Natural Products Inhibiting *Candida albicans* Secreted Aspartic Proteases from *Lycopodium cernuum*

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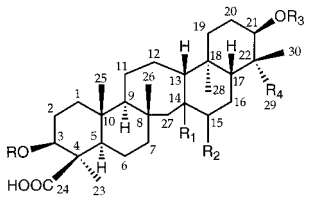
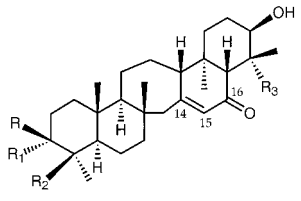
Activity-guided fractionation of an ethanol extract of *Lycopodium cernuum* for *Candida albicans* secreted aspartic proteases (SAP) inhibition resulted in the identification of six new (**1**−**6**) and four known (**7**−**10**) serratene triterpenes, along with the known apigenin-4′-*O*-(2″,6″-di-*O*-*p*-coumaroyl)-*β*-D-glucopyranoside (**11**). On the basis of spectroscopic analysis, the structures of **1**−**10** were established as 3*β*,14α,15α,21*β*,-29-pentahydroxyserratane-24-oic acid (lycernuic acid C, **1**), 3*β*,14α,15α,21*β*-tetrahydroxyserratane-24-oic acid (lycernuic acid D, **2**), 3*β*,14*β*,21*β*-trihydroxyserratane-24-oic acid (lycernuic acid E, **3**), 3*β*,21*β*,29-trihydroxy-16-oxoserrat-14-en-24-methyl ester (lycernuic ketone A, **4**), 3α,21*β*,29-trihydroxy-16-oxoserrat-14-en-24-methyl ester (lycernuic ketone B, **5**), 3α,21*β*,24-trihydroxyserrat-14-en-16-one (lycernuic ketone C, **6**), 3*β*,21*β*-dihydroxyserrat-14-en-24-oic acid (lycernuic acid A, **7**), 3*β*,21*β*,29-trihydroxyserrat-14-en-24-oic acid (lycernuic acid B, **8**), serrat-14-en-3*β*,21*β*-diol (**9**), and serrat-14-en-3*β*,21α-diol (**10**). The ^{13}C NMR data for the known compounds **7** and **8** are reported for the first time. Compounds **1** and **11** showed inhibitory effects against *C. albicans* secreted aspartic proteases (SAP) with IC_{50} of 20 and 8.5 *μ*g/mL, respectively, while the other compounds were inactive.

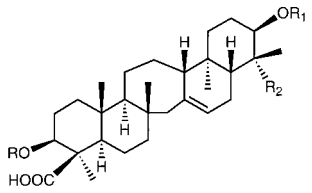
The secreted aspartic proteases (SAP) of *Candida albicans* have been shown to be a major virulence factor in *Candida* infections. Inhibition of SAP has been proposed as a new approach in the control of candidiasis.[1,2] As a part of our screening program searching for SAP inhibitors from higher plants,[3,4] we investigated the plant *Lycopodium cernuum* L. Previous work on this plant indicated the presence of alkaloids[5] and triterpenes of the serratene group.[5,6] Serratenes have been mainly isolated from conifers (Pinaceae and *Pinus* species) and club mosses (*Lycopodium* species).[7,8] The characteristic structural features of serratene triterpenes incorporate seven tertiary methyl groups (instead of eight methyls in common pentacyclic triterpenes), a central seven-membered C ring, and a double bond between C-14 and C-15. Bioassay-guided fractionation of an ethanol extract of a mixture of root-stem-leaf of *L. cernuum* L. (Lycopodiaceae) utilizing the SAP assay led to the isolation of several serratene triterpenes (**1**−**10**) and the known apigenin-4′-*O*-(2″,6″-di-*O*-*p*-coumaroyl)-*β*-D-glucoside (**11**).[9] We report herein the structure elucidation of the new triterpenes (**1**−**6**) and the biological evaluation of compounds **1**−**11**.



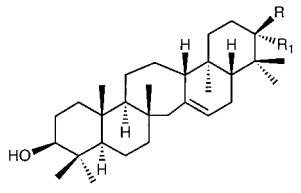
	R	R_1	R_2	R_3	R_4
1	H,	αOH,	αOH,	H,	CH_2OH
1a	Ac,	αOH,	αOAc,	Ac,	CH_2OAc
2	H,	αOH,	αOH,	H,	CH_3
3	H,	βOH,	H,	H,	CH_3
3a	Ac,	βOH,	H,	Ac,	CH_3
7b	Ac,	αOH,	αOH,	Ac,	CH_3
7c	Ac,	βOH,	βOH,	Ac,	CH_3



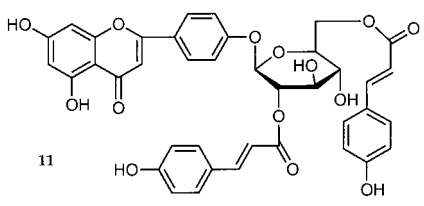
	R	R_1	R_2	R_3
4	OH,	H,	$COOCH_3$,	CH_2OH
5	H,	OH,	$COOCH_3$,	CH_2OH
6	H,	OH,	CH_2OH,	CH_3



7 R = H, R_1 = H, R_2 = CH_3
7a R = Ac, R_1 = Ac, R_2 = CH_3
7d R = Ac, R_1 = Ac, R_2 = CH_3 (Δ^{13-14})
8 R = H, R_1 = H, R_2 = CH_2OH



9 R = OH, R_1 = H
10 R = H, R_1 = OH



11

Results and Discussion

An ethanol extract of *Lycopodium cernuum* was dissolved in MeOH/H_2O (9:1) and then partitioned with *n*-hexane followed by $CHCl_3$. The activity resided in the MeOH/H_2O fraction. Repeated column chromatography (Experimental Section) of the aqueous methanol fraction resulted in the isolation of the serratene triterpenes (**1**, **2**, **6**−**8**) and apigenin-4′-*O*-(2″,6″-di-*O*-*p*-coumaroyl)-*β*-D-glucoside (**11**). Serratene triterpenes **3**, **4**, **5**, **9**, and **10** were obtained from the nonactive $CHCl_3$ fraction. The structures of the known compounds **7**−**10** were determined as 3*β*,21*β*-dihydroxyserrat-14-en-24-oic acid (lycernuic acid A, **7**),[6] 3*β*,21*β*,29-trihydroxyserrat-14-en-24-oic acid (lycernuic acid B, **8**),[6] serrat-14-en-3*β*,21*β*-diol,[10] and serrat-14-en-3*β*,21α-diol,[10] respectively, by comparison of the spectral data with published values. The ^{13}C NMR data for compounds **7** and

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Table 1. ^{13}C NMR Data of Compounds **1**, **1a**, **2**, **3**, **3a**, **4**−**7c**, and **8** (100 MHz in pyridine-d_5)[a]

C	1	1a	2	3	3a	4	5	6	7	7a	7b	7c	8
1	39.4	39.9	39.9	40.1	39.5	39.6	34.7	34.4	40.0	39.3	39.5	40.1	40.0
2	29.5	25.7	30.5	30.4	25.2	29.1	27.8	27.2[b]	29.8	25.2	25.5	25.8	29.7
3	78.4	80.2	78.9	78.9	80.4	78.2	70.4	70.3	78.5	80.2	81.4	81.6	78.4
4	49.7	49.3	49.5	49.8	48.7	50.0	48.8	44.6	49.7	49.1	49.0	49.0	49.7
5	56.8	57.6	57.6	57.6	57.0	56.9	49.5	50.5	57.3	57.1	57.3	57.7	57.3
6	21.7	21.5	22.0	22.0	23.7	21.1	21.0	19.9	21.6	21.3	22.0	21.3	21.6
7	44.7	46.2	45.5	46.0	46.0	45.4	45.6	46.1	45.9	45.6	44.7	47.2	46.0
8	38.0	38.0	38.0	38.3	38.4	38.1	38.4	38.6	37.6	37.5	37.3	37.2	37.6
9	59.8	61.5	59.7	60.4	60.6	62.4	62.4	63.0	62.9	62.8	59.9	63.4	62.9
10	39.2	39.3	39.3	39.4	39.2	38.9	39.3	38.9	39.3	39.1	39.0	39.3	39.2
11	25.7	24.8[b]	26.0[b]	26.2[b]	26.8	25.7	25.7	25.5	27.0	25.8	26.8	27.0	26.5
12	26.8[b]	26.9	26.5[b]	26.8[b]	30.4	30.3	27.1	27.1[b]	28.1	27.7	29.3	29.3[b]	28.1
13	59.0	59.1	59.5	60.3	59.2	59.4	59.5	59.4	57.6	57.4	58.9	61.1	57.8
14	78.2	76.8	78.1	75.7	75.4	164.4	164.8	164.1	139.3	139.1	78.3	79.4	139.2
15	77.0	80.0	76.7	46.0	44.7	129.3	129.2	129.3	123.2	122.8	76.6	73.2	123.3
16	28.4	24.9[b]	28.2	19.6	19.3	202.3	202.4	201.7	24.9	24.5	28.0	29.5[b]	24.9
17	47.3	48.9	46.5	49.8	51.2	60.6	60.6	59.9	44.1	45.1	48.0	40.9	45.1
18	39.0	39.0	39.0	39.4	39.0	45.1	45.1	45.0	36.7	36.5	38.6	38.9	36.7
19	34.1	33.8	33.7	33.8	34.4	32.3	32.3	32.4	32.2	32.4	34.4	36.3	32.2
20	26.6[b]	23.3	26.1[b]	26.6[b]	25.7	26.2	26.2	26.3	26.0	23.6	23.6	23.7	26.2
21	70.2	73.6	75.3	75.5	78.6	70.3	70.4	76.2	75.6	78.4	78.4	78.4	70.1
22	44.3	41.5	38.2	38.6	37.5	44.1	44.1	37.9	38.3	37.1	38.3	38.6	43.8
23	24.8	25.7	26.1	25.9	24.5	24.5	25.1	24.0	25.1	24.5	25.3	25.5	25.0
24	183.9	180.4	183.3	183.3	177.1	177.8	178.5	66.1	181.1	177.6	179.0	179.3	181.0
25	14.8	14.9	15.2	15.3	15.1	14.1	14.0	16.9	14.6	14.3	15.2	15.1	14.6
26	23.3	21.9	23.0	23.1	23.8	20.0	20.0	20.4	20.1	20.1	24.0	22.6	20.1
27	54.4	55.5	54.9	63.4	62.2	56.1	56.4	56.3	57.1	56.8	54.4	55.8	57.1
28	17.2	16.8	16.4	16.8	16.4	16.2	16.2	15.5	14.2	13.7	16.3	16.6	15.0
29	65.9	66.8	22.9	22.9	22.6	64.5	64.5	22.5	22.5	21.7	22.6	22.5	65.4
30	23.9	22.4	29.5	29.8	28.7	23.3	23.4	29.4	29.0	27.6	28.5	28.1	23.3
OCH_3						51.4	51.4						
C=O-3		171.8			171.3					171.5	171.5	171.9	
C=O-15		171.0											
C=O-21		170.8			170.8					170.8	170.8	170.9	
C=O-29		171.3											
CCH_3-3		21.6			21.7					21.6	21.8	21.9	
CCH_3-15		21.4											
CCH_3-21		21.4			21.6					21.4	21.6	21.4	
CCH_3-29		20.9											

[a] The assignment was based upon DEPT, COSY, HMQC, and HMBC experiments. [b] The data with the same superscript in each column may be interchangeable.

8 (Table 1) are reported for the first time. Compound **11** was identified as apigenin-4′-*O*-(2″,6″-di-*O*-*p*-coumaroyl-*β*-D-glucoside), by comparison of the spectral data with literature values.[9] In addition, alkaline hydrolysis of **11** afforded *trans*-*p*-coumaric acid (identified with an authentic sample) and apigenin-4′-*O*-*β*-D-glucoside (identified by comparison of its spectral data with literature values).[11] Acid hydrolysis of **11** yielded glucose detected by co-TLC with the authentic sugar D-glucose.

The molecular formula $C_{30}H_{50}O_7$ for **1**, established by positive-ion HRESIMS, was 34 mass units (2 × OH) higher than lycernuic acid B (**8**). Analysis of the ^{1}H and ^{13}C NMR spectra indicated that **1** was related to **8** (Tables 1 and 2). In the ^{13}C NMR spectrum of **8**, the double bond signals for C-14 (*δ* 139.2) and C-15 (*δ* 123.3) were replaced in **1** by signals at *δ* 78.2 (C-14) and 77.0 (C-15), clearly indicating the location of the two hydroxyl groups at C-14 and C-15. The ^{13}C NMR and DEPT 135 spectra showed 30 carbons for five methyls, 10 methylenes, four methines, five quaternary carbons, three oxymethines (*δ* 78.4, C-3; *δ* 77.0, C-15 and *δ* 70.2, C-21), one oxymethylene (*δ* 65.9, C-29), one oxyquaternary carbon (*δ* 78.2, C-14), and one carboxyl carbon (*δ* 183.9, C-24). The assignments of the carboxyl group to C-4, the four hydroxyl groups to carbons 3, 14, 15, and 21, and the oxymethylene to C-22 were based on HMBC correlations (Figure 1). The orientations of the carboxyl group at C-4 and the hydroxymethyl group at C-22 were deduced by comparison of their carbon chemical shifts with those of **8**. The H-3 in **1** is axial (on the α-face), as indicated by its ^{1}H NMR resonance at *δ* 3.27 (1 H, dd, *J* = 11 and 4 Hz), whereas H-21 appears to be equatorial (on the α-face) with a resonance at *δ* 4.50 (1H, br s).[10] These data were in agreement with those of **8**. The relative stereochemistry for OH-14 and OH-15 were deduced from **1a**, the corresponding tetraacetate of **1**, using a ROESY experiment (Figure 2). Correlations of the proton at H-15 (*δ* 5.03) with CH_3-26*β* (*δ* 1.07), H-13*β* (*δ* 1.43), and H-17*β* (*δ* 1.88) indicated that H-15 was *β*-oriented; hence OH-15 is α-oriented. On the other hand, correlation of OH-14 (*δ* 5.20) with CH_3-28α (*δ* 1.27) established the α-orientation of the OH-14. In the HMBC, the proton of the OH-14 (*δ* 5.20) displayed two clear cross-peaks with C-13 (*δ* 59.1) and C-14 (*δ* 76.8) (Figure 1).

To further confirm the stereochemistry for OH-14 and OH-15 in compound **1**, compound **7** was converted to its corresponding diacetate (**7a**), which was subjected to osmolation. Osmolation of **7a** gave two isomeric glycols, **7b** and **7c**.[12] The minor glycol **(7b)** was proven to be 14α,15α-diol, while the major one (**7c**) to be 14*β*,15*β*-diol.[12] The ^{13}C NMR spectra allowed for the differentiation of the isomers as the chemical shifts of carbons C-13, C-14, C-15, C-17, and C-27 of the α-isomer (**7b**) displayed significant differences of Δ*δ* −2.2 ppm, Δ*δ* −1.1 ppm, Δ*δ* +3.4 ppm, Δ*δ* +7.1 ppm, and Δ*δ* −1.4 ppm, respectively, when compared to those of the *β*-isomer (**7c**) (Table 1). The configuration of OH-15 was also indicated by the characteristic ^{1}H NMR signals at *δ* 3.60 (1H, t, *J* = 6.0 Hz, *β*H-15) for **7b** and *δ* 4.01 (1H, br t, αH-15) for **7c** (Experimental Section). The

Table 2. ^{1}H NMR Data of Compounds **1**−**8** (400 MHz in pyridine-d_5)[a]

H	1	2	3	4	5	6	7	8
1	1.10 (1H, m)	1.12 (1H, m)	1.05 (1H, m)	0.98 (1H, m)	1.60 (1H, m)	1.56 (1H, m)	1.09 (1H, m)	1.09 (1H, m)
	1.90 (1H, m)	1.91 (1H, m)	1.88 (1H, m)	1.84 (1H, m)	1.75 (1H, m)	1.83 (1H, m)	1.95 (1H, m)	1.94 (1H, m)
2	1.98 (1H, m)	2.00 (1H, m)	1.97 (1H, m)	1.94 (1H, m)	1.92 (1H, m)	1.91 (1H, m)	2.00 (1H, m)	1.99 (1H, m)
	2.45 (1H, m)	2.52 (1H, m)	2.55 (1H, m)	2.46 (1H, m)	2.43 (1H, m)	2.16 (1H, m)	2.51 (1H, m)	2.45 (1H, m)
3	3.27 (1H, dd, 12/4 Hz)	3.31 (1H, dd, 11.5/4.0 Hz)	3.24 (1H, dd, 12/4 Hz)	3.34 (1H, dd, 11/4 Hz)	4.49 (1H, br s)	4.45 (1H, br s)	3.38 (1H, dd, 12.0/4.2 Hz)	3.38 (1H, dd, 12/4 Hz)
5	1.07 (1H, m)	1.05 (1H, m)	1.00 (1H, m)	0.98 (1H, m)	1.99 (1H, m)	1.87 (1H, m)	1.05 (1H, m)	1.06 (1H, m)
6	1.92 (1H, m)	2.02 (1H, m)	2.03 (1H, m)	1.82 (2H, m)	1.78 (2H, m)	1.65 (2H, m)	2.00 (1H, m)	1.98 (1H, m)
	2.30 (1H, m)	2.56 (1H, m)	2.58 (1H, m)				2.35 (1H, m)	2.32 (1H, m)
7	1.49 (1H, m)	1.54 (1H, m)	1.49 (1H, m)	1.36 (1H, m)	1.22 (1H, m)	1.25 (1H, m)	1.25 (1H, m)	1.25 (1H, m)
	1.67 (1H, m)	1.63 (1H, m)	1.66 (1H, m)	1.40 (1H, m)	1.42 (1H, m)	1.38 (1H, m)	1.50 (1H, m)	1.44 (1H, m)
9	1.27 (1H, m)	1.30 (1H, m)	1.29 (1H, m)	0.81 (1H, m)	0.98 (1H, m)	1.03 (1H, m)	0.81 (1H, m)	0.78 (1H, m)
11	1.16 (1H, m)	1.18 (1H, m)	1.16 (1H, m)	1.10 (1H, m)	1.08 (1H, m)	1.10 (1H, m)	1.17 (1H, m)	1.15 (1H, m)
	1.76 (1H, m)	1.80 (1H, m)	1.80 (1H, m)	1.85 (1H, m)	1.80 (1H, m)	1.80 (1H, m)	1.86 (1H, m)	1.85 (1H, m)
12	1.98 (1H, m)	1.95 (1H, m)	1.93 (1H, m)	1.24 (1H, m)	1.10 (1H, m)	1.11 (1H, m)	1.99 (1H, m)	1.95 (1H, m)
	2.05 (1H, m)	2.10 (1H, m)	2.10 (1H, m)	1.31 (1H, m)	1.93 (1H, m)	1.90 (1H, m)	2.06 (1H, m)	2.01 (1H, m)
13	1.70 (1H, m)	1.70 (1H, m)	1.56 (1H, m)	2.54 (1H, m)	2.54 (1H, m)	2.48 (1H, m)	2.06 (1H, m)	2.08 (1H, m)
15	3.62 (1H, t, 7.9 Hz)	3.69 (1H, t, 8.0 Hz)	1.93 (2H, m)	5.96 (1H, s)	5.94 (1H, s)	5.94 (1H, s)	5.50 (1H, br s)	5.47 (1H, br s)
16	2.20 (2H, m)	2.10 (2H, m)	1.52 (1H, m)				2.05 (1H, m)	2.15 (1H, m)
			2.00 (1H, m)				2.16 (1H, m)	2.25 (1H, m)
17	2.08 (1H, m)	1.88 (1H, m)	1.80 (1H, m)	3.27 (1H, s)	3.26 (1H, s)	3.03 (1H, s)	2.15 (1H, m)	2.35 (1H, m)
19	1.65 (1H, m)	1.66 (1H, m)	1.66 (1H, m)	1.61 (1H, m)	1.56 (1H, m)	1.58 (1H, m)	1.70 (1H, m)	1.70 (1H, m)
	1.93 (1H, m)	1.88 (1H, m)	1.85 (1H, m)	2.29 (1H, m)	2.31 (1H, m)	2.25 (1H, m)	2.00 (1H, m)	2.03 (1H, m)
20	1.98 (1H, m)	1.95 (1H, m)	1.85 (1H, m)	1.96 (1H, m)	1.93 (1H, m)	1.90 (1H, m)	1.86 (1H, m)	1.85 (1H, m)
	2.05 (1H, m)	2.10 (1H, m)	2.10 (1H, m)	2.18 (1H, m)	2.19 (1H, m)	2.05 (1H, m)	2.05 (1H, m)	2.10 (1H, m)
21	4.50 (1H, br s)	3.66 (1H, br s)	3.65 (1H, br s)	4.58 (1H, br s)	4.56 (1H, br s)	3.59 (1H, br s)	3.68 (1H, br s)	4.56 (1H, br s)
23	1.64 (3H, s)	1.70 (3H, s)	1.66 (3H, s)	1.55 (3H, s)	1.53 (3H, s)	1.60 (3H, s)	1.72 (3H, s)	1.69 (3H, s)
24						3.86, 4.08 (each 1H, d, 10.5 Hz)		
25	1.04 (3H, s)	1.13 (3H, s)	1.11 (3H, s)	0.76 (3H, s)	0.80 (3H, s)	0.90 (3H, s)	1.05 (3H, s)	1.02 (3H, s)
26	0.98 (3H, s)	1.02 (3H, s)	0.99 (3H, s)	0.76 (3H, s)	0.81 (3H, s)	0.74 (3H, s)	0.89 (3H, s)	0.87 (3H, s)
27	1.78, 2.05	1.90, 2.05	1.52, 1.78	1.91, 2.36	1.87, 2.36	1.89, 2.34	1.87, 2.36	1.86, 2.32
28	1.36 (3H, s)	1.32 (3H, s)	1.33 (3H, s)	0.89 (3H, s)	0.87 (3H, s)	0.89 (3H, s)	0.85 (3H, s)	0.87 (3H, s)
29	3.92, 4.21 (each 1H, d, 10.9 Hz)	0.97 (3H, s)	0.95 (3H, s)	4.11, 4.76 (each 1H, d, 10.2 Hz)	4.09, 4.74 (each 1H, d, 10.7 Hz)	1.38 (3H, s)	0.96 (3H, s)	3.93, 4.16 (each 1H, d, 10.9 Hz)
30	1.63 (3H, s)	1.23 (3H, s)	1.23 (3H, s)	1.98 (3H, s)	1.97 (3H, s)	1.71 (3H, s)	1.16 (3H, s)	1.54 (3H, s)
OCH_3				3.63 (3H, s)	3.67 (3H, s)			

[a] The assignment was based upon COSY, HMQC, HMBC, and ROESY experiments.

chemical shifts for C-13, C-14, C-15, C-17, and C-27 for compound **1** are almost identical with those of **7b**, supporting the 14α,15α configuration for the OH groups at these positions. The ^{1}H and ^{13}C NMR assignments for compound **1** (Tables 1 and 2) were unambiguously made using HMQC and HMBC NMR correlations (Figure 1). On the basis of the above evidence, the structure of **1** was established as 3*β*,14α,15α,21*β*,29-pentahydroxyserratane-24-oic acid, a new natural product named lycernuic acid C.

Compound **2** was obtained as a colorless powder. Its molecular formula of $C_{30}H_{50}O_6$ was determined by HRESIMS and indicated that it had one less oxygen atom when compared with **1**. Examination of the NMR data of **2** showed that **2** is related to **7** in the same manner as **1** is to **8** (Tables 1 and 2). Detailed NMR analysis indicated that **2** differs from **1** only in ring E. In the ^{13}C NMR of **1**, the signal at *δ* 65.9 (t, C-29) was replaced in **2** by a signal at *δ* 22.9 (q), indicating that a methyl group was present at C-29. The ^{13}C NMR data for C-13, C-14, C-15, C-17, and C-27 of **2** are very close to those of **1** and **7b**, but differ from those of **7c**, proving the α-configuration for both OH-14 and OH-15 in **2**. On the basis of the above evidence, the structure of **2** was established as 3*β*,14α,15α,21*β*-tetrahydroxyserratane-24-oic acid, a new natural product named lycernuic acid D.

Compound **3** has a molecular formula of $C_{30}H_{50}O_5$, as deduced from the HRESIMS ion at *m*/*z* 513.3516 $[M + Na]^+$. The molecular formula is one oxygen atom less than that of **2**. The ^{13}C NMR and DEPT 135 spectra also showed that **3** has only three hydroxyl groups including two oxymethines and one oxyquaternary. HMBC correlations of **3** (Figure 1) established the three hydroxyl groups at C-3 (*δ* 78.9), C-14 (*δ* 75.7), and C-21 (*δ* 75.5). The NMR data (Tables 1 and 2) for A and E rings of **3** and **2** are very similar, indicating that both **3** and **2** have the same configurations for OH-3 and OH-21. Dehydration of **3a**, the corresponding diacetate of **3**, with thionyl chloride in pyridine yielded **7a** as a sole product, indicative of a *β*-configuration for OH-14. If the configuration of OH-14 was α, dehydration[13] of **3a** would produce a mixture of **7a** (Δ^{14}) and **7d** (Δ^{13}). Confirmation of the ^{1}H and ^{13}C NMR assignments was made with the analyses of HMQC and HMBC (Figure 1). Therefore, **3** was shown to be 3*β*,14*β*,-21*β*-trihydroxyserratane-24-oic acid, a new natural product named lycernuic acid E.

Compound **4** has molecular formula $C_{31}H_{48}O_6$, established by HRESIMS. The^{13}C NMR and DEPT 135 spectra of **4** showed 31 carbon signals, for a ketone group (*δ* 202.3), an ester carbonyl group (*δ* 177.8), a double bond (*δ* 164.4, C-14 and *δ* 129.3, C-15), two oxymethines (*δ* 78.2, C-3 and *δ* 70.3, C-21), one oxymethylene (*δ* 64.5), one carbomethoxy (*δ* 51.4), five methyls, nine methylenes, four methines, and five quaternary carbons. The positions of the ketone group at C-16, the carbomethoxy at C-4, and the oxymethylene at C-22 were established by HMBC correlations, as shown in Figure 1. The ^{13}C NMR downfield shift values (Table 1) for C-14 (Δ*δ* 25 ppm), C-15 (Δ*δ* 6 ppm), and C-17 (Δ*δ* 15

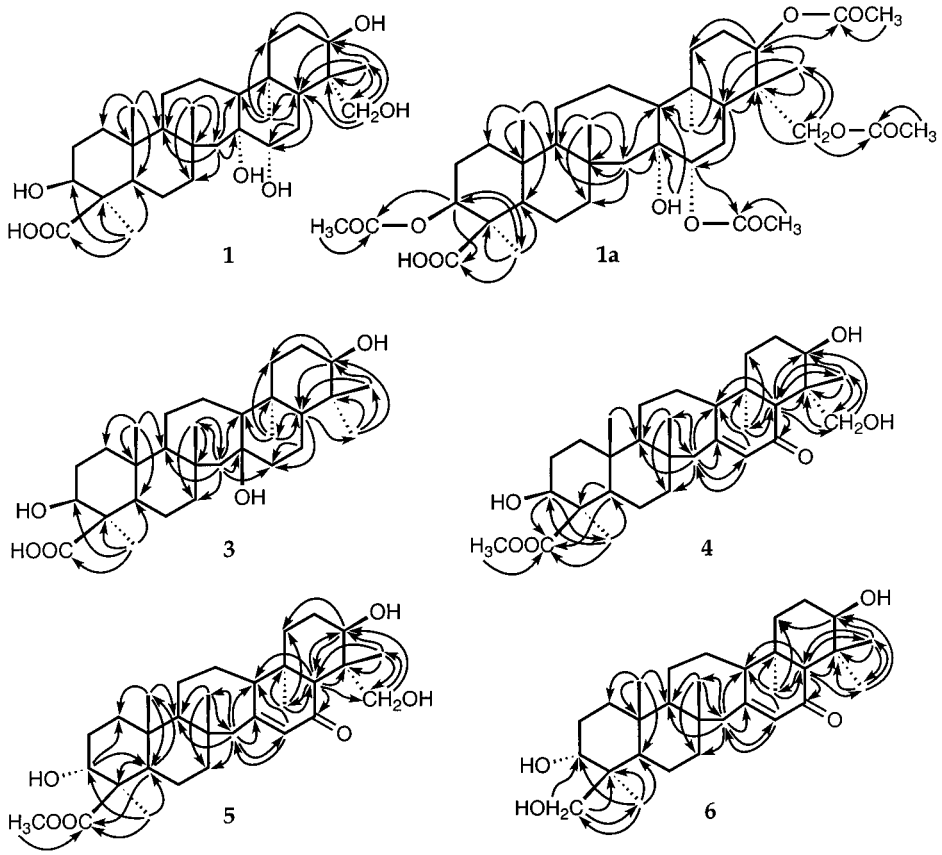

Figure 1. HMBC correlations for compounds **1**, **1a**, and **3**−**6**.

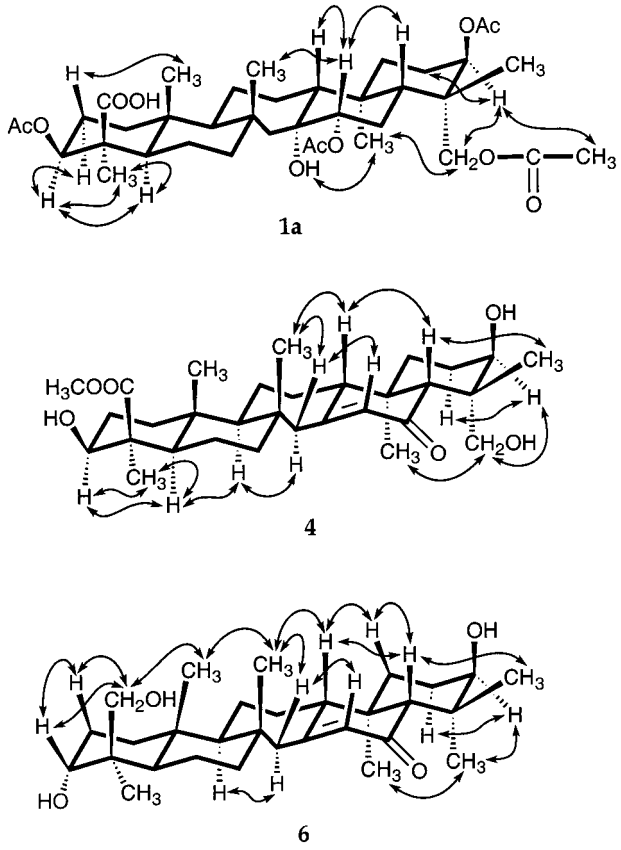

Figure 2. Selective ROESY correlations for **1a**, **4**, and **6**.

ppm), as well as the ^{1}H NMR downfield value for H-17 ($\Delta\delta$ 0.92 ppm), when compared with those of **8**, further confirmed the assigned ketone position. Both H-3 and H-21 were determined to have α-orientation, as indicated by their characteristic ^{1}H NMR signals at δ 3.34 (1H, dd, J = 11 and 4 Hz, H-3) and δ 4.58 (1H, br s, H-21).[10] The NOE correlations (Figure 2) of αH-3 with αH-5 and αH_3-23, and αH-5 with αH_3-23, as well as the αH-29 with αH-21 and αH_3-28, confirmed not only the α-orientation for H-3 and H-21, but also the α-orientation of CH_2OH-29 and the β-orientation of $COOCH_3$-24. Confirmation was made with the analyses of the spectra of **4** (Figure 1). On the basis of the above evidence, the structure of **4** was established as 3β,21β,29-trihydroxy-16-oxoserrat-14-en-24-methyl ester, a new natural product named lycernuic ketone A.

Compound **5** had the same molecular formula as **4**, $C_{31}H_{48}O_6$, established by HRESIMS. Detailed NMR comparison showed that **5** differs from **4** only in the configuration of OH-3. In compound **5**, the OH-3 was assigned an α-orientation, as indicated by the characteristic ^{1}H NMR signal[10] at δ_H 4.49 (1H, br s, H-3, β-epimer), as compared with the H-3 α-epimer (**4**) with a signal at δ_H 3.34 (1H, dd, J = 11 and 4 Hz). The significant chemical shift differences (Table 1) for carbons 1−5 between **5** and **4** further supported the α-configuration[14] for OH-3 in **5**. The ^{1}H and ^{13}C NMR assignments were unambiguously made using HMQC and HMBC NMR correlations (Figure 1). Therefore, **5** was established to be 3α,21β,29-trihydroxy-16-oxoserrat-14-en-24-methyl ester, a new natural product named lycernuic ketone B.

The HRESIMS of compound **6** displayed a molecular ion peak at m/z $[M + Na]^+$ 495.3474, supporting a molecular formula of $C_{30}H_{48}O_4$. The ^{1}H and ^{13}C NMR spectra of **6** are very similar to those of **5**. The ^{13}C NMR spectrum of **6** shows signals for a ketone group (δ 201.7), a double bond (δ 164.1, C-14 and δ 129.3, C-15), two oxymethines (δ 76.2, C-21 and δ 70.3, C-3), one oxymethylene (δ 66.1, C-24), six methyls, nine methylenes, four methines, and five quater-

nary carbons (assigned by DEPT spectrum). The ketone group at C-16 and the oxymethylene at C-4 were established by HMBC correlations as shown in Figure 1. The oxymethylene was shown to have a β-orientation by the presence of two key NOE correlations between H-24 (δ 3.86 and 4.08) and H_3-25 (δ 0.90). In the ^{1}H NMR spectrum of **6**, both 3β and 21α protons are shown to be equatorial, as indicated by broad singlets in the^{1}H NMR spectrum at δ 4.45 (H-3) and 3.59 (H-21), respectively.[10] ROESY correlations (Figure 2) confirmed these assignments. Furthermore, the ^{13}C NMR data for the A ring of **6** were very close to those of **5**, also indicating the α-configuration for OH-3. The ^{1}H and ^{13}C NMR signals (Tables 1 and 2) of **6**, a new natural product named lycernuic ketone C (3α,21β,24-trihydroxylserrat-14-en-16-one), were unambiguously assigned using HMQC and HMBC NMR correlations (Figure 1).

Crude extracts were initially tested in the *Candida*-secreted aspartic proteases (SAP) inhibition assay[3] at a single concentration of 200 μg/mL to identify active extracts ($\geq$80% inhibition). Active extracts were confirmed by retesting at three concentrations (50, 10, 2 μg/mL). The aspartic protease inhibitor pepstatin A was used as a positive control (IC_{50} 0.0015 μg/mL). The crude EtOH extract was tested in the SAP assay and was found to have an IC_{50} of 30 μg/mL. Only compounds **1** and **11** are active, with IC_{50}'s of 20 and 8.4 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and were uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Hewlett-Packard 8435 spectrometer. IR spectra were obtained using a ATI Mattson Genesis Series FTIR spectrometer. The NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. 2D NMR spectra were measured on a Bruker Avance DPX-500 operating at 500 MHz using standard pulse programs and acquisition parameters. HRESIMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. Si gel (40 μm, J. T. Baker) and RP Si gel (RP-18, 40 μm, J. T. Baker) were used for low-pressure chromatography. HPLC was performed using an ODS column (Phenomenex, Prodigy ODS prep, 21.2 mm i.d. $\times$ 250 mm, 10 μm). TLC was performed on Si gel 60 F_{254} (EM Science) using $CHCl_3$/MeOH (6:1, solvent A), toluene/EtOAc (1:3, solvent B), $CHCl_3$/MeOH (14:1, solvent C), or reversed-phase KC_{18} F Si gel 60 (Whatman) using MeOH/H_2O (70:30, solvent D), MeOH (100%, solvent E), and *trans-p*-coumaric acid (Sigma).

Plant Material. *Lycopodium cernuum* (root-stem-leaf) was collected in Peru. A voucher specimen is on deposit at the National Center for Natural Products Research, The University of Mississippi (voucher # IBE 9729).

Extraction and Isolation. The powdered material (445 g) was percolated with 95% EtOH (3000 mL $\times$ 4), and the alcoholic extracts were combined and evaporated to dryness (38.4 g). Part of the ethanol extract (30.0 g) was dissolved in MeOH/H_2O (9:1, 1000 mL) and then partitioned successively with hexane (600 mL $\times$ 3) and $CHCl_3$ (1000 mL) to give hexane (1.98 g), $CHCl_3$ (17.2 g), and MeOH/H_2O (11.0 g) fractions. Part of the MeOH/H_2O fraction (8.0 g) was passed over a Diaion HP 20 column (200 g) and then washed with 10%, 50%, 80%, and 100% MeOH (each 1000 mL). The activity resided in the 80% MeOH fraction (fraction A, 1.4 g, IC_{50} 30 μg/mL). Part of fraction A (1.1 g) was chromatographed over a low-pressure ODS column (40 g) using mixtures of MeOH/H_2O (30:70, 50:50, 80:20, 100:0 each 300 mL) to give fractions B (25 mg, 0−300 mL), C (26 mg, 300−450 mL), D (28 mg, 450−600 mL), E (930 mg, 600−900 mL, IC_{50} 20 μg/mL), and F (75 mg, 900−1200 mL). Part of fraction E (600 mg) was refractionated on a Si gel column (100 g) using $CHCl_3$/MeOH (9:1, 4:1, and 3:2, each 800 mL) to give a total of 25 fractions. Fractions were monitored by TLC and similar fractions combined to afford fractions E_1 (56 mg, 0−100 mL), E_2 (86 mg, 100−200 mL), E_3 (46 mg, 200−300 mL, IC_{50} 40 μg/mL), E_4 (25 mg, 300−400 mL, IC_{50} 15 μg/mL), E_5 (40 mg, 400−600 mL, IC_{50} 15 μg/mL), E_6 (10 mg, 600−700 mL), E_7 (86 mg, 700−900 mL), E_8 (86 mg, 900−1200 mL, IC_{50} 45 μg/mL), E_9 (56 mg, 1200−1500 mL), E_{10} (40 mg, 1500−1900 mL), and E_{11} (52 mg, 1900−2400 mL). Fraction E_5 was purified by HPLC to give **11** (10 mg, MeOH/H_2O, 70:30, 3 mL/min, UV 314 nm, t_R 45.1 min). Fractions E_3 and E_4 were combined and chromatographed over a low-pressure ODS column (30 g), which was eluted with MeOH/H_2O (40:60, 75:25, 100:0, each 100 mL) to give F_1 (7 mg, 0−120 mL), F_2 (25 mg, 120−160 mL), F_3 (30 mg, 220−260 mL), and F_4 (4 mg, 260−300 mL). Fraction F_3 was rechromatographed over a low-pressure ODS column (30 g) washing with MeOH/H_2O (70:30, 1500 mL) to give **7** (10.3 mg, 1060−1140 mL), **8** (6.0 mg, 940−1000 mL), and **11** (6.5 mg, 860−920 mL). F_2 was further separated by ODS column (30 mg) washing with MeOH/H_2O (70:30, 600 mL) to afford **2** (2.6 mg, 160−200 mL) and **6** (2.0 mg, 460−500 mL). Refractionation of fraction E_8 using a Si gel column (50 g) and eluting with $CHCl_3$/MeOH (7:1, 500 mL) furnished 25 fractions. Of these, fractions 10−14 contained **1** (10 mg, 180−280 mL).

Part of the $CHCl_3$ fraction (5.6 g) was applied on a Si gel column (200 g) eluting with $CHCl_3$/MeOH (9:1 and 4:1, 1000 mL, each) to give 10 fractions, G_1 (0.45 g, 0−200 mL), G_2 (0.26 g, 200−400 mL), G_3 (0.98 g, 400−600 mL), G_4 (0.31 g, 600−800 mL), G_5 (0.58 g, 800−1000 mL), G_6 (0.38 g, 1000−1200 mL), G_7 (0.29 g, 1200−1400 mL), G_8 (0.33 g, 1400−1600 mL), G_9 (0.57 g, 1600−1800 mL), and G_{10} (1.35 g, 1800−2000 mL). Part of G_1 (200 mg) was rechromatographed on a Si gel column (100 g) eluting with $CHCl_3$/MeOH (19:1, 1800 mL) to give **9** (12 mg, 450−550 mL), **10** (11 mg, 700−850 mL), and **7** (3 mg, 1400−1500 mL). Refractionation of part of fraction G_3 (400 mg) using an ODS column (15.0 g) and washing with MeOH/H_2O (75:25, 100 mL) and MeOH (100 mL) gave three fractions, H (3.0 mg, 0−30 mL), I (120 mg, 30−100 mL), and J (200 mg, 100−200 mL). Fraction H contained **3** (3.0 mg). Fraction I was repeatedly purified by preparative TLC (Si gel, $CHCl_3$/MeOH, 12:1), furnishing **4** (3.0 mg), **5** (12.0 mg), and **6** (12.0 mg).

Lycernuic acid C (**1**)**:** colorless powder; mp 265−266 °C (MeOH/$CHCl_3$); $[\alpha]^{22}_D$ −42.0° (*c* 0.20, MeOH); IR (KBr) ν_{max} 3397, 2930, 2855, 1697, 1568, 1447, 1414, 1245, 1168, 1025 cm^{-1}; ^{13}C and ^{1}H NMR data (Tables 1 and 2); HRESIMS *m*/*z* 505.3525 $[M - OH]^+$, 523.3629 $[M + H]^+$, 545.3447 $[M + Na]^+$, 561.3188 $[M + K]^+$, 1067.7000 [dimer + Na]$^+$ (calcd for $C_{30}H_{50}O_7$ 505.3523 $[M - OH]^+$, 523.3629 $[M + H]^+$, 545.3448 $[M + Na]^+$, 561.3188 $[M + K]^+$, 1067.7004 [dimer + Na]$^+$), R_f 0.26 and 0.07 (Si gel, solvents A and B, respectively), 0.77 (reversed-phase KC18 F, solvent D).

Lycernuic acid D (**2**)**:** colorless powder; mp 289−290 °C (MeOH/$CHCl_3$); $[\alpha]^{26}_D$ −35.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3422, 2928, 2865, 1695, 1567, 1457, 1386, 1247, 1064, 1025, 999 cm^{-1}; ^{13}C and ^{1}H NMR data (Tables 1 and 2); HRESIMS *m*/*z* 529.3474 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_6Na$ 529.3499); R_f 0.55 and 0.19 (Si gel, solvents A and B, respectively), 0.59 (reversed-phase KC_{18} F, solvent D).

Lycernuic acid E (3): colorless powder; mp 245−246 °C (MeOH/$CHCl_3$); $[\alpha]^{26}_D$ −46.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3426, 2932, 2867, 1702, 1562, 1455, 1385, 1245, 1066, 1000 cm^{-1}; ^{13}C and ^{1}H NMR data (Tables 1 and 2); HRESIMS *m*/*z* 473.3642 $[M - OH]^+$, 491.3828 $[M + H]^+$, 513.3516 $[M + Na]^+$, 529.3302 $[M + K]^+$, 1003.7219 [dimer + Na]$^+$ (calcd for $C_{30}H_{50}O_5$ 473.3625 $[M - OH]^+$, 491.3731 $[M + H]^+$, 513.3550 $[M + Na]^+$, 529.3290 $[M + K]^+$, 1003.7208 [dimer + Na]$^+$; R_f 0.59 and 0.31 (Si gel, solvents A and B, respectively), 0.21 (reversed-phase KC_{18} F, solvent D).

Lycernuic ketone A (**4**)**:** colorless powder; mp 176−177 °C (MeOH/$CHCl_3$); $[\alpha]^{26}_D$ −22.5° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.25) nm; IR (KBr) ν_{max} 3424, 2928, 1720, 1652,

1457, 1376, 1299, 1238, 1217, 1030 cm^{-1}; ^{13}C and ^1H NMR data (Tables 1 and 2); HRESIMS *m/z* 499.3437 $[M - OH]^+$, 517.3543 $[M + H]^+$, 539.3330 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_6$ 499.3418 $[M - OH]^+$, 517.3524 $[M + H]^+$, 539.3343 $[M + Na]^+$); R_f 0.65 and 0.29 (Si gel, solvents A and B, respectively), 0.19 (reversed-phase KC_{18} F, solvent D).

Lycernuic ketone B (5): colorless powder; mp 189−190 °C ($MeOH/CHCl_3$); $[\alpha]^{26}_D$ +51.7° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.30) nm; IR (KBr) ν_{max} 3420, 2936, 1724, 1649, 1455, 1223, 1029, 994 cm^{-1}; ^{13}C and ^1H NMR data (Tables 1 and 2); HRESIMS *m/z* 499.3436 $[M - OH]^+$, 517.3547 $[M + H]^+$, 539.3340 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_6$ 499.3418 $[M - OH]^+$, 517.3524 $[M + H]^+$, 539.3343 $[M + Na]^+$); R_f 0.60 and 0.27 (Si gel, solvents A and B, respectively), 0.31 (reversed-phase KC_{18} F, solvent D).

Lycernuic ketone C (6): colorless powder; mp 235−236 °C ($MeOH/CHCl_3$); $[\alpha]^{26}_D$ +28.3° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.09) nm; IR (KBr) ν_{max} 3395, 2934, 1653, 1455, 1383, 1241, 1060, 1028, 994 cm^{-1}; ^{13}C and ^1H NMR data (Tables 1 and 2); HRESIMS *m/z* 495.3474 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4$ Na 495.3445); R_f 0.63 and 0.24 (Si gel, solvents A and B, respectively), 0.15 (reversed-phase KC_{18} F, solvent D).

Lycernuic acid A (7): colorless powder; mp 322−324 °C ($MeOH/CHCl_3$); $[\alpha]^{26}_D$ −21.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3374, 2962, 2941, 2899, 2843, 1692, 1461, 1385, 1248, 1161, 1110, 1066, 1040, 982 cm^{-1}; ^{13}C and ^1H NMR data (Tables 1 and 2); HRESIMS *m/z* 471. 3483 $[M - H]^-$ (calcd for $C_{30}H_{47}O_4$, 471.3479); R_f 0.71 and 0.34 (Si gel, solvents A and B, respectively), 0.13 (reversed-phase KC_{18} F, solvent D).

Lycernuic acid B (8): colorless powder; mp 304−305 °C ($MeOH/CHCl_3$); $[\alpha]^{26}_D$ −31.0 ° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3457, 3411, 2955, 2931, 2900, 2840, 1694, 1455, 1382, 1247, 1160, 1110, 1067, 1036, 974 cm^{-1}; ^{13}C and ^1H NMR data (Tables 1 and 2); HRESIMS *m/z* 487.3430 $[M - H]^-$ (calcd for $C_{30}H_{47}O_5$, 487.3429); R_f 0.50 and 0.16 (Si gel, solvents A and B, respectively), 0.33 (reversed-phase KC_{18} F, solvent D).

Serrat-14-en-3β,21β-diol (9): colorless powder; mp 303−304 °C (MeOH) [lit.[10] mp 305−308 °C]; $[\alpha]^{26}_D$ −19.6° (*c* 0.3, $CHCl_3$); R_f 0.82 and 0.60 (Si gel, solvents B and C, respectively), 0.43 (reversed-phase KC_{18} F, solvent E). The ^{13}C and ^1H NMR data were identical to the reported values.[10]

Serrat-14-en-3β,21α-diol (10): colorless powder; mp 298−299 °C (MeOH) [lit.[15] mp 300 °C]; $[\alpha]^{26}_D$ +21.5° (*c* 0.30, $CHCl_3$) [lit.[15] $[\alpha]^{22}_D$ +19° (*c* 0.80, $CHCl_3$)]; R_f 0.78 and 0.55 (Si gel, solvents B and C, respectively), 0.38 (reversed-phase KC_{18} F, solvent E). The ^{13}C and ^1H NMR data were identical to the reported values.[10,15]

Apigenin-4′-*O*-(2′,6″-di-*O*-*p*-coumaroyl)-β-D-glucoside (11): yellow powder; mp 252−253 °C (MeOH); $[\alpha]^{22}_D$ −65.0° (*c* 0.30, MeOH) [lit.[9] mp 254 °C (MeOH), $[\alpha]^{22}_D$ −119.0° (*c* 0.13, pyridine)]; R_f 0.41 and 0.11 (Si gel, solvents A and B, respectively), 0.34 (reversed-phase KC_{18} F, solvent D). The UV, IR, and ^1H and ^{13}C NMR data were identical with the reported values.[9]

Acetylation of 1, 3, and 7. Compound **1** (6 mg) was dissolved in Ac_2O/pyridine (1:2, 3 mL), and the mixture was allowed to stand at room temperature for 24 h. After addition of 10 mL of H_2O, the resulting mixture was evaporated to dryness and then purified by preparative TLC (Si gel, toluene/EtOAc, 1:1) to afford **1a** (5.0 mg). By the same method, **3** (2.0 mg) afforded **3a** (2.0 mg), and **7** (10. 0 mg) afforded **7a** (9.8 mg).

Lycernuic acid C tetraacetate (1a): colorless powder; $[\alpha]^{26}_D$ −17.3° (*c* 0.20, MeOH); IR ν_{max} (KBr) cm^{-1} 2928, 1732, 1666, 1452, 1382, 1241, 1031, 991; ^1H NMR (400 MHz, pyridine-d_5) δ 1.07 (3H, s, CH_3-26), 1.08 (1H, m, H-5), 1.10 (3H, s, CH_3-30), 1.16 (3H, s, CH_3-25), 1.27 (3H, s, CH_3-28), 1.35 (1H, m, H-9), 1.43 (1H, m, H-13), 1.51 (3H, s, CH_3-23), 1.88 (1H, m, H-17), 1.90 (αH-2), 2.00 (3H, s, −CO−CH_3-29), 2.08 (3H, s, −CO−CH_3-15), 2.12 (3H, s, −CO−CH_3-21), 2.22 (3H, s, −CO−CH_3-3), 3.23 (1H, m, βH-2), 4.22 (1H, d, *J* = 11.3 Hz, H-29a), 4.50 (1H, d, *J* = 11.3 Hz, H-29b), 4.88 (1H, dd, *J* = 13.3/4.4 Hz, H-3), 5.03 (1H, dd, *J* = 10.9/4.1 Hz, H-15), 5.20 (1H, br s, HO-14), 5.33 (1H, br s, H-21); ESIMS *m/z* 713 $[M + Na]^+$; R_f 0.66 and 0.72 (Si gel, solvents B and C, respectively), 0.50 (reversed-phase KC_{18} F, solvent D).

Lycernuic acid E diacetate (3a): colorless powder; $[\alpha]^{26}_D$ −17.0° (*c* 0.20, MeOH); IR ν_{max} (KBr) cm^{-1} 2928, 2853, 1733, 1459, 1371, 1245, 1030; ^1H NMR (400 MHz, pyridine-d_5) δ 0.92 (3H, s, CH_3-29), 0.95 (3H, s, CH_3-30), 0.98 (3H, s, CH_3-26), 1.11 (3H, s, CH_3-25), 1.31 (3H, s, CH_3-28), 1.46 (3H, s, CH_3-23), 2.06 (3H, s, −CO−CH_3-21), 2.13 (3H, s, −CO−CH_3-3), 4.81 (1H, d, *J* = 8.5 Hz, H-3), 4.90 (1H, br s, H-21); ESIMS *m/z* 573 $[M - H]^-$; R_f 0.75 and 0.66 (Si gel, solvents B and C, respectively), 0.36 (reversed-phase KC_{18} F, solvent D).

Lycernuic acid A diacetate (7a): colorless powder; $[\alpha]^{26}_D$ −25.5° (*c* 0.20, MeOH); IR ν_{max} (KBr) cm^{-1} 2965, 2939, 1729, 1455, 1372, 1245, 1190, 1031, 998; ^1H NMR (400 MHz, pyridine-d_5) δ 0.74 (3H, s, CH_3-28), 0.81 (3H, s, CH_3-30), 0.83 (3H, s, CH_3-26), 0.87 (3H, s, CH_3-29), 1.05 (3H, s, CH_3-25), 1.51 (3H, s, CH_3-23), 2.08 (3H, s, −CO−CH_3-21), 2.09 (3H, s, −CO−CH_3-3), 4.87 (2H, br s, H-3, 21), 5.41 (1H, br s, H-15); ESIMS *m/z* 555 $[M - H]^-$; R_f 0.77 and 0.70 (Si gel, solvents B and C, respectively), 0.12 (reversed-phase KC_{18} F, solvent D).

Osmolation of 7a. A mixture of **7a** (9.8 mg) and OsO_4 (50 mg) in pyridine (3 mL) was kept in the dark for 28 days. An aqueous solution (18 mL) of $NaHSO_3$ (1.0 g) was added, and the mixture was stirred for 30 min at room temperature and then extracted with $CHCl_3$ (10 mL × 3).[12] The extract was washed with water (15 mL × 3) and evaporated to dryness in vacuo. The residue was dissolved in 1.5 mL of MeOH and then isolated by preparative TLC (Si gel TLC, $CHCl_3$/MeOH, 12:1) to give **7b** (1.5 mg) and **7c** (3.5 mg).

7b: colorless powder; $[\alpha]^{26}_D$ −59.0° (*c* 0.15, MeOH); IR ν_{max} (KBr) cm^{-1} 3447, 2945, 1722, 1569, 1452, 1376, 1247, 1026; ^1H NMR (400 MHz, pyridine-d_5) δ 0.92 (3H, s, CH_3-29), 0.95 (6H, s, CH_3-26, 30), 1.13 (3H, s, CH_3-25), 1.28 (3H, s, CH_3-28), 1.48 (3H, s, CH_3-23), 2.09 (3H, s, −CO−CH_3-21), 2.12 (3H, s, −CO−CH_3-3), 3.60 (1H, t, *J* = 6.0 Hz, H-15), 4.90 (2H, br s, H-3, 21); ESIMS *m/z* 589 $[M - H]^-$; R_f 0.62 and 0.72 (Si gel, solvents B and C, respectively), 0.57 (reversed-phase KC_{18} F, solvent D).

7c: colorless powder; $[\alpha]^{26}_D$ −45.0° (*c* 0.35, MeOH); IR ν_{max} (KBr) cm^{-1} 3443, 2948, 1724, 1569, 1452, 1374, 1261, 1031; ^1H NMR (400 MHz, pyridine-d_5) δ 0.90 (3H, s, CH_3-28), 0.95 (3H, s, CH_3-30), 0.97 (3H, s, CH_3-29), 1.13 (3H, s, CH_3-25), 1.34 (3H, s, CH_3-26), 1.48 (3H, s, CH_3-23), 1.96 (3H, s, −CO−CH_3-21), 2.13 (3H, s, −CO−CH_3-3), 4.01 (1H, br t, H-15), 4.90 (2H, br s, H-3, 21); ESIMS *m/z* 589 $[M - H]^-$; R_f 0.48 and 0.56 (Si gel, solvents B and C, respectively), 0.62 (reversed-phase KC_{18} F, solvent D).

Dehydration of Lycernuic Acid E Diacetate (3a) by Thionyl Chloride. A solution of **3a** (2.0 mg) in pyridine (1 mL) was treated with $SOCl_2$ (0.2 mL) at room temperature for 3 h. The mixture was poured into ice water and then passed over a small ODS column (6.0 g) washing with water (50 mL) and MeOH (50 mL). The MeOH solution was evaporated to give **7a** (1.5 mg).

Alkaline Hydrolysis of 11. Compound **11** (5 mg) was refluxed with 10 mL of 0.8% KOH at 60 °C for 30 min. After cooling, the reaction mixture was neutralized with 10% HCl and then extracted with EtOAc (10 mL × 3). The organic layers were combined and then evaporated to dryness in vacuo. The residue was purified by HPLC ($MeOH/H_2O$, 75:25, 2 mL/min, UV 312 nm) to afford *trans*-*p*-coumaric acid (1.6 mg, t_R 19.5 min) and apigenin-4′-*O*-β-D-glucoside (1.8 mg, t_R 25.5 min).

Acid Hydrolysis of 11. Compound **11** and the authentic sugar were spotted on a Si gel TLC plate and hydrolyzed in situ by exposure to HCl vapor at 70 °C for 25 min. The TLC plate was then developed with $CHCl_3$/MeOH/AcOH/H_2O (14:6:2:1) and sprayed with 10% H_2SO_4 for detection. Glucose was detected with a R_f value of 0.21 from **11**.

Biological Testing. The assay was conducted as previously described.[3] The *C. albicans* secreted aspartic proteases inhibitory activities expressed as IC_{50} were 20 and 8.5 μg/mL for **1** and **11**, respectively, while other compounds were inactive. Pepstatin A was used as a positive control (IC_{50} = 0.0015 μg/mL).

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