (30), 466 $[M - H_2O - CO_2]^+$ (9), 424 $[M - C_3H_4O_3]^+$ (11), 409 (12), 284 (21), 269 (15), 203 (40), 189 (100), 187 (45); ESIMS *m*/*z* 1256 $[2M]^+$.

3α,21α-Dihydroxyserrat-14-en-24-oic acid (4): white powder ($CHCl_3$−CH_3OH); mp 304−306 °C; $[\alpha]^{20}_D$ −0.6° (*c* 0.78, C_5D_5N); IR (KBr) ν_{max} 3365 (OH), 1699 (C=O), 1450, 1385, 1243, 993 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC (400 MHz, C_5D_5N) [C-1, Me-25, H-3*β*], [C-3, Me-23], [C-4, Me-23, H-5α], [C-5, Me-23, Me-25], [C-8, Me-26], [C-9, Me-26, Me-25], [C-10, Me-25], [C-13, Me-28], [C-17, Me-28, Me-29, Me-30], [C-18, Me-28, H-17*β*], [C-19, Me-28], [C-21, Me-29, Me-30], [C-22, Me-29, Me-30, H-17*β*], [C-23, H-5α], [C-24, Me-23, H-5α], [C-27, Me-26]; [C-30, Me-29, H-17*β*, H-21*β*]; NOESY (400 MHz, C_5D_5N) [Me-23, H-5α], [H-13*β*, H-17*β*], [Me-25, Me-24], [Me-28, Me-29]; EIMS *m*/*z* 472 $[M]^+$ (5), 454 $[M - H_2O]^+$ (20), 436 (18), 421 (16), 411 (22), 393 (36), 349 (18), 286 (4), 271 (9), 189 (38), 187 (68), 147 (80), 95 (100); HREIMS *m*/*z* 472.3560 $[M]^+$ (calcd for $C_{30}H_{48}O_4$, 472.3574).

16-Oxo-3α,21*β*-dihydroxyserrat-14-en-24-al (5): colorless prisms ($CHCl_3$−CH_3OH); mp 270−272 °C; $[\alpha]^{20}_D$ −23.7° (*c* 0.35, C_5D_5N); IR (KBr) ν_{max} 3439 (OH), 1716 (C=O), 1660 (C=C−C=O), 1454, 1384, 1328, 995 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC (400 MHz, C_5D_5N) [C-1, Me-25, H-5], [C-3, Me-23, H-24], [C-4, Me-23], [C-5, Me-23, Me-25, H-24], [C-8, Me-26], [C-9, Me-26, Me-25], [C-10, Me-25], [C-13, Me-28, H-15, H-17], [C-16, H-17], [C-17, Me-28, Me-29, Me-30], [C-18, Me-28], [C-19, Me-28], [C-21, Me-29, Me-30], [C-22, Me-29, Me-30, H-17], [C-24, Me-23, H-5], [C-27, Me-26, H-15]; [C-30, Me-29, H-17, H-21]; EIMS *m*/*z* 470 $[M]^+$ (25), 452 $[M - H_2O]^+$ (28), 437 (18), 407 (6), 391(6), 330 (8), 269 (10), 261 (9), 189 (51), 187 (39), 161 (50), 149 (73), 121 (90), 91 (100); HREIMS *m*/*z* 470.3389 $[M]^+$ (calcd for $C_{30}H_{46}O_4$, 470.3464).

16-Oxo-3α,21*β*-dihydroxyserrat-14-en-24-oic acid (6): white powder ($CHCl_3$−CH_3OH); mp 298−300 °C; $[\alpha]^{20}_D$ +10.5° (*c* 0.55, C_5D_5N); IR (KBr) ν_{max} 3500 (OH), 1718 (C=O), 1659 (C=C−C=O), 1452, 1384, 1201, 995, 873 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC (400 MHz, C_5D_5N) [C-1, Me-25], [C-3, Me-23], [C-4, Me-23, H-3*β*], [C-5, Me-23, Me-25, H-3*β*], [C-8, Me-26, H-27*β*], [C-9, Me-26, Me-25, H-27*β*], [C-10, Me-25], [C-13, Me-28, H-15, H-17*β*], [C-16, H-17*β*], [C-17, Me-28, Me-29, Me-30], [C-18, Me-28], [C-21, Me-29, Me-30], [C-22, Me-29, Me-30, H-17*β*, H-21α], [C-24, Me-23, H-3*β*], [C-27, Me-26, H-15], [C-30, Me-29, H-17*β*, H-21α]; NOESY (400 MHz, C_5D_5N) [Me-23, H-5α], [H-13*β*, H-17*β*], [Me-25, Me-24], [Me-28, Me-29]; EIMS *m*/*z* 486 $[M]^+$ (5), 468 $[M - H_2O]^+$ (67), 453 $[M - H_2O - Me]^+$ (43), 450 (16), 406 (41), 391(30), 324 (8), 283 (10), 269 (15), 243 (20), 217 (42), 189 (58), 187 (46), 175 (52), 135 (58), 122 (100); HREIMS *m*/*z* 486.3329 $[M]^+$ (calcd for $C_{30}H_{46}O_5$, 486.3313).

16-Oxo-21*β*-hydroxyserrat-14-en-3α-yl acetate (7): colorless needles ($CHCl_3$); mp 270−274 °C; $[\alpha]^{20}_D$ −24.1° (*c* 0.47, $CHCl_3$); IR (KBr) ν_{max} 3496 (OH), 1725 (COO−), 1661 (C=C−C=O), 1458, 1387, 1245, 986, 796 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC (400 MHz, C_5D_5N) [C-1, Me-25], [C-3, Me-23, Me-24], [C-4, Me-23, Me-24, H-3*β*], [C-5, Me-23, Me-24, Me-25], [C-8, Me-26], [C-9, Me-26, Me-25, H-27*β*], [C-10, Me-25], [C-13, Me-28, H-15, H-17*β*], [C-16, H-17*β*], [C-17, Me-28, Me-29, Me-30], [C-18, Me-28], [C-19, Me-28], [C-21, Me-29, Me-30], [C-22, Me-29, Me-30, H-17*β*, H-21α], [C-24, Me-23, H-5α], [C-27, Me-26, H-15], [C-30, Me-29, H-17*β*, H-21α], [δ_C 170.3, H-3*β*]; EIMS *m*/*z* 498 $[M]^+$ (60), 480 $[M - H_2O]^+$ (15), 438 $[M - AcOH]^+$ (44), 423 (36), 403 (38), 379 (100), 358 (16), 287 (14), 207 (35), 203 (52), 190 (100), 187 (74); HREIMS *m*/*z* 498.3693 $[M]^+$ (calcd for $C_{32}H_{50}O_4$, 498.3709).

16-Oxo-3α-hydroxyserrat-14-en-21*β*-ol (8): white powder ($CHCl_3$−CH_3OH); mp 314−316 °C; IR (KBr) ν_{max} 3475 (OH), 1660 (C=C−C=O), 1452, 1326, 1224, 993, 875 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N), *δ* 3.58 (1H, br s, H-3), 5.92 (1H, s, H-15), 3.01 (1H, s, H-17), 3.60 (1H, br s, H-21), 1.17 (3H, s, H_3-23), 0.88 (3H, s, H_3-24), 0.80 (3H, s, H_3-25), 0.72 (3H, s, H_3-26), 0.86 (3H, s, H_3-28), 1.35 (3H, s, H_3-29), 1.68 (3H, s, H_3-30); ^{13}C NMR (400 MHz, C_5D_5N, *δ*), C-1 (33.8), C-2 (26.7), C-3 (75.9), C-4 (38.6), C-5 (49.4), C-6 (19.1), C-7 (45.3), C-8 (38.2), C-9 (62.5), C-10 (38.2), C-11 (25.2), C-12 (26.7), C-13 (59.5), C-14 (163.6), C-15 (128.9), C-16 (201.3), C-17 (59.0), C-18 (44.7), C-19 (32.0), C-20 (25.9), C-21 (75.0), C-22 (37.6), C-23 (29.3), C-24 (22.6), C-25 (16.0), C-26 (20.1), C-27 (56.1), C-28 (15.2), C-29 (22.2), C-30 (29.1); EIMS *m*/*z* 456 $[M]^+$ (42), 438

(54), 423 (30), 397 (6), 357 (16), 284 (16), 269 (15), 203 (84), 189 (100), 187 (56).

Supporting Information Available: Scheme showing HREIMS fragments for compounds **4**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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