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Amarisolide F, an Acylated Diterpenoid Glucoside and Related Terpenoids from *Salvia amarissima*

Mabel Fragoso-Serrano,[†] Naytzé Ortiz-Pastrana,[‡] Norma Luna-Cruz,[§,⊥] Rubén A. Toscano,[∥] Angel G. Alpuche-Solís,[⊥] Alfredo Ortega,[∥] and Elihú Bautista*[,§]

[†]Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán, Ciudad de México 04510, México

[‡]Departamento de Química, Centro de Investigación y de Estudios Avanzados del IPN, Avenida IPN 2508, Col. San Pedro Zacatenco, Ciudad de México 07360, México

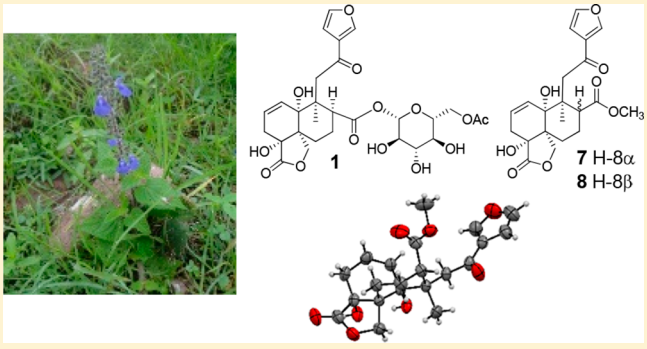
[§]CONACYT-Consorcio de Investigación, Innovación y Desarrollo para las Zonas Áridas (CIIDZA), Instituto Potosino de Investigación Científica y Tecnológica A. C., Camino a la Presa San José 2055, Lomas 4ta sección, San Luis Potosí 78216, México

[⊥]División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica A. C., Camino a la Presa San José 2055, Lomas 4ta sección, San Luis Potosí 78216, México

[∥]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán, Ciudad de México 04510, México

Supporting Information

ABSTRACT: Nine terpenoids were isolated from the leaves and flowers of *Salvia amarissima*, including a new acylated diterpenoid glucoside, amarisolide F (**1**), a new *neo*-clerodane diterpenoid, amarissinin D (**2**), which was isolated as an acetyl derivative (**2a**), and four known diterpenoids. The structure of amarisolide F (**1**) was elucidated by NMR and MS data analyses, as well as its methanolysis products **7** and **8**, which also constituted new diterpenoids, named amarissinin E and 8-*epi*-amarissinin E, respectively. The absolute configuration of compound **7** was established by single-crystal X-ray diffraction. The cytotoxicity and anti-MDR effect of **1** in three phenotypes of the MCF-7 cell lines were assayed. Compound **1** was 2−3.6-fold more active than amarissinins A (**3**) and B (**4**), but several orders of magnitude less active than teotihuacanin (**6**) and reserpine.



Salvia amarissima (Lamiaceae) is a herbaceous and perennial plant that is endemic to Mexico. The distribution of this plant is found in semiarid and wooded areas from the states of San Luis Potosi to Oaxaca.[1,2] This plant species is popularly known as "hierba del cancer", "insulina", "hierba azul", "chupona", "peludito", and "ñadri".[3,4] The common names for this plant species describe some of their medicinal uses to treat cancer and diabetes, as well as rheumatism.[3,5] In addition, this plant together with 17 other *Salvia* species constitute the group of medicinal plants called "chía".[6] On the other hand, it is noteworthy that similar to other *Salvia* species,[7] the metabolic profile of *S. amarissima* is susceptible to geographic and climate conditions. The phytochemical profiles of three populations of this plant species have been determined; from the first two populations, distributed in the states of Puebla and Oaxaca, the glucoside diterpenoids amarisolides A−E (**11**−**15**) were isolated.[5,8] From a third population, distributed in the valley of Teotihuacan, state of Mexico, the diterpenoids amarissinins A−C (**3**−**5**) and teotihuacanin (**6**) were isolated.[7,9] Furthermore, as a continuation of the studies of the phytochemistry of the *Salvia* genus and search for new bioactive natural products, scale-up of the isolation of teotihuacanin (**6**) to obtain semisynthetic derivatives as potential MDR modulators was performed. Herein, we describe the isolation and structural elucidation of a new diterpenoid glucoside (**1**), its methanolysis products (**7**/**8**), which also constituted new diterpenoids named amarissinin E/8-*epi*-amarissinin E, and the crystal structure of the *O*-acetyl derivative (**2a**) as a new diterpenoid, amarissinin D (**2**).

Chemical reinvestigation of an acetone-soluble extract of the leaves and flowers of *S. amarissima* in order to scale-up the isolation of teotihuacanin (**6**) led to the isolation of a new diterpenoid glucoside (**1**), named amarisolide F, together with the known diterpenoids amarissinin A (**3**), acetylamarissinin B

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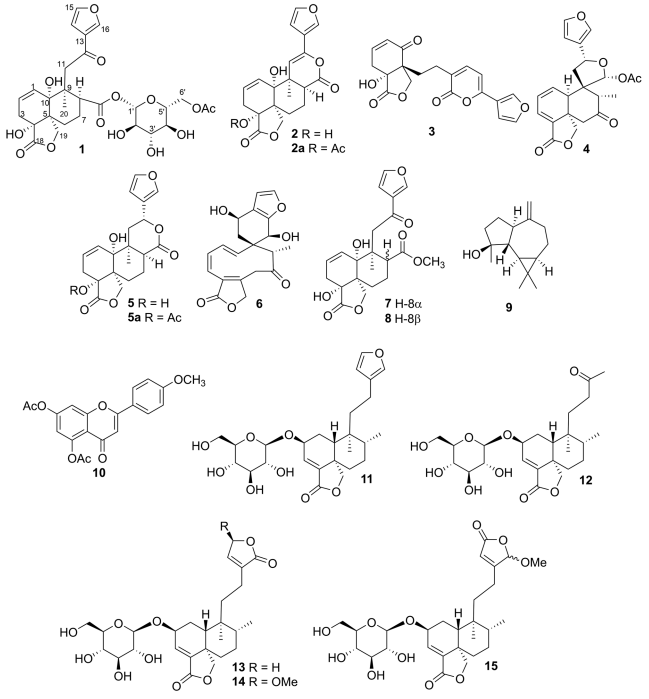
Chart 1



(**4**), and amarissinin C (**5**),[7,9] as well as spathulenol (**9**) and 5,7-*O*-diacetylacacetin (**10**) (Chart 1). Derivatives **2a** and **5a** resulted from the acetylation of amarissinin D (**2**) and amarissinin C (**5**), respectively. Compounds **7**/**8** were obtained as methanolysis products by the treatment of **1** with Na_2CO_3 in MeOH. The structures of these compounds also represent new diterpenoids, which were named amarissinin E/8-*epi*-amarissinin E.

Amarisolide F (**1**) was isolated as a beige, amorphous powder. Its molecular formula of $C_{28}H_{35}O_{14}$ was deduced from its HRESIMS (*m*/*z* 595.2046, calcd 595.2021), indicating 12 indices of hydrogen deficiency. The ESIMS/MS analysis showed potassium and sodium adduct ions at *m*/*z* 633 $[M + K]^+$ and 617 $[M + Na]^+$ and ions produced by the cleavage of a glycosidic fragment containing an acetyl group at *m*/*z* 429 [(M − 162 (hexose unit) − 42 (ketene)) + K]$^+$ and 413 [(M − 162 (hexose unit) − 42 (acetate)) + Na]$^+$.[10] The IR spectrum showed absorption bands of hydroxy groups (3408 cm^{-1}), a γ-lactone carbonyl (1742 cm^{-1}), a ketocarbonyl (1710 cm^{-1}), an ester carbonyl (1672 cm^{-1}), and a furan ring (872 cm^{-1}). These assignments were supported by analysis of the ^1H and ^{13}C NMR data (Table 1), which indicated the presence of a mono-substituted furan ring (δ_C 128.4, C-13; δ_H 8.15, br s, H-16; 7.44, d, *J* = 5.5 Hz, H-15; 6.77, br s, H-14) and an 18,19-γ-lactone (δ_C 174.9, C-18, 71.7, C-19; δ_H 4.94, d, *J* = 8.2 Hz, H-19α; 4.37, d, *J* = 8.2 Hz, H-19β). Additional signals in the ^1H and ^{13}C NMR spectra of **1** at δ_H 6.01 (d, *J* = 10.4 Hz) and 5.70 (br d, *J* = 10.4 Hz) and δ_C 76.8 (C) and 75.4 (C) were assigned to H-1 (δ_C 129.4), H-2 (δ_C 124.7), C-10, and C-4, respectively, and suggested the presence of a $\Delta^{1(2)}$ double bond, together with two tertiary hydroxy groups at C-4 and C-10, as has been described for amarissinin C (**5**) and infuscatin.[11] The signals for the glycosidic moiety were resolved and assigned by the identification of the anomeric carbon at δ_C 93.7 (CH, C-1′), its HSQC correlation with the anomeric proton (δ_H 5.46, d, *J* = 8.2 Hz, H-1′), and the subsequent analysis of the COSY and HSQC spectra of the resulting spin system (Table 1).[12] The coupling constants (8.2−9.3 Hz) for this sugar moiety (Table 1) suggest

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of Amarisolide F (1) and Amarissinin E/8-*epi*-Amarissinin E (7/8) in $CDCl_3$/DMSO-d_6, 3:1

	1		**7/8**[b,c,d]	
position	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C
1	6.01 d (10.4)	129.4, CH	5.95 ddd (10.3, 4.6, 1.9)/ 5.98 ddd (10.4, 4.8, 1.8)	130.2/128.7,[a] CH
2	5.70 br d (10.4)	124.7, CH	5.80 dt (10.3, 4.6)/5.92 ddd (10.4, 2.4, 1.0)	124.2/128.1,[a] CH
3a	2.42 dd (13.1, 10.1)[a]	29.2, CH_2	2.44 dd (4.6, 1.9)[a]/2.56 ddd (18.8, 4.8, 1.0)	30.0/30.4, CH_2
3b	2.42 dd (13.1, 10.1)[a]		2.44 dd (4.6, 1.9)[a]/2.37 ddd (18.8, 2.4, 1.8)[a]	
4		75.4, C		76.1/77.1, C
5		47.7, C		48.2/48.1, C
6a	2.01 d (6.8)	19.1, CH_2	1.99 m[a]/1.93 dd (13.4, 3.2)	19.9/21.1, CH_2
6b	1.92 m[a]		1.99 m[a]/1.72 m[a]	
7α	1.31 br d (10.3)[a]	24.4, CH_2	1.39 ddd (14.9, 4.5, 3.4)/ 1.22 m[a]	25.0/27.6, CH_2
7β	1.92 br d (9.7)[a]		1.73 m/1.56 dt (13.7, 3.3)	
8	3.42 br s	43.7, CH	2.96 t (3.4)/2.39 t (3.3)	48.4/50.0, CH
9		43.1, C		43.7/44.4, C
10		76.8, C		75.1/75.9, C
11a	3.74 d (18.3)	42.3, CH_2	3.51 d (17.6)/3.27 d (18.0)	45.7/47.6, CH_2
11b	3.21 d (18.3)		3.37 d (17.6)/3.20 d (18.0)	
12		194.8, C		196.2/196.5, C
13		128.4, C		128.1[a]/ 128.7,[a] C
14	6.77 br s	108.4, CH	6.78 dd (1.6, 1.0)/6.76 dd (1.6, 1.2)	108.7, CH
15	7.44 d (5.5)	143.6, CH	7.47 t (1.6)/7.49 t (1.6)	144.6/144.9, CH
16	8.15 br s	147.2, CH	8.11 t (1.0)/8.08 t (1.2)	147.9/148.7, CH
17		172.0, C		173.3/173.4, C
18		174.9, C		174.5, 174.3, C
19α	4.94 d (8.2)	71.7, CH_2[a]	4.98 dd (8.4, 2.2)/5.01 dd (8.8, 2.3)	71.2/70.6, CH_2
19β	4.37 d (8.2)		4.35 d (8.4)/4.26 d (8.8)	
20	1.32 s	21.9, CH_3	1.19 s/1.13 s	22.7/15.7, CH_3
1′	5.46 d (8.2)	93.7, CH		
2′	2.93 dd (8.7, 8.2)	71.7, CH[a]		
3′	3.39 dd (9.0, 8.7)	76.4, CH		
4′	3.16 dd (9.3, 9.0)	69.4, CH		
5′	3.52 dd (9.3, 3.9)	74.4, CH		
6′a	4.30 d (3.9)[a]	63.3, CH_2		
6′b	4.30 d (3.9)[a]			
1″		170.8, C		
2″	2.10 s	20.7, CH_3		

[a]Overlapped signal. [b]Determined in $CDCl_3$. [c]OCH_3 signals appeared at δ_H 3.61 (s)/3.75 (s), respectively. [d]OCH_3 signals appeared at δ_C 51.6/51.9.

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trans-diaxial dispositions of all the involved protons in the spin system, as is common for a β-glucopyranoside.[8] In addition, the NMR data of the sugar moiety and furan ring of **1** were similar to those of *O*-acetylglucoside diterpenoids from *Salvia chamaedryoides*.[10] The C-6 location of the acetoxy group in the sugar moiety was established by the HMBC correlations between H_2-6′ (δ_H 4.30, d, J = 3.9 Hz) and C-1″ (δ_C 170.8). The glycosidic linkage position of the aglycone was established at C-17 (δ_C 172.0) based on the HMBC correlations of this carbon with H-1′ and H-8 (δ_H 3.42, br s). The establishment of the relative configuration of **1** was carried out considering an α-disposition of H_3-20, which is common in *neo*-clerodane diterpenoids,[11] followed by the analysis of its NOESY spectrum. NOE correlations of H_3-20 with H-8 and H-19β determined their α-dispositions (Figure 1). However, the NOESY spectrum did not

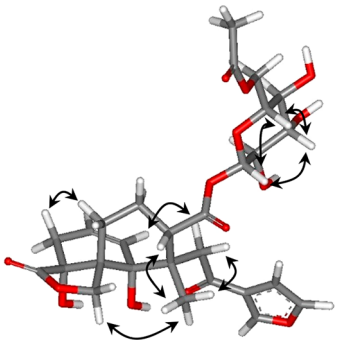

Figure 1. Key NOESY correlations of compound **1**.

permit definition of the orientation of the tertiary hydroxy groups and prompted formation of the aglycone using the published methodology.[13] Thus, treatment of **1** with Na_2CO_3 in MeOH gave two fractions, a nonpolar fraction containing the aglycone and a polar fraction containing mainly glucose, which was directly analyzed by HPLC and coeluted with a reference of D-glucose. In addition, the specific rotation of the polar fraction was closely similar ($[\alpha]^{20}_D$ +26) to the reference value.[5] The nonpolar fraction yielded an epimeric mixture of the methanolysis products **7**/**8** in a 55:45 relation. Several attempts to separate this mixture by chromatography were unsuccessful. Crystallization from acetone/hexanes gave crystalline **7**, which was used to perform a single-crystal X-ray diffraction study. The Flack parameter of 0.03(8)[14] permitted definition of the configuration as (4*R*,5*S*,8*S*,9*R*,10*S*) (Figure 2). Spectroscopic and spectrometric data of the remaining epimeric mixture of compounds **7**/**8** were acquired. The correct ^{1}H and ^{13}C NMR assignment (Table 1) for each compound was made by using the Mnova Spin Simulation software. A comparison of the ^{1}H NMR experimental spectrum of the mixture **7**/**8** and the simulated spectra are shown in Figure 3. The NOE correlations of H_3-20

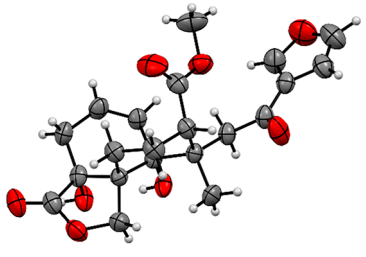

Figure 2. ORTEP drawing of compound **7**.

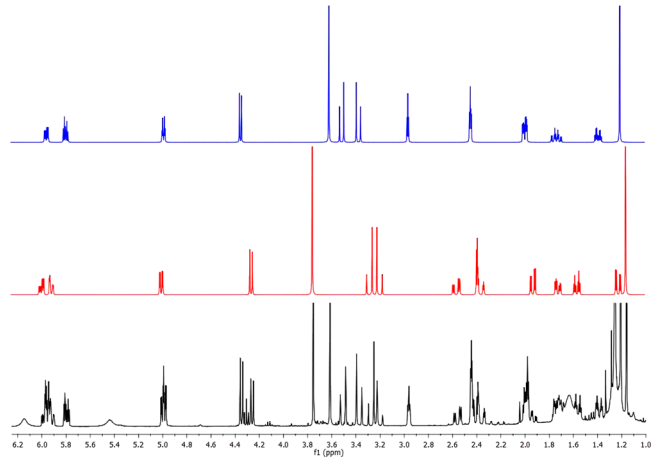

Figure 3. ^{1}H NMR spin analysis of compounds **7**/**8** (simulated of **7** in blue, simulation of **8** in red, experimental in black).

with H-8 and H-19β determined their α-dispositions (Figure 4a) in compound **7**. These correlations were the same as those

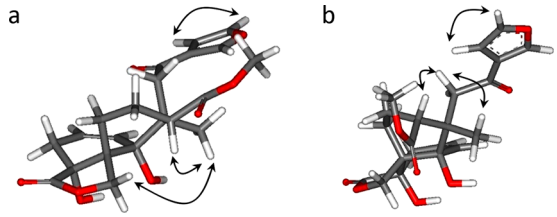

Figure 4. Key NOESY correlations of compound **7** (a) and **8** (b).

observed for compound **1** and indicated that the absolute configuration of the aglycone in **1** is the same as the one shown in **7**. The NOESY correlations observed for compound **8** of H-11b (δ_H 3.20, d, J = 18.0 Hz) with H_3-20 (δ_H 1.13, s) and H-8 (δ_H 2.39, t, J = 3.3 Hz) (Figure 4b) confirmed that H-8 is β-oriented.

Compound **2** was isolated as the *O*-acetyl derivative (**2a**) from a previously analyzed mixture by ^{1}H NMR (Figure S11, Supporting Information), which showed the presence of diagnostic signals (δ_H 7.71, 7.43, 6.55, 4.86, and 4.36) of a *neo*-clerodane diterpenoid[7,8] and was resolved by acetylation with Ac_2O in pyridine at room temperature to give crystalline compound **2a**. The structure of this compound was directly determined by a single-crystal X-ray diffraction analysis (Figure 5).

Compound **1** did not show cytotoxicity (IC_{50} > 42 μM) against five human cancer cell lines: HeLa, HCT-15, HCT-116,

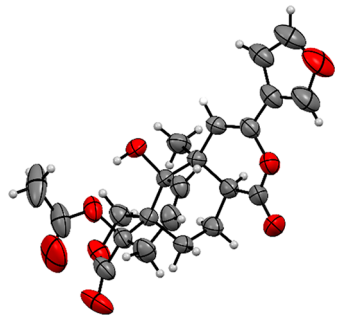

Figure 5. ORTEP drawing of compound **2a**.

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MCF-7, and MDA-MB-231. Therefore, its anti-MDR effect was tested in two phenotypes of the MCF-7 cell line,[15] which was at 42.1 μM ($RF_{MCF\text{-}7/Vin^-}$ 2.1 and $RF_{MCF\text{-}7/Vin^+}$ 12.0), 2−3.6-fold more active than amarissinin A (**3**, $RF_{MCF\text{-}7/Vin^-}$ 3.1 and $RF_{MCF\text{-}7/Vin^+}$ 5.8) and amarissinin B (**4**, $RF_{MCF\text{-}7/Vin^-}$ 54.0 and $RF_{MCF\text{-}7/Vin^+}$ 2.6) in phenotype MCF-7/Vin+, respectively, but exhibited a weak MDR modulatory effect in comparison to the most active diterpenoid from the plant (teotihuacanin, **6**; $RF_{MCF\text{-}7/Vin^-}$ 8437.5 and $RF_{MCF\text{-}7/Vin^+}$10703) and reserpine, used as positive control ($RF_{MCF\text{-}7/Vin^-}$ 5.0 and $RF_{MCF\text{-}7/Vin^+}$ 29.2).[7,9]

EXPERIMENTAL SECTION

General Experimental Procedures. The (uncorrected) melting points were measured on a Fisher-Johns apparatus. Optical rotations were measured on a PerkinElmer 343 polarimeter. The UV spectra were recorded on an Agilent Cary Series UV−vis−NIR spectrophotometer. IR spectra were obtained on a Shimadzu IRTracer-100 spectrometer. NMR experiments were performed on a Bruker Avance III 400 MHz or on a Varian Unity Plus 500 MHz. Chemical shifts were relative to tetramethylsilane, and J values are given in Hz. X-ray crystallographic data were obtained on a Bruker D8 Venture κ-geometry diffractometer with Cu Kα radiation (λ = 1.541 78 Å). HRESIMS data were recorded on a MALDI SYNAPT G2-Si mass spectrometer. Waters HPLC equipment was composed of a 600E multisolvent delivery system with a refractive index detector (Waters 2410). Control of the equipment, data acquisition, and processing of the chromatographic information were performed with the Empower 2 software (Waters). GC-MS was performed on an Agilent 7890B instrument coupled to an Agilent 5977A spectrometer. GC conditions: DB-5ht (5% phenyl)methylpolysiloxane column (30 m × 0.32 mm, film thickness 0.10 μm); He, 1.37 mL/min; 100 °C isothermal for 1 min, linear gradient to 350 °C at 9 °C/min; final temperature hold, 3 min. MS conditions: ionization energy, 70 eV; ion source temperature, 250 °C; interface temperature, 270 °C; scan speed, 2 scans s^{-1}; mass range, 14−600 amu. The Mass Hunter Wiley10 Nist11 G1035 (W10N11) was used for the identification of volatile compounds. Column chromatography (CC) was performed on silica gel 230−400 mesh (Macherey−Nagel). TLC was carried out on precoated Macherey−Nagel Sil G/UV254 plates of 0.25 mm thickness, and spots were visualized by UV light at 254 nm and sprayed with 3% $CeSO_4$ in 2 N H_2SO_4, followed by heating.

Chemicals, Cell Lines, and Cell Culture. The resistant MCF-7/Vin was developed through continuous exposure to vinblastine during five consecutive years as previously described.[15]

Plant Material. The leaves and flowers of *S. amarissima* were collected in the mountains that surround the Valley of Teotihuacan, in the state of Mexico, in August 2015. A voucher specimen was deposited (MEXU-1407290) at the National Herbarium, Instituto de Biología, Universidad Nacional Autónoma de México.

Extraction and Isolation. The dried and powdered leaves and flowers from *S. amarissima* (3.5 kg) were extracted by percolation with acetone (20 L). The extract was concentrated at reduced pressure to obtain 190 g of gummy residue, which was defatted by partition with hexanes (1 L) and MeOH/H_2O, 4:1 (3 × 1 L). The MeOH was evaporated from the aqueous alcohol fraction, water (1 L) was added, and the resulting mixture was partitioned with EtOAc (3 × 1 L) to give 46.3 g of residue. The EtOAc fraction was subjected to silica gel 60 G CC (4.5 × 15.0 cm, 500 mL) eluted with mixtures of hexanes/EtOAc and EtOAc/acetone as eluents. The fractions obtained from this column were analyzed by TLC and pooled as follows: fraction A (frs. 1−11, 3.1 g, hexanes/EtOAc, 4:1), fraction B (frs. 12−18, 14.5 g, hexanes/EtOAc, 7:3), fraction C (frs. 19−22, 2.6 g hexanes/EtOAc, 7:3), fraction D (frs. 23−26, 3.89 g, hexanes/EtOAc, 3:2), fraction E (frs. 27−37, 7.52 g, hexanes/EtOAc, 2:3), fraction F (frs. 38−49, 4.3 g, hexanes/EtOAc, 1:4), and fraction G (frs. 50−53, 10.3 g, EtOAc/acetone, 80:20). Constituents from fraction A were separated by silica gel CC (4.0 × 8.0 cm, 100 mL) as follows: A1 (frs. 1−12, hexanes/EtOAc, 70:30), A2 (frs. 13−20, hexanes/EtOAc, 65:35), A3 (frs. 21−27, hexanes/EtOAc, 60:40). Fraction A2 (0.9 g) showed a complex mixture by TLC and ^{1}H NMR, which was acetylated using Ac_2O (3 mL) in pyridine (1.5 mL) to give 1.1 g of reaction product. A mixture (150 mg) containing **9** as the major constituent precipitated, which was separated by filtering and directly analyzed by GC-MS. The supernatant (0.85 g) was subjected to silica gel CC (2.0 × 12.0 cm, 25 mL) eluted with hexanes/EtOAc of increasing polarity. Fractions eluted with hexanes/EtOAc, 70:30, gave 5.9 mg of **10**. Fractions eluted with hexanes-/EtOAc, 65:35, yielded 0.5 mg of **2a**. From fractions eluted with hexanes/EtOAc, 60:40, was obtained 11.6 mg of **4a**. Fraction A3 afforded compound **5** (101.6 mg) after crystallization from acetone/hexanes. From fraction B a solid was obtained and recrystallized from acetone/hexanes to give 2.45 g of **5**. Mother liquors (11.7 g) were subjected to silica gel CC (4.0 × 8.0, 100 mL) eluted with mixtures of hexanes/EtOAc, 65:35 (fr. B1, 0.4 g), hexanes/EtOAc, 60:40 (fr. B1, 4.12 g), and hexanes/EtOAc, 50:50 (fr. B3, 1.44 g). Fraction B1 yielded 350 mg of **5** by crystallization from EtOAc/hexanes. Fraction B2 was shown by TLC and ^{1}H NMR as a complex mixture, which was acetylated with Ac_2O (12.4 mL) in pyridine (6.2 mL) to give 4.4 g of reaction mixture. It was subjected to silica gel CC (4.0 × 8.0, 50 mL) developed with hexanes/EtOAc, 60:40, to give 61 mg of **4a** and with hexanes/EtOAc, 50:50, to yield 87 mg of **5a**. From fraction B3 43.0 mg of **3** was isolated by crystallization with acetone/hexanes. From fraction D (3.9 g), compounds **3** (365 mg) and **6** (214 mg) were obtained by successive silica gel CC eluted with mixtures of $CHCl_3$/MeOH of increasing polarity and finally crystallized from acetone/hexanes. Fraction E (7.52 g) was submitted to silica gel CC (4.0 × 8.0, 50 mL) eluted with hexanes/EtOAc, 1:1, to obtain 1.15 g of **3**. Fraction G (10.32 g) was subjected to silica gel CC (4.5 × 8.0, 100 mL) eluted with mixtures of hexanes/EtOAc and EtOAc/MeOH. The fractions obtained from this column were analyzed by TLC and pooled as follows: G1 (frs. 1−22, hexanes/EtOAc, 2:3), G2 (frs. 23−32, hexanes/EtOAc, 3:7), G3 (frs. 33−53, hexanes/EtOAc, 1:4), G4 (frs. 54−67, EtOAc/MeOH, 99:1), G5 (frs. 68−77, EtOAc/MeOH, 95:5), G6 (frs. 78−83, EtOAc/MeOH, 80:20). Fraction G5 (1.48 g) was subjected to silica gel CC (3.0 × 8.0, 50 mL) and eluted with mixtures of $CHCl_3$/MeOH of increasing polarity. The fractions eluted with $CHCl_3$/MeOH, 9:1, yielded 413.4 mg of **1**.

*Amarisolide F (**1**):* beige, amorphous powder; $[\alpha]^{25}_D$ −6 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.85), 250 (3.54); IR (ATR) ν_{max} 3408, 1742, 1710, 1672, 872 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$/DMSO-d_6) see Table 1; positive ESIMS m/z 633 $[M + K]^+$, 617 $[M + Na]^+$; HRESIMS m/z 595.2046 $[M + H]^+$ (calcd for $C_{28}H_{35}O_{14}$, 595.2021).

X-ray Single-Crystal Structure Determination of 4-*O*-acetylamarissinin D (2a). Formula: $C_{22}H_{22}O_7$, MW = 398.39, monoclinic, space group $P2_1$, unit cell dimensions a = 18.8711(11) Å, b = 9.2292(5) Å, c = 22.5216(13) Å, β = 105.731(2)°, V = 3775.6(4) Å^3, Z = 8, D_c = 1.402 g/cm^3, $F(000)$ = 1680. A total of 15 144 unique reflections were collected, with 14 448 reflections greater than $I \geq 2\sigma(I)$ (R_{int} = 0.0401). The structure was solved by direct methods and refined by full-matrix least-squares on F^2, with anisotropic displacement parameters for non-hydrogen atoms at final R indices [$I > 2\sigma(I)$], R_1 = 0.0319, wR_2 = 0.0814; R indices (all data), R_1 = 0.0339, wR_2 = 0.0833. Flack parameter = 0.04(3). Crystallographic data reported in this paper have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1482296). The data can be obtained free of charge via http://www.ccdc.ac.uk./data_request/cif.

*4-O-Acetylamarissinin C (**5a**):* colorless crystals, mp 198−201 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 (1H, t, J = 2.0 Hz, H-16), 7.37 (1H, dd, J = 2.0, 1.5 Hz, H-15), 6.40 (1H, t, J = 1.5 Hz, H-14), 6.12 (1H, ddd, J = 10.4, 2.4, 1.2 Hz, H-1), 5.86 (1H, ddd, J = 10.4, 5.0, 2.1 Hz, H-2), 5.67 (1H, dt, J = 7.3, 1.3 Hz, H-12), 4.81 (1H, dd, J = 8.6, 1.8 Hz, H-19β), 4.30 (1H, d, J = 8.6 Hz, H-19α), 3.44 (1H, s, OH-10), 3.12 (1H, dd, J = 16.0, 8.5 Hz, H-11α), 3.08 (1H, ddd, J = 19.7, 5.0, 1.2 Hz, H-3α), 2.54 (1H, ddd, J = 19.7, 5.4, 2.8 Hz, H-3β), 2.09 (1H, overlapped, H-7a), 2.08 (3H, s, H_3-2′), 1.94 (1H, dd, J = 16.3, 1.2 Hz, H-11β), 1.69 (2H, overlapped, H-7b, H-6a), 1.40 (1H, dd, J = 9.8, 2.7 Hz, H-6b), 1.29 (3H, s, H_3-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 172.2 (C, C-17), 169.3 (C, C-18), 166.9 (C, C-1′), 144.4 (CH, C-16), 138.7 (CH,C-15),

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129.8 (CH, C-1), 127.2 (C, C-13), 126.3 (CH, C-2), 108.7 (CH, C-14), 84.5 (C, C-4), 75.4 (C, C-5), 71.0 (CH, C-12), 70.2 (CH_2, C-19), 49.4 (C, C-5), 42.9 (CH, C-8), 41.5 (C, C-9), 34.0 (CH_2, C-11), 27.4 (CH_3, C-20), 25.9 (CH_2, C-3), 24.0 (CH_2, C-6), 21.6 (CH_3, C-2′), 16.4 (CH_2, C-7).

Amarissinin E/-8-epi-Amarissinin E (7/8). To a solution of **1** (50.4 mg) in MeOH (1.5 mL) was added Na_2CO_3 (54 mg). The resulting mixture was stirred at room temperature for 12 h. The supernatant was filtered and concentrated under reduced pressure to afford a residue, which was successfully extracted with 10 mL of EtOAc and 10 mL of $CHCl_3$ to give a nonpolar fraction (29.8 mg), which was extracted with 10 mL of MeOH to give a polar fraction (22.1 mg). An epimeric mixture of **7**/**8** was obtained directly from the nonpolar fraction. Crystallization of this mixture from acetone/hexanes yielded **7** as a pink solid, mp 203−205 °C; $[\alpha]^{25}_D$ −2 (*c* 0.2, Me_2CO); UV (MeOH) λ_{max} (log ε) 204 (4.02), 234 (3.29), 250 (3.45); IR (KBr) ν_{max} 3310, 2921, 1776, 1709, 1671, 875 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$/DMSO-d_6) see Table 1; positive HRESIMS *m*/*z* 427.1382 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_8Na$, 427.1363).

X-ray Single-Crystal Structure Determination of Amarissinin E (7). Formula: $C_{21}H_{24}O_8$, MW = 404.40, orthorhombic, space group $P2_12_12_1$, unit cell dimensions a = 9.7873(3) Å, b = 10.4684(3) Å, c = 18.7272(6) Å, V = 1918.74(10) $Å^3$, Z = 4, D_c = 1.400 g/cm^3, $F(000)$ = 856. A total of 4082 unique reflections were collected, with 3739 reflections greater than $I \geq 2\sigma(I)$ (R_{int} = 0.0675). The structure was solved by direct methods and refined by full-matrix least-squares on F^2, with anisotropic displacement parameters for non-hydrogen atoms at final R indices $[I > 2\sigma(I)]$, R_1 = 0.0374, wR_2 = 0.0956; R indices (all data), R_1 = 0.0417, wR_2 = 0.0995. Flack parameter = 0.03(8). Crystallographic data reported in this paper have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1843755). The data can be obtained free of charge via http://www.ccdc.ac.uk./data_request/cif.

Sugar Analysis. The polar fraction (22.1 mg) obtained by the reaction of **1** with Na_2CO_3 in MeOH was analyzed directly by HPLC on a YMC aminopropyl column (5 μm, 10 × 150 mm) with an isocratic elution with CH_3CN/H_2O, 75:25, and a flow rate of 2 mL/min. The resulting chromatogram and the specific rotation {$[\alpha]^{25}_D$ +26 (*c* 0.2, MeOH)]} of the polar fraction were compared with those obtained from an authentic sample of D-glucose.

Cytotoxicity and Anti-MDR Effect of 1. Cytotoxicity was determined by following the protocols that are established by the NCI.[15] Cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and cultured at 37 °C in 5% CO_2 in air (100% humidity). MCF-7/Vin+ cells were cultured in medium containing 211.2 μM vinblastine. A stock of MCF-7/Vin cells was also maintained in vinblastine-free medium (MCF-7/Vin−). Cells at log phase were treated in triplicate with **1** (0.4−42.1 μM) and incubated for 72 h.

For the reversal effects, sensitive MCF-7 and MDR MCF-7/Vin cells were seeded into 96-well plates and treated with various concentrations of vinblastine (0.7 nM−11 μM) in the presence or absence of compound **1** (42.1 μM) for 72 h. The ability of compound **1** to potentiate vinblastine cytotoxicity was measured by calculating the IC_{50}.[15]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00565.

CIF file of **2a** (CIF)

CIF file of **7** (CIF)

NMR spectra for compounds **1** and **7**/**8** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +52 444 834 2000. Fax: +52 444 834 2010. E-mail: francisco.bautista@ipicyt.edu.mx (E. Bautista).

ORCID

Elihú Bautista: 0000-0002-7050-9182

Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Dr. Rachel Mata, National Autonomous University of Mexico, Mexico City, Mexico, for her pioneering work on bioactive natural products.

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