A Novel Constituent from *Rollinia mucosa*, Rollicosin, and a New Approach to Develop Annonaceous Acetogenins as Potential Antitumor Agents

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Rollicosin (**1**), a new Annonaceous acetogenin, was isolated from the unripe fruits of *Rollinia mucosa*. Rollicosin (**1**) is the first compound of this type to contain lactone moieties on both sides of the aliphatic chain and to lack either tetrahydrofuran or tetrahydropyran rings. Its structure was determined on the basis of spectroscopic analyses. This compound may serve as a new prototype molecule to develop Annonaceous acetogenins as potential antitumor agents.

Since the first bioactive Annonaceous acetogenin, uvaricin, was found from the roots of *Uvaria accuminata* (Annonaceae) in 1982,[1] more than 350 Annonaceous acetogenins have been isolated from various parts of this family of plants in the past two decades.[2−7] The common structural features of Annonaceous acetogenins are a terminal γ-lactone ring and a terminal aliphatic side chain connected with some oxygen-bearing moieties, such as zero to three tetrahydrofuran (THF) and/or tetrahydropyran (THP) rings and several hydroxyl groups.[2−7] Depending on these structural features, Annonaceous acetogenins are classified into several subtypes, i.e., acetogenins with a mono-THF ring, adjacent bis-THF rings or nonadjacent bis-THF rings, nonadjacent THF rings or THP rings, adjacent tris-THF rings, and without a THF or THP ring, but including epoxides, hydroxyls, and/or double bonds.

Generally speaking, Annonaceous acetogenins possess a broad spectrum of biological effects, such as anticancer, antiparasitic, insecticide, and immunosuppressive activities. To investigate the mechanism of action within membranes of these compounds, several research groups have proposed various hypotheses.[8−15] Most interestingly, in 1998, McLaughlin et al. proposed a model in which, at the molecular level, the lactone ring alone interacts directly with a binding site in mitochondrial complex I, while the THF moiety with flanking OH groups function simply as a hydrophilic anchor at the membrane surface that allows lateral diffusion (or random distribution) of the lactone ring into the membrane interior.[16] To verify the model, Kuwabara et al. synthesized a series of analogues with two terminal γ-lactone rings.[17] However, the bioassay results did not show that these analogues worked twice as effectively as the natural Annonaceous acetogenins as one might have predicted.

Among these hypotheses, a series of rules about the structure−activity relationships (SAR) of Annonaceous acetogenins have evolved.[18−20] For example, Landolt et al., in 1995, concluded that the bis-adjacent THF or the bis-nonadjacent THF acetogenins were approximately 10 times more active than the mono-THF ones by measuring the inhibition of oxygen uptake by intact rat liver mitochondria.[18] Oberlies et al., in 1997, first proposed that a spacing of 13 carbons between the flanking hydroxyl of the THF ring system and the γ-unsaturated lactone seems to be optimal after measuring the ability of 14 diverse Annonaceous acetogenins to inhibit the growth of Adriamycin-resistant human mammary adenocarcinoma (MCF-7/Adr) cells.[21] Miyoshi et al., in 1998, presented several conclusions on the basis of the purified mitochondrial NADH oxidase activity by these compounds.[22] First, the adjacent bis-THF ring moiety is not an essential structural factor, and the mono-THF ring compounds can also exhibit potent activity. Second, the ring stereochemical factor is not essential for potent activity irrespective of the number (one or two) of THF rings. Third, the THF rings of the acetogenins gave strong interactions with the interface of lipid bilayers irrelevant to the stereochemistry of the THF region. Fourth, the spacer moiety is very important for potent activity. Takada et al., in 2000, further indicated that the γ-lactone ring and the tetrahydrofuran ring should act in a cooperative manner on the enzyme with the support of some conformation of the spacer and that the optimal length of the alkyl spacer is 13 carbon atoms.[23] These results seem to support the conclusions of Oberlies et al.[21] and Miyoshi et al.[22]

After summarizing the SAR data of Annonaceous acetogenins at the molecular level, it is clear that most researchers have focused on three features, the terminal γ-lactone ring, the THF ring, and the space between them. However, the role of the terminal aliphatic side chain of Annonaceous acetogenins against cancer cell lines has been rarely considered except for the observations of McLaughlin et al. in 1998.[16]

Previously, we reported various types of Annonaceous acetogenins isolated from the unripe fruit of *Rollinia mucosa* Baill. (Annonaceae).[24−26] Through our continued investigation of acetogenins from this plant, we have now isolated a new compound, rollicosin (**1**). This compound is the first example of an acetogenin containing two lactone moieties on both sides of an aliphatic chain. Moreover, this compound may help investigate the role that the terminal aliphatic side chain of Annonaceous acetogenins plays in their bioactivity.

Rollicosin (**1**) (2 mg) was obtained as a white amorphous powder with $[\alpha]^{24}_{D}$ −26.0° (*c* 0.05, $CHCl_3$). The MH^+ peak at *m*/*z* 397 in the FABMS and an exact mass at *m*/*z* 396.2524 (calcd 396.2512, Δ +3.0 ppm) in the HREIMS of the molecular ion confirmed the molecular formula to be $C_{22}H_{36}O_6$. The UV absorption at 225 nm and the IR absorption at 1760 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone. The typical form of a lactone tail with a hydroxyl at C-4 was confirmed by the comparison of the ^1H and ^{13}C NMR data with those in the literature[27,28] (see Table 1). The absolute stereochemistry at C-4 and C-21

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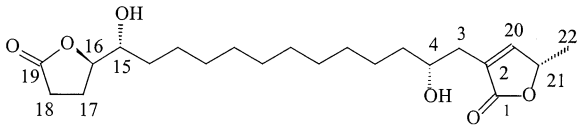
Table 1. NMR Data of Compound **1** in $CDCl_3$

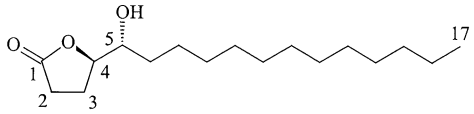
position	δ_C[a]	δ_H[b]	mult., *J* (Hz)
1	174.6		
2	131.2		
3	37.2	2.44	dd (15.2, 3.6, H-3a)
		2.32	dd (15.2, 8.0, H-3b)
4	69.7	3.75	dd (8.0, 3.6)
5	33.1	1.46−1.22	
6−13	25.4−30.8	1.46−1.22	
14	32.8	1.46−1.22	
15	73.3	3.48 m	
16	83.3	4.39	ddd (7.6, 6.8, 4.0)
17	24.0	2.20	m (H-17a)
		2.08	m (H-17b)
18	28.7	2.57	ddd (18.2, 10.4, 5.2)
19	177.1		
20	152.0	7.16	d (1.5)
21	78.1	5.02	qd (6.8, 1.5)
22	18.9	1.38	d (6.8)

[a] Run at 100 MHz. [b] Run at 400 MHz.

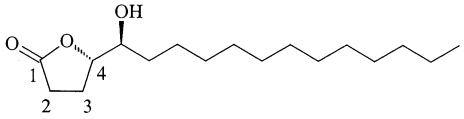
of **1** was assigned in the *R*- and *S*-configurations due to a positive $\pi-\pi^*$ Cotton effect ($\Delta\epsilon > 0$) in the CD spectrum.[29]



Rollicosin (**1**)



Muricatacin (**2**)



4-(1-hydroxy-dodecane)-butyrolactone (**3**)

The signals at δ 4.39 (1H, ddd, $J = 7.6, 6.8, 4.0$ Hz, H-16), 3.48 (1H, m, H-15), 2.57 (2H, ddd, $J = 18.2, 10.4, 5.2$ Hz, H-18), 2.20 (1H, m, H-17a), and 2.08 (1H, m, H-17b) in the ^{1}H NMR spectrum and the signals at δ 177.1 (C-19), 83.3 (C-16), 73.3 (C-15), 28.7 (C-18), and 24.0 (C-17) in the ^{13}C NMR spectrum indicated the presence of a lactone with a flanking hydroxyl group at the other terminal end of the aliphatic chain.[30] For the absolute stereochemistry between C-15 and C-16, comparison was made with muricatacin (**2**), on a (4*R*,5*R*)-configuration ($[\alpha]^{20}_D$ −13.3°, $CHCl_3$),[30] which was isolated from the MeOH extract of the seeds of *Annona montana*,[31] and with 4-(1-hydroxydodecane)butyrolactone (**3**), with a (4*S*,5*S*)-configuration ($[\alpha]^{24}_D$ +21.2°, $CHCl_3$),[32] which was synthesized, suggesting that compound **1** could be determined as 15*R*, 16*R*. This is quite similar to the fragment in which murisolin[33] has lost the terminal aliphatic chain by cleavage between C-19 and C-20 or the one in which bullatacin (rolliniastatin 2) has lost a THF ring, a hydroxyl group, and an 11-membered hydrocarbon chain.[34,35]

In the low-resolution EIMS of **1**, the fragment peaks were present at *m/z* 311 for [$M^+ - \gamma$-lactone], *m/z* 293 for [$M^+ - \gamma$-lactone − H_2O], *m/z* 285 for [$M^+ - CH_2 - \alpha,\beta$-unsaturated-$\gamma$-lactone], and *m/z* 267 for [$M^+ - CH_2 - \alpha,\beta$-unsaturated-$\gamma$-lactone − H_2O] (Figure 1). The fragmentation of **1** suggested two hydroxyl groups located at C-15 and C-4, respectively. From the above data, the structure and absolute stereochemistry of rollicosin (**1**) were determined as 4*R*, 15*R*, 16*R*, 21*S*.

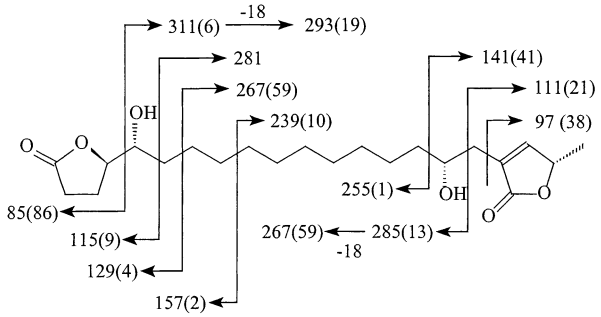


Figure 1. EIMS fragmentation (*m/z* values) of rollicosin (**1**).

In three-day cytotoxicity bioassays, compound **1** exhibited significant inhibitory activity against two cancer cell lines, Hep G_2 (human hepatoma cells) and Hep 2,2,15 (human hepatoma cells transfected with hepatitis B virus) with IC_{50} values of 1.0×10^{-1} and 2.1×10^{-2} μg/mL, respectively.[36,37] The positive control, adriamycin, had an IC_{50} value of 4.5×10^{-1} μg/mL. Because of the resemblance of **1** to the partial Annonaceous acetogenin structure, we have compared the cytotoxicity and obtained the mechanism of action of this isolate and other Annonaceous acetogenins from the same plant.[26] We prefer to re-think the McLaughlin model.[16] Could the THF ring moiety be replaced by another lactone ring moiety so as to act as an anchor to the polar groups of the membrane phospholipids as indicated in McLaughlin's model? Could both of the two lactone ring moieties work as the active species to react with the same binding sites without the adherence of the THF ring in the membrane? On the other hand, this compound perhaps helps to explain the observations of Kuwabara et al.[17] in that the inhibitory potency of those synthesized analogues with two terminal lactone ring moieties was neither reduced nor increased.

Compound **1** is the first example of an Annonaceous acetogenin that contains two lactone moieties on both sides of an aliphatic chain. This unusual structure may promote a new abbreviated approach to develop synthetic analogues of the Annonaceous acetogenins as new bioactive agents.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. CD spectra were measured on a JASCO J-720 spectropolarimeter. The IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra (all in $CDCl_3$) were recorded with Varian NMR spectrometers, using TMS as internal standard. LRFABMS and LREIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer or a Quattro GC/MS spectrometer having a direct inlet system. HRFABMS were measured on a JEOL JMS-HX 110 mass spectrometer. Si gel 60 (Macherey-Nagel, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Macherey-Nagel, SIL G-25 UV_{254}, 0.25 mm) were used for analytical TLC. The spots were detected by spraying with Kedde's reagent, Dragendorff's reagent, or 50% H_2SO_4 and then heating on a hot plate. HPLC was performed on a Shimadzu LC-10AT apparatus equipped with a Shimadzu SPD-10A UV−vis detector. Develosil ODS-5 (250 × 4.6 mm i.d.) and preparative ODS-5 (250 × 20 mm i.d.)

columns were used for analytical and preparative HPLC purposes, respectively.

Plant Material. Fresh, unripe fruits of *Rollinia mucosa* were collected from Chia-Yi City, Taiwan, in June 1994. A voucher specimen (Annona-18) is deposited in the Graduate Institute of Natural Products, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. Fresh fruits (11.0 kg) were extracted repeatedly with EtOAc at room temperature. The combined EtOAc extract was partitioned with chloroform and water to yield chloroform and aqueous layers. The $CHCl_3$ layer was concentrated and then partitioned between *n*-hexane and MeOH. After concentration, the MeOH layer afforded a waxy extract (30.6 g), positive to Kedde's reagent. The MeOH layer was eluted to produce 14 fractions by Si gel column chromatography with a *n*-hexane−$CHCl_3$−MeOH gradient system. Rollicosin (**1**) (2.0 mg, 0.000018%) was separated from the ninth fraction, eluted with $CHCl_3$−MeOH (97:3), and further purified by preparative reversed-phase HPLC (MeOH−H_2O 80:20, detection at 225 nm) .

Rollicosin: white waxy solid (>99% pure by HPLC); $[\alpha]^{24}_D$ −26.0° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (3.93) nm; IR (KBr) ν_{max} 3470 (OH), 2912, 2840, 1760 (OC=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; FABMS *m*/*z* 397; HREIMS *m*/*z* 396.2524 (calcd 396.2512 for $C_{22}H_{36}O_6$).

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