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Teotihuacanin, a Diterpene with an Unusual Spiro-10/6 System from *Salvia amarissima* with Potent Modulatory Activity of Multidrug Resistance in Cancer Cells

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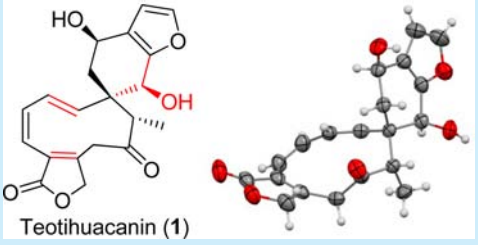
Supporting Information

ABSTRACT: Teotihuacanin (**1**), an unusual rearranged clerodane diterpene with a new carbon skeleton containing a spiro-10/6 bicyclic system, was isolated from the leaves and flowers of *Salvia amarissima*. Its structure was determined through spectroscopic analyses. Its absolute configuration was established by single-crystal X-ray diffraction. Compound **1** showed potent modulatory activity of multidrug resistance in vinblastine-resistant MCF-7 cancer cell line (reversal fold, $RF_{MCF\text{-}7/Vin+} > 10703$) at 25 μg/mL.



In tumor cells, the gradual loss of hypersensitivity to anticancer drugs results in multidrug resistance (MDR), one of the main causes of failure in cancer chemotherapy. MDR is a serious concern even if the treatment consists of a combination of drugs with multiple targets.[1,2] Several mechanisms of resistance have been described.[3] Resistance conferred by ATP-binding cassette (ABC) transporters, including efflux mediated by P-glycoprotein (ABCB1), is one of the most common mechanisms.[4]

Diterpenoids derived from the *Salvia* species have shown the capacity to cross membranes and the blood−brain barrier due to their lipophilic properties.[5] These features facilitate interactions with molecular targets of therapeutic interest, such as P-glycoprotein (e.g., salvinorin A)[6] and the antiapoptotic protein Hsp-27 (e.g., 1,10-dehydrosalviarin).[7] In addition, these compounds have shown cytotoxicity activity against human cancer cells.[8,9] Another remarkable fact of the chemistry of the *Salvia* genus is the reported isolation of diterpenes with unconventional carbon skeletons produced through the rearrangement of clerodanes and abietanes through pericyclic reactions.[10−12]

S. amarissima is a herbaceous plant endemic to and widely distributed throughout Mexico.[13] Previous study of its chemical composition led to the isolation of amarisolide, a neoclerodane glycoside.[14] The pharmacological properties of compounds isolated from this species have not been described to date. In our continuing efforts in the search for new bioactive natural products from the genus *Salvia*,[9,15] a reinvestigation of the chemical composition of *S. amarissima* was undertaken. Aerial portions of this plant were collected in 2014 from the mountains surrounding the Teotihuacan Valley. A voucher specimen was deposited at the National Herbarium (MEXU-1407290).

Teotihuacanin (**1**) was isolated from *S. amarissima* as yellowish-white crystals.[16] Its molecular formula ($C_{20}H_{20}O_6$) was deduced from the pseudomolecular ion peak observed at m/z 357.13406 (HRDARTMS, calcd 357.13381), indicating 11 degrees of unsaturation. The ^{13}C NMR spectrum showed 20 signals, which were resolved through DEPT experiment as one methyl, three methylenes, nine methines, and seven non-protonated carbon atoms. In the IR spectrum, bands corresponding to hydroxyl groups (3524 and 3479 cm^{-1}), γ-lactone and ketone carbonyl groups (1689 and 1744 cm^{-1}), and a furan ring (853 cm^{-1}) were observed. The ^1H and ^{13}C NMR spectra (Table 1) confirmed the presence of the lactone (δ_C 172.4, C-18; 73.6, C-19; δ_H 5.24, d, J = 18.0 Hz, H-19$_{Pro\text{-}R}$; 4.65, d, J = 18.0 Hz, H-19$_{Pro\text{-}S}$), as well as a disubstituted furan ring (δ_C 150.9, C-16; 123.7, C-13; δ_H 7.26, d, J = 1.9 Hz, H-15; 6.35, d, J = 1.9 Hz, H-14). In the ^1H NMR spectrum (Table 1), four signals could be attributed to vinylic protons at δ_H 6.38 (dd, J = 10.2, 8.5 Hz), 5.93 (d, J = 10.6 Hz), δ_H 5.52 (d, J = 17.2 Hz), and 5.82 (dd, J = 17.2, 8.1 Hz) and were assigned to H-2, H-3, H-1, and H-10 on the basis of COSY correlations (Figure 1).

These data, together with the presence of two quaternary carbons at δ_C 157.6 (C-5, C) and 123.9 (C-4, C), indicated that **1** was 5,10-*seco* in the clerodane framework.[17] This was confirmed by the HMBC correlations of H-10 with C-1 (δ_C 129.5) and C-9 (δ_C 47.2) and of H-19$_{Pro\text{-}R}$ and H-6α (δ_H 3.76, d, J = 15.2 Hz) with C-5 (Figure 1). The position of the ketone carbonyl (δ_C 208.3) was established at C-7 by its HMBC

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data for 1 in $CDCl_3$–DMSO-d_6 (δ in ppm)

position	δ_H mult (J, Hz)	δ_C
1	5.82 dd (17.2, 8.5)	129.5, CH
2	6.38 dd (10.6, 8.5)	131.5, CH
3	5.93 d (10.6)	118.8, CH
4		123.9, C
5		157.6, C
6α	3.76 d (15.2)	41.0, CH_2
6β	2.85 d (15.2)[a]	
7		208.3, C
8	2.86 q (7.0)[a]	59.8, CH
9		47.2, C
10	5.52 d (17.2)	133.9, CH
11α	2.35 dd (13.8, 5.8)	33.5, CH_2
11β	1.81 dd (13.8, 9.7)	
12	4.27 dd (9.4, 6.1)	61.9, CH
13		123.7, C
14	6.35 d (1.9)	108.7, CH
15	7.26 d (1.9)	142.2, CH
16		150.9, C
17	1.24 d (7.0)	9.1, CH_3
18		172.4, C
19-*Pro R*	5.24 d (18.0)	73.6, CH_2
19-*Pro S*	4.65 d (18.0)	
20	4.43 s	65.0, CH

[a]Overlapped.

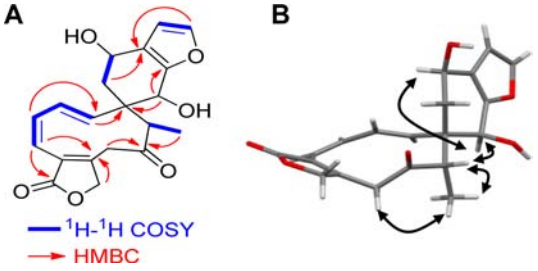
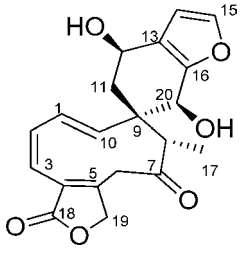


Figure 1. (A) Key ^{1}H–^{1}H COSY and HMBC correlations of **1**. (B) Key NOESY correlations of **1**.



correlations with H-6α and H-8 (δ_H 2.86, d, J = 6.9 Hz). In the ^{1}H NMR spectrum (Table 1), a signal at δ_H 4.27 (dd, J = 9.4, 6.1 Hz) was assigned to H-12 by its HMBC correlations with C-13 and C-16 (Figure 1).

The signal for H-12 also showed cross peaks in the COSY spectrum with two signals at δ_H 2.35 (dd, J = 13.8, 5.8 Hz) and 1.81 (dd, J = 13.8, 9.7 Hz), assigned to H-11α and H-11β, respectively. Additional HMBC correlations observed for the signal attributed to a geminal proton with a hydroxyl group at δ_H 4.43 (s) allowed its assignment as H-20, as confirmed by its cross peaks with C-8, C-9, C-10, C-11, C-13, and C-16 (Figure 1). This is only possible if there is a C–C bond between C-16 and C-20. This information indicated that **1** possesses a spiro ring as part of a new carbon skeleton, which has been named amarisane.

At this point, we can assume that **1** is biosynthetically related to the clerodane diterpenes and can consider its stereochemistry to be very similar. Thus, the relative configuration of this compound was deduced by NOESY correlations (Figure 1) taking into account the α-disposition of the methyl at C-17.

The NOESY correlation of H_3-17 with H-8 and H-20 and of H-8 with H-12 indicated that both hydroxyl groups in the spiro-six-membered ring system possess a β-*cis* orientation.

To verify these assumptions, the absolute configuration of **1** was determined through single-crystal X-ray diffraction using Cu Kα radiation (Flack parameter = −0.09(10); λ = 1.54178 Å).[18] An ORTEP drawing of the crystallographically determined structure of **1** is depicted in Figure 2.

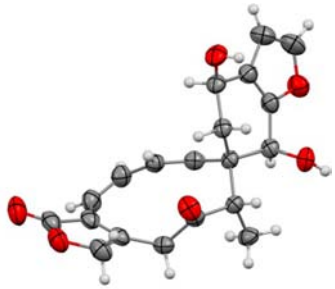
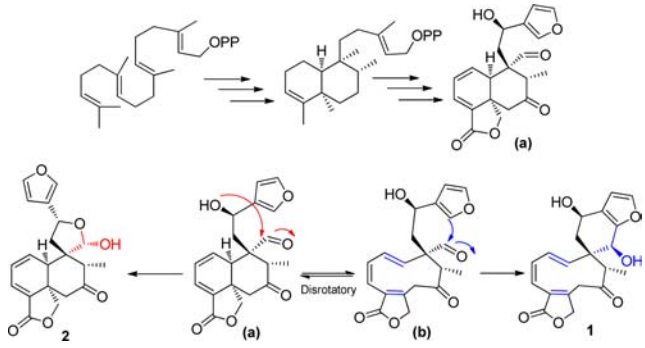


Figure 2. ORTEP drawing of **1**.

A plausible biosynthetic route for this new framework, represented by teotihuacanin (**1**), is the initial formation of intermediate (**a**), in which the hydroxyl group at C-12 can react with the carbonyl at C-20 to give the spiro compound **2**, which is closely related to dugesin F (Scheme 1).[19] This intermediate

Scheme 1. Plausible Biosynthetic Route for 1



(**a**) can play a role in a second reaction sequence involving the electrocyclic opening of the decalin ring system to produce (**b**), followed by the addition of the furan ring to the carbonyl at C-20 to give **1**. This biosynthetic hypothesis is supported by the concomitant existence of clerodanes with a 1,3-diene in the decalin ring and 5,10-*seco*-clerodanes bearing a 10,2,4-triene, both isolated from other *Salvia* species.[20,21]

The cytotoxicity of teotihuacanin (**1**) was tested against cancer cell lines of the human breast (MCF-7 and MDA-MB-231), colon (HCT-15 and HCT-116), and cervix (HeLa).[22] The results of these assays showed **1** to exhibit moderate cytotoxicity against the following cell lines: MDA (IC_{50} 12.3 ± 1.5 μg/mL), HeLa (IC_{50} 13.7 ± 4.9 μg/mL), HCT-15 (IC_{50}

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Table 2. Modulation of Vinblastine Cytotoxicity in Drug-Sensitive MCF-7 and Multidrug-Resistant MCF-7/Vin by Compound 1

	IC_{50} (μg/mL)			reversal fold[d]		
compd[a]	MCF-7/Vin$^-$	MCF-7/Vin$^+$	MCF-7 sens	$RF_{MCF\text{-}7/Vin^-}$	$RF_{MCF\text{-}7/Vin^+}$	$RF_{MCF\text{-}7\ sens}$
vinblastine	1.08 ± 0.06	1.37 ± 0.23	0.047 ± 0.01			
teotihucanin (**1**)	<0.000128	<0.000128	<0.000128	8437.5	10703.1	367.2
teotihucanin (**1**)[b]	0.15 ± 0.08	0.19 ± 0.05	0.02 ± 0.01	7.2	7.2	2.4
reserpine[c]	0.037 ± 0.01	0.31 ± 0.19	0.003 ± 0.001	29.2	4.4	15.7

[a]Serial dilutions from 0.000128 to 10 μg/mL of vinblastine in the presence or absence of diterpene (25 μg/mL). [b]Diterpene = 5 μg/mL. Each value represents the mean ± SD from three independent experiments. [c]Reserpine = 5 μg/mL as positive control. [d]RF = IC_{50} vinblastine/IC_{50} vinblastine in the presence of diterpene (**1**).

12.9 ± 1.7 μg/mL), and HCT-116 (IC_{50} 10.9 ± 1.0 μg/mL). No cytotoxicity was demonstrated against the MCF-7 cell line (IC_{50} > 20 μg/mL). Therefore, the compound **1** is a potential modulator of multidrug resistance (MDR) in MCF-7 cancer cells.[23] The results of the modulatory assay are shown in Table 2. Reversal fold ($RF_{MCF\text{-}7/Vin+}$ > 10703 at 25 μg/mL and $RF_{MCF\text{-}7/Vin+}$ 7.2 at 5 μg/mL) reveals that **1** is more potent than the positive control (reserpine, $RF_{MCF\text{-}7/Vin+}$ 4.4 at 5 μg/mL). Thus, compound **1** represents a new class of potent MDR modulators, with effects even visible in the sensitive counterpart ($RF_{MCF\text{-}7\ sens}$ > 367 at 25 μg/mL). In addition, the reversal fold value of **1** is higher than those described for purgin II ($RF_{MCF\text{-}7/Vin+}$ > 2140)[24] and jalapinoside ($RF_{MCF\text{-}7/Vin+}$ > 1906),[25] two potent modulators of MDR derived of plant origin (*Ipomoea* species). As a result of these findings, teotihuacanin (**1**) can be considered a scaffold for the development of more potent MDR modulators for cancer chemotheraphy.

■ ASSOCIATED CONTENT

Supporting Information

Experimental general procedures, 1D and 2D NMR spectra, and X-ray crystallographic data (CIF file) of **1**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01320.

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Notes

The authors declare no competing financial interest.

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(16) Teotihuacanin (**1**): yellowish white crystals; mp 256−258 °C; $[\alpha]^{22}_D$ +209 (*c* 0.10, Me_2CO); UV (MeOH) λ_{max} (log ε) 224 (4.39); IR (KBr) ν_{max} 3524, 3479, 1744, 1689, 853 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$−DMSO-d_6) see Table 1; DARTMS *m*/*z* 357 $[M + H]^+$; HRDARTMS *m*/*z* 357.13406 (calcd for $C_{20}H_{21}O_6$ 357.13381).
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