Mucocin: A New Annonaceous Acetogenin Bearing a Tetrahydropyran Ring

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The acetogenins are a class of potent bioactive compounds found in various plant species in the Annonaceae.[1] Motivated by their promising potential as new antitumor drugs,[1] we have now investigated the bioactive leaves of *Rollinia mucosa* (Jacq.) Baill. (Annonaceae). Some initial results have been reported elsewhere.[2] Herein, we report the isolation, absolute structure determination, and bioactivity of mucocin (**1**, Figure 1). The previously known acetogenins are usually characterized (among other features) by their bearing one to three tetrahydrofuran (THF) rings.[1] **1** is the first annonaceous acetogenin to be reported that bears a hydroxylated tetrahydropyran (THP) ring along with a THF ring. This finding adds a new skeletal type to the family of annonaceous acetogenins.

The isolation of **1** was guided by the brine shrimp lethality test (BST)[3] using repetitive open column and HPLC chromatography to separate the partitioned ethanol extract.[2,4] The molecular formula of **1** was determined as $C_{37}H_{66}O_8$ by HRFABMS (MH^+, *m/z* found 639.4828, calcd 639.4836). A DEPT experiment revealed the presence of two methyl, 10 methine, and two quaternary carbons (some methylene carbon peaks overlapped at δ 29.5). The planar structure was established by means of COSY, EIMS, and EIMS of its TMS derivative (**1a**, Figure 1). The cross peak corresponding to H-20/23 was successfully observed in the double-relayed COSY spectrum of **1**. The existence of the methylated α,β-unsaturated γ-lactone with a 4-OH was confirmed by comparing the ^1H and ^{13}C NMR data (Table 1) with those of known annonaceous acetogenins.[1]

The relative stereochemistry of **1** was established as follows. Applying Born's rule,[5] the δ value of H-16 indicated a *threo* relationship at C-15/16. The configurational assignment of the THF ring as *trans* was suggested by the close match of the ^{13}C NMR data (from C-12 to C-16) with those of synthetic model

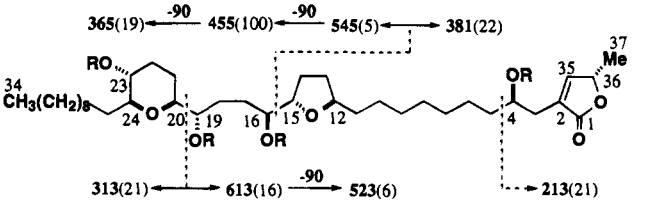

Figure 1. Chemical structures of **1** (R = H), **1a** (R = TMS), **1r** (R = (*R*)-MTPA), and **1s** (R = (*S*)-MTPA). The EIMS *m/z* data of **1a** are shown (numbers in parentheses are percent intensities).

Table 1. NMR Data of Mucocin (**1**) (^1H 500 MHz, ^{13}C 75 MHz, in $CDCl_3$)

position	δ_H	δ_C (mult)[a]	position	δ_H	δ_C (mult)[a]
1		174.6 (s)	18	1.62	28.8 (t)
2		131.2 (s)	19	3.48	73.5 (d)
3	2.40, 2.53	33.3 (t)	20	3.15	80.1 (d)
4	3.84	69.9 (d)	21	1.45, 1.70	26.9 (t)
5	1.47	37.3 (t)	22	1.43, 2.10	31.9 (t)
6−10	1.10−1.35	26−33 (t)	23	3.28	70.5 (d)
11	1.46	35.6 (t)	24	3.05	82.0 (d)
12	3.88	79.3 (d)	25	1.41	25.5 (t)
13	1.49, 2.02	32.4 (t)	26−33	1.10−1.35	26−33 (t)
14	1.62, 1.98	28.3 (t)	34	0.88	14.1 (q)
15	3.80	81.9 (d)	35	7.19	151.8 (d)
16	3.43	73.8 (d)	36	5.06	78.0 (d)
17	1.52	28.7 (t)	37	1.43	19.1 (q)

[a] Assigned by HMQC and HMBC (multiplicity determined by DEPT).

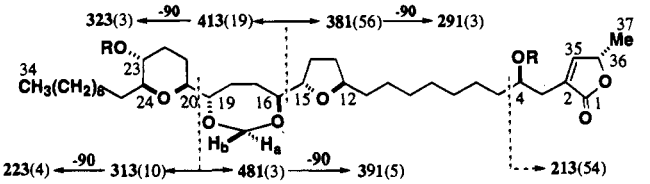

Figure 2. Chemical structures of **2** (R = H), **2a** (R = TMS), **2r** (R = (*R*)-MTPA), and **2s** (R = (*S*)-MTPA). The EIMS *m/z* data of **2a** are shown (numbers in parentheses are percent intensities).

compounds,[6] the characteristic δ value of H-15,[7] and the relatively large δ differences between the two geminal protons at the C-13 and C-15 positions.[1c,2] The relative stereochemistry between the 16-OH and 19-OH was solved by preparing the formaldehyde acetal derivative (**2**, Figure 2).[8] The NOESY spectrum of **2** clearly showed two cross peaks at H-16/H_a and H-19/H_b, which was evidence for a *trans*-1,4-diol (i.e., the configurations at C-16/19 must be either *S/S* or *R/R*).[2] If Born's rule[5] should hold true for a hydroxyl-flanked THP ring system, we may predict a *threo* relationship at C-19/20. The *cis* stereochemistry (referring to the side chains at C-20 and C-24) was assigned to the THP ring because the cross peak at H-20/24 was observed in the NOSEY spectra of both **1** and **2**; the two side chains at C-20 and C-24 should both then assume the equatorial positions which are energetically favorable. Homo decoupling performed at δ 1.4 (H-25) simplified the H-24 signal to a doublet with J = 9 Hz (a typical a−a coupling constant), and this constant was repeated by H-23; thus, the 23-OH was assumed to be equatorial.

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(4) **1**: 25 mg was isolated; mp 57−58 °C; $[\alpha]^{23}_D = -10.8°$, IR (film on KBr plate) 3419, 2925, 2853, 1748, 1716, 1456, and 1066 cm^{-1}; UV (in MeOH) λ_{max} = 207 nm (log ϵ' = 3.84 cm^{-1} M^{-1}).

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Table 2. Partial[a] ^{1}H NMR Data of **2**, **2s**, and **2r** (500 MHz in $CDCl_3$)

	δ_H					δ_H			
H no.	**2**	**2s**	**2r**	$\Delta\delta(S-R)$	H no.	**2**	**2s**	**2r**	$\Delta\delta(S-R)$
25	1.45	1.28	1.44	−0.16	37	1.43	1.28	1.31	−0.03
24	2.99	3.21	3.26	−0.05	36	5.06	4.86	4.90	−0.04
23	3.30	4.71	4.69	*R*[b]	35	7.19	6.72	6.98	−0.26
22a	2.10	2.31	2.26	+0.05	3a	2.53	2.60	2.68	−0.08
22b	1.44	1.61	1.46	+0.15	3b	2.40	2.57	2.60	−0.03
21a	1.72	1.79	1.78	+0.01	4	3.84	5.31	5.38	*R*[b]
21b	1.58	1.59	1.59	0	5	1.45	1.66	1.62	+0.04
20	3.27	3.30	3.30	0					

[a] From H-12 to H-19, $\Delta\delta = 0$. [b] The absolute configuration.

Table 3. ED_{50} (μg/mL) Values of **1** and **2** against Six Human Tumor Cell Lines[a]

	A-549	MCF-7	HT-29	A-498	PC-3	PACA-2
1	1.0×10^{-6}	1.8	9.4×10^{-1}	2.6	1.6×10^{-1}	4.7×10^{-7}
2	4.8×10^{-8}	3.3	1.3	>10	8.9×10^{-1}	3.3×10^{-4}
Adr[b]	4.0×10^{-3}	4.7×10^{-1}	2.8×10^{-2}	4.7×10^{-3}	4.1×10^{-2}	2.1×10^{-3}

[a] All samples were tested in the same run for accurate comparisons. [b] Adriamycin was used for the standard positive control.

The absolute stereochemistry of **1** was determined by advanced Mosher ester methodology.[9] The (*R*)- and (*S*)-di-MTPA esters of **2** (**2r** and **2s**, Figure 2) were prepared, their ^{1}H NMR signals were assigned by the COSY spectra, and the corresponding $\Delta\delta(S-R)$ values were calculated (Table 2). The data indicated that the respective absolute configurations at C-23, C-4, and C-36 were *R*, *R*, and *S*.[10] When this information was combined with the relative stereochemistry, as previously established, the absolute configurations of **1** at the remaining stereocenters were assigned as 12(*R*), 15(*S*), 16(*S*), 19(*S*), 20-(*S*), and 24(*S*).

Concerned with the legitimacy of extrapolating Born's rule[5] from THF to THP systems, we have taken special caution to secure the *threo* assignment at C-19/20. If this assignment were to be wrong, we would have seen reversed absolute configurations from C-12 to C-19. We, therefore, made the (*R*)- and (*S*)-tetra-MTPA esters of **1** (**1r** and **1s**, Figure 1) to check independently the configuration at C-16. Despite the expected severe interference between the MTPA groups at C-16 and C-19, a $\Delta\delta(S-R)$ value of +0.06 at H-12 was observed and confirmed the configuration at C-16 as *S* as previously predicted.[2] Also, the $\Delta\delta(S-R)$ values of +0.05, +0.14, −0.02, and −0.09 (corresponding to H-22a, H-22b, H-24, and H-25, respectively) were in good accordance with the already established *R* assignment at C-23.

Both **1** and **2** were quite active in the BST assay[3] (LC_{50} 1.3 and 1.5 μg/mL, respectively) and showed selective inhibitory effects against A-549 (lung cancer) and PACA-2 (pancreatic cancer) in a panel of six human solid tumor cell lines (Table 3),[11] The selective potencies were up to 10 000 times that of adriamycin. The annonaceous acetogenins inhibit cancerous cells by the blockage of mitochondrial complex I (NADH-ubiquinone oxidoreductase)[12] and also through the inhibition of the plasma membrane NADH oxidase.[13] These mechanisms deplete ATP and likely induce apoptosis (programmed cell death),[14] **1** inhibited oxygen uptake by rat liver mitochondria (IC_{50} 18 nM/mg protein;bullatacin was used in the same run as a positive standard, IC_{50} 9 nM/mg protein);[15] this experiment demonstrated that the introduction of the THP ring had not induced a new mode of action to these compounds. The biogenetic pathway of **1**, however, seems to be dissimilar to those proposed for known nonadjacent bis-THF annonaceous acetogenins.[2,7]

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Supporting Information Available: Copies of ^{1}H, ^{13}C, and 2D NMR spectra of **1** and **2** (18 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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