Three New Serratane Triterpenoids from *Phlegmariurus squarrosus*

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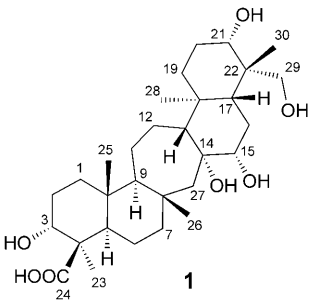
Three new serratane triterpenoids, (3α,14α,15α,21α)-3,14,15,21,29-pentahydroxyserratan-24-oic acid (**1**), (3α,21β)-serrat-14-ene-3,21,24,30-tetraol (**2**), and (3α,21α)-serrat-14-ene-3,21,24,29-tetraol (**3**), were isolated from *Phlegmariurus squarrosus*, together with eight known compounds. Their chemical structures were elucidated on the basis of in-depth spectroscopic analyses.

Introduction. – Recent extensive studies on *Lycopodium*, *Huperzia*, and *Picea* plants have revealed some genera-characteristic serratane-type triterpenoids, the common structural feature being a seven-membered *C*-ring [1–3], some of which were found to be highly bioactive [4–7]. In continuation of our studies on these species, we now report three new serratane[1]) derivatives (**1**–**3**), which were isolated from *Phlegmariurus squarrosus* (FORST.) LÖVE, together with the following eight known constituents: (3α,21β)-serrat-14-ene-3,21,24,29-tetraol (**4**), serratenediol-3-acetate, 21-episerratenediol, serratenediol, serrat-14-ene-3β,21α,24-triol, 3α,21α-dihydroxyserrat-14-en-24-oic acid, 21α-hydroxyserrat-14-en-3β-yl propanedioic acid monoester, and 21α-hydroxyserrat-14-en-3β-yl dihydrocaffeate.



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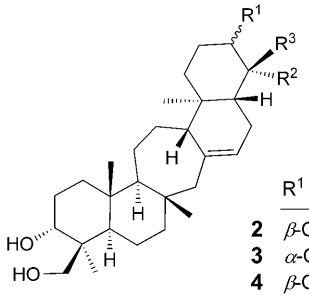

	R^1	R^2	R^3
2	β-OH	Me	CH_2OH
3	α-OH	CH_2OH	Me
4	β-OH	CH_2OH	Me

Results and Discussion. – The molecular formula $C_{30}H_{50}O_7$ was established for **1** by FAB-MS, combined with HR-FAB-MS (*m*/*z* 521.3469 ($[M-H]^-$, $C_{30}H_{49}O_7^-$; calc.

[1]) Serratane = (4a*S*,6a*S*,9a*S*,13a*R*,13b*S*,15a*S*,15b*S*)-docosahydro-4,4,6a,10,10,13a,15b-heptamethyl-1*H*-cyclohepta[1,2-*a*:5,4-*a*′]dinaphthalene.

521.3478). In the ^{13}C-NMR spectrum of **1** (*Table*), the signals at δ(C) 77.8 (C(14)) and 76.4 (C(15)) indicated the presence of OH groups at C(14) and C(15). Analysis of the ^{1}H- and ^{13}C-NMR spectra showed that **1** was structurally related to lycernuic acid C [8], except for the configurations at C(3) and C(21). The ^{13}C-NMR (DEPT) spectrum showed 30 C-atoms: five quaternary C-atoms, five Me, ten CH_2 (including a characteristic serratane signal at δ(C) 54.9 (C(27))), and four CH groups, as well as three oxygenated CH (δ(C) 70.8 (C(3)), 76.4 (C(15)), 73.3 (C(21))), one oxygenated CH_2 (δ(C) 67.6 (C(29))), one oxygenated quaternary C-atom (δ(C) 77.8 (C(15))), and one COOH group at δ(C) 181.0 (C(24)).

The positions of the functional groups were deduced from HMBC experiments. The orientation of the COOH group at C(4) was derived by comparison of the ^{13}C-NMR chemical shifts with those of lycernuic acid C [8] and 3α,21α-dihydroxyserrat-14-en-24-oic acid [9]. H–C(3) in **1** was axial (β-face), as indicated by its ^{1}H-NMR resonance at δ(H) 4.68 (br. *s*, 1 H), whereas H–C(21) appeared to be equatorial (β-face), resonating at δ(H) 4.28 (*dd*, $J = 10.8, 4.6$, 1 H) [9–11]. The α-orientations of the 14- and 15-OH groups were deduced by comparison of their ^{13}C-NMR chemical shifts with those of lycernuic acid C [8], and by means of NOE experiments. The orientation of the CH_2OH group at C(22) was established by a ROESY experiment (*Figure*). ROESY Correlations between H–C(21), H–C(17), and Me(30), and between CH_2(29) and Me(28) indicated that C(29) was α-configured.

On the basis of the above evidence, the structure of compound **1** was established as (3α,14α,15α,21α)-3,14,15,21,29-pentahydroxyserratan-24-oic acid, and was named *lycernuic acid F.*

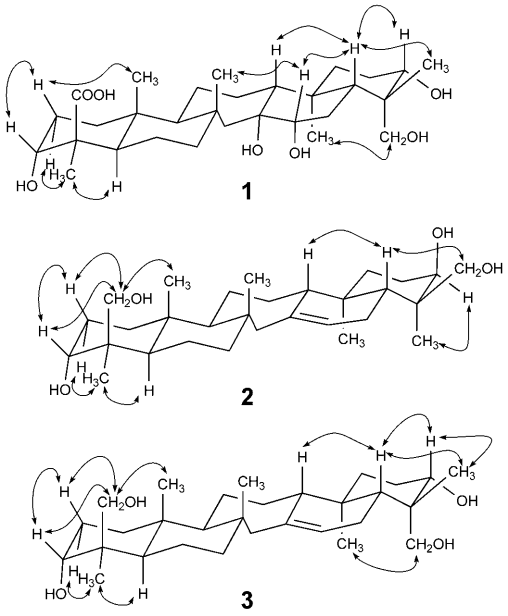

Figure. *Key ROESY correlations for compounds* **1**–**3**

Table. *^{13}C- and ^{1}H-NMR Data for* **1**–**3**. Recorded at 100 or 125 MHz (^{13}C), and at 400 or 500 MHz (^{1}H) in C_5D_5N; δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	34.7 (*t*)	1.88–1.94 (*m*)	34.3 (*t*)	1.40–1.51 (*m*), 1.68–1.72 (*m*)	34.3 (*t*)	1.39–1.51 (*m*), 1.68–1.78 (*m*)
2	27.9 (*t*)	2.02–2.10 (*m*)	26.8 (*t*)	1.85–1.95 (*m*)	26.8 (*t*)	1.85–1.95 (*m*)
3	70.8 (*d*)	4.68 (br. *s*)	70.1 (*d*)	4.45 (br. *s*)	70.1 (*d*)	4.60 (br. *s*)
4	48.7 (*s*)	–	44.3 (*s*)	–	44.3 (*s*)	–
5	49.5 (*d*)	2.14–2.16 (*m*)	50.4 (*d*)	1.85–1.95 (*m*)	50.4 (*d*)	1.84–1.96 (*m*)
6	21.2 (*t*)	2.43–2.48 (*m*), 1.86–1.90 (*m*)	19.7 (*t*)	1.59–1.64 (*m*)	19.7 (*t*)	1.59–1.64 (*m*)
7	45.0 (*t*)	1.62–1.70 (*m*)	46.0 (*t*)	1.20–1.30 (*m*), 1.35–1.45 (*m*)	46.0 (*t*)	1.25–1.35 (*m*)
8	38.6 (*s*)	–	37.7 (*s*)	–	37.4 (*s*)	–
9	59.7 (*d*)	1.19–1.22 (*m*)	63.1 (*d*)	0.95–1.05 (*m*)	63.0 (*d*)	0.95–1.05 (*m*)
10	38.8 (*s*)	–	38.2 (*s*)	–	38.6 (*s*)	–
11	26.2 (*t*)	1.22–1.28 (*m*), 1.86–1.90 (*m*)	25.6 (*t*)	1.05–1.15 (*m*)	25.6 (*t*)	1.05–1.13 (*m*)
12	27.8 (*t*)	2.13 –2.17 (*m*)	27.6 (*t*)	1.05–1.16 (*m*)	27.7 (*t*)	1.05–1.16 (*m*)
13	59.3 (*d*)	1.85–1.93 (*m*)	57.4 (*d*)	2.00–2.10 (*m*)	57.6 (*d*)	2.00–2.15 (*m*)
14	77.8 (*s*)	–	139.2 (*s*)	–	138.0 (*s*)	–
15	76.4 (*d*)	3.88–3.91 (*m*)	122.5 (*d*)	5.40 (br. *s*)	122.7 (*d*)	5.40 (br. *s*)
16	25.5 (*t*)	1.79–1.86 (*m*)	24.4 (*t*)	1.55–1.62 (*m*)	24.6 (*t*)	1.55–1.62 (*m*)
17	45.5 (*d*)	1.55–1.62 (*m*)	38.6 (*d*)	2.57–2.61 (*m*)	43.4 (*d*)	2.50 (*dd*, J=7.0, 4.0)
18	38.1 (*s*)	–	36.1 (*s*)	–	36.3 (*s*)	–
19	38.8 (*t*)	1.20–1.24 (*m*), 1.80–1.90 (*m*)	31.7 (*t*)	1.61–1.70 (*m*), 1.92–1.99 (*m*)	37.3 (*t*)	1.90–1.96 (*m*)
20	28.0 (*t*)	1.25–1.29 (*m*), 2.00–2.05 (*m*)	26.7 (*t*)	1.90–1.97 (*m*)	28.2 (*t*)	2.00–2.06 (*m*)
21	73.3 (*d*)	4.28 (*dd*, J=10.8, 4.6)	75.7 (*d*)	4.04 (br. *s*)	73.4 (*d*)	4.44 (*dd*, J=2.3, 11.2)
22	43.2 (*s*)	–	40.8 (*s*)	–	43.1 (*s*)	–
23	25.3 (*s*)	1.12 (*s*)	23.6 (*s*)	1.59 (*s*)	23.1 (*s*)	1.59 (*s*)
24	181.0 (*s*)	–	65.8 (*t*)	4.10 (*d*, J=10.8), 3.86 (*d*, J=10.8)	65.5 (*t*)	3.86 (*d*, J=10.7), 4.25 (*d*, J=10.7)
25	14.4 (*s*)	1.17 (*s*)	16.7 (*s*)	0.92 (*s*)	14.4 (*s*)	0.82 (*s*)
26	23.2 (*s*)	1.70 (*s*)	20.2 (*s*)	0.82 (*s*)	20.3 (*s*)	0.87 (*s*)
27	54.9 (*t*)	1.90–1.99 (*m*)	56.9 (*t*)	2.20–2.31 (*m*), 1.86–1.90 (*m*)	56.7 (*t*)	2.20–2.31 (*m*), 1.82–1.95 (*m*)
28	13.3 (*s*)	1.09 (*s*)	14.7 (*s*)	0.80 (*s*)	12.2 (*s*)	0.89 (*s*)
29	67.6 (*t*)	4.20 (*d*, J=10.2), 3.75 (*d*, J=10.2)	17.6 (*s*)	0.83 (*s*)	67.0 (*t*)	3.65 (*d*, J=10.5), 4.27 (*d*, J=10.5)
30	16.9 (*s*)	1.12 (*s*)	70.7 (*t*)	3.64 (*d*, J=10.9), 3.84 (*d*, J=11.0)	16.7 (*s*)	0.93 (*s*)

The ESI mass spectrum of compound **2** displayed the $[M+Na]^+$ peak at m/z 497, supporting the molecular formula $C_{30}H_{50}O_4$. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table*) were very similar to those of the known compound (3α,21β)-serrat-14-ene-3,21,24,29-tetraol (**4**) [12][13], apart from the C(22) resonances. The ^{13}C-NMR

(DEPT) spectrum of **2** showed signals for a C=C bond at δ(C) 139.2 (C(14)) and 122.5 (C(15)), two oxygenated CH (δ(C) 70.1 (C(3)) and 75.7 (C(21))), two oxygenated CH_2 (δ(C) 65.8 (C(24)) and 70.7 (C(30))), five Me, eleven CH_2, and four CH groups, as well as five quaternary C-atoms.

The CH_2OH groups at C(4) and C(22) were established by HMBC correlations. In the ^{1}H-NMR spectrum of **2**, both H–C(3) and H–C(21) appeared as broad signals, suggesting that they were in β- and α-position, respectively. The CH_2OH group at C(4) was shown to be in β-orientation, as deduced by two key NOE correlations between CH_2(24) (δ(H) 4.10, 3.86) and Me(25) (δ(H) 0.92). The CH_2OH function at C(22) was also in β-orientation, with NOE correlations between CH_2(30) (δ(H) 3.84, 3.64) and H–C(17) (δ(H) 2.59), as further corroborated by ROESY experiments (*Figure*). The ^{13}C-NMR signal for C(17) at δ(C) 38.6 was shifted upfield by 4.9 ppm compared to that in **4** [12] due to a γ-gauche effect from the additional CH_2OH group. Owing to intramolecular H-bonding between the 21-OH and 30-OH groups, the chemical shift for C(21) (δ(C) 75.7) was shifted downfield by 7.6 ppm relative to that in **4** [12], which confirmed that the second OH group was at C(30), rather than at C(29).

From the above data, in combination with further HMQC, HMBC, and ROESY experiments, the structure of compound **2** was unambiguously assigned as (3α,21β)-serrat-14-ene-3,21,24,30-tetraol, and named *phlegmariurol A*.

Compound **3** had the same molecular formula, $C_{30}H_{50}O_4$, as **2** and **4**, and the IR spectrum indicated OH groups. The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table*) were basically identical with those of **4**, except for the signals of H–C(21), with a C=C bond, two oxygenated CH, two oxygenated CH_2, five Me, eleven CH_2, four CH, and five quaternary C-atoms. Therefore, compounds **3** and **4** were supposed to be 21-epimers. The ^{1}H-NMR signal for H–C(21) (*dd*, J = 2.3, 11.2 Hz) of **3** indicated that the OH group was in α-position. In the HMBC spectrum of **3**, long-range correlations were observed between CH_2(29) (δ(H) 3.65 (*d*, J = 10.5), 4.27 (*d*, J = 10.5)) and both C(21) (δ(C) 73.4) and Me(30) (δ(C) 16.7), between CH_2(24) (δ(H) 3.86 (*d*, J = 10.7), 4.25 (*d*, J = 10.7)) and both C(3) (δ(C) 70.1) and Me(23) (δ(C) 23.1). In the ROESY spectrum (*Figure*), significant NOEs were observed between CH_2(29) and Me(28), and between H–C(17) and Me(30), which suggested that the 21-OH and the 22-CH_2OH groups were β- and α-configured, respectively. Since we observed no γ-gauche effect, the chemical shift at C(17) (δ(C) 43.4) was similar as that in **4** [12], which corroborated a 29-OH group.

From the above considerations, the chemical structure of compound **3** was established as (3α,21α)-serrat-14-ene-3,21,24,29-tetraol, and named *phlegmariurol B*.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, *Qingdao Marine Chemical, Inc.*), *Lichroprep RP-18* (40–63 μm, *Merck*), or *Sephadex LH-20* (*Pharmacia*). TLC: Spots were visualized by spraying with 10% aq. H_2SO_4 soln., followed by heating. HPLC: *Agilent 1100* instrument. Melting points (m.p.): *X-4* apparatus; uncorrected. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra:

Shimadzu IR-450 instrument, with KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AV-400* or *DRX-500* instruments; chemical shifts δ in ppm rel. to Me_4Si, coupling constants J in Hz. FAB-MS and HR-EI-MS: *VG Autospec-3000* spectrometer; in m/z (rel. %).

Plant Material. Phlegmariurus squarrosus (FORST.) LÖVE was collected in Xishuangbanna, Yunnan Province, China, in May 2003. The plant was identified by Prof. *Baogui Li*, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (KUN No. 200304022) was deposited at the State Key Laboratory of Phytochemistry, Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The powdered plant material of *P. squarrosus* (850 g) was exhaustively extracted with 90% aq. EtOH (4×3 l) at reflux. The extracts were combined and evaporated to dryness. The residue (239 g) was dissolved in MeOH/H_2O 9:1 (2 l) and extracted with AcOEt (4×1 l), which afforded 70 g of material. Part of the AcOEt extract was adsorbed on SiO_2 (100 g) and subjected to CC (1000 g SiO_2; $CHCl_3$/Me_2CO 10:0, 9:1, 8:2, 7:3, 6:4, and 0:10) to afford five fractions (*Fr.*): *Fr. 1* (oil), *Fr. 2* (25 g), *Fr. 3* (10 g), *Fr. 4* (20 g), and *Fr. 5* (5 g). *Fr. 2* was subjected to CC (SiO_2; $CHCl_3$/MeOH 40:1, 30:1, 20:1) to afford four subfractions (*Fr. 2.1–2.4*). *Fr. 2.1* was further purified by repeated CC (SiO_2; $CHCl_3$/MeOH 60:1, 50:1) to yield serratenediol-3-acetate (500 g) and 21-episerratenediol (2 g). *Fr.* 2.2 was further purified by CC (SiO_2; $CHCl_3$/MeOH 35:1) to provide serratenediol (1 g). *Fr. 3* was repeatedly purified by CC (SiO_2; $CHCl_3$/MeOH 20:1) to afford serrat-14-ene-3β,21α,24-triol (300 mg), 3α,21α-dihydroxyserrat-14-en-24-oic acid (200 mg), and a residue. The latter was purified by HPLC (*SHIMADZU PRC-ODS (K)*, 30.0 mm i.d.×25 cm; MeOH/H_2O 65:35) to provide **2**. *Fr. 4* was rechromatographed (SiO_2; $CHCl_3$/MeOH 25:1, 20:1, 15:1), which gave two subfractions (*Fr. 4.1* and *4.2*) and 21α-hydroxyserrat-14-en-3β-yl propanedioic acid monoester (100 mg). *Fr. 4.1* was subjected to CC (Silicagel; $CHCl_3$/MeOH 20:1) to yield 21α-hydroxyserrat-14-en-3β-yl dihydrocaffeate (500 mg) and **1** (10 mg). Finally, *Fr. 4.2* was subjected to HPLC (*ZORBAX® ODS* 21.2 mm i.d.×25 cm; MeOH/H_2O 60:40) to give **2** (30 mg) and **3** (15 mg).

Lycernuic Acid F (=*(3α,14α,15α,21α)-3,14,15,21,29-Pentahydroxyserratan-24-oic Acid*; **1**). Colorless powder. M.p. 244°. $[\alpha]_D^{23} = -25°$ ($c = 0.3$, MeOH). IR (KBr): 3395, 2930, 2855, 1695, 1568, 1445, 1245, 1168, 1025. ^{1}H- and ^{13}C-NMR: see the *Table*. FAB-MS: 521. HR-FAB-MS: 521.3469 ($[M-1]^-$, $C_{30}H_{49}O_7^-$; calc. 521.3478).

Phlegmariurol A (=*(3α,21β)-Serrat-14-ene-3,21,24,30-tetraol*; **2**). Colorless powder. M.p. >350°. $[\alpha]_D^{23} = -13.1$ ($c = 0.91$, EtOH). IR (KBr): 3405, 2935, 1570, 1448, 1250, 1168, 1030. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS: 497 (20, $[M+Na]^+$), 456 (10, $[M-OH]^+$), 325 (5), 298 (10), 212 (20), 197 (55), 102 (65), 74 (100). HR-ESI-MS: 497.3610 (10, $[M+Na]^+$, $C_{30}H_{50}NaO_4^+$; calc. 497.3606).

Phlegmariurol B (=*(3α,21α)-Serrat-14-ene-3,21,24,29-tetraol*; **3**). Colorless powder. M.p. 310°. $[\alpha]_D^{23} = -14.4$ ($c = 0.81$, EtOH). IR (KBr): 3405, 2920, 1570, 1450, 1250, 1168, 1025. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS: 474 (25, M^+), 456 (20, $[M-OH]^+$), 325 (15), 298 (10), 212 (20), 197 (55), 102 (65), 74 (100). HR-ESI-MS: 474.7235 (M^+, $C_{30}H_{50}O_4^+$; calc. 474.7230).

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