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Identification and Evaluation of Antiepileptic Activity of C_{21} Steroidal Glycosides from the Roots of *Cynanchum wilfordii*

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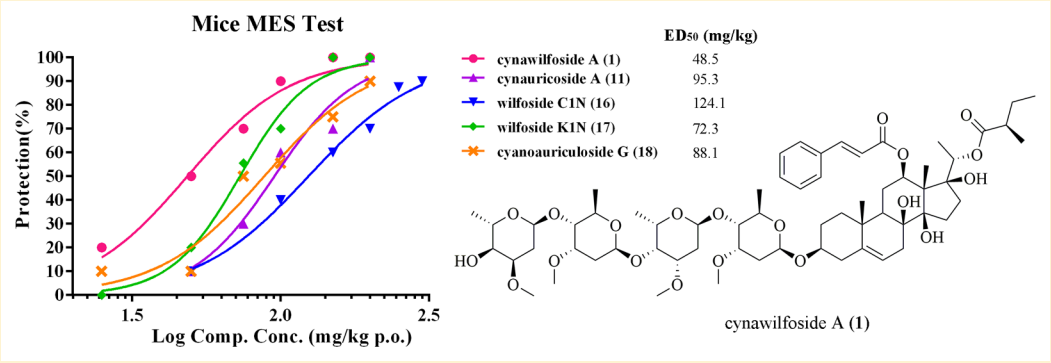
Supporting Information



ABSTRACT: Nine new C_{21} steroidal glycosides, named cynawilfosides A–I (**1**–**9**), along with 12 known compounds were isolated from the roots of *Cynanchum wilfordii*. The structures of the new compounds were elucidated by spectroscopic analysis and chemical methods. The five major components, cynawilfoside A (**1**), cynauricoside A (**11**), wilfoside C1N (**16**), wilfoside K1N (**17**), and cyanoauriculoside G (**18**), exhibited significant protection activity in a maximal electroshock (MES)-induced mouse seizure model with ED_{50} values of 48.5, 95.3, 124.1, 72.3, and 88.1 mg/kg, respectively.

Epilepsy is a brain disorder associated with significant psychological consequences, such as increased levels of anxiety and depression and poor self-esteem, which may lead to certain social consequences.[1] Around 50 million people suffer from epilepsy, and this number is increasing year by year. Although epilepsy is not necessarily a fatal disease, it carries an increased risk of premature death, with an overall mortality rate 2–3 times that of the general population.[2] Pharmacological treatment for epilepsy does not cure the disease, but it can control the condition by decreasing the frequency, duration, and severity of seizures. Traditional antiepileptic drugs (AEDs) were discovered in a serendipitous fashion, usually by confirming their efficacy in established animal models.[3−5] The maximal electroshock (MES)-induced seizure provides a model for single, acute seizures.[6] In the MES test, electrical stimulus treatment of mice or rats elicits behavioral and neuropathological changes similar to epileptic seizures in humans.[7] It is probably the best-validated preclinical test to predict the efficacy of compounds against generalized seizures of the tonic-clonic type.[8] A series of AEDs, such as oxcarbazepine,[9] zonisamide,[10] and retigabine,[11] have been proven to be effective in the MES test. Thus, in spite of the continuous search for new models closer to the human epilepsy phenomenon, the MES test in rodents persists as a useful tool.[12]

Cynanchum wilfordii (Maxim.) Hemsl. (Asclepiadaceae) is widely distributed in the People's Republic of China, and the roots have been used to treat impotency, neurasthenia, lumbago, and abscesses.[11] Previous phytochemical studies have revealed C_{21} steroidal glycosides as the major secondary metabolites of this plant species.[13−17] The bioactivity evaluation of these glycosides was earlier focused on antitumor and anti-inflammatory aspects.[17−19] Recently, neuroprotective,[20,21] antiaging,[22] and antidepressant[23] effects of C_{21} pregnane glycosides were also reported. In our previous work, three C_{21} glycosides were found to exhibit more potent antiseizure-like locomotor activity than phenytoin sodium in the zebrafish model.[24] Herein, the isolation and identification of nine new C_{21} steroidal glycosides, named cynawilfosides A–I (**1**–**9**), along with 12 known ones from the roots of *C. wilfordii*, as well as the antiepileptic activities of several abundant isolates using the mouse MES test are discussed.

RESULTS AND DISCUSSION

The molecular formula of compound **1** was determined to be $C_{63}H_{96}O_{20}$ based on the sodium adduct ion at m/z 1195.6365 in the HRESIMS (calcd 1195.6387) and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **1** showed the characteristic signals for the aglycone of C_{21} steroidal glycosides isolated from *Cynanchum* botanicals,[13,25] i.e., two angular methyl groups, CH_3-18 and CH_3-19 (δ_H 1.49, 1.15; δ_C 10.4, 18.2); a secondary

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds 1−6 (δ ppm, J in Hz, $CDCl_3$; 500 MHz)

position	1	2	3	4	5	6
1	1.06, m; 1.83, m	1.05, m; 1.85, m	1.05, m; 1.86, m	1.10, m; 1.84, m	1.08, m; 1.85, m	1.10, m; 1.89, m
2	1.58, m; 1.88, m	1.58, m; 1.93, m	1.58, m; 1.92, m	1.62, m; 1.92, m	1.47, m; 1.90, m	1.60, m; 1.91, m
3	3.53, m	3.55, m	3.54, m	3.59, m	3.55, m	3.55, m
4	2.27, m; 2.38, m	2.30, m; 2.35, m	2.05, m; 2.34, m	2.28, m; 2.34, m	2.29, m; 2.38, m	2.33, m; 2.40, m
6	5.37, brs	5.37, brs	5.37, brs	5.34, brs	5.36, brs	5.36, brs
7	2.20, m	2.17, m	2.17, m	2.20, m	2.28, m	2.20, m
9	1.50, m	1.40, m	1.40, m	1.50, m	1.46, m	1.58, m
11	1.94, m	1.97, m	1.97, m	1.89, m	1.92, m	1.89, m
12	4.80, m	3.47, m	3.47, m	4.57, m	3.70, m	4.80, m
15	1.82, m; 2.22, m	1.82, m; 2.22, m	1.80, m; 2.23, m	1.95, m	1.94, m	1.99, m
16	1.99, m	1.74, m; 2.30, m	1.76, m; 2.28, m	1.86, m; 2.80, m	1.97, m; 2.75, m	1.92, m; 2.86, m
18	1.49, s	1.32, s	1.32, s	1.40, s	1.24, s	1.52, s
19	1.15, s	1.15, s	1.15, s	1.13, s	1.15, s	1.11, s
20	4.74, q (6.5)	5.21, q (6.5)	5.20, q (6.5)			
21	1.16, d (6.5)	1.27, d (6.5)	1.23, d (6.5)	2.17, s	2.34, s	2.07, s
2′	6.30, d (15.9)			5.52, s		
3′	7.63, d (15.9)					7.85, d (8.8)
4′				2.33, m		6.86, d (8.8)
5′	7.37, m			1.06, d (6.8)		
6′	7.51, m			1.06, d (6.8)		
7′	7.37, m			2.12, s		
2″	2.36, m	5.64, brs	2.05, m; 2.35, m			
3″	1.43, m; 1.74, m		1.91, m			
4″	0.93, t (7.5)	2.35, m	1.58, m			
5″	1.15, m	1.05, d (6.5)	0.83, d (6.8)			
6″		1.05, d (6.5)	0.87, d (6.9)			
7″		2.12, s	0.88, d (6.8)			
	Cym	Cym	Cym	Cym	Cym	Digit
1‴	4.83, dd (1.8, 9.6)	4.83, dd (1.8, 9.6)	4.84, dd (1.8, 9.6)	4.84, dd (2.0, 9.8)	4.83, dd (2.1, 9.7)	4.95, dd (2.1, 9.8)
2‴	1.58, m; 2.20, m	1.58, m; 2.20, m	1.58, m; 2.20, m	1.60, m; 2.18, m	1.58, m; 2.19, m	1.73, m; 2.11, m
3‴	3.71, dd (2.9, 5.9)	3.69, dd (3.1, 6.5)	3.70, dd (3.1, 6.3)	3.72, dd (3.1, 6.0)	3.70, dd (3.1, 6.5)	4.25, brd (6.3)
4‴	3.29, dd (2.9, 9.5)	3.29, dd (3.1, 9.5)	3.29, dd (3.1, 9.8)	3.26, dd (2.8, 9.4)	3.24, dd (3.1, 9.6)	3.23, dd (3.0, 9.5)
5‴	3.81, dq (6.5, 9.5)	3.81, dq (6.4, 9.5)	3.82, dq (6.4, 9.8)	3.83, dq (6.3, 9.4)	3.82, dq (6.3, 9.6)	3.82, dq (6.3, 9.5)
6‴	1.24, d (6.5)	1.24, d (6.4)	1.24, d (6.4)	1.24, d (6.3)	1.25, d (6.3)	1.26, d (6.3)
3‴-OMe	3.46, s	3.46, s	3.46, s	3.43, s	3.42, s	
	Digin	Digin	Digin	Digin	Digin	Ole
1⁗	4.98, d (3.0)	4.98, d (3.0)	4.98, d (3.0)	4.99, d (3.6)	4.98, d (3.6)	4.56, dd (2.1, 9.8)
2⁗	1.80, m; 2.01, m	1.82, m; 2.01, m	1.80, m; 2.00, m	1.63, m; 1.92, m	1.82, m; 2.00, m	1.48, m; 2.35, m
3⁗	3.61, dd (3.1, 4.7)	3.61, dd (3.8, 7.6)	3.61, dd (3.9, 7.4)	3.80, brd (2.4)	3.61, dd (2.8, 4.8)	3.17, dd (4.8, 9.0)
4⁗	3.83, brd (2.5)	3.84, m	3.84, brd (3.1)	3.68, dd (2.4, 5.2)	3.87, brd (2.8)	3.13, t (9.0)
5⁗	3.94, q (6.4)	3.96, q (6.9)	3.95, q (6.7)	4.01, q (6.8)	3.97, q (6.6)	3.33, dq (6.2, 9.0)
6⁗	1.21, d (6.4)	1.22, d (6.9)	1.22, d (6.7)	1.30, d (6.8)	1.22, (6.6)	1.32, d (6.2)
3⁗-OMe	3.41, s	3.41, s	3.41, s	3.39, s	3.41, s	3.40, s
	Cym	Cym	Cym		Cym	
1⁗′	4.75, dd (1.8, 9.7)	4.75, dd (1.8, 9.7)	4.76, dd (2.0, 9.5)		4.68, dd (1.9, 9.9)	
2⁗′	1.22, m; 1.76, m	1.17, m; 1.76, m	1.17, m; 1.77, m		1.64, m; 2.22, m	
3⁗′	3.77, dd (3.3, 5.1)	3.77, dd (3.3, 5.1)	3.77, dd (3.0, 6.2)		3.65, dd (3.1, 6.5)	
4⁗′	3.30, dd (5.8, 9.6)	3.29, dd (2.8, 9.3)	3.30, dd (3.1, 9.6)		3.29, dt (3.1, 9.8)	
5⁗′	3.84, dq (6.2, 9.6)	3.84, m	3.85, dq (6.3, 9.6)		3.55, dq (6.1, 9.8)	
6⁗′	1.26, d (6.2)	1.26, m	1.26, d (6.3)		1.30, d (6.1)	
3⁗′-OMe	3.41, s	3.41, s	3.41, s		3.42, s	
	Cym	Cym	Cym			
1⁗″	4.78, d (3.0)	4.78, d (3.0)	4.78, d (3.0)			
2⁗″	1.88, m; 2.58, m	1.80, m; 2.44, m	1.73, m; 2.42, m			
3⁗″	3.58, dd (3.2, 6.4)	3.58, m	3.58, m			
4⁗″	3.26, brd (9.5)	3.26, dd (3.5, 9.2)	3.27, dd (3.6, 9.6)			
5⁗″	4.03, dq (6.2, 9.5)	4.03, dq (6.2, 9.2)	4.03, dq (6.3, 9.6)			
6⁗″	1.25, d (6.2)	1.25, d (6.2)	1.25, d (6.3)			
3⁗″-OMe	3.39, s	3.39, s	3.39, s			

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methyl group, CH_3-21 (δ_H 1.16; δ_C 15.1); a $\Delta^{5,6}$ double bond (δ_H 5.37; δ_C 139.7, 118.6); three methines, CH-3, CH-12, and CH-20 (δ_H 3.53, 4.80, and 4.74; δ_C 78.0, 73.8, and 74.1) attached to oxygen; and three oxygenated tertiary carbons, C-8, C-14, and C-17 (δ_C 74.2, 88.0, and 87.9). Analysis of the ^{1}H−^{1}H COSY, HSQC, and HMBC data of **1** (Figures S1E−S1G, Supporting Information) led to the establishment of an ester-substituted sarcostin analogue.[26] In addition, a cinnamoyl group [δ_H 6.30 (1H, d, J = 15.9 Hz), 7.63 (1H, d, J = 15.9 Hz), 7.37 (3H, m), and 7.51 (2H, m); δ_C 166.9, 119.1, 144.7, 134.7, 128.2, 128.9, and 130.3] and a 2-methylbutanoyl moiety [δ_H 2.36 (1H, m), 1.74 (1H, m), 1.43 (1H, m), 1.15 (3H, m), and 0.93 (3H, t, J = 7.5 Hz); δ_C 174.5, 41.5, 26.4, 11.9, and 16.7] were also observed. HMBC correlations between H-12 (δ_H 4.80) and C-1′ (δ_C 166.9) and between H-20 (δ_H 4.74) and C-1″ (δ_C 174.5) revealed the placement of the two ester groups at C-12 and C-20. In addition to the aforementioned signals of the aglycone moiety, the ^{1}H NMR spectrum of **1** exhibited four resonances for anomeric protons at δ_H 4.98 (d, J = 3.0 Hz), 4.78 (d, J = 3.0 Hz), 4.75 (dd, J = 1.8, 9.7 Hz), and 4.83 (dd, J = 1.8, 9.6 Hz), corresponding to carbon signals at δ_C 101.0, 98.8, 99.5, and 95.8, respectively (Tables 1 and 3) in the HSQC spectrum. Selective irradiation of the signals at δ_H 4.83 (H-1‴), 4.98 (H-1⁗), 3.77 (H-3⁗′), and 4.03 (H-5⁗″) in the 1D TOCSY experiment revealed the chemical shifts and coupling constants of H-1−H-4 of a β-cymaropyranosyl, H-1−H-5 of a β-cymaropyranosyl and an α-diginopyranosyl, and H-1−H-6 of an α-cymaropyranosyl residue (Figure S1I, Supporting Information). ^{1}H−^{13}C long-range correlations between H-1‴ (δ_H 4.83) and C-3 (δ_C 78.0), between H-1⁗ (δ_H 4.98) and C-4‴ (δ_C 81.9), between H-1⁗′ (δ_H 4.75) and C-4⁗ (δ_C 74.5), and between H-1⁗″ (δ_H 4.78) and C-4⁗′ (δ_C 82.0) revealed the placement of each sugar moiety. Acid hydrolysis of **1** followed by column chromatography yielded diginose and cymarose. Their specific rotation values ([α]$_D$ = −44.6 and +15.5, respectively) were obtained from an aqueous solution after a 24 h equilibration period. The diginose was L configured, and the mixture of D- and L-cymaroses were in the ratio 2:1 based on comparison of experimental and reported [α]$_D$ values.[27] In addition, the ^{13}C NMR spectroscopic data of the sugar moiety of **1** matched well with those of wilfoside M1N (**14**),[28] tanwanoside C (**15**),[29] wilfoside C1N (**16**),[30] and wilfoside K1N (**17**)[28] isolated from *Cynanchum* plants. In order to define the absolute configuration of C-2″ in the side chain, compound **1** was subjected to alkaline hydrolysis aiming to obtain the 2-methylbutyric acid. With the authentic samples [racemic- and (*S*)-2-methylbutyric acid] in hand, chiral GC analysis was carried out to establish the absolute configuration of C-2″ in **1**. A major GC peak (t_R = 17.778 min) in the hydrolysis mixture of **1** matched the (*R*)-2-methylbutyric acid peak in the GC spectrum of the racemate, indicating a 2″*R* absolute configuration for compound **1** (Figure S1J and K, Supporting Information). Thus, the structure of **1** was defined as 12-*O*-cinnamyl-20-*O*-[(*R*)-2-methylbutanoyl]sarcostin 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside and named cynawilfoside A.

Compounds **1**−**3** possessed identical saccharide moieties according to their NMR data (Tables 1 and 3). The molecular formula of cynawilfoside B (**2**) was deduced as $C_{56}H_{92}O_{19}$ on the basis of an [M + Na]$^+$ ion at m/z 1091.6099 in the HRESIMS (calcd 1091.6125) and ^{13}C NMR data. The NMR data of the aglycone of **2** were similar to those of **1**, except for the absence of the cinnamoyl and 2-methylbutanoyl groups and the presence of an ikemaoyl group [δ_H 5.64 (1H, brs), 2.35 (1H, m), 2.12 (3H, s), and 1.05 (6H, d, J = 6.5 Hz); δ_C 166.9, 165.9, 113.5, 38.3, 21.0, 21.0, and 16.6]. The HMBC correlations from H-20 (δ_H 5.21) to C-1″ (δ_C 166.9) enabled location of the ikemaoyl group at C-20. Thus, the structure of **2**

Table 2. ^{1}H NMR Spectroscopic Data for Compounds 7−9 (δ ppm, J in Hz, $CDCl_3$; 500 MHz)

position	7	8	9
1	1.10, m; 1.84, m	1.08, m; 1.88, m	1.07, m; 1.90, m
2	1.62, m; 1.90, m	1.61, m; 1.91, m	1.63, m; 1.91, m
3	3.58, m	3.56, m	3.59, m
4	2.25, m; 2.40, m	2.28, m; 2.39, m	2.29, m; 2.41, m
6	5.36, brs	5.37, brs	5.36, brs
7	2.18, m	2.20, m	2.20, m
9	1.57, m	1.52, m	1.52, m
11	1.89, m	1.83, m	1.85, m
12	4.80, m	4.55, m	4.52, m
15	1.99, m	1.94, m	1.97, m
16	1.93, m; 2.84, m	2.20, m; 2.85, m	1.82, m; 2.85, m
18	1.52, s	1.40, s	1.40, s
19	1.09, s	1.12, s	1.12, s
21	2.07, s	2.17, s	2.17, s
2′		5.52, s	5.51, s
3′	7.81, d (7.9)		
4′	6.82, d (7.9)	2.34, m	2.34, m
5′		1.06, d (6.8)	1.06, d (6.8)
6′		1.06, d (6.8)	1.06, d (6.8)
7′		2.12, s	2.12, s
	Digit	Cym	Digit
1‴	4.94, dd (2.0, 9.5)	4.84, dd (2.0, 9.7)	4.92, dd (2.0, 9.9)
2‴	1.67, m; 2.24, m	1.58, m; 2.08, m	1.71, m; 2.10, m
3‴	4.25, dd (3.8, 7.6)	3.79, dd (2.9, 6.8)	4.22, dd (2.6, 5.8)
4‴	3.22, dd (3.1, 9.7)	3.23, dd (2.9, 9.7)	3.21, dd (2.6, 9.5)
5‴	3.81, dq (6.2, 9.7)	3.86, dq (6.5, 9.7)	3.81, dq (6.5, 9.5)
6‴	1.24, d (6.2)	1.22, d (6.5)	1.25, d (6.5)
3‴-OMe		3.44, s	
	Can	Ole	Ole
1⁗	4.57, dd (2.0, 9.5)	4.46, dd (1.8, 9.8)	4.51, dd (2.0, 9.8)
2⁗	1.60, m; 2.27, m	1.54, m; 2.35, m	1.51, m; 2.35, m
3⁗	3.60, ddd (5.4, 9.0, 11.6)	3.25, ddd (5.1, 9.1, 10.7)	3.26, m
4⁗	2.99, t (9.0)	3.10, t (9.1)	3.10, t (9.0)
5⁗	3.35, dq (6.4, 9.4)	3.25, m	3.26, dq (6.4, 9.4)
6⁗	1.28, d (6.4)	1.24, d (6.3)	1.24, d (6.4)
3⁗-OMe		3.35, s	3.36, s
	Ole	Cym	Cym
1⁗′	4.49, dd (2.0, 9.5)	4.86, d (3.2)	4.86, d (3.0)
2⁗′	1.49, m; 2.38, m	1.73, m; 2.25, m	1.73, m; 2.24, m
3⁗′	3.18, dd (2.9, 9.8)	3.59, dd (3.4, 6.7)	3.58, dd (3.5, 7.0)
4⁗′	3.16, brd (9.8)	3.27, brd (9.4)	3.27, dd (3.5, 9.4)
5⁗′	3.43, dq (6.0, 9.8)	4.08, dq (6.4, 9.4)	4.07, dq (6.2, 9.4)
6⁗′	1.36, d (6.0)	1.25, d (6.4)	1.25, d (6.2)
3⁗′-OMe	3.41, s	3.37, s	3.37, s

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Table 3. ^{13}C NMR Spectroscopic Data for Compounds 1−9 (δ ppm, $CDCl_3$; 125 MHz)

position	1	2	3	4	5	6	7	8	9
1	38.9	39.0	39.0	38.9	38.9	38.8	38.9	38.9	38.9
2	29.1	29.2	29.2	29.0	29.1	29.0	28.9	29.0	29.0
3	78.0	78.1	78.0	78.0	78.0	78.0	78.0	78.0	78.0
4	38.9	38.9	38.9	38.9	38.9	38.9	38.8	38.9	38.8
5	139.7	139.7	139.7	140.8	140.5	140.6	140.5	140.8	140.8
6	118.6	118.7	118.6	117.8	118.0	117.9	117.9	117.8	117.8
7	34.6	34.6	34.6	34.4	34.3	34.4	34.4	34.4	34.3
8	74.2	74.2	74.2	74.4	74.4	74.4	74.4	74.4	74.4
9	43.5	43.8	43.8	43.8	44.2	43.7	43.7	43.8	43.8
10	37.1	37.0	37.0	37.3	37.2	37.3	37.2	37.3	37.2
11	25.0	29.2	29.3	24.4	28.1	24.4	24.4	24.4	24.4
12	73.8	70.6	70.5	71.7	69.6	72.9	72.9	71.8	71.7
13	56.3	58.1	58.0	58.1	61.0	58.5	58.5	58.1	58.0
14	88.0	88.0	87.9	88.1	87.8	88.2	88.2	88.1	88.1
15	31.9	32.3	32.3	33.3	33.5	33.4	33.4	33.2	33.2
16	33.4	33.3	33.3	32.0	32.5	32.0	32.0	32.0	32.0
17	87.9	87.9	87.9	91.6	91.9	91.6	91.6	91.6	91.6
18	10.4	9.0	9.0	9.5	7.8	9.7	9.7	9.5	9.6
19	18.2	18.2	18.2	18.7	18.8	18.7	18.7	18.7	18.7
20	74.1	73.8	74.6	209.0	214.0	209.9	209.9	209.1	209.1
21	15.1	15.3	15.0	27.3	28.5	27.6	27.6	27.3	27.3
1′	166.9			167.1		165.3	165.3	167.1	167.1
2′	119.1			113.1		122.1	122.0	113.1	113.1
3′	144.7			166.1		132.0	132.0	166.1	166.1
4′	134.7			38.3		115.4	115.4	38.3	38.3
5′	128.2			21.1		160.7	160.8	21.1	21.1
6′	128.9			21.0				21.0	21.0
7′	130.3			16.7				16.7	16.7
1″	174.5	166.9	172.5						
2″	41.5	113.5	39.6						
3″	26.4	165.9	35.6						
4″	11.9	38.3	32.2						
5″	16.7	21.0	16.1						
6″		21.0	18.4						
7″		16.6	19.9						
	Cym	Cym	Cym	Cym	Cym	Digit	Digit	Cym	Digit
1‴	95.8	95.7	95.7	95.9	95.8	95.9	95.8	96.2	95.9
2‴	34.2	34.3	34.4	34.3	34.3	37.2	37.2	36.0	37.2
3‴	77.2	77.2	77.2	77.2	77.1	66.7	66.9	77.1	66.7
4‴	81.9	82.0	82.0	81.9	82.0	82.9	82.8	82.7	82.9
5‴	68.7	68.9	68.9	69.0	69.0	68.1	68.1	68.5	68.0
6‴	18.3	18.3	18.3	18.5	18.4	18.3	18.3	18.3	18.3
3‴-OMe	57.1	57.1	57.1	57.0	57.1			58.4	
	Digin	Digin	Digin	Digin	Digin	Ole	Can	Ole	Ole
1⁗	101.0	101.0	101.0	100.6	101.0	100.5	100.5	101.5	100.4
2⁗	31.7	31.7	31.8	30.0	31.8	35.4	38.4	36.1	36.1
3⁗	73.9	74.0	74.0	67.7	74.0	80.5	69.5	78.9	78.8
4⁗	74.5	74.6	74.6	74.4	74.4	75.4	88.1	81.7	81.6
5⁗	66.9	66.9	66.9	66.1	66.9	71.9	70.6	71.8	72.0
6⁗	18.2	18.4	18.4	17.2	18.2	18.1	18.0	18.5	18.5
3⁗-OMe	55.7	55.7	55.7	55.7	55.7	56.6		56.4	56.5
	Cym	Cym	Cym		Cym		Ole	Cym	Cym
1⁗′	99.5	99.5	99.5		99.2		101.1	97.1	97.2
2⁗′	34.4	34.4	34.4		34.4		35.2	31.1	31.1
3⁗′	77.3	77.3	77.3		77.5		80.4	75.1	75.1
4⁗′	82.0	82.0	82.0		72.2		75.1	72.3	72.2
5⁗′	69.2	69.2	69.1		71.2		72.2	65.2	65.3
6⁗′	18.4	18.3	18.3		17.7		17.8	17.9	17.9
3⁗′-OMe	57.6	57.6	57.6		57.1		56.7	56.4	56.4
	Cym	Cym	Cym						
1⁗″	98.8	98.7	98.7						

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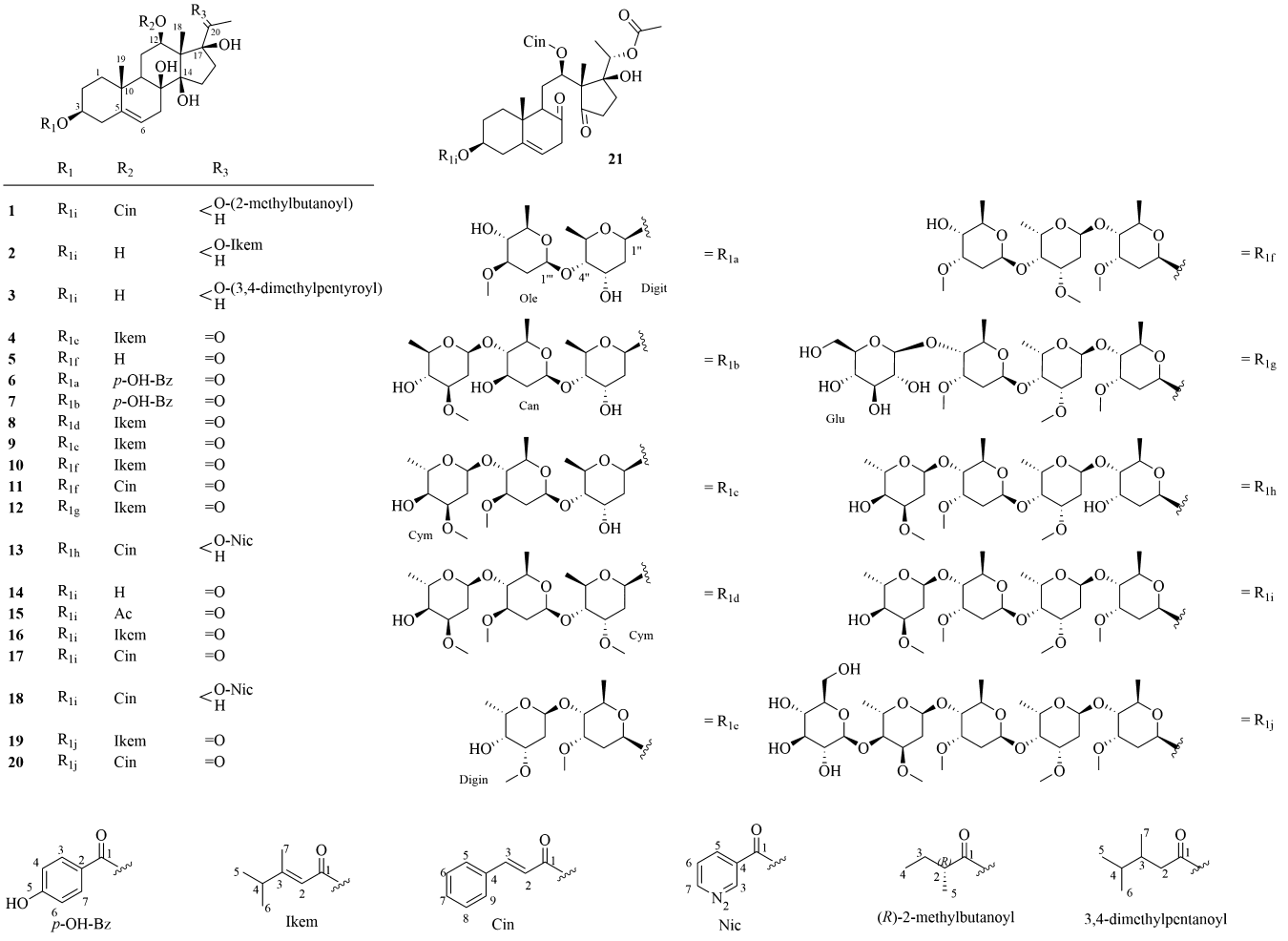
Table 3. continued

position	1	2	3	4	5	6	7	8	9
2′′′′′	31.2	31.1	31.2						
3′′′′′	74.9	74.9	74.9						
4′′′′′	72.1	72.1	72.1						
5′′′′′	65.7	65.7	65.7						
6′′′′′	17.6	17.6	17.7						
3′′′′′-OMe	56.5	56.5	56.5						

Chart 1



	R_1	R_2	R_3
1	R_{1i}	Cin	O-(2-methylbutanoyl)
2	R_{1i}	H	O-Ikem
3	R_{1i}	H	O-(3,4-dimethylpentyroyl)
4	R_{1c}	Ikem	=O
5	R_{1f}	H	=O
6	R_{1a}	*p*-OH-Bz	=O
7	R_{1b}	*p*-OH-Bz	=O
8	R_{1d}	Ikem	=O
9	R_{1c}	Ikem	=O
10	R_{1f}	Ikem	=O
11	R_{1f}	Cin	=O
12	R_{1g}	Ikem	=O
13	R_{1h}	Cin	O-Nic
14	R_{1i}	H	=O
15	R_{1i}	Ac	=O
16	R_{1i}	Ikem	=O
17	R_{1i}	Cin	=O
18	R_{1i}	Cin	O-Nic
19	R_{1j}	Ikem	=O
20	R_{1j}	Cin	=O

was characterized as 20-*O*-ikemaoylsarcostin 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

The positive mode HRTOFMS of cynawilfoside C (**3**) showed an $[M + Na]^+$ ion at *m*/*z* 1093.6312, revealing its molecular formula to be $C_{56}H_{94}O_{19}$. Comparison of the ^1H and ^{13}C NMR data of **3** and **2** showed that compound **3** differs from **2** only in the structure of the ester group at C-20. Analysis of the HMBC correlations between H-2″ (δ_H 2.35, 2.05) and C-1″ (δ_C 172.5), C-3″ (δ_C 35.6), C-4″ (δ_C 32.2), and C-7″ (δ_C 19.9); between H-5″ and H-6″ (δ_H 0.83, 0.87) and C-3″ (δ_C 35.6) and C-4″ (δ_C 32.2); and between H-7″ (δ_H 0.88) and C-3″ (δ_C 35.6) identified the 3,4-dimethylpentanoyl substituent. Therefore, the structure of **3** was identified as 20-*O*-(3,4-dimethylpentanoyl)sarcostin 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Cynawilfoside D (**4**) was isolated as a white, amorphous powder, and its molecular formula was deduced as $C_{42}H_{66}O_{13}$ by a sodium adduct ion at *m*/*z* 801.4390 in the HRTOFMS and ^{13}C NMR data. The aglycone of **4** was defined as caudatin based on the characteristic NMR signals of caudatin 3-*O*-glycosides isolated from *Cynanchum*.[13,24] Two anomeric proton signals at δ_H 4.99 (1H, d, *J* = 3.6 Hz) and 4.84 (1H, dd, *J* = 2.0, 9.8 Hz) corresponding to two anomeric carbon signals at δ_C 100.6 and 95.9 indicated a saccharide chain containing two sugar units. Further analysis of the coupling constant of each sugar proton using $^1H-^1H$ COSY and 1D-TOCSY data (Figure S4G and H, Supporting Information) showed the two sugar moieties as α-diginopyranosyl and β-cymaropyranosyl, respectively. The structure of the saccharide chain was established according to the HMBC correlation between H-1‴ (δ_H 4.99) and C-4‴ (δ_C 81.9) and between H-1′′′ (δ_H 4.84) and C-3 (δ_C 78.0). The L configuration of the diginopyranosyl and D

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configuration of the cymaropyranosyl moieties were determined on the basis of their specific rotation values ($[\alpha]_D$ = −42.4 and +47.6, respectively).[24,27] Thus, **4** was characterized as caudatin 3-*O*-*α*-L-diginopyranosyl-(1→4)-*β*-D-cymaropyranoside.

Compound **5** gave a sodium adduct ion at *m*/*z* 835.4464 in the HRTOFMS, revealing its molecular formula to be $C_{42}H_{68}O_{15}$. The ^{1}H and ^{13}C NMR data of the aglycone part of **5** were similar to those of **4** (Tables 1 and 3), except for the absence of signals due to the ikemaoyl group and the shielding of H-12 (δ_H 3.70) and C-12 (δ_C 69.6), which indicated the aglycone of **5** to be deacetylmetaplexigenin.[24] Three glycosyl units are present in the structure of **5** according to three anomeric proton signals at δ_H 4.98 (1H, d, *J* = 3.6 Hz), 4.83 (1H, dd, *J* = 2.1, 9.7 Hz), and 4.68 (1H, dd, *J* = 1.9, 9.9 Hz) and corresponding anomeric carbon resonances at δ_C 101.0, 95.8, and 99.2, respectively. The sugar moieties were determined to be two *β*-cymaropyranosyl and one *α*-diginopyranosyl by analyzing the coupling constants (Table 1). The connection of these glycosyl units was established by the HMBC correlations between H-1‴ (δ_H 4.83) and C-3 (δ_C 78.0), between H-1⁗ (δ_H 4.98) and C-4‴ (δ_C 82.0), and between H-1⁗′ (δ_H 4.68) and C-4⁗ (δ_C 74.4). The D configuration of the two cymaropyranosyl units and L configuration of the diginopyranosyl unit were confirmed by the specific rotation values of the purified saccharides ($[\alpha]_D$ = +49.6 and −41.8) obtained via acid hydrolysis of **5**.[24,27] Thus, **5** was characterized as deacetylmetaplexigenin 3-*O*-*β*-D-cymaropyranosyl-(1→4)-*α*-L-diginopyranosyl-(1→4)-*β*-D-cymaropyranoside and named cynawilfoside E.

The molecular formula of cynawilfoside F (**6**) was determined to be $C_{41}H_{58}O_{14}$ from the $[M - H]^-$ ion at *m*/*z* 773.3747 in the HRTOFMS and ^{13}C NMR data. A pair of *ortho*-coupled aromatic proton signals were observed at δ_H 7.85 (2H, d, *J* = 8.8 Hz) and 6.86 (2H, d, *J* = 8.8 Hz) in its ^{1}H NMR spectrum (Figure S6C, Supporting Information), which indicated the presence of a *para*-disubstituted benzene ring. Analysis of its ^{13}C NMR, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra showed the aglycone of **6** to be qingyangshengenin.[24] Two anomeric proton signals at δ_H 4.95 (1H, dd, *J* = 2.1, 9.8 Hz) and 4.56 (1H, dd, *J* = 2.1, 9.8 Hz) corresponding to two anomeric carbon signals at δ_C 95.9 and 100.5 revealed that **6** contained two saccharide moieties with *β* glycosidic linkages. The coupling constants of H-3‴ (δ_H 4.25, brd, *J* = 6.3 Hz), H-4‴ (δ_H 3.23, dd, *J* = 3.0, 9.5 Hz), and H-5‴ (δ_H 3.82, dq, *J* = 6.3, 9.5 Hz); H-3⁗ (δ_H 3.17, dd, *J* = 4.8, 9.0 Hz), H-4⁗ (δ_H 3.13, t, *J* = 9.0 Hz), and H-5⁗ (δ_H 3.33, dq, *J* = 6.2, 9.0 Hz) indicated the presence of digitoxopyranose and oleandropyranose in the saccharide chain. ^{1}H−^{13}C long-range correlation signals between δ_H 4.95 (H-1‴) and δ_C 78.0 (C-3) and between δ_H 4.56 (H-1⁗) and δ_C 82.9 (C-4‴) revealed the linkage of the digitoxopyranosyl unit to C-3 and the oleandropyranosyl unit to C-4‴. The D configurations of the two monosaccharides were determined on the basis of their specific rotation values ($[\alpha]_D$ = +47.4 and −12.3, respectively).[24] Thus, **6** was characterized as qingyangshengenin 3-*O*-*β*-D-oleandropyanosyl-(1→4)-*β*-D-digitoxopyranoside.

Cynawilfoside G (**7**) exhibited an $[M - H]^-$ ion at *m*/*z* 903.4380 in the HRTOFMS, indicative of a molecular formula of $C_{47}H_{68}O_{17}$. Analyses of its ^{1}H and ^{13}C NMR data (Tables 2 and 3) indicated that **7** possessed the same aglycone moiety as **6**. Three saccharide units with *β* glycosidic linkages were present based on the three anomeric proton signals at δ_H 4.94 (1H, dd, *J* = 2.0, 9.5 Hz), 4.57 (1H, dd, *J* = 2.0, 9.5 Hz), and 4.49 (1H, dd, *J* = 2.1, 9.5 Hz). Irradiation of H-1⁗ (δ_H 4.57) in the 1D TOCSY experiment revealed the chemical shifts and coupling constants of H-1−H-5 of the canaropyranosyl unit (Figure S7H, Supporting Information). The other two sugar units were confirmed to be digitoxopyransyl and oleandropyranosyl moieties by analyzing their coupling constants of H-3, H-4, and H-5 according to the ^{1}H and ^{1}H−^{1}H COSY spectra. The HMBC correlations between H-1‴ (δ_H 4.94) and C-3 (δ_C 78.0); between H-1⁗ (δ_H 4.57) and C-4‴ (δ_C 82.8); and between H-1⁗′ (δ_H 4.49) and C-4⁗ (δ_C 88.1) revealed the structure of the sugar chain. Acid hydrolysis of **7** yielded digitoxose, canarose, and oleandrose. The D configurations of the three monosaccharide moieties were identified according to their specific rotation values ($[\alpha]_D$ = +48.4, +22.3, and −11.7, respectively).[24,31] Thus, the structure of **7** was identified as qingyangshengenin 3-*O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-canaropyranosyl-(1→4)-*β*-D-digitoxopyranoside.

Comparison of NMR data of **4**, **8**, and **9** indicated caudatin as their common aglycone. The molecular formula of cynawilfoside I (**8**) was determined to be $C_{49}H_{78}O_{16}$ from the sodiated adduct ion $[M + Na]^+$ at *m*/*z* 945.5214. Irradiation of H-3‴ (δ_H 3.79), H-1⁗ (δ_H 4.46), and H-3⁗′ (δ_H 3.59) in the 1D TOCSY experiments gave the chemical shifts and coupling constants of H-1−H-6 of two cymarose residues and H-1−H-5 of an oleandrose residue (Figure S9G, Supporting Information). The 1→4 connection of the three saccharide units was deduced via the HMBC correlations between H-1⁗ (δ_H 4.46) and C-4‴ (δ_C 82.7) and between H-1⁗′ (δ_H 4.86) and C-4⁗ (δ_C 81.7). Acid hydrolysis of **8** and subsequent purification afforded cymarose and oleandrose. The oleandrose was D-configured based on comparison of experimental ($[\alpha]_D$ = −11.3) and reported ($[\alpha]_D$ = −11.7) specific rotation values,[24] while cymarose was obtained as a 1:1 racemate. Thus, the structure of **8** was characterized as caudatin 3-*O*-*α*-L-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-cymaropyranoside. Cynawilfoside H (**9**) had the molecular formula $C_{48}H_{76}O_{16}$ as deduced by a sodiated molecular ion peak at *m*/*z* 931.5037 in the HRTOFMS. The ^{1}H and ^{13}C NMR data of **9** differ from those of **8** in terms of an additional methoxy group (Tables 2 and 3), which is in accordance with the molecular mass difference of 14 amu between **9** and **8**. The saccharide moiety was determined to consist of digitoxopyranosyl, cymaropyranosyl, and oleandropyranosyl units by irradiation of glycosyl proton signals at δ_H 4.86, 4.51, and 4.22 in the 1D TOCSY experiments (Figure S8G, Supporting Information). Further analysis of its ^{1}H−^{1}H COSY, HSQC, and HMBC spectra enabled assignment of all the proton and carbon signals and connection of glycosyl units of **9**. Thus, the structure of **9** was identified as caudatin 3-*O*-*α*-cymaropyranosyl-(1→4)-*β*-oleandropyranosyl-(1→4)-*β*-digitoxopyranoside.

Twelve known compounds were identified as cynauricoside A (**10**),[27] wilfoside C3N (**11**),[28] caudatin 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*α*-L-diginopyranosyl-(1→4)-*β*-D-cymaropyranoside (**12**),[32] gagaminin 3-*O*-*α*-L-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*α*-L-diginopyranosyl-(1→4)-*β*-D-digitoxopyranoside (**13**),[33] wilfoside M1N (**14**),[28] taiwanoside C (**15**),[29] wilfoside C1N (**16**),[30] wilfoside K1N (**17**),[28] cyanoauriculoside G (**18**),[33] wilfoside C1G (**19**),[30] cynauricuoside A (**20**),[30] and wilfoside G (**21**)[13] by comparing their experimental and reported spectroscopic and spectrometric data.

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The antiepileptic activity of compounds **1**, **11**, **12**, **14**−**19**, and **21** were evaluated using the mouse MES model after oral administration. At a dose of 100 mg/kg, the seizure protection rate of **1**, **11**, and **16**−**18** was 90.0%, 60.0%, 40.0%, 70.0%, and 55.5%, respectively. Protection of mice against MES was achieved at rates of 0%, 20%, 50%, 70%, 90%, and 100% when vehicle or 25, 50, 75, 100, and 150 mg/kg of compound **1** were administered, respectively. The ED_{50} value of **1** was calculated to be 48.5 mg/kg (Figure 1, Table 4). In parallel experiments

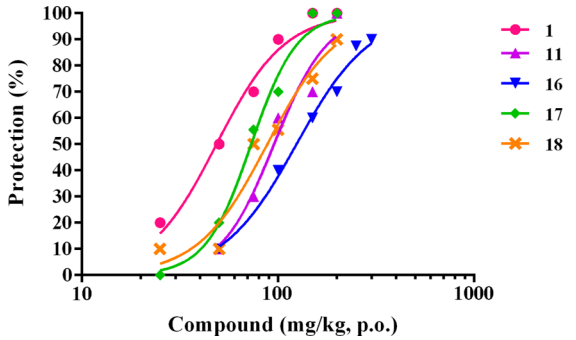

Figure 1. Effects of compounds **1**, **11**, and **16**−**18** in the mouse MES-induced seizure model.

Table 4. ED_{50} Values of Compounds 1, 11, 12, 14−19, and 21 in Mouse MES-Induced Seizure Assays

compound	ED_{50} (mg/kg)	95% confidence interval
1	48.5	41.4−56.9
11	95.3	75.7−120.0
12	>200	
14	>200	
15	>200	
16	124.1	111.6−138.0
17	72.3	65.5−81.1
18	88.1	71.5−108.5
19	>200	
21	>200	
retigabine[34]	15.0	

under identical conditions, the antiepileptic activity of compounds **11** and **16**−**18** was evaluated and the ED_{50} values were calculated to be 95.3, 124.1, 72.3, and 88.1 mg/kg, respectively (Figure 1, Table 4). Under identical conditions, the ED_{50} value of retigabine, a first-line antiepilepsy drug, was 15.0 mg/kg.[34] The MES test results suggested that these C_{21} steroidal glycosides deserve further evaluation as potential candidates for the development of therapeutic agents against epilepsy. Detailed MES experimental data of these compounds are available in Table S1 (Supporting Information).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 341 polarimeter (PerkinElmer, Waltham, MA, USA). HRTOFMS analyses were performed on an LCT Premier XE mass spectrometer; HRESIMS analyses were performed on a Micromass Ultra Q-TOF mass spectrometer (Waters, Milford, MA, USA). IR spectra were recorded on a PerkinElmer 577 spectrometer by using KBr disks. NMR experiments were performed on a Varian-MERCURY Plus-400 (Varian, Palo Alto, CA, USA) or Bruker Advance III 500 (Bruker, Ettlingen, Germany) spectrometer with tetramethylsilane as an internal standard. Preparative HPLC was performed on a Unimicro EasySep-1010 binary pump system (Unimicro, Shanghai, People's Republic of China) with a Unimicro EasySep-1010 detector by using a YMC-Pack ODS-A (250 × 20 mm, 5 μm; YMC Co., Ltd., Kyoto, Japan) column. Silica gel (300−400 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China) and C_{18} reversed-phase (RP-18) silica gel (150−200 mesh; Merck, Whitehouse Station, NJ, USA) were used for column chromatography (CC). Precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co., Ltd.) were used for TLC detection.

Plant Material. The roots of *Cynanchum wilfordii* were collected in November 2012 from Xianfeng County, Hubei Province, People's Republic of China, and identified by Prof. Xin-Sheng Qin of College of Forestry, South China Agricultural University. A voucher specimen (No. WMZ-201211-GSX) has been deposited at Shanghai Institute of Materia Medica.

Extraction and Isolation. Air-dried roots of *C. wilfordii* (5.0 kg) were powdered and percolated with 95% EtOH (3 × 10 L) at room temperature to afford the crude extract, which was suspended in 2 L of H_2O and extracted with EtOAc (3 × 1 L). The EtOAc extract was applied to a silica gel column using petroleum ether−acetone (from 10:1 to 0:1, v/v) as eluent to yield nine fractions (Fr.1−9). Fr.6 was further applied to silica gel CC (petroleum ether−acetone, 5:1−2:1) to afford four subfractions (Fr.6a−6d). Compound **1** (1.195 g) was isolated from Fr.6b by using preparative HPLC (70% CH_3CN); Fr.6c was subjected to an RP-18 column and eluted with MeOH−H_2O (from 40% to 100% v/v) to obtain three subfractions (Fr.6c1−6c3). Fr.6c2 was separated by preparative HPLC (60% CH_3CN) to yield **2** (0.02 g), **3** (0.014 g), and **15** (0.189 g); Fr.6c3 was purified by preparative HPLC to yield **4** (0.012 g) and **11** (0.098 g); compounds **10** (0.288 g) and **16** (3.025 g) were isolated from Fr.6d by using preparative HPLC (55% CH_3CN); Fr.7 was subjected to silica gel CC (petroleum ether−acetone, 5:1 to 3:1, v/v) and further purified by preparative HPLC to yield compounds **6** (0.011 g), **7** (0.134 g), **8** (0.004 g), **9** (0.021 g), **17** (2.53 g), and **21** (0.23 g); Fr.8 was chromatographed over silica gel CC (petroleum ether−acetone, 3:1 to 0:1, v/v) and further purified by preparative HPLC to yield **5** (0.009 g), **13** (0.006 g), **14** (0.312 g), and **18** (0.283 g); Fr.8 was separated using the RP-18 column (from 50% to 60% MeOH) and further purified by preparative HPLC to yield **12** (0.073 g), **19** (0.12 g), and **20** (0.054 g).

*Cynawilfoside A (**1**):* white, amorphous powder, $[\alpha]_D^{25}$ +5 (*c* 0.1, MeOH); IR (KBr) ν_{max} 2937, 2827, 1714, 1452, 1365, 1309, 1164, 1083, and 989 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1−3; HRESIMS *m*/*z* 1195.6365 $[M + Na]^+$ (calcd for $C_{63}H_{96}O_{20}Na$, 1195.6387).

*Cynawilfoside B (**2**):* white, amorphous powder, $[\alpha]_D^{25}$ −19 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3428, 2933, 1698, 1644, 1455, 1371, 1230, 1166, 1085, 1066, and 989 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1−3; HRESIMS *m*/*z* 1091.6099 $[M + Na]^+$ (calcd for $C_{56}H_{92}O_{19}Na$, 1091.6125).

*Cynawilfoside C (**3**):* white, amorphous powder, $[\alpha]_D^{25}$ −28 (*c* 0.1, MeOH); IR (KBr) ν_{max} 2940, 2888, 2832, 1718, 1457, 1365, 1087, and 991 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1−3; HRTOFMS *m*/*z* 1093.6312 $[M + Na]^+$ (calcd for $C_{56}H_{94}O_{19}Na$, 1093.6287).

*Cynawilfoside D (**4**):* white, amorphous powder, $[\alpha]_D^{25}$ −11 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3462, 2963, 2931, 1712, 1644, 1461, 1384, 1364, 1223, 1166, 1093, 1068, 1005, and 988 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1−3; HRTOFMS *m*/*z* 801.4390 $[M + Na]^+$ (calcd for $C_{42}H_{66}O_{13}Na$, 801.4401).

*Cynawilfoside E (**5**):* white, amorphous powder, $[\alpha]_D^{25}$ +12 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3454, 2970, 2932, 1704, 1648, 1448, 1378, 1366, 1351, 1166, 1147, 1087, 1005, and 988 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1−3; HRTOFMS *m*/*z* 835.4464 $[M + Na]^+$ (calcd for $C_{42}H_{68}O_{15}Na$, 835.4456).

*Cynawilfoside F (**6**):* white, amorphous powder, $[\alpha]_D^{25}$ −10 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3423, 2942, 2908, 1704, 1610, 1594, 1457, 1276, 1238, 1165, 1064, and 985 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1−3; HRTOFMS *m*/*z* 773.3747 $[M - H]^-$ (calcd for $C_{41}H_{57}O_{14}$, 773.3748).

*Cynawilfoside G (**7**):* white, amorphous powder, $[\alpha]_D^{25}$ −15 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3390, 2985, 2942, 2904, 1714, 1612, 1455,

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1373, 1278, 1234, 1165, 1062, and 991 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRTOFMS m/z 903.4380 $[M - H]^-$ (calcd for $C_{47}H_{67}O_{17}$, 903.4378).

*Cynawilfoside H (**8**):* white, amorphous powder, $[\alpha]_D^{25}$ −37 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3450, 2969, 2935, 1712, 1644, 1456, 1367, 1222, 1061, 1103, 1057, and 989 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRTOFMS m/z 945.5214 $[M + Na]^+$ (calcd for $C_{49}H_{78}O_{16}Na$, 945.5188).

*Cynawilfoside I (**9**):* white, amorphous powder, $[\alpha]_D^{25}$ −36 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3472, 2969, 2934, 1712, 1644, 1451, 1383, 1224, 1162, 1109, 1058, 1012, and 988 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRTOFMS m/z 931.5037 $[M + Na]^+$ (calcd for $C_{48}H_{76}O_{16}Na$, 931.5031).

Acidic Hydrolysis of Compounds 1 and 4−8. One drop of 2 N HCl was added to a solution of **1** (10 mg) in dioxane (3 mL), and the reaction mixture was stirred at 60 °C for 1 h. After cooling, H_2O (10 mL) was added, and the mixture was extracted with EtOAc (10 mL). The aqueous phase was dried to afford a crude sugar fraction, which was subjected to silica gel CC, eluting with gradient DCM−MeOH to afford cymarose (70:1, v/v) and diginose (50:1, v/v). Compounds **4**−**8** were hydrolyzed using the same method mentioned above. The identity of the monosaccharides was defined by co-TLC with authentic samples, and their absolute configurations were determined based on comparison of experimental and reported $[\alpha]_D$ values.[27]

Alkaline Hydrolysis of Compound 1. A portion of cynawilfoside A (**1**, 50 mg) was dissolved in EtOH (2 mL) and treated with 10% NaOH in H_2O (2 mL), with stirring at room temperature for 3 h. The reaction mixture was concentrated and partitioned between EtOAc and H_2O (1:1, v/v). The aqueous layer was acidified with HCl to pH 3.0 and extracted with CH_2Cl_2 (5 mL × 2). The CH_2Cl_2 layer was combined and concentrated to afford the alkaline hydrolysate.

Mouse MES-Induced Seizure Assays.[34] KM male mice weighing 20 ± 2 g were purchased from the Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, People's Republic of China). At the conclusion of each experiment, animals were euthanized in accordance with the guidelines established by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, strictly following protocols that were approved by the institutional animal care and use committees. Electroconvulsions were produced by an alternating current (5.4 s stimulus duration, fixed current intensity of 4 mA, maximum stimulation voltage of 160 V) delivered via ear-clip electrodes by a physiologic and pharmacologic electronic stimulator (Jinan, Shandong, People's Republic of China). The criterion for the occurrence of seizure was the tonic hindlimb extension. The day before the experiment, the mouse generalized tonic-clonic seizure induced by MES was selected for the test of the anticonvulsant effects of the compounds. Groups of 8 to 10 mice were given solvent or different dosages of experimental compounds (po) 1 h before the electrical stimulation. Protection was defined as the absence of tonic hindlimb extension. The half-maximal effective dose (ED_{50}) and 95% confidence intervals in animal experiments were determined by the Hill equation using Graphpad Prism 6.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00766.

NMR spectra of all new compounds together with bioassay results of tested compounds (PDF)

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Notes

The authors declare no competing financial interest.

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