Rollinecins A and B: Two New Bioactive Annonaceous Acetogenins from *Rollinia mucosa*

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Two new mono-THF ring acetogenins, rollinecins A (**1**) and B (**2**), were isolated from the partitioned ethanolic extracts of the leaves of *Rollinia mucosa* (Annonaceae) by activity-directed fractionation. **1** and **2** are epimeric at the C-14 carbinol stereocenter. Their absolute stereochemical structures were solved by preparing their respective per-Mosher ester derivatives. **1** and **2** showed equivalent and selective *in vitro* activities against several human solid tumor cell lines.

In our continued efforts to find new bioactive leads from the leaves of *Rollinia mucosa* (Jacq.) Baill. (Annonaceae), two new and closely related bioactive acetogenins, rollinecins A (**1**) and B (**2**), were isolated. **1** and **2** are epimers which differ from each other only at the C-14 carbinol center (Figure 1). The structural similarities between **1** and **2** made their separation quite difficult, but satisfactory results were obtained by using reversed-phase chromatography with a suitable eluant system (see Experimental Section). In addition to **1** and **2**, three known acetogenins, 12,15-*cis* bullatalicin[1] and (2,4)-*cis*- and *trans*-bullatalicinones,[2] were also isolated for the first time from this species. Our earlier activity-directed work on this plant yielded other bioactive acetogenins which have been reported elsewhere.[3−5]

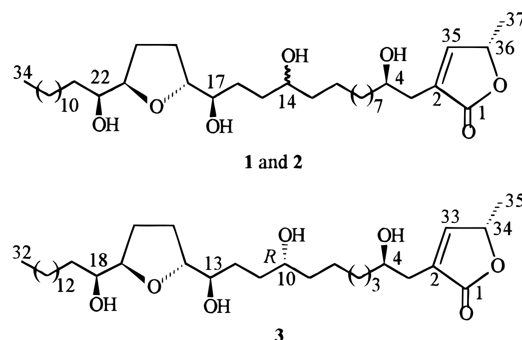

Figure 1. Chemical structures of rollinecin A (**1**, 14*R*), rollinecin B (**2**, 14*S*), and longicin (**3**).

The molecular formulas for **1** and **2** were both established as $C_{37}H_{68}O_7$ by HRCIMS (see Experimental Section). It was obvious from the ^{1}H and ^{13}C NMR data that in both **1** and **2** the α,β-unsaturated γ-lactone unit was present along with a hydroxyl at the C-4 position.[6] The ^{1}H NMR spectra of these two compounds were so similar (Table 1) that we wondered why one compound would have yielded two distinctive peaks in the same HPLC run. More careful examinations, however, revealed that the ^{1}H NMR signal for H-14 (Table 1, the rationale for chemical shift assignments will be explained later) in **1** was downshifted by 0.1 ppm compared with the same proton in **2**, suggesting that the two molecules might be epimeric at the C-14 carbinol center. This notion was supported by their ^{13}C NMR data (Table 1) in which **1** and **2** appeared to be very much alike except that, for **1**, the respective δ values of C-14 and C-17 were 71.72 and 74.59 and, for **2**, the corresponding δ values were 71.90 and 74.67. The slight difference in the C-17 δ values could be satisfactorily explained as the perturbation at the OH-17 caused by the configurational change of OH-14 via hydrogen bonding. It was expected, then, that **1** and **2** should afford the same EIMS pattern. Indeed, exactly the same EIMS peaks were obtained from **1** and **2**, as well as from their respective TMSi derivatives, **1a** and **2a** (Figure 2). At this point, a common planar structure for **1** and **2** was unambiguously established.

In Annonaceous acetogenins, isolated carbinol methine protons, which may be easily distinguished by the appropriate ^{1}H−^{1}H COSY spectra, usually resonate at around δ 3.6; corresponding carbinol carbons should resonate at *ca.* δ 71−72,[6] and this empirical rule was applied to the NMR assignments of C-14 and H-14 of **1** and **2**. The bis-hydroxyl-flanked mono-THF ring subunit, located from C-17 to C-22, was proposed to have *threo-trans-erythro* relative stereochemistry by applying Born's rule[7] and by comparing the ^{13}C NMR data of **1** and **2** with those of Fujimoto's model compounds.[8] The location of the *threo* assignment should be at the C-17/18 position rather than at the C-21/22 junction because the δ value of the *threo* carbinol methine was 3.44; if, on the contrary, the reverse were to be true, this value would have been 3.40.[6]

The structures of **1** and **2** are closely related to longicin (**3**, Figure 1), a C_{35} acetogenin that we have recently isolated from *Asimina longifolia*.[9] It was noticed that, except for the increase of two methylene units and an oxygenation placement frame shift along the aliphatic chain, **1** and **2** resembled **3** in other structural aspects. Particularly, the stereochemistry around the THF ring (from C-17 to C-22) in **1** and **2**

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Table 1. The NMR Peaks for **1** and **2** (500 MHz for ^{1}H and 75 MHz for ^{13}C, in $CDCl_3$)

pos.	rollinecin A (**1**) ^{1}H	rollinecin A (**1**) ^{13}C	rollinecin B (**2**) ^{1}H	rollinecin B (**2**) ^{13}C
1		174.6		174.6
2		131.2		131.2
3	2.40 m, 2.53 m	22.7−37.7	2.40 m, 2.53 m	22.7−37.7
4	3.84 m	69.9	3.84 m	69.9
5	1.40−1.60 m	37.4	1.40−1.60 m	37.4
6−13	1.20−1.40 m	22.7−37.7	1.20−1.40 m	22.7−37.7
14	3.63 m	71.72	3.62 m	71.90
15−16	1.40−1.72 m	22.7−37.7	1.40−1.70 m	22.7−37.7
17	3.44 m	74.59	3.44 m	74.67
18	3.85 m	83.10	3.85 m	83.11
19−20	1.45−2.10 m	22.7−37.7	1.45−2.10 m	22.7−37.7
21	3.86−3.90 m	82.18	3.86−3.90 m	82.19
22	3.86−3.90 m	71.55	3.86−3.90 m	71.54
23	1.70−1.90 m	22.7−37.7	1.70−1.90 m	22.7−37.7
24−33	1.20−1.40 m	22.7−37.7	1.20−1.40 m	22.7−37.7
34	0.88 t (J = 7.0 Hz)	14.1	0.88 t (J = 7.0 Hz)	14.1
35	7.19 q (J = 1.4 Hz)	151.8	7.19 q (J = 1.4 Hz)	151.8
36	5.06 qq (J = 6.9, 1.4 Hz)	78.0	5.06 qq (J = 6.9, 1.4 Hz)	78.0
37	1.43 d (J = 6.9 Hz)	19.1	1.43 d (J = 6.9 Hz)	19.1

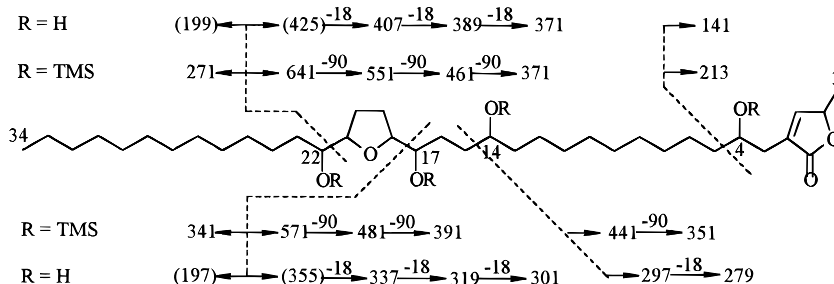

Figure 2. Diagnostic EIMS peaks for **1** and **2** (R = H) and their respective tetra-TMSi derivatives (R = TMSi). Peaks in parentheses were not observed.

Table 2. Some Selected ^{1}H δ Values of Tetra-MTPA Derivatives of Compounds **1**−**3**

	H-23	H-22	H-21	H-18	H-17	H-14	H-5	H-4	H-3a	H-3b	H-35	H-36	H-37
1s	1.46	5.19	3.91	3.82	4.92	5.02	1.66	5.31	2.60	2.57	6.72	4.86	1.28
1r	1.55	5.26	3.96	3.69	4.92	4.91	1.62	5.38	2.68	2.60	6.98	4.90	1.31
2s	1.46	5.20	3.93	3.89	5.04	5.11	1.66	5.31	2.60	2.57	6.72	4.86	1.28
2r	1.55	5.26	3.95	3.64	4.89	5.00	1.62	5.38	2.68	2.60	6.98	4.90	1.31
3s	1.46	5.19	3.91	3.82	4.92	5.02	1.66	5.31	2.60	2.57	6.72	4.86	1.28
3r	1.55	5.26	3.96	3.69	4.92	4.91	1.62	5.38	2.68	2.60	6.98	4.90	1.31

was most likely to be the same as, or, very unlikely from the biogenetic point of view, the mirror image of that in the corresponding segment (from C-13 to C-18) of **3**. As anticipated, the ^{1}H NMR spectra of **1**−**3** were all very similar. A more careful inspection found that **1** was slightly closer to **3** in terms of the H-14 (vs H-10) δ values. Unfortunately, a comparison among their ^{13}C NMR spectra did not offer much help for this purpose, presumably because the frame shift had slightly influenced the ^{13}C NMR δ values.

It would be reasonable to suggest that the configurational difference between two diastereomeric acetogenins, as reflected in their ^{1}H NMR spectra, will be much more manifested in their respective *R*- or *S*-per-Mosher ester derivatives. In other words, the odds that the same ^{1}H NMR spectra might be observed for the per-Mosher esters derived from a pair of diastereomeric acetogenins, each of which possesses four stereogenic carbinols, is slim enough to be ruled out for practical purposes. Although, strictly speaking, **3** was not diastereomeric to either **1** or **2** because of the difference in aliphatic chain length, the above argument, nonetheless, was valid in light of the large degree of separation between the terminal unit (including the 4-OH) and the rest of the functionalities in these molecules.

Hence, the *R*- and *S*-tetra-Mosher ester derivatives of **1**−**3** (**1r**−**3r** and **1s**−**3s**, respectively) were prepared and their ^{1}H NMR spectra were compared (Table 2). To our satisfaction, **1r** and **3r** could not be distinguished by their ^{1}H NMR spectra, which, on the other hand, were clearly dissimilar with that of **2r**. Likewise, **1s** and **3s** afforded the same ^{1}H NMR spectra which were unlike that of **2s**. This piece of evidence was decisive enough to prove that **1** had the same absolute stereochemical structure from C-14 through C-22 as that of the corresponding section from C-10 through C-18 in **3** (*i.e.*, 14*R*,17*R*,18*R*,21*R*,22*S*). It followed by the same argument that the configurations of the C-4 and C-36 stereocenters in **1** and **2** should be the same as that of C-4 and C-34 in **3**, which are *R* and *S*, respectively. The notion that the only difference between **1** and **2** was the absolute configuration at the C-14 stereocenter was further substantiated because the differences of ^{1}H δ values between **1s** and **2s**, as well as those between **1r** and **2r**, were the greatest at H-14 and gradually tapered off toward the left of the molecules (essentially no

Table 3. Bioactivities[a] of **1** and **2**

	BST[b] LC_{50}	A-549[c] ED_{50}	MCF-7[d] ED_{50}	HT-29[e] ED_{50}	A-498[f] ED_{50}	PC-3[g] ED_{50}	PACA-2[h] ED_{50}
1	3.1×10^{-1}	1.14×10^{-4}	1.44	1.60	7.25×10^{-4}	2.62×10^{-4}	3.47×10^{-5}
2	1.3×10^{-1}	4.23×10^{-4}	2.72	1.44	2.29×10^{-4}	3.62×10^{-4}	2.53×10^{-4}
Adr[i]		1.57×10^{-3}	9.68×10^{-2}	1.93×10^{-2}	1.90×10^{-3}	2.81×10^{-2}	2.24×10^{-2}

[a] Results are reported in μg/mL, and all samples were tested in the same run in each cytotoxicity bioassay (*c*–*h*). [b] Brine shrimp lethality test. [c] Human lung carcinoma. [d] Human breast carcinoma. [e] Human colon adenocarcinoma. [f] Human renal carcinoma. [g] Human prostatic adenocarcinoma. [h] Human pancreatic carcinoma. [i] Adriamycin was used as the standard positive control in the same run.

difference was noticed at H-22 and H-23 or around the terminal lactone unit). Thus, the absolute configuration of C-14 in **2** was assigned as *S*.

The brine shrimp lethality (BST)[10] and antitumor activity assay results of **1** and **2** (Table 3) showed that the tumor inhibition potencies of **1** and **2** were more selective and generally more potent, in the most susceptible cell lines, than that of adriamycin by 1–3 orders of magnitude. No significant differences in activity between **1** and **2**, themselves, were observed. The SAR's of Annonaceous acetogenins, at the levels of relative and absolute stereochemistries, appear to be very complicated, and no systematic study of these factors has been reported so far.[11] We have noticed, however, that in some cases, two acetogenins, which differ in just one or a few stereocenters, can have striking differences in their antitumor activity profiles. For example, bullatanocin and bullatalicin differ from each other only by their absolute configuration at C-24 (and there are nine stereocenters in each of these molecules); nonetheless, bullatanocin is about 10^6 times as potent as bullatalicin against the human colon adenocarcinoma (HT-29) cell line, while bullatalicin is about 10^5 times as potent as bullatanocin against the human prostatic adenocarcinoma (PC-3) cell line.[1] This suggests that the C-24 hydroxyls in bullatalicin and bullatanocin may play a key role in the binding with their receptors[12] in various cancerous cell lines. It might be implied, then, that the 14-OHs in **1** and **2** are not as likely to be involved in the drug-receptor binding in a critical way.

The Annonaceous acetogenins act, at least in part, to limit ATP production via inhibition of NADH:ubiquinone oxidoreductase in mitochondria complex I of the electron transport system[12a] and by inhibition of the ubiquinone-linked NADH oxidase peculiar to the plasma membrane of cancerous cells.[12b] Rapid progress in the discovery of new Annonaceous acetogenins, which have excellent potential for development as mechanistically novel anti-neoplastic drugs, calls for more intensive research to achieve a better understanding of their drug-receptor interaction mechanisms. It is our prediction that future investigations in this field will be very fruitful resulting in the identification of specific acetogenins which most favorably attack specific tumor cell types.

Experimental Section

Instruments. The instruments used were as described previously.[3,5]

Plant Material. The plant material used was as described previously.[3,5]

Extraction and Purification Procedures. The general procedures have been described previously.[3,5] **1** (3 mg) and **2** (3 mg) were isolated from the fractions F-(13,16)-30 to F-(13,16)-69 using repetitive reversed-phase HPLC chromatography on a C18 column with the CH_3CN:H_2O (65%:35%, isocratic) eluant system. The typical elution times for **1** and **2** in the above experimental setup were around 2 h. 12,15-*cis* Bullatalicin[1] and (2,4)-*cis*- and *trans*-bullatalicinones[2] were concurrently isolated in the above experimental procedure and were identified by comparing their NMR spectra with those reported.

Procedures of Chemical Derivatizations and Bioassays. The procedures were as described previously.[3,5]

Rollinecin A (1): whitish waxy solid; mp 61–62 °C; $[\alpha]^{25}_D$ +10.0° (CH_2Cl_2); UV (MeOH) λ_{max} = 227 nm (log ϵ = 3.80); IR (dry film) 3400, 2910, 2800, 1745, 1665, 1075 cm^{-1}; HRCIMS $(M + H)^+$ found *m*/*z* 625.5061, calcd 625.5043 for $C_{37}H_{69}O_7$; EIMS (Figure 1); EIMS of its tetra-TMSi derivatives (Figure 1, intensities are indicated in parentheses) 641 (3.0), 571 (10.2), 551 (22.4), 481 (100), 461 (16.7), 441 (28.4), 391 (3.2), 371 (6.0), 351 (2.8), 341 (21.5), 271 (20.0), 213 (10.2); ^1H and ^{13}C NMR (Table 1).

Rollinecin B (2): whitish waxy solid; mp 61–62 °C; $[\alpha]^{25}_D$ +12.7° (CH_2Cl_2); UV (MeOH) λ_{max} = 227 nm (log ϵ = 3.80); IR (dry film) 3400, 2910, 2800, 1745, 1665, 1075 cm^{-1}; HRCIMS $(M + H)^+$ found *m*/*z* 625.5062, calcd 625.5043 for $C_{37}H_{69}O_7$; EIMS (Figure 1); EIMS of its tetra-TMSi derivatives (Figure 1, intensities are indicated in parentheses) 641 (4.4), 571 (11.3), 551 (19.4), 481 (100), 461 (4.0), 441 (21.6), 391 (7.4), 371 (2.9), 351 (5.0), 341 (10.7), 271 (25.8), 213 (13.5); ^1H and ^{13}C NMR (Table 1).

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