New Annonaceous Acetogenins from *Rollinia mucosa*

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Four new compounds, a mixture of 20,23-*cis*-2,4-*trans*-bullatalicinone (**1**) and 20,23-*cis*-2,4-*cis*-bullatalicinone (**2**), rollimusin (**3**), and rolliacocin (**4**), along with eight known acetogenins, were isolated from an ethyl acetate extract of the unripe fruits of *Rollinia mucosa*. The structures and stereochemistry of **1**−**4** were determined on the basis of spectral data and chemical evidence.

The genus *Rollinia* (Annonaceae) consists of about 65 species altogether, and some of these plants have been used in traditional medicine for the treatment of tumors.[1] Among the constituents of these materials, annonaceous acetogenins, known to have potent anticancer activities, are regarded as the major active principles. Our laboratory has been investigating the acetogenins from the unripe fruits of the Formosan plant, *Rollinia mucosa* Baill. (Annonaceae). In a previous report, five acetogenins[2] had been identified from this species. Among these, a mixture of epomusenin A and epomusenin B[2] contained one epoxy group and one double bond in place of the usual tetrahydrofuran (THF) moieties. In this paper, we report four new annonaceous acetogenins. A mixture of 20,23-*cis*-2,4-*trans*-bullatalicinone (**1**) and 20,23-*cis*-2,4-*cis*-bullatalicinone (**2**), along with five known compounds, rolliniastatin-1,[3,4] bullatalicin,[5] desacetyluvaricin,[6] bullatalicinone,[7] and sylvaticin,[8] were purified by normal-phase column chromatography. Rollimusin (**3**) and rolliacocin (**4**), together with three known acetogenins, rollitacin,[9] annoglaucin,[10] and 10-hydroxyasimicin,[11] were further purified by reversed-phase HPLC.

Results and Discussion

20,23-*cis*-2,4-*trans*-Bullatalicinone (**1**) and 20,23-*cis*-2,4-*cis*-bullatalicinone (**2**) were isolated in a mixture as a white powder (4:1). The chemical shifts of protons in the ^{1}H NMR spectrum, especially at δ 4.54 and 4.39 (4:1), indicated the existence of two steric isomers of acetogenins with the ketolactone terminal.[12,13] Annonaceous acetogenins with the ketolactone terminal almost always exist as (2,4)-*cis*/*trans* mixtures in natural sources.[12,13] Because of their chemical similarities, purification of such compounds is difficult. The HRFABMS gave the ion $[M + H]^+$ at *m*/*z* 639.4852 (calcd 639.4836), corresponding to the molecular formula, $C_{37}H_{67}O_8$. The UV spectral absorption at 202 nm and the IR spectral absorptions at 1700 and 1750 cm^{-1} indicated the presence of a carbonyl group and a γ-lactone group, respectively. The ^{1}H NMR signals at δ 2.19 (3H, H-37), 2.59 (1H, H-35b), 3.01 (1H, H-35a), 3.02 (1H, H-2), and 4.39 (1H, H-4) and the other signals at δ 2.19 (3H, H-37), 2.68 (1H, H-35b), 3.00 (1H, H-2), 3.08 (1H, H-35a), and 4.54 (1H, H-4) indicated the presence of the *cis* and *trans* lactone moieties of the ketolactone terminal, respectively.[14] According to the work of Fujimoto,[15] a series of model mono-THF ring compounds with all the possible relative stereochemistries has been prepared, and the ^{1}H and ^{13}C NMR data were reported. The ^{1}H NMR data of C-12 through C-16 of mixtures **1** and **2** at δ 3.92−3.79 (2H, H-12, 15), 3.43 (1H, H-16), 1.99 (2H, H-13a, -14a), and 1.66 (2H, m, H-13b, -14b) matched well with those of the model single-hydroxyl-flanked THF ring with a *trans*/*threo* relative configuration. The peak at δ 79.2 for C-12 in ^{13}C NMR spectrum also supported the presence of the *trans* type of THF moiety.[15] The signals of C-19 through C-24 at δ 3.92−3.79 (3H, H-20, -23, -24), 3.48 (1H, H-19), 1.99 (2H, H-21a, -22a), 1.75 (2H, H-21b, -22b) agreed well with those of a mono-THF ring having a flanking hydroxyl on both sides with a *threo*/*cis*/*erythro* or an *erythro*/*cis*/*threo* configuration.[15] Moreover, the ^{13}C NMR data of the mixture of **1** and **2** were compared with those of model compounds, and the results are listed in Tables 2 and 3. In the FABMS, three successive losses of water from the molecular ion $[M + H]^+$ suggested that the molecules of **1** and **2** contain three hydroxyls. The locations of these hydroxyls, as well as the planar structures of the molecules, were established by close examination of the EIMS fragmentation (Scheme 1). Compared with all the spectral data and the EIMS fragmentation of bullatalicinone,[7] the mixture of **1** and **2** was concluded to be closely related. Thus, the structures of **1** and **2** were determined as shown and named as 20,23-*cis*-2,4-*trans*-bullatalicinone (**1**) and 20,23-*cis*-2,4-*cis*-bullatalicinone (**2**).

Rollimusin (**3**) was isolated as a white waxy solid, $[\alpha]^{25}_D$ −7.8° (*c* 0.05, $CHCl_3$). The UV spectral absorption at 208 nm and the IR spectral absorption at 1747 cm^{-1} indicated the presence of an α,β-unsaturated γ-lactone group, positive to Kedde's reagent. The prominent molecular adduct peak at *m*/*z* 661 $[M + Na]^+$ in the FABMS indicated the molecular weight as 638. The HREIMS gave the $[M]^+$ ion at *m*/*z* 638.4750 (calcd 638.4758), corresponding to a molecular formula, $C_{37}H_{66}O_8$. The EIMS (Scheme 2) exhibited four successive losses of water from the peak at *m*/*z* 553 (C-28/C-29 cleavage), indicating the presence of four hydroxyl groups. Analysis of the UV, IR, and ^{1}H and ^{13}C NMR spectra of **3** and comparison with literature values[1] suggested that **3** belongs to the group of adjacent bis-THF acetogenins. The ^{1}H NMR peaks (Table 4) at δ 6.98 (1H, H-35), 4.99 (1H, H-36), and 1.40 (3H, H-37), as well as the ^{13}C NMR resonances (Table 4) at δ 173.9 (C-1), 148.9 (C-35), 134.3 (C-2), 77.4 (C-36), and 19.2 (C-37), matched well with the published data of annonaceous acetogenins with an α,β-unsaturated γ-lactone ring.[12,16] The ^{13}C NMR spectrum showed four resonances at δ 83.1, 82.8, 82.5, and 82.2 (C-16, -19, -20, and -23) due to the oxygen-bearing methines of THF rings, as well as δ 74.0 (C-15) and 71.9 (C-24) for the corresponding hydroxylated carbons. These ^{13}C NMR signals were correlated to multiplets at δ 3.86 (5H, H-16, -19, -20, -23, and -24), 3.43 (1H, H-15) in the ^{1}H NMR

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Table 1. ^{1}H and ^{13}C NMR Chemical Shifts of Compounds **1** and **2**

	(2,4)-*trans*		(2,4)-*cis*	
position	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1		178.7		178.2
2	3.00	34.4	3.02 m	36.6
3	1.22−1.63	35−22	1.22−1.63	35−22
4	4.54 m	78.8	4.39 m	79.2
5−11	1.22−1.63	35−22	1.22−1.63	35−22
12	3.92−3.79 m	79.1	3.92−3.79 m	79.2
13, 14	1.99 m H-13a, -14a	32.2−22.5	1.99 m H-13a, -14a	32.2−22.5
	1.60 m H-13b, -14b		1.60 m H-13b, -14b	
15	3.92−3.79 m	81.8	3.92−3.79 m	81.8
16	3.43 m	74.1	3.43 m	74.1
17−18	1.22−1.63	35−22	1.22−1.63	35−22
19	3.48 m	73.9[c]	3.48 m	73.9[c]
20	3.92−3.79 m	82.2[d]	3.92−3.79 m	82.2[d]
21, 22	1.99 m H-21a, -22a	32.2−22.5	1.99 m H-21a, -22a	32.2−22.5
	1.75 m H-21b, -22b		1.75 m H-21b, -22b	
23	3.92−3.79 m	82.8[d]	3.92−3.79 m	82.8[d]
24	3.92−3.79 m	72.1[c]	3.92−3.79 m	72.1[c]
25−33	1.22−1.63	35−22	1.22−1.63	35−22
34	0.88 t (6.8)	13.9	0.88 t (6.8)	13.9
35	3.08 dd H-35a	44.1	3.01 dd H-35a	43.6
	2.68 dd H-35b		2.59 dd H-35b	
36	1.22-1.63	205.4	1.22−1.63	205.5
37	2.19 s	29.8	2.19 s	29.8

[a] Measured at 400 MHz, in $CDCl_3$. Chemical shifts are in δ values. [b] Measured at 100 MHz, in $CDCl_3$. Chemical shifts are in δ values. [c,d] Assignments may be interchangeable.

Table 2. ^{13}C NMR (100 MHz, $CDCl_3$) Data of the C-11/C-16 THF Subunit of Compounds **1** and **2** and Model Compounds[a]

carbon	11	12	13	14	15	16
1, 2	35.4	79.2	32.2	28.4	81.8	74.1
t/t[a]	35.7	79.3	32.4	28.4	81.9	74.2
t/c[a]	36.1	79.9	31.4	27.8	82.2	74.5

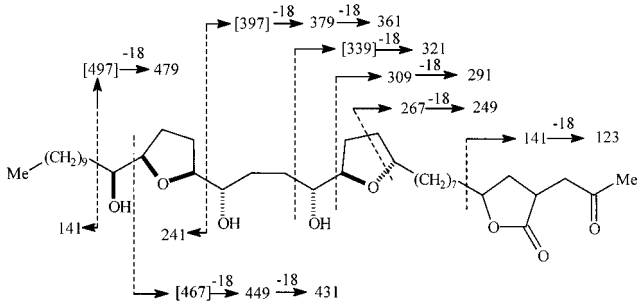
[a] Data of model compounds taken from Fujimoto et al.[15]

Table 3. ^{13}C NMR (100 MHz, $CDCl_3$) Data of the C-19/C-26 THF Subunit of Compounds **1** and **2** and Model Compounds[a]

carbon	19	20	21	22	23	24	25	26
1, 2	73.9	82.2	28.2	23.9	82.8	72.1	33.1	25.9
t/c/e[a]	74.2	82.3	28.4	24.1	82.8	72.1	33.1	25.9
t/t/e[a]	74.3	83.3	28.6	25.1	82.2	71.6	32.5	25.9

[a] Data of model compounds taken from Fujimoto et al.[15]

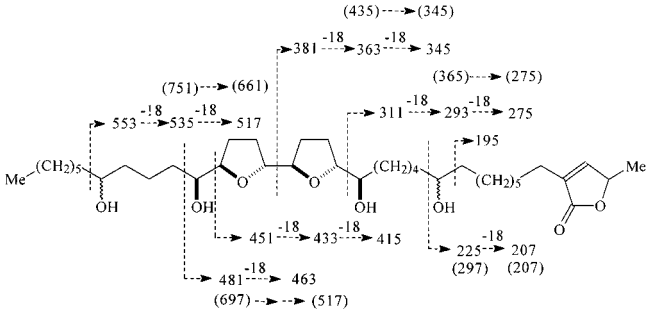
Scheme 1. EIMS Fragmentations (*m*/*z*) of Compounds **1** and **2**[a]



[a] Data in parentheses refer to peaks that were not observed.

spectrum.[13,17] According to the work of McLaughlin[1] and Fujimoto,[16] these data indicated that **3** was an acetogenin of the bullatacin or squamocin-I type. The ^{1}H NMR signals at δ 1.90 (4H, H-17a, -18a, -21a, and -22a) and 1.62 (4H, H-17b, -18b, -21b, and -22b) suggested the relative stereochemistries of the bis-THF rings as *threo*/*trans*/*threo*/*trans*/*erythro*, or *erythro*/*trans*/*threo*/*trans*/*threo*.[1,18] The ^{1}H NMR signal at δ 3.61 (2H) and the ^{13}C NMR signals at δ 71.8 and 71.5 indicated the presence of two free hydroxyls. The structure of the molecule was also established by close

Scheme 2. EIMS Fragmentations (*m*/*z*) of Rollimusin (**3**) and Its TMS Derivative (in parentheses)



examination of the EIMS fragmentation of **3** and its trimethylsilyl derivative (Scheme 2). The significant peaks at *m*/*z* 433 (cleavage between C-23/C-24−H_2O), 363 (C-19/C-20−H_2O), and 293 (C-15/C-16−H_2O) allowed placement of the THF system between C-15 and C-24. The fragment ions at *m*/*z* 225, 207, and 195 suggested that a free hydroxyl group should be located at C-10, and the fragment ions at *m*/*z* 553, 535, and 517 suggested that the last free hydroxyl group should be located at C-28. By HOHAHA NMR, a correlation between δ 3.86 (H-24) and 3.61 (H-28) was observed, but no correlation between δ 3.43 (H-15) and 3.61 (H-10) was seen. Thus, we could confirm the configuration between C-15 and C-24 as a bullatacin-type pattern, *threo*/*trans*/*threo*/*trans*/*erythro*. Therefore, the structure of **3** was determined as shown and named as rollimusin (**3**).

Rolliacocin (**4**) was isolated as a white waxy solid, $[\alpha]^{25}_D$ +11.8° (*c* 0.33, $CHCl_3$). The $[M + Na]^+$ peak in the FABMS at *m*/*z* 619 established the molecular weight as 596. The HREIMS gave the $[M]^+$ ion at *m*/*z* 596.4661 (calcd 596.4652), corresponding to the molecular formula, $C_{35}H_{64}O_7$. The proton signals in the ^{1}H NMR spectrum at δ 7.18 (1H, H-35), 5.07 (1H, H-36), 3.82 (1H, H-4), 2.53 (1H, H-3a), 2.39 (1H, H-3b), and 1.42 (3H, H-37) indicated the presence of an α,β-unsaturated γ-lactone with a hydroxyl group at C-4 position.[12] The normal-form tail[1] of **4** was corroborated by the absorptions in the IR at 1750 cm^{-1}, the UV maximum at 210 nm, and a positive reaction to Kedde's reagent. The

Table 4. ^{1}H and ^{13}C NMR Chemical Shifts of Compound **3**

position	δ_H[a]	δ_C[b]
1		173.9
2		134.3
3	2.26 t (8.1)	25.7−25.1
4−8	1.27−1.61	33−22
9	1.27−1.61	37.5−37.2
10	3.61 br s	74.0
11	1.24−1.61	37.5−37.2
12−14	1.24−1.61	33−22
15	3.43 br s	71.9[c]
16	3.86 m	83.1[d]
17−18	1.62, 1.90	33−22
19	3.86 m	82.5[d]
20	3.86 m	82.2[d]
21−22	1.62, 1.90	33−22
23	3.86 m	82.8[d]
24	3.86 m	71.5[c]
25−26	1.27−1.61	33−22
27	1.27−1.61	37.5−37.2
28	3.61 br s	71.8[c]
29	1.27−1.61	37.5−37.2
30−33	1.27−1.61	33−22
34	0.88 t (7.2)	14.0
35	6.98 d (1.2)	148.9
36	4.99 dq (1.2, 6.4)	77.4
37	1.40 d (6.4)	19.2

[a] Measured at 400 MHz, in $CDCl_3$. Chemical shifts are in δ values. [b] Measured at 100 MHz, in $CDCl_3$. Chemical shifts are in δ values. [c,d] Assignments may be interchangeable.

Table 5. ^{1}H and ^{13}C NMR Chemical Shifts of Compound **4**

position	δ_H[a]	δ_C[b]
1		174.6
2		131.2
3	2.53 dd (1.6, 15.2) 2.39 dd (8, 15.2)	37.3
4	3.82 m	70.0
5	1.22−1.67	35−22
10	1.22−1.67	36.7[c]
11	3.62 br s	71.1
12	1.22−1.67	37.3[c]
13−14	1.22−1.67	35−22
15	3.41 m	74.1[d]
16	3.82 m	82.5
17−18	1.67, 1.97	35−22
19	3.82 m	82.5
20	3.41 m	74.4[d]
21−31	1.22−1.67	35−22
32	0.89 t (6.6)	14.1
33	7.18 d (1.3)	151.8
34	5.07 dq (1.3, 6.8)	78.0
35	1.42 d (6.8)	19.1

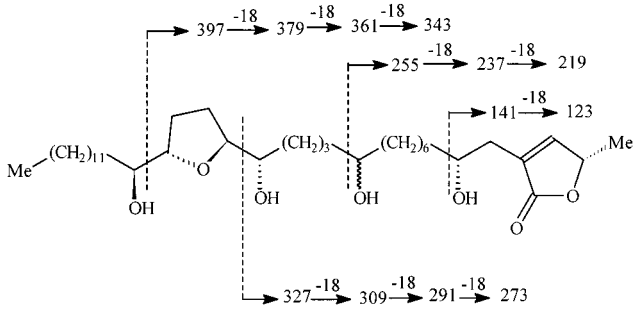
[a] Measured at 400 MHz, in $CDCl_3$. Chemical shifts are in δ values. [b] Measured at 100 MHz, in $CDCl_3$. Chemical shifts are in δ values. [c,d] Assignments may be interchangeable.

signals in the ^{1}H NMR spectrum at δ 3.82 (2H, H-16, -19), 3.41 (2H, H-15, -20), 1.97 (2H, H-17a, -18a), and 1.67 (2H, H-17b, -18b), as well as ^{13}C NMR peaks at δ 82.5 (C-16), 82.5 (C-19), 74.4 (C-20), and 74.1 (C-15), indicated the presence of a single THF ring with two flanking hydroxyls in the relative configuration *threo*/*trans*/*threo*.[16,19] By making the (*R*)- and (*S*)-Mosher ester derivatives and Hoye's methodology,[20,21] the absolute stereochemistries at C-4, -15, -20, and -34 of compound **4** could be confirmed as *R*, *S*, *S*, and *S*. (see Table 6). The ring was placed between C-15 and C-20, based on the EIMS fragments at *m*/*z* 379 and 309 (Scheme 3). The position of the fourth hydroxyl group was established by examination of the EIMS fragmentation pattern. A peak at *m*/*z* 255 (cleavage between C-12/C-11−H_2O) and its daughter peak at *m*/*z* 237 (C-12/C-11−H_2O) suggested that the final hydroxyl group should be assigned

Table 6. ^{1}H NMR Data (400 MHz, $CDCl_3$) of the Diagnostic Protons from the (*S*)- and (*R*)-per-MTPA Mosher Derivatives of Compound **4**

position	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta_{S-R}$	configuration
34	4.86	4.88	−0.02	*S*
33	6.72	6.95	−0.23	
3	2.57	2.58	−0.01	
4	5.33	5.33		*R*
5	1.80−1.10	1.80−1.10		
11	5.04	5.02−4.94		
14	1.80−1.10	1.80−1.10		
15	4.99	5.02−4.94		*S*
16	3.95, 3.91	3.84, 3.81	+0.11, 0.10	
19	3.95, 3.91	3.84, 3.81	+0.11, 0.10	
20	4.99	5.02−4.94		*S*
21	1.80−1.10	1.80−1.10		

Scheme 3. EIMS Fragmentations (*m*/*z*) of Rolliacocin (**4**)



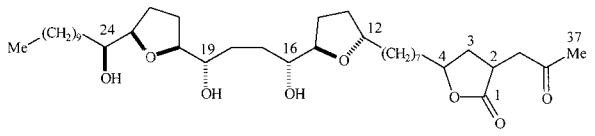
to C-11. Finally, we also determined the absolute configuration at C-34 of **4** by the CD method.[22] According to a positive $\pi-\pi^*$ Cotton effect ($\Delta\epsilon > 0$), it clearly indicated the stereochemistry at the C-34 on the γ-lactone fragment had the (*S*)-configuration. Therefore, the structure of **4** was determined as shown and has been named as rolliacocin (**4**).

The identities of the known acetogenins were verified by comparing UV, IR, ^{1}H NMR, ^{13}C NMR, and MS data with published values of rolliniastatin-1,[3,4] bullatalicin,[5] desacetyluvaricin,[6] bullatalicinone,[7] sylvaticin,[8] rollitacin,[9] annoglaucin,[10] and 10-hydroxyasimicin.[11]

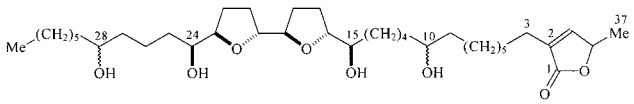
In a 3-day bioassay against the cancer cell line, Hep 2,2,15 (human hepatoma cell transfected HBV), the mixtures of **1** and **2**, **3** and **4**, as well as annoglaucin and 10-hydroxyasimicin, exhibited significant cytotoxicities, with IC_{50} values as low as 10^{-2} μg/mL. The IC_{50} values of the mixtures of **1** and **2**, **3** and **4** were 5.7×10^{-2}, 8.6×10^{-2}, and 7.0×10^{-2} μg/mL, respectively. The positive control drug, Adriamycin, showed an IC_{50} value of 4.5×10^{-1} μg/mL.

Experimental Section

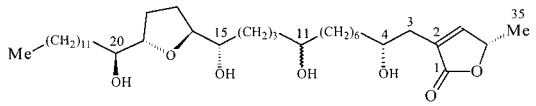
General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Melting points were determined using a Yanagimoto micro-melting point apparatus and were uncorrected. The IR spectra were measured on a Hitachi 260−30 spectrophotometer. ^{1}H NMR (400 MHz) and ^{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. CD data were measured on a JASCO DIP 370 polarimeter. 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, and precoated Si gel plates (Macherey−Nagel,



1 *erythro*/cis/threo** *threo/trans* *trans*
2 *erythro*/cis/threo** *threo/trans* *cis*
* the configurations maybe interchangeable.



3 *erythro/trans/threo/trans/threo*



4 *threo/trans/threo*

SIL G/UV_{254}, 0.25 mm) were used for preparative TLC. The spots were detected by spraying with Dragendorff's reagent or 50% H_2SO_4 and then heating on a hot plate. HPLC was performed on a JASCO PU-980 apparatus equipped with a UV-970 detector. Develosil ODS-5 (250 × 4 mm i.d.) and preparative ODS-5 (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

Plant Material. Fresh unripe fruits of *R. mucosa* were collected from Chia-Yi City, Taiwan, in June 1994. A voucher specimen 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 extracts were evaporated and partitioned to yield $CHCl_3$ and aqueous extracts. 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. It was further separated by column chromatography on Si gel with gradient systems of *n*-hexane−$CHCl_3$ (*n*-hexane−$CHCl_3$ 4:1 to pure $CHCl_3$) and $CHCl_3$−MeOH (pure $CHCl_3$ to $CHCl_3$−MeOH 10:1). Rolliniastatin-1,[3,4] bullatalicin,[5] and desacetyluvaricin[6] were purified from the eighth and ninth fractions, respectively. The mixture of 20,23-*cis*-2,4-*trans*-bullatalicinone (**1**) and 20,23-*cis*-2,4-*cis*-bullatalicinone (**2**), along with bullatalicinone[7] and sylvaticin,[8] were afforded from the twelfth fraction. Then, the remnants of the eighth and ninth fractions purified by column chromatography were collected and further separated with reversed-phase HPLC. From this material, rollimusin (**3**) and rolliacocin (**4**), along with rollitacin,[9] annoglaucin,[10] and 10-hydroxyasimicin,[11] were purified by preparative reversed-phase HPLC (ODS-5 column) with 87:13 MeOH−H_2O (flow rate of 2 mL/min; UV detector set at 225 nm).

Mixture of 20,23-*cis*-2,4-*trans*-Bullatalicinone (1) and 20,23-*cis*-2,4-*cis*-Bullatalicinone (2): obtained as white amorphous powder; mp 95−96 °C; UV (MeOH) λ_{max} (log ϵ) 202 (3.54) nm; IR (KBr) ν_{max} 3450 (OH), 1750 (OC═O), 1700 (C═O), 1210, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; FABMS *m/z* 639 $[M + H]^+$; EIMS (70 eV) 479 (1), 449 (8), 431 (5), 379 (25), 361 (24), 309 (24), 291(7), 267 (26), 249 (7), 241 (7), 141 (12) and 123 (15), see Scheme 1; HRFABMS *m/z* 639.4852 (calcd for $C_{37}H_{67}O_8$, 639.4836).

Rolliniastatin-1: obtained as colorless oil; $[\alpha]^{25}_D$ +28.7° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (3.91) nm; MS and ^1H and ^{13}C NMR data were identical with published values.[3,4]

Bullatalicin: obtained as colorless oil; $[\alpha]^{25}_D$ +13.5° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 212 (3.85) nm; MS and ^1H and ^{13}C NMR data were identical with published values.[5]

Desacetyluvaricin: obtained as colorless oil; $[\alpha]^{25}_D$ +30.3° (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (3.84) nm; MS and ^1H and ^{13}C NMR data were identical with published values.[6]

Bullatalicinone: obtained as white powder; mp 125−126 °C; $[\alpha]^{25}_D$ +23.4° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (3.84) nm; MS and ^1H and ^{13}C NMR data were identical with published values.[7]

Sylvaticin: obtained as colorless oil; $[\alpha]^{25}_D$ +5.5° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 224 (3.84) nm; MS and ^1H and ^{13}C NMR data were identical with published values.[8]

Rollimusin (3): obtained as white waxy solid; $[\alpha]^{25}_D$ −7.8° (*c* 0.55, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (3.84) nm; IR (KBr) ν_{max} 3417 (OH), 1747 (OC═O), 1215, 1063 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 4; FABMS *m/z* 661 $[M + Na]^+$; EIMS (70 eV) 517 (1), 499 (1), 481 (1), 463 (0.5), 433 (1), 415 (1), 397 (5), 363 (3), 345 (10), 293(17), 275 (19), 239 (20), 225 (9), and 195 (14), see Scheme 2; HREIMS *m/z* 638.4750 (calcd for $C_{37}H_{66}O_8$, 638.4758).

TMSi Derivative of 3. The TMSi derivative was prepared by treatment of **3** with bis-(trimethylsilyl) acetamide in the presence of pyridine. A small amount (ca. 500 μg) of **3** was treated with 200 μL pyridine/150 μL of bis-(trimethylsilyl) acetamide and heated at 70 °C for 30 min to yield the tetra-TMSi derivative. For the EIMS and FABMS measurements of this derivative, see Scheme 2; EIMS (70 eV) 751 (12), 697 (10), 661 (20), 517 (2), 435 (4), 345 (6), 297 (7), 275 (15), 207 (5).

Rolliacocin (4): obtained as waxy solid; $[\alpha]^{25}_D$ +11.8° (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (3.84) nm; IR (KBr) ν_{max} 3421 (OH), 1750 (OC═O), 1261, 1215, 1096 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 5; FABMS *m/z* 619 $[M + Na]^+$; EIMS (70 eV) 379 (1), 361 (9), 343 (2), 309 (40), 291 (6), 273 (3), 255 (10), 237 (12), 219 (15), 141 (10) and 123 (11), see Scheme 3; HREIMS *m/z* 596.4661 (calcd for $C_{35}H_{64}O_7$, 596.4652).

(*R*)- and (*S*)-MTPA Derivatives of 4. Compound **4** (1 mg) was dissolved in 0.5 mL of dry CH_2Cl_2, and 0.2 mL of pyridine, and 0.5 mg of 4-(dimethylamino)pyridine, and 25 mg of (*R*)−(−)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride were introduced to this solution sequentially. After the reaction mixture was allowed to sit for more than 6 h at room temperature (the reaction progress could be conveniently monitored by TLC), saturated $NaHCO_3$ (~3 mL) and Et_2O (~3 mL) were added. The organic phase was removed, and the aqueous phase was then extracted with Et_2O (~5 mL, 2 ×). The organic phases were combined, washed three times with $NaHSO_4$ (5% aqueous solution, to remove pyridine) and brine, dried ($MgSO_4$), and concentrated under reduced pressure to leave a crude yellow oil.[20] The crude oil was purified by preparative TLC to give the (*S*)-MTPA esters. The (*R*)-MTPA esters were prepared in the same way using (*S*)−(+)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride reagent.

Rollitacin: obtained as white waxy solid; $[\alpha]^{25}_D$ +18.0° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 224 (3.54) nm; UV, MS, and ^1H and ^{13}C NMR data were identical with published values.[9]

Annoglaucin: obtained as waxy solid; $[\alpha]^{25}_D$ +15.5° (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (3.98) nm; UV, MS, and ^1H and ^{13}C NMR data were identical with published values.[10]

10-Hydroxyasimicin: obtained as waxy solid; $[\alpha]^{25}_D$ +10.8° (*c* 0.60, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (3.78) nm; UV, MS, and ^1H and ^{13}C NMR data were identical with published values.[11]

Bioassays. The 3-day bioassay against Hep 2,2,15 was carried out according to procedures described in the literature.[23,24]

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References and Notes

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