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Jimenezin, a Novel Annonaceous Acetogenin from the Seeds of *Rollinia mucosa* Containing Adjacent Tetrahydrofuran−Tetrahydropyran Ring Systems[†,1]

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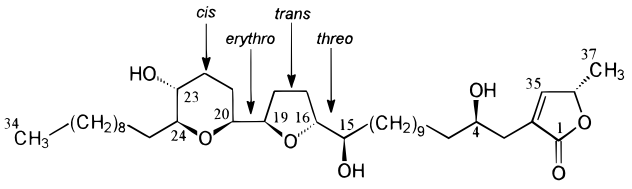
Abstract: A new cytotoxic acetogenin, jimenezin (**1**), containing a hydroxylated tetrahydropyran ring along with an adjacent tetrahydrofuran ring and representing a novel carbon skeleton, was isolated from the seeds of *Rollinia mucosa*. The structure was elucidated by means of chemical and spectral methods including MS and NMR measurements. Compound **1** exhibited potent cytotoxic activity against six human solid tumor cell lines.

As part of our ongoing investigation on biologically active compounds from Mexican medicinal plants, we have studied the seeds of *Rollinia mucosa* (Jacquin) Baillon (syn. *Rollinia jimenezii* Safford)[2] (Annonaceae) and isolated the novel acetogenin **1**, which was given the trivial name jimenezin. In this paper, we describe the structure elucidation and cytotoxic activity of compound **1**.



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Jimenezin (**1**) belongs to the rare type of acetogenins containing a tetrahydropyran (THP) ring. The first THP ring acetogenin, named mucocin, was discovered by McLaughlin's group in 1995.[3] Mucocin is an acetogenin that bears a hydroxylated THP ring, as well as a nonadjacent tetrahydrofuran (THF) ring. Later on, the same group described another related acetogenin,[4] muconin, that contains a nonhydroxylated THP ring along with an adjacent THF ring. We have now isolated a closely related acetogenin possessing a hydroxylated THP ring along with an adjacent THF ring and one flanking hydroxyl group on the THF side.

Table 1. ^{1}H (500 MHz, $CDCl_3$, *J* (Hz)) and ^{13}C NMR (125 MHz, $CDCl_3$) Data of **1**[a]

position	δ_H	δ_C	COSY[b]	HMBC[c]
1		174.6		
2		131.2		
3a	2.53 dddd (15.1, 3.2, 1.5, 1.5)	33.3	H-4, H-35	C-1, C-2,
3b	2.40 dddd (15.1, 8.2, 1.5, 1.5)			C-4, C-35
4	3.85 m	70.0	H-3a,b, H-5	
5	1.47 m	37.4		
6−13	1.2−1.4 m	25−33		
14	1.47 m	34.9		
15	3.35 m	73.9	H-14, H-16	C-16
16	3.90 m	82.4	H-15, H-17a,b	C-15
17	1.62 m, 1.93 m	28.0		
18	1.60 m, 2.0 m	28.3		
19	3.94 m	80.9	H-18a,b, H-20	C-20
20	3.24 ddd (11.5, 2.3, 2.3)	79.0	H-19, H-21a,b	C-19
21	1.64 m, 1.77 m	27.9	H-20, H-22a,b	
22	1.45 m, 2.13 m	32.9	H-21a,b, H-23	
23	3.28 ddd (10.8, 9.2, 4.6)	70.6	H-22a,b, H-24	C-24
24	3.00 ddd (9.2, 9.0, 2.1)	82.3	H-23, H-25	C-23, C-25
25	1.42 m	25.5		
26−31	1.2−1.4 m	25−33		
32	1.2−1.4 m	31.9		
33	1.2−1.4 m	22.7		
34	0.88 t (6.8)	14.1		
35	7.18 ddd (1.5, 1.5, 1.5)	151.7	H-3a,b, H-36	C-1, C-36
36	5.06 qq (6.8, 1.5)	78.0	H-3a,b, H-37	
37	1.43 d (6.8)	19.1		

[a] Assigned by HMQC, HMBC, and DEPT spectra. [b] Key ^{1}H−^{1}H correlations. [c] Important long-range ^{1}H-^{13}C correlations.

Compound **1** was isolated from the cytotoxic $CHCl_3$−MeOH (1:1) extract of the seeds of *R. mucosa*.[5,6] The brine shrimp lethality test[7] was employed to direct the fractionation of the active extract.

Jimenezin (**1**)[8] was obtained as a yellow oil. Its molecular formula was established as $C_{37}H_{66}O_7$ by HRFABMS.[9] The IR spectrum[9] contained absorptions for hydroxyl (3418 cm^{-1}) and α,*β*-unsaturated *γ*-lactone (1751 cm^{-1}) functionalities. Sequential losses of three molecules of H_2O from the MH^+ in the FABMS[9] as well as the formation of the tri-TMSi derivative **1a** confirmed the existence of three hydroxyl groups in compound **1**.

The presence in jimenezin (**1**) of an α,*β*-unsaturated methyl *γ*-lactone with a hydroxyl group at C-4 was suggested by the ^{1}H NMR[9] resonances at *δ* 7.18 (H-35), 5.06 (H-36), 3.85 (H-4), 2.53 (H-3a), 2.40 (H-3b), and 1.43 (H-37) corresponding in the ^{13}C NMR[9] spectrum to the resonances at *δ* 174.6 (C-1), 151.7 (C-35), 131.2 (C-2), 78.0 (C-36), 70.0 (C-4), 33.3 (C-3), and 19.1 (C-37) (Table 1).[10] In addition to the resonance attributable to H-4, the ^{1}H NMR spectrum (Table 1) of compound **1** exhibited six well-defined signals in the range δ_H 3.0−4.0. Those

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Table 2. Partial ^{1}H NMR Data of the Mosher Esters of **1s** and **1r**[a]

proton	δ_H **1s**	δ_H **1r**	$\Delta\delta_{S-R}$
14	1.62	1.51	+0.11
15	5.14	5.20	*R*[b]
16	4.01	4.02	−0.01
17a	1.81	1.91	−0.10
17b	1.46	1.70	−0.24
18a	1.90	1.91	−0.01
18b	1.52	1.79	−0.27
19	3.93	3.91	+0.02
20	3.28	3.21	+0.07
21a	1.81	1.61	+0.20
21b	1.54	1.53	+0.01
22a	2.32	2.24	+0.08
22b	1.62	1.41	+0.21
23	4.71	4.75	*R*[b]
24	3.23	3.26	−0.03
25	1.30	1.49	−0.19
35	6.78	7.03	−0.25
36	4.86	4.92	−0.06
3a	2.54	2.62	−0.08
3b	2.62	2.69	−0.07
4	5.36	5.43	*R*[b]
5	1.69	1.65	+0.04

[a] Obtained in $CDCl_3$ at 500 MHz. [b] Absolute configuration.

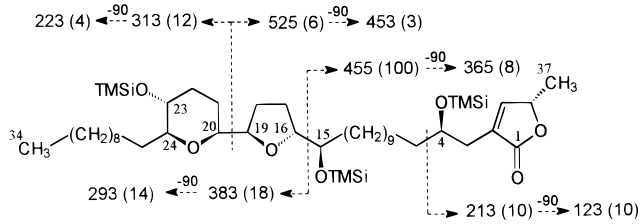

Figure 1. Diagnostic EIMS fragment ions of the TMSi derivative **1a** (shown as *m*/*z* values, intensities indicated in parentheses).

at δ 3.75 (H-15), 3.90 (H-16), and 3.94 (H-19) were assigned to the THF ring with a flanking hydroxyl group.[10] The remaining signals at δ 3.00 (H-24), 3.24 (H-20), and 3.28 (H-23) were consistent with the presence of a hydroxylated THP ring in the molecule.[3] The COSY NMR relationships found between H-20/H-19, H-24/H-23, H-23/H-22, H-22/H-21, and H-21/20 not only confirmed the presence of a hydroxylated THP moiety but also established that this was adjacent to the THF ring that was itself flanked by a hydroxyl group.

The disposition of the adjacent THF−THP unit along the aliphatic chain was determined by the analysis of the fragmentation pattern displayed by the tri-TMSi derivative[11] **1a** (Figure 1). Thus, the intense fragment ions peaks at *m*/*z* 455 (cleavage at C-15/16) and *m*/*z* 525 (cleavage at C-19/20) allowed placement of the THF ring with a flanking hydroxyl between C-15 and C-19 and of the hydroxylated THP ring between C-20 and C-24.

The relative stereochemistry at C-15/C-16 was assigned according to Born's rule.[12] Thus, the chemical shift values of both C-15 (δ 73.9) and H-15 (δ 3.35) indicated a threo relationship. On the other hand, the relative configuration of the chiral carbon centers of the THF moiety was determined as trans by comparing the NMR information with that of muconin.[4] The cis stereochemistry of the THP ring was established by interpreting the NOESY spectrum that exhibited an intense cross-peak between H-20 (δ 3.24) and H-24 (δ 3.00). The trans-diaxial relationship of H-23 and H-24 was determined by a homodecoupling NMR experiment. Thus, irradiation of the resonance at δ 1.42 (H-25) simplified the signal assignable to H-24 to a doublet (J = 9.2 Hz). The magnitude of the coupling constant observed for this doublet was consistent with the trans relationship between H-23 and H-24 and, therefore, with the equatorial orientation of the hydroxyl group at C-23.

The absolute configuration of the stereogenic carbinol centers was established using Mosher ester methodology.[13] Analysis of the $\Delta\delta_{S-R}$ data (Table 2) of the per-(*S*)- and per-(*R*)-MTPA Mosher ester derivatives[14] **1s** and **1r** showed that the absolute stereochemistry of the chiral centers at C-15, C-23, and C-4 were *R*, *R*, and *R*, respectively. Thus, the absolute stereochemistry for C-16, C-19, C-20, and C-24 was deduced as *R*, *R*, *S*, and *S*, respectively. The erythro relationship at C-19/C-20 was confirmed according to these results. Finally, the *S* configuration at C-36 was established by the negative Cotton effect at 238 nm.[15,16]

Acetogenin **1** was significantly active in the brine shrimp lethality test and was also cytotoxic for six human solid tumor cell lines in a 7-day MTT test[17] using adriamycin as the positive control (Table 3).

The proposed sequence of the THP ring and the adjacent THF ring in jimenezin (**1**) is opposite to the order of those moieties in muconin.[4] Therefore, the type of acetogenin skeleton exhibited by jimenezin (**1**) is new. Accordingly, three annonaceous acetogenins bearing a THP ring have now been isolated from *R. mucosa*.[3,4]

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Table 3. Brine Shrimp Lethality and Cytotoxicity Data for Compound **1**

compd	BST[a] (μg/mL)]	tumor cell line [ED_{50} (μg/mL)] A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	PACA-2[g]
1	5.7×10^{-3}	1.64×10^{-2}	$>10^{-1}$	4.25×10^{-3}	4.94×10^{-2}	2.77×10^{-4}	1.69×10^{-4}
adriamycin		4.47×10^{-3}	8.97×10^{-2}	1.62×10^{-2}	1.10×10^{-3}	2.13×10^{-2}	2.88×10^{-3}

[a] Brine shrimp lethality test. [b] Human lung carcinoma. [c] Human breast carcinoma. [d] Human colon adenocarcinoma. [e] Human kidney carcinoma. [f] Human prostate adenocarcinoma. [g] Human pancreatic carcinoma.

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(5) The seeds of *R. mucosa* (Annonaceae) were collected in September 1994 in Catemaco, Veracruz, México. A voucher specimen of the plant (no. CA94-3) is preserved in the Herbarium of the Instituto de Ecología (XAL), Xalapa, Veracruz, México.

(6) The air-dried seeds of *R. mucosa* (663 g) were pulverized in a Wiley 4 mill. The pulverized seeds were extracted with $CHCl_3$−MeOH (1:1). The extract (241 g, brine shrimp lethality test LC_{50} = 0.41 μg/mL) was partitioned between hexane−MeOH (10% water). The methanolic fraction (26 g, brine shrimp lethality test $LC_{50} = 6 \times 10^{-2}$ μg/mL) was subjected to open column chromatography (531 g of Si gel Kieselgel 60 Merck, 0.063 mm, 230 mesh ASTM) and eluted with a gradient of increasing polarity with hexane/$CHCl_3$/MeOH. Altogether, 230 fractions were collected and combined according to their similar TLC patterns to yield 14 primary fractions. Purification of the active pool F_6 (500 mg, brine shrimp lethality test $LC_{50} = 7 \times 10^{-3}$ ppm) on a normal-phase silica gel column [7.5 mL/min, hexane−*i*-PrOH−MeOH (90:5:5)] yielded **1** (16 mg; retention time 45.0 min).

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(8) Jimenezin (1): yellow oil; $[\alpha]^{20}_D$ +8.3° (*c* 1.2 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 210.5 (3.97) nm; CD (MeOH) $\Delta\epsilon$ (nm) -1.7×10^3 (238); IR ν max (film) 3100−3650, 3023, 2928, 1750, 1641, 1423, 1215, 1028, 930 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS (glycerol) *m*/*z* $\{MH\}^+$ 623; HRFABMS (NBA) *m*/*z* 623.4877 $[M + H]^+$, calcd for $C_{37}H_{66}O_7$ + H 623.4887; EIMS of the TMSi derivative **1a**, see Figure 1.

(9) The optical rotation was taken on a JASCO DIP-360 polarimeter. The UV spectrum was obtained on a Shimadzu 160 UV spectrometer in MeOH solutions, and the CD spectrum was performed on a JASCO 720 spectropolarimeter at 25 °C in MeOH solution. The IR spectrum (film) was measured on a Perkin-Elmer 599 spectrometer. The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra (all in $CDCl_3$) were obtained either on a Bruker DMX500 or a Varian UNITY PLUS 500 spectrometer. The FABMS data were recorded using a glycerol matrix on a JEOL DX300 mass spectrometer, with the HRFABMS obtained in a JEOL JX102A mass spectrometer, and the EIMS of the TMSi derivative performed on a JEOL JMS-AX505HA mass spectrometer. HPLC was carried out with a Waters HPLC instrument equipped with Waters UV photodiode array detector (900) set at 209−220 nm, using a silica gel column (19 i.d. × 300 mm). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the millennium 2000 software program (Waters).

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(11) A small amount (0.5 mg) of compound **1** was treated with 100 μL of Sigma-Sil-A (trimethylchlorosilane−hexadimethylsilane−pyridine 1:3:9) and heated at 60 °C for 10 min to yield the TMSi derivative **1a**.

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(14) To a solution of **1** (1.5 mg in 0.5 mL of $CDCl_3$ in a NMR tube) was sequentially added pyridine-d_5 (100 μL), 4-(dimethylamino)-pyridine (0.5 mg), and (*R*)-(−)α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (25 mg). The mixture was heated at 50 °C for 4 h under a N_2 atmosphere to give the *S*-Mosher ester (**1s**). Treatment of **1** (1.5 mg) with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as described above yielded the *R*-Mosher ester **1r**.

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(17) Cytotoxicity against human solid tumors cells was measured in a 7-day MTT assay at the Purdue Cell Culture Laboratory using adriamycin as a positive control.

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