Antioxidative Cassane Diterpenoids from the Seeds of *Caesalpinia minax*

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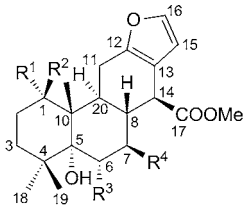
Five new cassane diterpenoids, caesalmins I – M (**1** – **5**), and 23 known analogs were isolated from the seeds of *Caesalpinia minax*. Their structures were elucidated by spectroscopic methods and comparison with reported data. The antioxidant properties of **1** – **28** were determined by the method of oxygen radical absorbance capacity of fluorescein (ORAC-FL), and 14 compounds exhibited good antioxidant activities with ORAC-FL values of 2.24 – 4.89 *Trolox* equivalents. The structure–activity relationship of the active compounds was also discussed.

Introduction. – *Caesalpinia minax* (Fabaceae), endemic to the southwest and southeast of China, is known as 'ku shi lian' in traditional Chinese medicine for the treatment of common cold, fever, and dysentery [1]. Previous chemical investigation on this species revealed a number of cassane diterpenoids with the structural modification usually occurring on the substitutions at C(1), C(6), C(7), C(14), and the furan ring of the diterpene core [2 – 4]. Recently, cassane diterpenoids have attracted considerable attention for their broad range of bioactivities, such as antiproliferative [5], antimalarial [6], and antibacterial activities [7]. In our continuing search for structurally diverse and biologically significant metabolites from medical plant resources [8 – 10], five new cassane diterpenoids and 23 known analogs (*Fig. 1*) were isolated from the seeds of *C. minax*. All compounds were evaluated for their anti-oxidative activities using oxygen radical absorbance capacity of fluorescein (ORAC-FL) assays, and compounds **1** – **4**, **6** – **9**, and **23** – **28** exhibited good antioxidant properties with ORAC-FL values of 2.24 – 4.89 *Trolox* equivalents. Herein, we report the details of the isolation, structural elucidation, and antioxidative evaluation of these compounds.

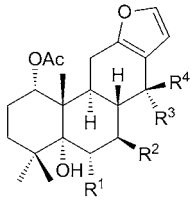
Results and Discussion. – The air-dried powder of seeds of *C. minax* was extracted with 95% EtOH at room temperature to give a crude extract, which was suspended in H_2O and successively partitioned with petroleum ether and AcOEt. Various column chromatographic separations of the AcOEt extract afforded compounds **1** – **28**.

Compound **1**, a white amorphous powder, had the molecular formula $C_{21}H_{30}O_6$ as determined by the HR-ESI-MS ion at *m*/*z* 401.1923 ($[M+Na]^+$, $C_{21}H_{30}NaO_6^+$; calc. 401.1940). The IR spectrum exhibited absorption bands for OH (3192 cm^{-1}) and CO (1722 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (*Table 1*) of **1** showed three Me

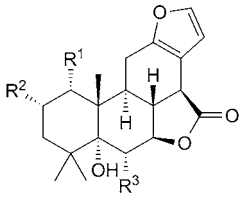
[1]) These authors have contributed equally to this work.



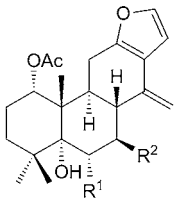
	R^1	R^2	R^3	R^4
1	OH	H	H	OH
2	=O		H	OH
3	OH	H	AcO	OH
4	OH	H	OH	AcO
6	=O		H	AcO
7	AcO	H	H	AcO
8	OH	H	H	AcO
9	AcO	H	H	H



	R^1	R^2	R^3	R^4
5	H	OH	H	COOBu
10	AcO	AcO	Me	OH
11	H	OH	Me	H
12	H	OH	H	H
13	AcO	AcO	OH	MeO
14	OH	AcO	Me	MeO
15	AcO	H	Me	H
16	AcO	AcO	Me	MeO
17	AcO	AcO	Me	BuO



	R^1	R^2	R^3
18	OH	OH	AcO
19	OH	H	AcO
20	AcO	H	AcO
21	AcO	H	H
22	OH	H	H



	R^1	R^2
23	H	OH
24	AcO	AcO
25	OH	AcO
26	AcO	OH
27	H	AcO
28	AcO	H

Fig. 1. *The structures of compounds* **1**–**28**

*singlet*s (δ(H) 1.01 (*s*, Me(18)), 1.07 (*s*, Me(19)), and 1.09 (*s*, Me(20)), a MeO group (δ(H) 3.74 (*s*)), two O-bearing CH groups (δ(H) 3.77 (*t*, $J = 2.8$, H–C(1)) and 3.94 (*td*, $J = 10.9$, 5.5, H–C(7)), two *ortho*-positioned furan H-atoms (δ(H) 6.16 (*d*, $J = 1.8$, H–C(15)) and 7.23 (*d*, $J = 1.8$, H–C(16)), and a series of aliphatic CH_2 *multiplet*s. The ^{13}C-NMR spectrum (*Table 1*) in combination with DEPT experiments resolved 21 C-atom resonances attributable to a COOMe group, a furan ring, three sp^3 quaternary C-atoms (one O-bearing), five sp^3 CH groups (two O-bearing), four sp^3 CH_2 groups, and three Me groups. The above-mentioned data implied that **1** possessed a cassane diterpenoid skeleton and showed high similarity to those of caesalpine A (**8**) [11], a co-isolated metabolite in the current study. In comparison to **8**, the structural difference of **1** was due to the absence of the AcO group in **1**, indicating that **1** was a deacetylated derivative of **8**. The upfield-shifted H–C(7) signal in **1** with respect to that in **8** (δ(H) 3.94 in **1**; δ(H) 5.13 in **8**) indicated that the AcO group at C(7) was removed in **1**. The

Table 1. *^{1}H- and ^{13}C-NMR Data* (in $CDCl_3$; 400 and 100 MHz, resp.) *of* **1**–**3** (δ in ppm, J in Hz)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	3.77 (*t*, *J* = 2.8)	72.5		214.0	3.69 (br. *s*)	72.5
2	2.04–2.09 (*m*), 1.61–1.65 (*m*)	26.2	2.85–2.95 (*m*), 2.17–2.20 (*m*)	35.5	1.98–2.04 (*m*), 1.61–1.67 (*m*)	25.7
3	2.00–2.04 (*m*), 1.10–1.12 (*m*)	29.7	2.00–2.05 (*m*), 1.64–1.71 (*m*)	38.0	1.96–2.02 (*m*), 1.03–1.06 (*m*)	31.9
4		38.5		38.8		38.7
5		79.6		83.4		80.7
6	1.96–1.99 (*m*), 1.64–1.68 (*m*)	36.7	1.94–1.99 (*m*), 1.82–1.90 (*m*)	36.6	5.33 (*d*, *J* = 9.5)	76.6
7	3.94 (*td*, *J* = 10.9, 5.5)	73.7	3.79–3.87 (*m*)	72.6	3.80 (*dd*, *J* = 10.3, 9.5)	77.2
8	2.17–2.26 (*m*)	42.3	2.10–2.16 (*m*)	43.3	2.35–2.42 (*m*)	41.4
9	2.77–2.86 (*m*)	36.4	2.66–2.74 (*m*)	37.5	2.82–2.90 (*m*)	35.8
10		43.4		55.7		45.0
11	2.70–2.77 (*m*), 2.46–2.54 (*m*)	21.5	3.17 (br. *d*, *J* = 15.0), 2.37 (*dd*, *J* = 15.0, 12.2)	23.8	2.75–2.83 (*m*), 2.43–2.50 (*m*)	21.6
12		150.7		151.3		150.5
13		113.5		112.6		113.4
14	3.45–3.48 (*m*)	46.6	3.47 (*d*, *J* = 8.8)	46.5	3.47 (*d*, *J* = 8.2)	46.3
15	6.16 (*d*, *J* = 1.8)	108.6	6.13 (br. *s*)	108.4	6.16 (*d*, *J* = 1.8)	108.5
16	7.23 (*d*, *J* = 1.8)	141.2	7.21 (br. *s*)	141.0	7.23 (*d*, *J* = 1.8)	141.4
17		176.4		176.5		175.7
18	1.01 (*s*)	27.8	1.02 (*s*)	25.6	1.07 (*s*)	30.4
19	1.07 (*s*)	24.9	1.30 (*s*)	27.1	1.11 (*s*)	24.5
20	1.09 (*s*)	17.6	1.45 (*s*)	16.4	1.12 (*s*)	17.0
MeO	3.74 (*s*)	52.2	3.72 (*s*)	52.2	3.72 (*s*)	52.2
AcO					2.12 (*s*)	171.2
						21.7

structure of **1** was further corroborated by detailed 2D analyses (^{1}H,^{1}H-COSY, HSQC, and HMBC). The relative configuration of **1** was determined to be the same as that of **8** by a NOESY experiment and by comparison of their ^{13}C-NMR data. The *trans*-fused *A*/*B* ring system and α-orientation of OH at C(1) was assigned to be the same as that of **8** by their identical 1D-NMR data regarding C(1) (δ(C) 72.5 in **1**; δ(C) 72.3 in **8**), C(5) (δ(C) 79.6 in **1**; δ(C) 79.7 in **8**), and C(10) (δ(C) 43.4 in **1**; δ(C) 43.5 in **8**). The NOESY correlations of Me(20)/H–C(8), H–C(7)/H–C(9) and H–C(14), indicated that Me(20), H–C(7), H–C(8), and H–C(9) were adopted the axial bonds of the chair-conformational *B*-ring, and the *B*/*C* ring was *trans*-fused. Thus, H–C(7) and H–C(14) were co-facial and assigned in α-orientation (*Fig. 2*). With these observations, compound **1** was given the trivial name caesalmin I.

Compound **2** displayed the HR-ESI-MS ion at *m*/*z* 399.1756 ($[M+Na]^+$), consistent with a molecular formula of $C_{21}H_{28}O_6$, two mass units less than that of **1**. The ^{1}H- and ^{13}C-NMR data of **2** (*Table 1*) were very similar to those of **1**, except for the presence of a ketone group (δ(C) 214.0) and the absence of the O-bearing CH group (δ(H) 3.77, H–C(1); δ(C) 72.5, C(1)) in **1**, indicating that **2** was a C(1)-oxidized

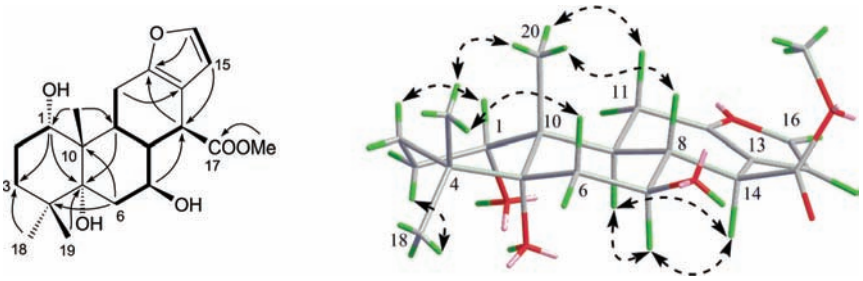


Fig. 2. *Selected* $^1H,^1H$*-COSY* (—), *HMBC* (→), *and NOESY* (⇢) *correlations of* **1**

derivative of **1**. This was supported by the downfield-shifted signals of C(2) and C(10) in **2** (*ca.* 10 ppm) as compared to those in **1**. The HMB correlation from Me(20) to the ketone group further confirmed the location of the ketone group at C(1). The relative configuration of **2** was assigned to be the same as that of **1** by comparing their 1D-NMR data and by analyzing its NOESY data. Thus, compound **2** was deduced as shown and given the trivial name caesalmin J.

The molecular formula for compound **3** was established as $C_{23}H_{32}O_8$ by its HR-ESI-MS. The ^1H- and ^{13}C-NMR signals (*Table 1*) of **3** were similar to those of **1**, except for the presence of an O-bearing CH group (δ(H) 5.33 (*d*); δ(C) 76.6) and an AcO group in **3** instead of a CH_2 group in **1**. A $^1H,^1H$-COSY correlation between H–C(6) (δ(H) 5.33) and H–C(7) (δ(H) 3.80) together with a HMB correlation from H–C(6) to the AcO CO group (δ(C) 171.2) further located the AcO group at C(6). The relative configuration of **3** was assigned to be the same as that of **1** by comparing their 1D-NMR data and NOESY data. Therefore, compound **3** was given the trivial name caesalmin K.

Compound **4** showed the same molecular formula as that of **3** based on its HR-ESI-MS data, which suggested that it was an isomer of **3**. Comparison of the ^1H- and ^{13}C-NMR signals (*Table 2*) with those of **3** indicated that the AcO group at C(6) and the OH group at C(7) in **3** were interchanged to a OH group at C(6) and an AcO group at C(7) in **4**. This was supported by the $^1H,^1H$-COSY correlation between H–C(7) (δ(H) 5.17) and H–C(8) (δ(H) 2.51–2.58), together with the HMB correlation from H–C(7) (δ(H) 5.17) to the AcO CO C-atom (δ(C) 172.7). The relative configuration of **4** was assigned to be the same as that of **3** by comparing their 1D-NMR and NOESY data. Accordingly, compound **4** was established and named caesalmin L.

Compound **5**, a white powder, had the molecular formula of $C_{26}H_{38}O_7$, as determined by the HR-ESI-MS data. The NMR spectra of **5** highly resembled those of bonducellpin C, a cassane diterpenoid previously reported from the same plant [12]. The structural difference of the two compounds came from the replacement of a MeO group in bonducellpin C by a BuO group in **5**. The BuO group was located at C(17) by the HMB correlation from H–C(1′) (δ(H) 4.15) to C(17) (δ(C) 175.7). The relative configuration of **5** was assigned to be the same as that of bonducellpin C by a NOESY experiment and by comparing their 1D-NMR data. Thus, compound **5** was assigned caesalmin M.

The known compounds caesalpinin F_1 (**6**) [13], 7-acetoxybonducellpin C (**7**) [12], caesalpine A (**8**) [11], caesalpinin MB (**9**) [14], caesalmin E (**10**) [2], caesalpinin K (**11**)

Table 2. *^{1}H- and ^{13}C-NMR Data* (in $CDCl_3$; 400 and 100 MHz, resp.) *of* **4** *and* **5** (δ in ppm, J in Hz)

Position	**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	3.70 (br. *s*)	72.5	4.91 (br. *s*)	75.6
2	1.97–2.03 (*m*), 1.61–1.68 (*m*)	25.9	1.97–2.01 (*m*), 1.75–1.78 (*m*)	22.6
3	1.98–2.04 (*m*), 1.03–1.07 (*m*)	31.9	1.72–1.75 (*m*), 1.14–1.17 (*m*)	30.0
4		39.0		38.4
5		80.4		78.5
6	3.97 (*d*, J = 9.1)	74.6	2.06–2.12 (*m*), 1.62–1.65 (*m*)	36.2
7	5.17 (*dd*, J = 10.6, 9.1)	81.1	3.99 (*ddd*, J = 14.5, 10.0, 4.6)	73.5
8	2.51–2.58 (*m*)	38.1	2.20–2.26 (*m*)	42.4
9	2.85–2.92 (*m*)	35.8	2.56–2.65 (*m*)	36.6
10		44.4		43.6
11	2.78–2.83 (*m*), 2.42–2.50 (*m*)	21.5	2.45–2.53 (*m*), 2.25–2.30 (*m*)	21.5
12		150.5		150.0
13		113.0		113.8
14	3.40 (*br. d*, J = 3.7)	46.0	3.45 (*d*, J = 8.6)	46.5
15	6.10 (*d*, J = 1.6)	108.3	6.17 (*d*, J = 1.8)	108.6
16	7.22 (*d*, J = 1.6)	141.5	7.23 (*d*, J = 1.8)	141.3
17		174.6		175.7
18	1.22 (*s*)	31.1	1.05 (*s*)	27.9
19	1.18 (*s*)	24.7	1.09 (*s*)	25.0
20	1.10 (*s*)	16.9	1.19 (*s*)	17.7
MeO	3.72 (*s*)	52.0		
AcO	2.05 (*s*)	172.7, 21.0	2.08 (*s*)	169.0, 21.5
BuO				
1′			4.15 (*t*, J = 6.8)	65.0
2′			1.65–1.67 (*m*), 1.12–1.15 (*m*)	30.7
3′			1.40–1.43 (*m*)	19.2
4′			0.94 (*t*, J = 7.4)	13.7

[15], caesalpinin N (**12**) [15], caesalmin D (**13**) [2], caesalmin E_2 (**14**) [13], 6-acetylcaesaldekarin D (**15**) [16], caesalmin F (**16**) [2], caesalminaxin L (**17**) [4], caesalmin A (**18**) [17], bonducellpin D (**19**) [18], caesalpinin D (**20**) [19], caesalmin B (**21**) [17], caesalmin G (**22**) [2], caesall D (**23**) [20], caesalmin C (**24**) [2], ζ-caesalpin (**25**) [6], bonducellpin G (**26**) [21], caesalpinin MJ (**27**) [22], and caesalpinin MK (**28**) [22], were identified by comparison of their NMR data with those in the literature.

The Antioxidant Activity. The antioxidant activities of **1**–**28** were determined by the method of oxygen radical absorbance capacity of fluorescein (ORAC-FL). The vitamin E analog, *Trolox*, was used as standard, and the antioxidant activity was expressed as *Trolox* equivalents (μM of *Trolox*/μM of tested compound). As shown in *Table 3*, compounds **1**–**4**, **6**–**9**, and **23**–**28** showed good antioxidant activity with ORAC-FL values of 2.24–4.89 *Trolox* equivalents, and compound **28** represented the most active antioxidant (ORAC-FL = 4.89). By a structure–activity relationship (SAR) analysis, it was found that the exocyclic C=C-bond or COOMe group at C(14) were essential for the antioxidant activity, as compounds **1**–**4**, **6**–**9**, and **23**–**28** with such structural features exhibited good activities, while compounds without these moieties lost their activity. The substituents at C(1), C(6), and C(7) could not in-

Table 3. *Antioxidant Activity of Compounds*

Compound	*Trolox* equiv.[a])	Compound	*Trolox* equiv.[a])	Compound	*Trolox* equiv.[a])
1	2.24 ± 0.03	**7**	4.12 ± 0.02	**25**	4.43 ± 0.04
2	2.43 ± 0.07	**8**	3.37 ± 0.02	**26**	2.25 ± 0.03
3	3.29 ± 0.05	**9**	4.07 ± 0.03	**27**	4.07 ± 0.02
4	4.01 ± 0.01	**23**	4.17 ± 0.02	**28**	4.89 ± 0.03
6	2.82 ± 0.02	**24**	3.09 ± 0.01		

[a]) Data were expressed as μM of *Trolox* equivalent μM^{-1} of tested compounds (means ± SD of three experiments).

fluence the activity, as the active compounds exhibited various substituents in these places.

Oxidative stress plays an important role in the progress of inflammation, and the antioxidant agents which scavenge the free radical could resist the oxidative stress and relief the inflammation [23–25]. Thus, the antioxidant diterpenoids isolated in the current study may explain the efficacy of *C. minax* in the treatment of inflammation-related diseases in the traditional Chinese medicine. However, the exact mechanisms require further investigation.

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Experimental Part

General. All solvents used were of analytical grade (*Guangzhou Chemical Reagents Co., Ltd.*). Column chromatography (CC): Silica gel (SiO_2, 300–400 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), reversed-phase C_{18} (*Rp-C*$_{18}$) silica gel (12 nm, *S-50* μm, *YMC Co., Ltd.*), *Sephadex LH-20* gel (*Amersham Biosciences*), and *MCI* gel (*CHP20P*, 75–150 μm, *Mitsubishi Chemical Industries Ltd.*). Semiprep. HPLC: *YMC-pack ODS-A* column (250 × 10 mm, *S-5* μm, 12 nm) on a *Shimadzu LC-20 AT* equipped with a *SPD-M20A* PDA detector. Thin layer chromatograchy (TLC): silica gel *GF*$_{254}$ precoated plates (0.20–0.25 mm; *Qingdao Haiyang Chemical Co., Ltd.*); visualization by heating SiO_2 plates, followed by spraying with 10% H_2SO_4 in EtOH. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-2450* spectrophotometer. IR Spectra: *Bruker Tensor 37* infrared spectrophotometer. NMR Spectra: *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Finnigan LCQ Deca* instrument, and HR-ESI-MS: *Waters Micromass Q-TOF*. Disodium fluorescein, *Trolox* and 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH): *Sigma–Aldrich*. *Spectrafluor Plus* plate reader: *Tecan, Crailsheim*.

Plant Material. The seeds of *C. minax* were collected in Yulin, Guangxi Province, in September, 2013, and were identified by Assoc. Prof. *Lin Jiang* of Sun Yat-sen University. A voucher specimen (accession number: KSL201309) has been deposited with the School of Pharmaceutical Sciences, Sun Yat-sen University.

Extraction and Isolation. The air-dried powder of the seeds of *C. minax* (5.0 kg) was extracted with 95% EtOH (3 × 10 l) at r.t. to give 477 g of crude extract, which was suspended in H_2O (1 l) and successively partitioned with petroleum ether (PE; 3 × 1 l) and AcOEt (3 × 1 l). The AcOEt extract (57 g) was subjected to CC (*MCI* gel; MeOH/H_2O 3:7 to 10:0) to afford five fractions (*I*–*V*). *Fr. II* (5 g) was applied to CC (SiO_2; hexane/AcOEt 10:1 to 1:1) to afford three fractions (*Frs. IIa*–*IIc*). *Fr. IIb*

(470 mg) was separated on CC (SiO_2; CH_2Cl_2/MeOH 150:1 to 40:1), further purified on HPLC (*ODS*; MeCN/H_2O 80:20 (*v*/*v*) 3 ml · min^{-1}) to afford **1** (t_R 8.5 min, 11 mg), **7** (t_R 11 min, 6 mg), and **9** (t_R 13.5 min, 18 mg). *Fr. IIa* (1.2 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 40:1) to give four fractions (*Frs. IIa1 – IIa4*). *Fr. IIa2* (170 mg) was further separated by CC (*Rp-C_{18}* SiO_2; MeOH/H_2O 6:4 to 10:0 and *Sephadex LH-20*) to afford **15** (22 mg), **16** (30 mg), **24** (15 mg), and **2** (7 mg). *Fr. IIc* (0.9 g) was applied to CC (SiO_2; hexane/AcOEt 10:1 to 1:1), further purified on HPLC (*ODS*; MeOH/H_2O 80:20 (*v*/*v*) 3 ml · min^{-1}) to afford **3** (t_R 12.5 min, 15 mg), **4** (t_R 15 min, 6 mg), and **26** (t_R 16.5 min, 26 mg). *Fr. III* (8.6 g) was applied to CC (SiO_2; hexane/AcOEt 10:1 to 1:2), to give eight fractions (*Frs. IIIa – IIIh*). *Fr. IIIc* (1.9 g) was subjected to CC (*Rp-C_{18}* SiO_2; MeOH/H_2O 6:4 to 10:0), followed by CC (SiO_2; hexane/AcOEt 10:1 to 1:1) to afford **19** (50 mg), **20** (122 mg), **23** (35 mg), and **25** (35 mg). *Fr. IIIe* (230 mg) was separated by CC (SiO_2; CH_2Cl_2/MeOH 200:1) to yield **5** (3.9 mg) and **8** (24 mg). *Fr. IIIe* (560 mg) was subjected to CC (SiO_2; hexane/AcOEt 10:1 to 1:1) to afford **6** (5.6 mg), **10** (15 mg), and **14** (24 mg). *Fr IV* (6.6 g) was subjected to CC (*Rp-C_{18}* SiO_2; MeOH/H_2O 5:5 to 10:0), followed by CC (SiO_2; hexane/AcOEt 10:1 to 1:1) to afford seven fractions (*Frs. IVa – Fr. IVg*). *Fr. IVc* (560 mg) was subjected to CC (*Rp-C_{18}* SiO_2; MeOH/H_2O 6:4 to 10:0), followed by CC (SiO_2; hexane/AcOEt 10:1 to 1:1) to afford **11** (16 mg), **17** (15 mg), **18** (15 mg), and **27** (24 mg). *Fr. IVe* (560 mg) was separated by CC (SiO_2; hexane/AcOEt 10:1 to 1:1) further purified on HPLC (*ODS*, MeOH/H_2O 85:15 (*v*/*v*) 3 ml · min^{-1}) to afford **12** (t_R 6.5 min, 15 mg), **13** (t_R 8 min, 6 mg), and **28** (t_R 10.5 min, 26 mg). *Fr. IVf* (40 mg) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 150:1) to yield **21** (6.9 mg) and **22** (14 mg). The purity of compounds **1** – **28** was greater than 95% as determined by ^{1}H-NMR spectra.

Caesalmin I (= *Methyl 1α,5α,7β-Trihydroxyvouacapan-17β-oate* = *Methyl (1*S*,4a*R*,6*S*,6a*R*,7*S*,11a*S*,11b*S*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-Dodecahydro-1,4a,6-trihydroxy-4,4,11b-trimethylphenanthro[3,2-*b*]furan-7-carboxylate*; **1**). White amorphous powder. $[\alpha]_D^{20} = +13.20$ ($c = 0.32$, MeOH). UV (MeOH): 215 (3.50). IR (KBr): 3694, 3192, 1722, 1267. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 401.1923 ($[M+Na]^+$, $C_{21}H_{30}NaO_6^+$; calc. 401.1940).

Caesalmin J (= *Methyl 5α,7β-Dihydroxy-1-oxovouacapan-17β-oate* = *Methyl (4a*R*,6*S*,6a*R*,7*S*,11a*S*,11b*R*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-Dodecahydro-4a,6-dihydroxy-4,4,11b-trimethyl-1-oxophenanthro[3,2-*b*]furan-7-carboxylate*; **2**). White amorphous powder. $[\alpha]_D^{20} = -3.56$ ($c = 0.20$, MeOH). UV (MeOH): 218 (3.38). IR (KBr): 3401, 2959, 1713, 1432, 1262. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 399.1756 ($[M+Na]^+$, $C_{21}H_{28}NaO_6^+$; calc. 399.1784).

Caesalmin K (= *Methyl 1α,5α,7β-Trihydroxy-6α-acetoxyvouacapan-17β-oate* = *Methyl (1*S*,4a*R*,5*S*,6*R*,6a*R*,7*S*,11a*S*,11b*S*)-5-(Acetyloxy)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-1,4a,6-trihydroxy-4,4,11b-trimethylphenanthro[3,2-*b*]furan-7-carboxylate*; **3**). White amorphous powder. $[\alpha]_D^{20} = -4.10$ ($c = 0.17$, MeOH). UV (MeOH): 212.5 (3.18). IR (KBr): 3435, 2941, 1730, 1248. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 437.2160 ($[M+H]^+$, $C_{23}H_{33}O_8^+$; calc. 437.2175).

Caesalmin L (= *Methyl 1α,5α,6α-Trihydroxy-7β-acetoxyvouacapan-17β-oate* = *Methyl (1*S*,4a*R*,5*S*,6*R*,6a*R*,7*S*,11a*S*,11b*S*)-6-(Acetyloxy)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-1,4a,5-trihydroxy-4,4,11b-trimethylphenanthro[3,2-*b*]furan-7-carboxylate*; **4**). White amorphous powder. $[\alpha]_D^{20} = -10.50$ ($c = 0.51$, MeOH). UV (MeOH): 215.5 (3.36). IR (KBr): 3394, 2932, 1733, 1245. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 459.1979 ($[M+Na]^+$, $C_{23}H_{32}NaO_8^+$; calc. 459.1995).

Caesalmin M (= *Butyl 5α,7β-Dihydroxy-1α-acetoxyvouacapan-17β-oate* = *Butyl (1*S*,4a*R*,6*S*,6a*R*,7*S*,11a*S*,11b*S*)-1-(Acetyloxy)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4a,6-dihydroxy-4,4,11b-trimethylphenanthro[3,2-*b*]furan-7-carboxylate*; **5**). White amorphous powder. $[\alpha]_D^{20} = +2.55$ ($c = 0.16$, MeOH). UV (MeOH): 212 (3.07). IR (KBr): 3478, 2958, 1735, 1235. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 463.2675 ($[M+H]^+$, $C_{26}H_{39}O_7^+$; calc. 463.2696).

The Antioxidant Activity Assay. The antioxidant activity was determined by the ORAC-FL assay [26 – 28]. All the assays were under 75 mM phosphate buffer (pH 7.4) and the final mixture was 200 µl. Antioxidant (20 µl) and fluorescein (120 µl, 70 nM final concentration) were placed in the wells of a black 96-well plate and the mixture was incubated for 10 min at 37°. Then, the AAPH soln. (60 µl, 12 mM final concentration) was added rapidly. The plate was immediately placed into a *Spectrafluor Plus* plate reader and the fluorescence was measured every 2 min for 4 h with excitation at 485 nm and emission at 520 nm. *Trolox* was used as standard (1 – 10 µM, final concentration). Fluorescence decay of sodium fluorescin without AAPH was used as a control, and fluorescence decay induced by AAPH without tested samples

served as a blank. All tests were performed in triplicate. Fluorescence measurements were normalized to the curve of the blank. The values were calculated as described in the reference [28], and the final results were in μM of *Trolox* equivalent/μM of pure compound.

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