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Dihydroagarofuran Derivatives from the Dried Roots of *Tripterygium wilfordii*

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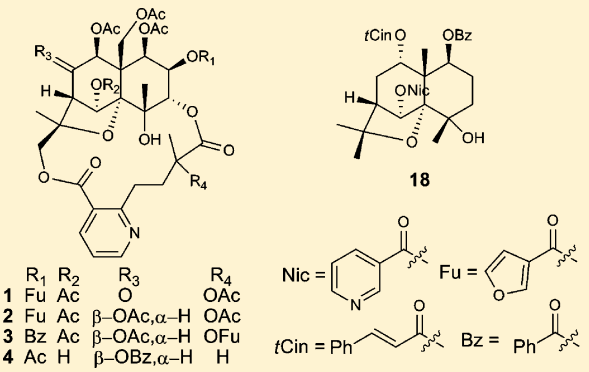
Supporting Information

ABSTRACT: Five new sesquiterpene derivatives, including dihydroagarofuran pyridine macrolides **1**−**4** and dihydroagarofuran ester **18**, and 13 known dihydroagarofuran derivatives were isolated from the aqueous EtOH extract of the dried roots of *Tripterygium wilfordii*. An in vitro antiherpetic activity assay indicated that compounds **11** and **17** displayed weak and moderate inhibition against herpes simplex virus type II, respectively.



Tripterygium wilfordii Hook. f. (Celastraceae) is a perennial woody vine that is native to China, Japan, and Korea.[1] Its air-dried root, known as thunder god vine ("lei gong teng" in Chinese), is a traditional Chinese medicine and has been used to treat inflammation and cancers, as well as autoimmune diseases including rheumatoid arthritis, multiple sclerosis, and lupus for more than 400 years.[2] The herb is considered poisonous because of its severe side effects.[1−3] Previous chemical investigations of this plant showed the presence of sesquiterpenes, diterpenes, triterpenes, and steroids with some exhibiting beneficial and/or adverse effects.[4] For instance, triptolide, a diterpene triepoxide, displayed multiple pharmacological activities including anti-inflammatory, immune modulation, antiproliferative, and proapoptotic activity.[5] However, it causes severe side effects, such as reproductive toxicity in rats, which limit its clinical applications.[5,6] Many investigations have been conducted to decrease the toxicity of these pharmaceutical candidates for clinical use.[5] A novel derivative of triptolide has recently been approved for phase I clinical trials for the treatment of prostate cancer.[5]

We performed extensive chemical and bioactivity investigations on the aqueous EtOH extract of the dried roots of *T. wilfordii*. The extract displayed weak inhibitory activity against herpes simplex virus type II (HSV2).[7] Guided by the antiherpetic activity assay, the $CHCl_3$-soluble fraction of the extract was separated over a silica gel column to produce six fractions. The fourth fraction was selected for further investigations, as it displayed antiherpetic activity. Partition, column chromatography, and semipreparative HPLC separation of the fraction produced new sesquiterpene pyridine alkaloids **1**−**4** and sesquiterpene ester **18**, as well as known sesquiterpene pyridine alkaloids **5**−**16** and sesquiterpene ester **17**. Among them, an in vitro antiherpetic activity assay indicated that compounds **11** and **17** displayed weak and moderate inhibition against herpes simplex virus type II, respectively.

Compound **1** was isolated as a white solid. Its molecular formula, $C_{41}H_{45}NO_{20}$, was provided by the $[M + Na]^+$ ion at m/z 894.2428 in its HRESIMS. Its IR spectrum displayed absorption bands at 3446, 1755, and 1586 cm^{-1} for hydroxyl, carbonyl, and aromatic groups, respectively. The NMR data (Tables 1 and 2) revealed the presence of five acetyl, two oxygenated methylene, two aliphatic methylene, one aliphatic methine, five oxygenated methine, three methyl, one 2,3-disubstituted pyridine, and one 3-furanoyl groups. In addition to the signals of the aforementioned groups, the signals for one keto carbonyl, two ester carbonyls, four oxygenated quaternary carbon, and one aliphatic quaternary carbon were observed in the ^{13}C NMR spectrum of **1**. A polyoxygenated dihydroagarofuran skeleton was determined by the $^1H-^1H$ COSY cross signals for H-1/H-2/H-3 and H-5/H-6 coupling systems and the following HMBC correlations: H-1/C-9, C-11; H-3/C-4, C-10; H-5/C-7, C-10, C-13; H-6/C-5, C-7, C-8, C-13; H-8/C-7, C-9, C-11; H-11/C-9; H-12/C-3, C-10; H-14/C-6,C-13, C-15; and H-15/C-13, C-14 (Figure 1A in the Supporting Information). A 2-(3-carboxy-3-hydroxybutyl)nicotinic acid moiety was established from the 2,3-disubstituted pyridine and $-CH_2-CH_2-$ moieties by the HMBC correlations of H-4′/C-2′, C-6′, C-12′; H-5′/C-3′, C-6′; H-6′/C-4′; H-8′/C-2′; and H-10′/C-8′, C-9′, C-11′. Thus **1** might be a sesquiterpene pyridine alkaloid.[8] The HMBC cross signals of H-3/C-11′ and H-15/C-12′confirmed the C-3−O−C-11′ and C-15−O−C-12′ linkages between the polyoxygenated dihydroagarofuran and the substituted nicotinic acid moieties, respectively. A free hydroxy group

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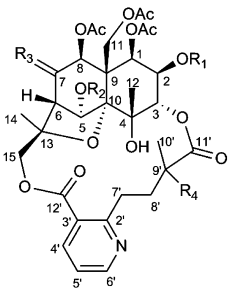
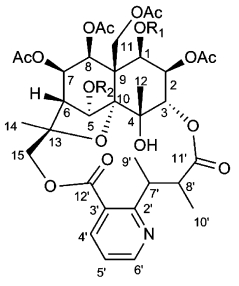
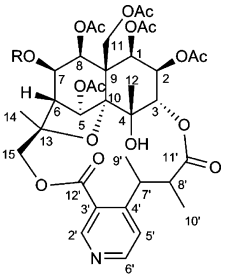


	R_1	R_2	R_3	R_4
1	Fu	Ac	O	OAc
2	Fu	Ac	β–OAc, α–H	OAc
3	Bz	Ac	β–OAc, α–H	OFu
4	Ac	H	β–OBz, α–H	H
5	Fu	Ac	β–OAc, α–H	OH
6	Fu	Ac	β–OAc, α–H	H
7	Bz	Ac	β–OAc, α–H	OH
8	Bz	Ac	β–OAc, α–H	H
9	Ac	Ac	β–OAc, α–H	H
10	H	Ac	β–OAc, α–H	OH

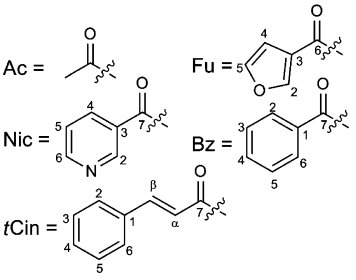


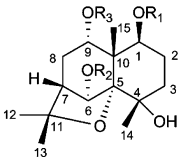
	R_1	R_2
11	Ac	Bz
12	Bz	Bz
13	Ac	H
14	Ac	Ac



15 R = Ac

16 R = Nic





	R_1	R_2	R_3
17	Bz	Nic	Bz
18	Bz	Nic	*t*Cin

Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−4 in $CDCl_3$

	1	2	3	4
position	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)
1	5.65, d (3.5)	5.54, d (4.0)	5.62, d (4.1)	5.77, d (3.5)
2	5.17, t (3.1)	5.18, dd (3.7, 2.3)	5.46, m	5.50, t (3.1)
3	4.95, d (2.8)	4.93, d (2.2)	5.05, d (2.2)	5.14, d (2.7)
5	6.65, s	6.90, s	6.90, s	5.32, d (3.0)
6	2.96, s	2.29, d (3.9)	2.27, m	2.46, d (4.0)
7		5.52, dd (5.6, 4.2)	5.51, dd (5.9, 4.0)	5.52, dd (5.5, 4.2)
8	5.47, s	5.20, d (5.9)	5.20, d (6.0)	5.38, d (5.7)
11	4.82, d (13.1) 4.44, d (13.1)	5.15, d (13.5) 4.47, d (13.5)	5.47, d (13.4) 4.28, d (13.4)	5.43, d (13.0) 4.47, d (13.0)
12	1.63, s	1.56, s	1.59, s	1.99, s
14	1.35, s	1.35, s	1.16, s	1.67, s
15	5.78, d (12.0) 3.79, d (12.0)	5.60, d (12.0) 3.80, d (12.0)	5.59, d (12.1) 3.75, d (12.0)	5.87, d (12.4) 3.74, d (12.4)
4′	8.16, dd (7.9, 1.7)	8.14, dd (7.8, 1.6)	8.11, dd (7.9, 1.7)	8.36, dd (8.0, 1.7)
5′	7.32, dd (7.9, 4.8)	7.32, m	7.33, dd (7.9, 4.8)	7.29, dd (8.0, 4.7)
6′	8.76, dd (4.8, 1.7)	8.76, dd (4.8, 1.6)	8.81, dd (4.8, 1.8)	8.77, dd (4.7, 1.7)
7′	3.82, ddd (13.8, 10.2, 5.6) 2.97, ddd (13.8, 10.1, 5.8)	3.75, m 2.96, m	3.76, dd (12.0, 7.0) 2.98, m	4.08, m 2.88, m
8′	2.69, ddd (13.6, 10.2, 5.8) 2.28, ddd (13.6, 10.1, 5.6)	2.81, m 2.27, m	3.00, m 2.35, m	2.35, m 1.88, m
9′				2.36, m
10′	1.81, s	1.79, s	1.84, s	1.21, d (6.4)
4-OH	4.66, s	4.22, s	4.14, s	6.37, s

was located at C-4 by a comparison of the ^{13}C NMR chemical shift of C-4 (δ_C 70.3) with those reported.[8] The OAc-1, OAc-5, OAc-8, OAc-11, and OFu-2 localities were determined by the corresponding HMBC correlations of H-1/OAc-1, H-5/OAc-5, H-8/OAc-8, H-11/OAc-11, and H-2/C-6$_{Fu}$. The OAc-9′ was assigned by a comparison of the ^{13}C NMR chemical shift of C-9′ (δ_C 81.5) with that reported.[8c] The relative configuration of **1** was established by the NOESY correlations of H-8/H-1, H-14; H-5/H-11, H-12; and H-11/H-12 (Figure 1B in the Supporting Information). Thus, the structure of compound **1** was determined to be 9′-*O*-acetyl-7-deacetoxy-7-oxowilfortrine.

Compound **2** was isolated as a white solid. Its molecular formula, $C_{43}H_{49}NO_{21}$, was calculated from the $[M + Na]^+$ ion at *m*/*z* 938.2679 in its HRESIMS. The NMR data (Tables 1 and 2) were similar to those of **5**, except that **2** contained one extra acetyl group. Analysis of the 1- and 2-D NMR data of **2**

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Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−4 in $CDCl_3$

	1	2	3	4
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	70.2, CH	72.1, CH	72.0, CH	73.6, CH
2	69.5, CH	69.4, CH	69.5, CH	69.3, CH
3	77.7, CH	78.5, CH	78.2, CH	75.1, CH
4	70.3, C	69.9, C	69.7, C	71.8, C
5	73.8, CH	73.9, CH	73.7, CH	74.2, CH
6	62.2, CH	50.9, CH	50.9, CH	52.4, CH
7	195.5, C	68.6, CH	68.4, CH	69.9, CH
8	79.4, CH	71.8, CH	71.8, CH	71.2, CH
9	52.5, C	52.2, C	52.2, C	50.7, C
10	94.9, C	93.5, C	93.4, C	92.7, C
11	60.2, CH_2	60.2, CH_2	60.6, CH_2	61.1, CH_2
12	24.1, CH_3	23.3, CH_3	23.5, CH_3	23.8, CH_3
13	86.3, C	84.3, C	84.2, C	85.1, C
14	18.1, CH_3	17.4, CH_3	17.4, CH_3	18.2, CH_3
15	69.9, CH_2	69.7, CH_2	69.3, CH_2	71.2, CH_2
2′	162.1, C	161.7, C	161.8, C	165.1, C
3′	125.0, C	125.5, C	125.8, C	123.9, C
4′	138.3, CH	138.3, CH	138.3, CH	138.6, CH
5′	121.3, CH	121.1, CH	120.9, CH	121.2, CH
6′	152.5, CH	152.3, CH	152.3, CH	153.5, CH
7′	30.9, CH_2	30.6, CH_2	30.6, CH_2	33.0, CH_2
8′	37.9, CH_2	37.7, CH_2	37.8, CH_2	33.4, CH_2
9′	81.5, C	81.4, C	81.7, C	38.2, CH
10′	22.2, CH_3	22.7, CH_3	23.1, CH_3	18.9, CH_3
11′	171.2, C	171.6, C	171.7, C	175.3, C
12′	167.4, C	167.7, C	167.9, C	167.1, C

confirmed that **2** possessed the same skeleton and substitution mode as **5** (Figure 1A in the Supporting Information). The OH-4 was determined by the HMBC cross signal of δ_H 4.22 (s, OH)/δ_C 23.3 (C-12). The OAc-1, OAc-5, OAc-7, OAc-8, OAc-11, and OFu-2 localities were assigned by the corresponding HMBC correlations of H-1/OAc-1, H-5/OAc-5, H-7/OAc-7, H-8/OAc-8, H-11/OAc-11, and H-2/C-6_{Fu}. The OAc-9′ was determined by a comparison of the ^{13}C NMR chemical shift of C-9′ (δ_C 81.4) with those reported.[8c] The relative configuration of **2** was established by the NOESY correlations of H-8/H-1, H-14; H-7/H-14; H-5/H-11, H-12; and H-11/H-12 (Figure 1B in the Supporting Information). Thus, the structure of compound **2** was elucidated as 9′-*O*-acetylwilfortrine.

Compound **3**, a white solid, had the molecular formula $C_{48}H_{51}NO_{21}$, calculated from the $[M + Na]^+$ ion at m/z 1000.2837 in its HRESIMS. Compound **3** displayed similar NMR data (Tables 1 and 2) to those of **7**, except that **3** contained an extra 3-furanoyl group. Analysis of the NMR data of **3** established its basic structure and substitution mode (Figure 1A in the Supporting Information). The OH-4 was concluded by the HMBC correlations of δ_H 4.14 (s, OH)/C-4, C-12. The OAc-1, OAc-5, OAc-7, OAc-8, OAc-11, and OBz-2 localities were assigned by the corresponding HMBC correlations of H-1/OAc-1, H-5/OAc-5, H-7/OAc-7, H-8/OAc-8, H-11/OAc-11, and H-2/C-6_{Bz}. The OFu-9′ was assigned in view of the ^{13}C NMR chemical shift of C-9′ (δ_C 81.7). The relative configuration of **3** was established by the NOESY correlations of H-8/H-1, H-14; H-7/H-14; H-5/H-11, H-12; and H-11/H-12 (Figure 1B in the Supporting Information). Thus, the structure of compound **3** was identified as 9′-*O*-furanoylwilfordine.

Compound **4**, a white solid, had the molecular formula $C_{41}H_{47}NO_{17}$ calculated from the $[M + Na]^+$ ion at m/z 848.2720 in its HRESIMS. The polyoxygenated dihydroagarofuran and 2,3-disubstituted pyridine moieties were determined by $^1H-^1H$ COSY and HMBC correlations (Figure 1A in the Supporting Information). A $-CH_2-CH_2-CH(CH_3)-$ moiety in **4** instead of $-CH_2-CH_2-$ in **1**−**3** was deduced from the $^1H-^1H$ COSY correlations. A 2-(3-carboxybutyl)nicotinic acid moiety was established by the HMBC correlations of H-4′/C-2′, C-6′, C-12′; H-5′/C-3′; H-6′/C-4′; H-8′/C-2′; and H-10′/C-8′, C-9′, C-11′. The HMBC cross signals of H-3/C-11′ and H-15/C-12′ confirmed the C-3−O−C-11′ and C-15−O−C-12′ linkages between the polyoxygenated dihydroagarofuran and the substituted nicotinic acid moieties, respectively. The OAc-1, OAc-2, OAc-8, OAc-11, and OBz-7 localities were assigned by their corresponding HMBC correlations of H-1/OAc-1, H-2/OAc-2, H-8/OAc-8, H-11/OAc-11, and H-7/C-7_{Bz}. A free hydroxy group at δ_H 6.37 was assigned as OH-4 by the HMBC correlations of δ_H 6.37 (s, OH)/C-4, C-12. The remaining free hydroxy group at δ_H 6.00 was assigned as OH-5 by a comparison of the ^1H NMR chemical shift of H-5 (δ 5.32) and the ^{13}C NMR chemical shift of C-5 (δ_C 74.2) with the sesquiterpene pyridines bearing a free OH-5.[8a] The relative configuration of **4** was established by the NOESY correlations of H-8/H-1, H-14; H-7/H-14; H-5/H-11, H-12; H-11/H-12; and OH-4/OH-5 (Figure 1B in the Supporting Information). Thus, the structure of compound **4** was elucidated as 7-*O*-benzoyl-5,7-dideacetylwilformine.

Dihydroagarofuran pyridine alkaloids **1**−**4** possessed the same relative configurations of the polyoxygenated dihydroagarofuran moiety as the known sesquiterpene pyridine alkaloids **5**−**16**. An X-ray crystallographic analysis of **8** (Supporting Information) confirmed the relative configurations of its polyoxygenated dihydroagarofuran moiety established by the NOESY data. However, the relative configuration of C-9′ in **1**−**4** remained undefined, as in the known sesquiterpene pyridine alkaloids **5**−**10**.

Compound **18** was isolated as an yellowish solid. Its molecular formula, $C_{37}H_{39}NO_8$, was calculated from the $[M + Na]^+$ ion at m/z 648.2581 in its HRESIMS. Its IR spectrum displayed absorption bands at 3436, 1720, 1635, and 1592 cm^{-1} for hydroxyl, carbonyl, conjugated double bond, and aromatic groups, respectively. The ^1H NMR data revealed the presence of four methyl, four methine, three methylene, one benzoyl, one *trans*-cinnamoyl, and one nicotinoyl group. In addition to the signals of these groups, the signals for one aliphatic quaternary carbon and three oxygenated quaternary carbons were observed in the ^{13}C NMR spectrum. The NMR data were similar to those of regelidine (**17**),[9] indicating that **18** could be a sesquiterpene ester derivative. The sesquiterpene skeleton was elaborated from the $^1H-^1H$ COSY correlations of the H-1/H-2/H-3 and H-6/H-7/H-8 coupling systems and the HMBC correlations of H-3/C-2, C-4; H-5/C-7, 10, 13; H-8/C-6, C-10; H-11/C-1, C-8, C-9, C-10; H-12/C-4, C-10; H-14/C-6, C-13; and H-15/C-13 (Figure 2 in the Supporting Information). The free hydroxy group at δ 3.05 was located at C-4 according to the HMBC correlations of OH-4/C-4, C-14. The ONic-6 and OCin-9 moieties were determined by the HMBC correlations of H-6/C-7_{Nic} and H-9/C-7_{Cin}, respectively. The benzoyl group was assigned as OBz-1. The relative configuration of **18** was established by the NOESY correlations of H-1/H-3; H-6/H-14, H-15; and H-15/H-8, H-9 (Figure 2 in the Supporting Information). The structure of

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compound **18** was elucidated as 9-*O*-*trans*-cinnamoyl-9-debenzoylregelidine.

By comparing the spectroscopic and physicochemical data of the reported compounds, the 13 known dihydroagarofuran derivatives were identified as wilfortrine (**5**),[10] wilforgine (**6**),[10] wilfordine (**7**),[10,11] wilforine (**8**),[11b,12] wilformine (**9**),[13] wilforidine (**10**),[14] cangorinine E-1 (**11**),[15] ebenifoline E-II (**12**),[16] neoeuonymine (**13**),[11b,13b] euonymine (**14**),[8b] peritassin A (**15**),[17] wilfornine G (**16**),[18] and regelidine (**17**).[9]

The dihydroagarofuran derivatives **1**−**18** were evaluated for their in vitro inhibitory activity against HSV2.[7] Acyclovir was used as a positive control. Compound **11** displayed 22.3% inhibitory activity at 0.5 mg/mL, and acyclovir 66.3% inhibitory activity at 0.5 mg/mL. Compound **17** showed 31.7% inhibitory activity at 0.25 mg/mL, while acyclovir displayed 60.6% inhibitory activity at 0.25 mg/mL. The remaining compounds were inactive at 0.5 mg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an X-6 apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 341 automatic polarimeter. UV spectra were recorded in MeOH (1 mg/50 mL) on a Perkin-Elmer Lambda 35 spectrometer. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer using a KBr disk with ν_{max} given in cm^{-1}. NMR spectra were recorded on an Avance Bruker 600 spectrometer at room temperature. The chemical shifts (δ) are reported in ppm using TMS as an internal standard, and the coupling constants (J) are given in hertz (Hz). HRESIMS and ESIMS spectra were obtained on a Bruker Bio TOF IIIQ (quadrupole time-of-flight) mass spectrometer. CC was performed on self-packed open columns with silica gel from Qingdao Haiyang Chemical Co., Ltd. (QHCC, PR China). TLC analyses were conducted on plates precoated with 10−40 μm of silica gel GF_{254} from QHCC and detected under a UV lamp at 254 or 365 nm and visualized by spraying 8% phosphomolybdic acid−EtOH solution (w/v) or 5% vanillin−H_2SO_4 (w/v) followed by heating, or visualized by iodine (I_2). Semipreparative HPLC was equipped with a Perkin-Elmer Series 200 pump, a Perkin-Elmer Series UV/vis detector, a 200 μL manual injector, and a Kromasil C_{18} column (10 × 250 mm, 5 μm). Fractions from all columns were generally collected by an autocollecting apparatus and were combined according to the TLC analyses. All other solvents were commercially purchased and distilled under normal atmospheric pressure prior to use.

Plant Material. The air-dried roots of *T. wilfordii* (product no. 0905107) were purchased from Sichuan Neautus Traditional Chinese Medicine Co. Ltd. (Chengdu, P.R. China). A voucher specimen (973-06) was identified by Prof. Fading Fu at Chengdu Institute of Biology of the Chinese Academy of Sciences (CIBCAS) and was deposited in the Herbarium of CIBCAS.

Extraction and Isolation. A total of 80 L of 70% EtOH(aq) was added to the air-dried and powdered roots of *T. wilfordii* (10 kg). The mixture was heated and boiled for 1 h and cooled to room temperature. The process was repeated twice more. After removing the EtOH under vacuum, 930 g of crude extract was obtained. The crude extract was suspended in H_2O (3 L) and extracted with $CHCl_3$ (5 × 3 L). The $CHCl_3$-soluble extract (40 g) was separated by CC (silica gel, 160−200 mesh) eluted with $CHCl_3$−acetone (10:1, v/v) to produce six fractions. The fourth fraction (20.7 g) was dissolved in MeOH (60 mL) and extracted with petroleum ether (4 × 100 mL) to afford the petroleum ether- and MeOH-soluble fractions. The MeOH-soluble fraction (7.2 g) was separated by CC (silica gel, 200−300 mesh), eluted with petroleum ether−$CHCl_3$−acetone (4:0.8:0.5, v/v/v), to afford subfractions B1−B20. Fraction B3 (340 mg) was separated by semipreparative HPLC using an MeOH−H_2O (78:22, v/v) solvent system at a flow rate of 4 mL/min, and the peaks were detected at 268 nm. The fraction with a retention time of 27.0 min gave compound **18** (3 mg). Fraction B5 (420 mg) was separated by semipreparative HPLC using an MeOH−H_2O (72:28, v/v) solvent system at a flow rate of 4 mL/min, and the peaks were detected at 268 nm. The fraction with a retention time of 34.0 min afforded compound **17** (2 mg). Fraction B7 (340 mg) was separated on a semipreparative HPLC using an MeOH−H_2O (75:25, v/v) solvent system at a flow rate of 3 mL/min, and the peaks were detected at 208 nm. The fractions with retention times of 18.0 and 26.0 min afforded compounds **11** (10 mg) and **12** (5 mg), respectively. Fraction B9 (170 mg) was separated by semipreparative HPLC using an MeOH−H_2O (62:38, v/v) solvent system at a flow rate of 4 mL/min, and the peaks were detected at 268 nm. The fraction with a retention time of 18.2 min afforded compound **13** (3 mg). Fraction B10 (210 mg) was separated by semipreparative HPLC using an MeOH−H_2O (80:20, v/v) solvent system at a flow rate of 4 mL/min, and the peaks were detected at 268 nm. The fraction with a retention time of 5.4 min gave compound **14** (76 mg). The solid (220 mg) that precipitated from the $CHCl_3$−MeOH solution of fraction B12 (460 mg) was separated by semipreparative HPLC using an MeOH−H_2O (71:29, v/v) solvent system at a flow rate of 3 mL/min, and the peaks were detected at 208 nm. The fractions with retention times of 12.9 and 20.4 min afforded compounds **6** (100 mg) and **8** (117 mg), respectively. The mother liquid of fraction B12 was also subjected to separation by semipreparative HPLC using an MeOH−H_2O (69:31, v/v) solvent system at a flow rate of 3 mL/min, and the peaks were detected at 208 nm. The fractions with retention times of 10.5, 28.5, and 33.9 min gave compounds **9** (2 mg), **4** (3 mg), and **3** (3 mg), respectively. Fraction B13 (400 mg) was separated on a semipreparative HPLC using an MeOH−H_2O (62:38, v/v) solvent system at a flow rate of 3 mL/min, and the peaks were detected at 208 nm. The fractions with retention times of 34.0, 40.0, and 45.0 min afforded compounds **15** (19 mg), **1** (8 mg), and **2** (8 mg), respectively. Fraction B16 (70 mg) was separated on a semipreparative HPLC using an MeOH−H_2O (68:32, v/v) solvent system at a flow rate of 3 mL/min, and the peaks were detected at 208 nm. The fractions with retention times of 14.3, 17.7, and 23.0 min gave compounds **5** (5 mg), **16** (7 mg), and **7** (23 mg), respectively. Fraction B20 (110 mg) was separated by semipreparative HPLC using an MeOH−H_2O (56:44, v/v) solvent system at a flow rate of 4 mL/min, and the peaks were detected at 268 nm. The fraction with a retention time of 10.8 min gave compound **10** (1 mg).

*9′-O-Acetyl-7-deacetoxy-7-oxowilfortrine (**1**):* white solid; mp 136−138 °C ($CHCl_3$); $[\alpha]_D^{20}$ −33 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 257 (4.91) nm; IR (KBr) ν_{max} 3446, 2925, 2854, 1755, 1632, 1586, 1509, 1456, 1376, 1319, 1296, 1220, 1177, 1134, 1083, 1043, 1008, 970, 915, 874, 765, 746, 628 cm^{-1}; ^1H NMR, see Table 1 and OAc-1, δ 2.16 (3H, s), OAc-5, δ 2.21 (3H, s), OAc-8, δ 2.03 (3H, s), OAc-11, δ 1.99 (3H, s), OAc-9, δ 1.80 (3H, s), OFu-2, δ 7.80 (1H, brs, H-2_{Fu}), 6.59 (1H, d, J = 3.6, 1.2 Hz, H-4_{Fu}), 7.31 (1H, dd, J = 3.6, 1.2 Hz, H-5_{Fu}); ^{13}C NMR, see Table 1 and δ 20.4, 168.0 (OAc-1), 21.0, 169.2 (OAc-5), 20.0, 168.7 (OAc-8), 20.2, 169.6 (OAc-11), 21.4, 168.0 (OAc-9), 162.4 (C-6_{Fu}), 149.0 (C-2_{Fu}), 118.7 (C-3_{Fu}), 110.2 (C-4_{Fu}), 143.3 (C-5_{Fu}); ESIMS m/z 894 $[M + Na]^+$; HRESIMS m/z 894.2428 (calcd for $C_{41}H_{45}NO_{20}Na$, 894.2427, error −0.1 ppm).

*9′-O-Acetylwilfortrine (**2**):* white solid; mp 138−140 °C ($CHCl_3$); $[\alpha]_D^{20}$ −42 (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 258 (3.96) nm; IR (KBr) ν_{max} 3453, 2926, 2854, 1756, 1632, 1585, 1509, 1441, 1373, 1320, 1231, 1259, 1175, 1158, 1132, 1096, 1054, 1007, 937, 898, 875, 845, 764, 710, 603 cm^{-1}; ^1H NMR, see Table 1 and OAc-1, δ 1.78 (3H,s), OAc-5, δ 2.17 (3H, s), OAc-7, δ 2.16 (3H, s), OAc-8, δ 1.93 (3H, s), OAc-11, δ 2.26 (3H, s), OAc-9′, δ 2.13 (3H, s), OFu-2, δ 7.75 (1H, brs, H-2_{Fu}), 6.54 (1H, m, H-4_{Fu}), 7.31 (1H, m, H-5_{Fu}); ^{13}C NMR, see Table 2 and δ 20.2, 168.0 (OAc-1), 21.5, 169.8 (OAc-5), 21.3, 168.2 (OAc-7), 20.3, 168.6 (OAc-8), 20.9, 170.2 (OAc-11), 21.0, 169.9 (OAc-9′), 149.0 (C-2_{Fu}), 118.8 (C-3_{Fu}), 110.2 (C-4_{Fu}), 143.2 (C-5_{Fu}), 162.2 (C-6_{Fu}); ESIMS m/z 938 $[M + Na]^+$; HRESIMS m/z 938.2679 (calcd for $C_{43}H_{49}NO_{21}Na$, 938.2689, error 1.1 ppm).

*9′-O-Furanoylwilfordine (**3**):* white solid; $[\alpha]_D^{20}$ −47 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 251 (5.03) nm; IR (KBr) ν_{max} 3436, 2925, 2854, 1748, 1633, 1586, 1455, 1376, 1288, 1231, 1133, 1094, 897, 761, 717, 600 cm^{-1}; ^1H NMR, see Table 1 and OAc-1, δ 1.90 (3H, s), OAc-5, δ 2.16 (3H, s), OAc-7, δ 1.69 (3H, s), OAc-8, δ 2.12 (3H, s),

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OAc-11, δ 2.26 (3H, s), OBz-2, δ 7.72 (2H, d, J = 7.2 Hz, H-2_{Bz}, 6_{Bz}), 7.34 (2H, d, J = 7.2 Hz, H-3_{Bz}, 5_{Bz}), 7.50 (1H, m, H-4_{Bz}), OFu-9′, δ 8.31 (1H, brs, H-2_{Fu}), 6.89 (1H, m, H-4_{Fu}), 7.49 (1H, m, H-5_{Fu}); ^{13}C NMR, see Table 2 and δ 20.3, 168.6 (OAc-1), 21.5, 169.9 (OAc-5), 20.2, 167.9 (OAc-7), 21.2, 169.8 (OAc-8), 21.0, 170.7 (OAc-11), 148.6 (C-2_{Fu}), 118.6 (C-3_{Fu}), 109.9 (C-4_{Fu}), 144.1 (C-5_{Fu}), 160.6 (C-6_{Fu}), 129.8 (C-1_{Bz}), 130.3 (C-2_{Bz}, 6_{Bz}), 128.1 (C-3_{Bz}, 5_{Bz}), 133.0 (C-4_{Bz}), 165.6 (C-7_{Bz}); ESIMS m/z 1000 $[M + Na]^+$; HRESIMS m/z 1000.2837 (calcd for $C_{48}H_{51}NO_{21}Na$, 1000.2846, error 0.8 ppm).

*7-O-Benzoyl-5,7-dideacetylwilformine (**4**):* white solid; $[\alpha]_D^{20}$ +53 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 224 (4.38), 258 (2.92) nm; IR (KBr) ν_{max} 3432, 2925, 2854, 1748, 1632, 1586, 1455, 1374, 1270, 1254, 1230, 1175, 1157, 1105, 1071, 1047, 1002, 874, 766, 713, 600 cm^{-1}; ^{1}H NMR, see Table 1 and OAc-1, δ 1.85 (3H,s), OAc-2, δ 2.16 (3H, s), OAc-8, δ 1.89 (3H, s), OAc-11, δ 1.89 (3H, s), OBz-7, δ 8.03 (2H, d, J = 7.2 Hz, H-2_{Bz}, 6_{Bz}), 7.50 (2H, t, J = 7.8 Hz, H-3_{Bz}, 5_{Bz}), 7.62 (1H, d, J = 7.4 Hz, H-4_{Bz}), 5-OH, δ 6.00 (1H, d, J = 3.5 Hz); ^{13}C NMR, see Table 2 and δ 20.9, 169.8 (OAc-1), 20.6, 170.0 (OAc-7), 20.4, 168.9 (OAc-8), 20.9, 169.7 (OAc-11), 129.0 (C-1_{Bz}), 129.7 (C-2_{Bz}, 6_{Bz}), 128.8 (C-3_{Bz}, 5_{Bz}), 133.7 (C-4_{Bz}), 165.0 (C-7_{Bz}); ESIMS m/z 848 $[M + Na]^+$; HRESIMS m/z 848.2720 (calcd for $C_{41}H_{47}NO_{17}Na$, 848.2736, error 1.9 ppm).

*9-O-trans-Cinnamoyl-9-debenzoylregelidine (**18**):* yellowish solid; mp 89−91 °C ($CHCl_3$); $[\alpha]_D^{20}$ +104 (c 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 274 (4.46) nm; IR (KBr) ν_{max} 3436, 2925, 2855, 1720, 1635, 1592, 1497, 1452, 1386, 1366, 1334, 1314, 1282, 1202, 1165, 1113, 1072, 1026, 997, 973, 950, 892, 868, 815, 768, 743, 714, 689, 661, 617 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.39 (s, 1H, H-2_{Nic}), 8.81 (s, 1H, H-6_{Nic}), 8.52 (d, J = 8.0 Hz, 1H, H-4_{Nic}), 7.76 (d, J = 7.2 Hz, 2H, H-2_{Bz}, 6_{Bz}), 7.46 (m, 1H, H-4_{Bz}), 7.44 (m, 2H, H-3_{Bz}, 5_{Bz}), 7.42 (m, 1H, H-5_{Nic}), 7.38 (m, 2H, H-2_{Cin}, 6_{Cin}), 7.36 (m, 1H, H-4_{Cin}), 7.32 (d, J = 16.0 Hz, 1H, H-β_{Cin}), 7.29 (t, J = 8.0 Hz, 2H, H-3_{Cin}, 5_{Cin}), 6.23 (d, J = 16.0 Hz, 1H, H-α_{Cin}), 5.70 (s, 1H, H-6), 5.67 (dd, J = 12.3, 4.2 Hz, 1H, H-1), 4.97 (d, J = 6.8 Hz, 1H, H-9), 3.05 (s, 1H, OH), 2.56 (ddd, J = 10.4, 7.0, 3.3 Hz, 1H, H-8a), 2.36 (m, 1H, H-7), 2.27 (dd, J = 16.4, 3.0 Hz, 1H, H-8b), 2.04 (m, 1H, H-3a), 2.02 (m, 1H, H-2a), 1.79 (m, 1H, H-2b), 1.64 (m, 1H, H-3b), 1.56 (s, 3H, H-15), 1.54 (s, 3H, H-13), 1.53 (s, 3H, H-12), 1.39 (s, 3H, H-14); ^{13}C NMR ($CDCl_3$) δ 165.8 (C-7_{Cin}), 165.7 (C-7_{Bz}), 164.9 (C-7_{Nic}), 153.8 (C-6_{Nic}), 151.6 (C-2_{Nic}), 145.3 (C-β_{Cin}), 137.6 (C-4_{Nic}), 134.4 (C-1_{Cin}), 132.8 (C-4_{Bz}), 130.3 (C-1_{Bz}), 130.2 (C-4_{Cin}), 129.3 (C-2_{Bz}, 6_{Bz}), 128.7 (C-2_{Cin}, 6_{Cin}), 128.2 (C-3_{Bz}, 5_{Bz}), 128.1 (C-3_{Cin}, 5_{Cin}), 125.8 (C-3_{Nic}), 123.5 (C-5_{Nic}), 117.7 (C-α_{Cin}), 91.6 (C-5), 84.4 (C-11), 80.9 (C-6), 72.9 (C-9), 72.8 (C-1), 70.7 (C-4), 51.7 (C-10), 49.1 (C-7), 38.9 (C-3), 31.9 (C-8), 29.8 (C-15), 25.8 (C-13), 23.9 (C-14), 23.7 (C-2), 20.0 (C-12); ESIMS m/z 648 $[M + Na]^+$; HRESIMS m/z 648.2581 (calcd for $C_{37}H_{39}NO_8Na$, 648.2568, error −2.0 ppm).

Anti-HSV2 Activity. According to the reported procedure,[6] the extract and compounds **1**−**18** were tested against HSV2. Acyclovir was used as a positive control. The initial concentrations of acyclovir and the dihydroagarofurans **1**−**12**, **14**−**16**, and **18** were 0.5 mg/mL. For compounds **13** and **17**, the initial concentrations were 0.25 mg/mL. These dihydroagarofurans displayed no cytotoxicity at the aforementioned concentration. The samples were assayed at different concentration levels. At each concentration level, four independent experiments were carried out. An MTT assay was carried out as described.[6] The optical density was measured on a Thermo Scientific VarioSkan Flash spectral scanning multimode reader. All data were presented as mean ± SD, and the differences between groups were assessed with the Student t-test.

■ ASSOCIATED CONTENT

Supporting Information

Figures for selected key 2-D NMR correlations, 1- and 2-D NMR spectra for compounds **1**−**4** and **18**, and X-ray crystallographic data of **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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