ABSOLUTE STEREOCHEMISTRIES OF SYLVATICIN AND 12, 15-*CIS*- SYLVATICIN, BIOACTIVE C-20, 23-*CIS* NON-ADJACENT BIS-TETRAHYDROFURAN ANNONACEOUS ACETOGENINS, FROM *ROLLINIA MUCOSA*

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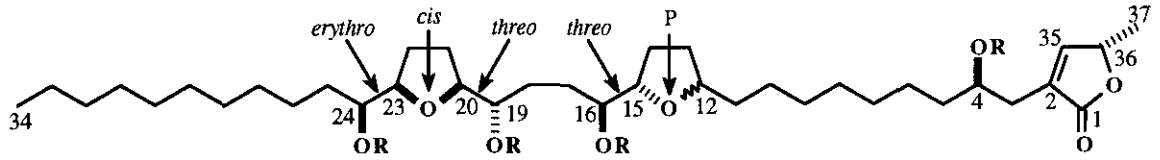
Abstract-A new acetogenin, 12, 15-*cis*-sylvaticin (**2**), and its stereoisomer, sylvaticin (**1**), a known acetogenin whose stereochemical structure has remained unsolved until now, were isolated, using activity-directed fractionation, from the leaf extracts of *Rollinia mucosa* (Jacq.) Baill. (Annonaceae). **1** is *cis* at C-20, 23 and *trans* at C-12, 15; **2** is *cis* at C-20, 23 and at C-12, 15. A key step in solving their absolute stereochemistries was the determination of the relative configurations (*cis* or *trans*) of their 1, 4-diols; this was achieved by a new procedure using the pattern recognition of the NOSEY spectra of their 1, 4-diol formaldehyde acetal derivatives (**1b** and **2b**). Both **1** and **2** showed potent and selective cytotoxicities, against the A-549 lung cancer and the PACA-2 pancreatic cancer, in a panel of six human solid tumor cell lines.

Annonaceous acetogenins have attracted considerable attention as a rapidly growing class of new compounds; these natural polyketides, among other significant bioactivities, have excellent potential as new antitumor agents.[1] Structurally, most of the acetogenins found to date may be classified into three major groups, i.e., the mono-tetrahydrofuran (THF), adjacent bis-THF, and nonadjacent bis-THF subclasses.[1] The last is also the most recent subclass and, so far, has about 20 members; some of them, e.g., bullatalicin, show promising *in vivo* antitumor efficacy although their potencies are less than those of the adjacent bis-THF compounds.[2] The work described in this paper reports, for the first time, two rare compounds (**1** and **2**) in this subclass which have been confirmed to possess a *cis*-THF ring at their respective C-20, 23 positions. Sylvaticin (**1**) and 12, 15-*cis*-sylvaticin (**2**), along with two known acetogenins, bullatalicin[3] and muricatetrocin B,[4] were isolated, using fractionation directed by brine shrimp lethality tests (BST), from the leaf extracts of *Rollinia mucosa* (Jacq.) Baill., a tropical fruit tree

native to the West Indies and Central America. Previous phytochemical studies have been reported on the bark,[5] and the seeds yielded rolliniastatin 1, an adjacent bis-THF acetogenin with *cis*-THF rings.[6]



	1	2	2_R	2_S
R	H	H	(R)-MTPA	(S)-MTPA
P	*trans*	*cis*	*cis*	*cis*

Figure 1. Chemical structures of **1**, **2**, $\mathbf{2_R}$ and $\mathbf{2_S}$ showing all absolute configurations.

Both **1** and **2** were isolated as colorless, waxy, solids, which had very close retention times on both normal and reverse phase hplc (**1** being slightly more polar), identical mps (63-64°C), almost identical $[\alpha]_D^{23}$ (**1** = +5.0°, **2** = +5.2°, in CH_2Cl_2) and the same *m/z* values for the HRFABms MH^+ ions (**1** = 639.4849, **2** = 639.4829, calc.= 639.4836) corresponding to the molecular formulae of $C_{37}H_{66}O_8$. For both compounds, a broad ir^{film} absorption band around 3450 cm^{-1} suggested the presence of free hydroxyl groups, and a sharp ir^{film} absorption band at 1752 cm^{-1} as well as the uv λ_{max} at 224 nm were indicative of an α, β-unsaturated γ-lactone ring, which is a common structural feature in all of the acetogenins found to date.[1] Indeed, the ^{1}H-nmr peaks (Table 1) at δ 7.19 (1H, q, J = 1.4, H-35), 5.06 (1H, qq, J = 6.8, 1.5, H-36), 1.43 (3H, d, J = 6.9, H-37), 2.53 (1H, ddt, J = 15.1, 3.2, 1.6, H-3a), 2.40 (1H, ddt, J = 15.1, 8.2, 1.3, H-3b), as well as the C^{13} -nmr resonances (Table 1) at δ 174.6 (C-1), 131.1 (C-2), 33.3 (C-3), 151.8 (C-35), 77.9 (C-36), and 19.1 (C-37), matched well with all the published data[1] corresponding to acetogenins with an α, β-unsaturated γ-lactone ring and a hydroxyl at the C-4 position. Having established this unit and excluding the possible existence of double bonds and extra carbonyls (in the ^{1}H-nmr spectra no other signals appeared further downfield than 4 ppm, and in the ^{13}C-nmr spectra no other signals appeared further downfield than 160 ppm), the remaining two units of unsaturation in each molecular formula were attributed to two THF rings in the respective structures. These structural features were confirmed and their precise positions (as well as those of the hydroxyl groups) were established by the EIms of **1** and **2** and their respective TMS derivatives (**1a** and **2a**)(Figure 2). **1** was identified as sylvaticin by comparing the ^{1}H- and ^{13}C-nmr data with those published; the original compound was isolated from *Rollinia sylvatica* St. Hil.[7] Although the planar structure of **1** was correctly established at the time of its publication, none of its nine stereocenters was assigned and the C-20, 23 THF ring was speculated to be *trans*. We also noticed that some of the original assignments of the nmr data needed to be revised.

We approached this stereochemical problem by first solving the relative stereochemistries around the THF rings of **1**. The most obvious information from the ^{1}H-nmr spectrum was that among the three hydroxyls flanking the THF rings, two of them must be *threo* and the third *erythro*. This was known for certain because two carbinol proton peaks at δ 3.44 and 3.51 (later assigned as H-16 and H-19, repective-

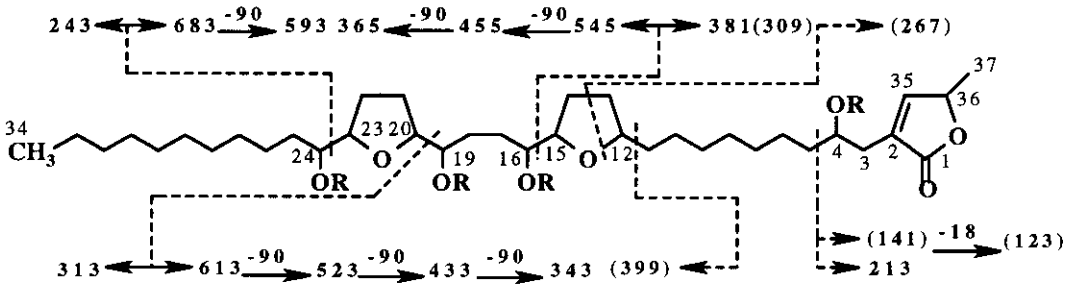


Figure 2. Diagnostic EIms fragment ions of **1** or **2** (R = H) and **1a** or **2a** (R = TMS). The numbers shown in parentheses are for underivatized **1** and **2**.

ly) were in the chemical shift range (*ca.* 3.4 - 3.6 ppm) which is indicative of a *threo* relative configuration between the hydroxyl and its adjacent THF ring (an *erythro* environment, on the other hand, should give the corresponding carbinol proton signal *ca.* 3.8 ppm).[8] However, just by examining the ^{1}H-nmr data, we did not know exactly which position, among three possible ones (i.e., at C-15/16, 19/20 or 23/24), was *erythro*. The *erythro* position was assigned later at C-23/24 based on the evidence obtained from the formal acetal and MTPA derivatives.[9]

A piece of less obvious information from the ^{1}H-nmr data was used to propose the relative stereochemistry of the two THF rings, themselves. We have noticed that the δ difference between the two THF geminal methylene protons is quite indicative of the ring configuration.[10] This difference is greater in the *trans* THF ring system (*ca.* 0.35 - 0.45 ppm) than in the *cis* THF ring system (*ca.* 0.15 - 0.25 ppm). The δ values of H-13a, 13b, 14a, 14b and H-21a, 21b, 22a, 22b (Table 1), suggested that the C-12, 15 ring was *trans*, and the C-20, 23 ring was *cis*. To this point, we had tentatively established the overall relative stereochemistries of **1** as follows: from C-12 to C-16, *trans/threo*, and from C-19 to C-24, *threo/cis/erythro*. Applying the same reasoning, we tentatively assigned the relative stereochemistry of **2** as follows: from C-12 to C-16, *cis/threo* and from C-19 to C-24, *threo/cis/ erythro*.

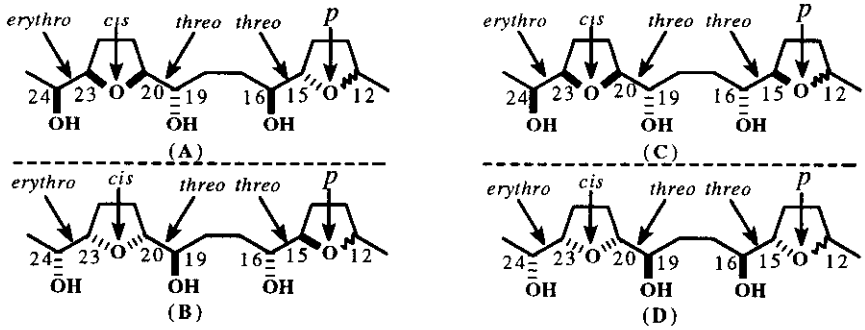


Figure 3. Four possible alternative structures (**A**-**D**) having the same relative stereochemistry (from C-12 to C-24) as described in the text. Sylvaticin (**1**) and 12, 15-*cis*-sylvaticin (**2**) correspond to the cases where *p* = *trans* and *cis*, respectively. Dashed lines reflect the structure pairs which are enantiomeric.

Table 1. Nmr resonances of sylvaticin (**1**) and 12,15 - *cis*-sylvaticin (**2**).

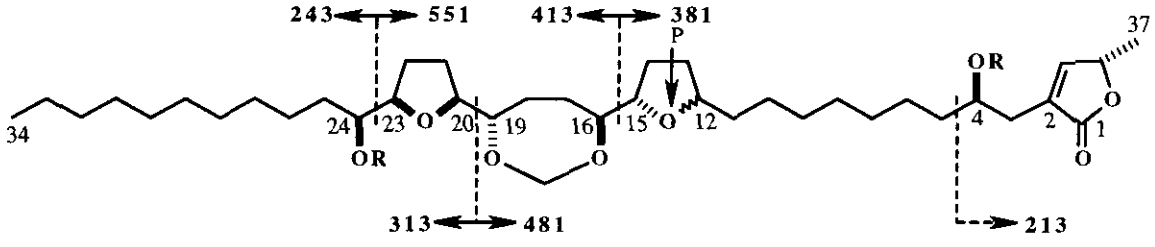
H/C No.	**1** ^{1}H (500 MHz) [a]	**1** ^{13}C (125 MHz) [a]	**2** ^{1}H (500 MHz) [a]	**2** ^{3}C (125 MHz) [a]
1		174.59		174.63
2		131.07		131.18
3a	2.53 (ddt, 15.1, 3.2, 1.6)	33.24	2.53 (ddt, 15.1, 3.2, 1.6)	33.32
3b	2.40 (ddt, 15.1, 8.2, 1.3)	33.24	2.40 (ddt, 15.1, 8.2, 1.3)	33.32
4	3.84 m	69.80	3.84 m	69.91
5	1.30 - 1.50 m [b]	37.31	1.30 - 1.50 m [b]	37.38
6 - 10, 26 - 33	1.20 - 1.40 m [b]	22.6-32.4 [b]	1.20 - 1.40 m [b]	22.7-31.9 [b]
11	1.30 - 1.50 m [b]	35.47	1.30 - 1.50 m [b]	35.93
12	3.89 m	79.25	3.88 m	80.05
13	1.99 m, 1.60 m	22.6-32.4 [b]	1.98 m, 1.86 m	22.7-31.9 [b]
14	1.97 m, 1.60 m	22.6-32.4 [b]	1.91 m, 1.65 m	22.7-31.9 [b]
15	3.80 (q, 6.9)	81.80	3.71(q, 6.9)	82.08
16	3.44 m	74.30	3.41 m	74.89
17	1.49 m	22.6-32.4 [b]	1.52 m	22.7-31.9 [b]
18	1.72 m	22.6-32.4 [b]	1.72 m	22.7-31.9 [b]
19	3.51 m	74.04	3.51 m	74.23
20	3.87 m	82.34	3.86 m	82.45
21	1.98 m, 1.86 m	22.6-32.4 [b]	1.98m, 1.86m	22.7-31.9 [b]
22	1.98 m, 1.77 m	22.6-32.4 [b]	1.98m, 1.77m	22.7-31.9 [b]
23	3.93 m	82.93	3.93 m	82.99
24	3.87 m	72.29	3.86 m	72.51
25	1.30 - 1.50 m [b]	33.04	1.30 - 1.50 m [b]	33.08
34	0.88 (t, 7.0)	14.06	0.88 (t, 7.0)	14.06
35	7.19 (q, 1.4)	151.80	7.19 (q, 7.0)	151.81
36	5.06 (qq, 6.8, 1.5)	77.93	5.06 (qq, 6.8, 1.5)	77.97
37	1.43 (d, 6.9)	19.04	1.43 (d, 6.9)	19.11

a) Assigned by ^{1}H-^{1}H COSY, HETCOR and HMQC; δ and J are in the units of ppm and Hz, respectively; b) the precise values not assigned in that region.

These assignments were directly supported by the NOESY experiments. For **1**, the cross peak corresponding to the H-20/H-23 correlation was present; and, for **2**, two cross peaks corresponding to the H-20/H-23 correlation, as well as to the H-12/H-15 correlation, were clearly seen. Also, we compared the ^{13}C-nmr data with those of hydroxyl-flanked mono-THF model compounds, which were synthesized by the group of Fujimoto,[11] and found that for both **1** and **2**, the proposed structures matched very well with the synthetic subunits having the same relative stereochemistries. Finally, the ^{1}H-nmr spectra of **1** and **2** featured the familiar H-15 quartet shift from *ca.* δ 3.8 to 3.7, with the concommitant change of the C-12,

15 THF ring configuration from *trans* to *cis*. This unique nmr behaviour of H-15 has been high-lighted in our previous communication.[12] Having collected all of this mutually supportive evidence, we believed that the relative configurations from C-12 to C-24 in both **1** and **2** had been firmly established.

In order to identify the correct absolute stereochemistries among the four alternatives having the same relative configurations (Figure 3), we first synthesized the intramolecular formal (formaldehyde) acetal derivatives (**1b** and **2b**) from the respective parent compounds (**1** and **2**). The HRFABms and EIms of **1b** and **2b**, as well as the EIms of their TMS derivatives (**1c** and **2c**), confirmed that the formal acetals had formed between the two hydroxyl groups at C-16 and C-19 (Figure 4). The reaction, using DMSO and Me_3SiCl as reagents, was first used to convert monoalcohols into intermolecular formal acetals.[13] It has been modified by our group to convert 1, 2-, 1, 4- and 1, 5-diols of appropriate acetogenins into cyclic intramolecular formal acetals.[3b]

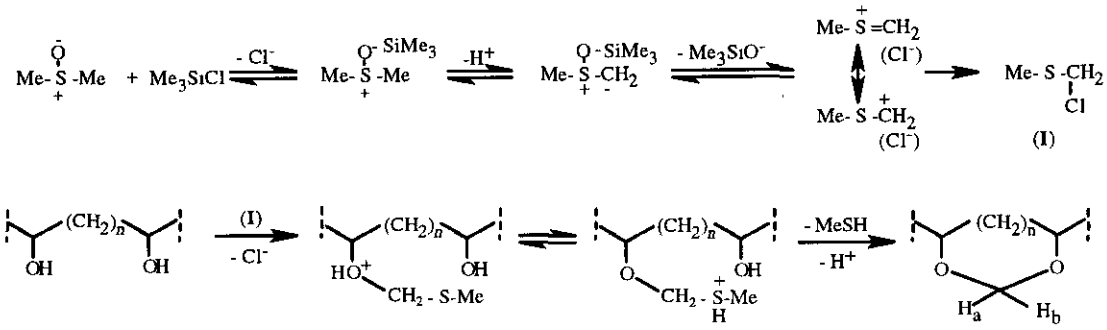


	1b	2b	1c	2c	$1b_R$	$1b_S$	$2b_R$	$2b_S$
R	H	H	TMS	TMS	(R)-MTPA	(S)-MTPA	(R)-MTPA	(S)-MTPA
P	*trans*	*cis*	*trans*	*cis*	*trans*	*trans*	*cis*	*cis*

Figure 4. EIms fragment ions of **1c** and **2c**. EIms results of **1b** and **2b** were consistent with these structures (data not shown). HRFABms MH^+ ions: m/z = 651.4823 (**1b**), 651.4836 (**2b**), calcd = 651.4836. The structures of $\mathbf{1b_R}$, $\mathbf{1b_S}$, $\mathbf{2b_R}$ and $\mathbf{2b_S}$ are also presented.

Although no intermediate(s) of this reaction has (have) been captured, as yet, it has been shown that by starting from D_6-DMSO, the acetal methylene groups are labeled.[13] Based on this experimental fact, we proposed that the reaction could possibly procede *via* a Pummerer type[14] step (Scheme 1).

Since the configurations of the two carbinol centers involved in the reaction are retained, the relative stereochemistry regarding the C-16 and C-19 hydroxyls (either *cis* or *trans*) may be deduced from the cyclic product. Our group has proposed that the chemical shift difference of the two methylene protons, H_a and H_b (labeled in Scheme 1), can be used to determine the relative configuration of the dioxacycloheptane ring in question.[3b, 12] More specifically, if H_a and H_b have the same or very close chemical shifts (A_2 or AB spin system), then the ring is *trans*; if H_a and H_b appear to be a pair of well separated doublets (AX spin system), then the ring is *cis*. This, we believe, is of little doubt when the two side chains attached to the dioxacycloheptane ring are very similar in their chemical nature. For the extreme situation where both the side groups are identical, e.g., in the case of *trans*-4, 7-dimethyl-1, 3-dioxacycloheptane, H_a and H_b give the A_2 nmr spectrum of a singlet at δ 5.30; while the *cis* isomer gives the AX nmr pattern, i.e., a pair of coupled doublets at δ 5.16 and 5.47.[15] However, little data has been reported, so far, to support this notion in a more general case where the two side chains attached to



Scheme 1. Proposed mechanism for the formation of the intramolecular formal acetals.[3b] In general cases, n = 0 - 3; with regard to the text, n =2.

the dioxacycloheptane ring are not structurally similar to each other. Intuitively, one may argue that the more different these side chain groups may become, the more diastereotopic the relationship between H_a and H_b will be, and, hence, it is more likely to see H_a and H_b as two well separated doublets even if the ring has the *trans* configuration. In our case, we found two very close doublets corresponding to H_a and H_b in both **1b** ($\Delta\delta$ = 0.03, J = 4.9) and **2b** ($\Delta\delta$ = 0.04, J = 4.9) (Table 2). Clearly, a more sophisticated analysis was needed to make a substantiated configuration assignment. We noticed that conformational analyses of *trans*- and *cis*-4, 7-disubstituted 1, 3-dioxacycloheptane had been extensively studied by Gianni *et al.*[15] Their work suggests that, for such a ring system, chair conformations are preferred over twist-chair conformations, and, especially when the substitution groups are big enough to cause severe steric interactions, these groups will be locked in the equatorial positions. Among the numerous possible chair conformations (which are equilibrated by pseudorotations), we selected those having both of their substitution groups in the equatorial positions and analyzed the proton through-space correlation patterns with regard to the *trans* and *cis* isomers (Figure 5).

We envisioned that if NOESY experiments were run, the *cis* and *trans* isomers would give different and characteristic correlation patterns. As pictured in Figure 5, the *cis* isomer should show two cross-peaks corresponding to (H_b, H_1) and (H_b, H_2), but not to (H_a, H_1) and/or (H_a, H_2); while the *trans* isomer should give two cross-peaks at (H_a, H_1) and (H_b, H_2), but not at (H_b, H_1) and/or (H_a, H_2). This implies that the differentiation between the *trans* and *cis* isomers can be achieved by the pattern recognition of their NOESY spectra. One merit of this method is that the exchangeable assignments between H_a and H_b, and/or between H_1 and H_2, are tolerated. Moreover, a detectable chemical shift difference between H_1 and H_2 (which are H-19 and H-16 in **1b** and **2b**, in that order) is not a requirement. Although the method does require some chemical shift difference between H_a and H_b, this seldom becomes a serious problem owing to the improved resolution power of a 2D nmr experiment. Also it should be pointed out that although we have limited our discussions, so far, to the most stable chair conformations depicted in Figure 5, we have checked all possible chair conformations *via* pseudorotations and found that the end result was the same. In other words, this method does not require the presence of bulky substitution groups (i.e., R_1 and R_2) as a prerequisite.

The NOESY spectra of both **1b** and **2b** matched the pattern that is characteristic to the *trans* configura-

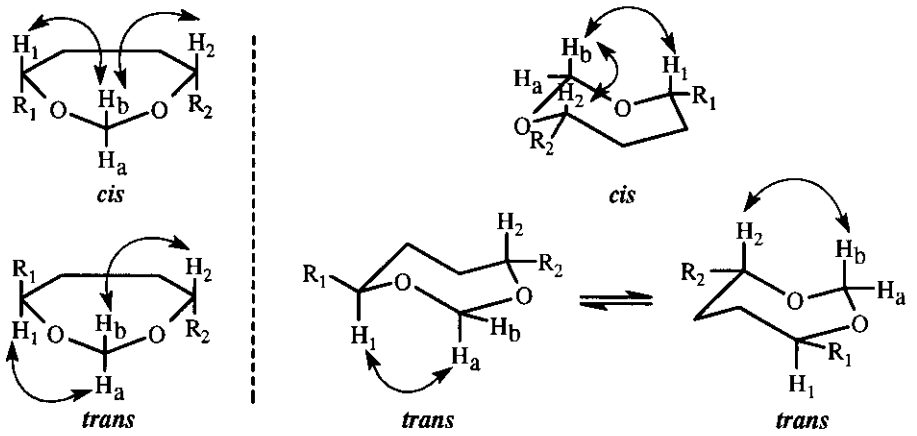


Figure 5. The most stable chair conformations for *trans*- and *cis*- 4, 7-disubstituted 1, 3-dioxacycloheptanes. Only one enantiomer is represented in each case. Planar drawings are shown at the left. Arrows indicate the proton pairs which are close enough to show NOE effects.

tion: two cross-peaks were clearly seen at the δ coordinates of (H-16, H_b) and (H-19, H_a) (δ values are listed in Table 2). Also differential NOE experiments confirmed the above correlations (4% enhancement of H-16 intensity and 3% enhancement of H-19 intensity, upon the selective irradiation of H_b and H_a, respectively). In order to make a comparison, we ran the parallel experiments using the formal acetal derivative of bullatalicin,[3b] whose H_a and H_b resonate at δ 5.29, 4.64, respectively, and because of this large δ difference, it was previously assigned to be *cis* with regard to the C-16, 19 hydroxyls. We found that in this case, H_a had no cross peak with H-16 or H-19 (they happen to have the same δ at 3.62), but H_b had cross peaks with H-16 and/or H-19. The differential NOE experiments confirmed the above correlations, and, moreover, the large intensity increase (*ca.* 10%) of the signals at δ 3.62 upon the irradiation of H_b further suggested that H_b actually correlated with both H-16 and H-19. This matched the picture we predicted for the *cis* isomer. Based on these experimental results, we determined with confidence that in both **1b** and **2b**, the C-16 and C-19 hydroxyls were *trans* to each other, and, thus, the relative configurations at C-16 and C-19 must be either *S*/*S* or *R*/*R*. These results were of key importance in the whole structural elucidation process because they immediately allowed us to rule out two of the alternative structures, i.e., (**C**) and (**D**), in Figure 3.

The determination of the absolute stereochemistries of **1b** and **2b** was achieved by using Mosher ester methodology.[16] (R-) and (S-) di-Mosher esters (di-MTPA) ($\mathbf{1b_R}$ and $\mathbf{1b_S}$, repectively) were prepared from **1b**, and (R-) and (S-) di-Mosher esters ($\mathbf{2b_R}$ and $\mathbf{2b_S}$, respectively) were prepared from **2b** (Figure 4). The ^{1}H-^{1}H COSY spectra of the products were analyzed, and the δ_H values are tabulated in Table 2. From the $\Delta\delta_{S-R}$ values we determined that the absolute configurations of both **1b** and **2b**, and hence of both **1** and **2**, at positions C-4, C-24 and C-36 were: 4R, 24S, and 36S. The determination of the C-36 configuration was based on the comparison of $\Delta\delta_H(\delta_S - \delta_R)$ values at positions C-35, 36, 37 with the published data of chiral model compounds.[17] Combining the prior established relative stereochemistry in the region from C-12 to C-24, we finally defined the absolute configurations of **1** and **2** as (shown in

Figure 1) 4*R*, 12*R*, 15*S*, 16*S*, 19*S*, 20*S*, 23*R*, 24*S*, 36*S* for **1** and 4*R*, 12*S*, 15*S*, 16*S*, 19*S*, 20*S*, 23*R*, 24*S*, 36*S* for **2**. 12, 15-*cis*-sylvaticin (**2**) is the first example of a nonadjacent bis-THF acetogenin which has two *cis*-THF rings.

Table 2. ^{1}H Nmr data of **1b**, **2b** and their respective (R)-, (S)-di-MTPA esters (**1b$_R$**, **1b$_S$**, **2b$_R$**, **2b$_S$**).

H No.	**1b**	**1b$_R$**	**1b$_S$**	$\Delta\delta_{S-R}$[a]	**2b**	**2b$_R$**	**2b$_S$**	$\Delta\delta_{S-R}$[b]
37	1.43	1.31	1.28	- 0.03	1.43	1.31	1.28	- 0.03
36	5.06	4.90	4.86	- 0.04(**S**[d])	5.06	4.90	4.86	- 0.04(**S**[d])
35	7.19	6.98	6.72	- 0.26	7.19	6.98	6.72	- 0.26
3a	2.53	2.68	2.60	- 0.08	2.53	2.68	2.60	- 0.08
3b	2.40	2.60	2.57	- 0.03	2.40	2.60	2.57	- 0.03
4	3.84	5.38	5.31	**R**[d]	3.83	5.38	5.31	**R**[d]
5	c	1.62	1.66	+0.04	c	1.62	1.66	+0.04
25	c	1.70	1.61	- 0.09	c	1.68	1.61	- 0 07
24	3.93	5.16	5.19	**S**[d]	3.86	5.18	5.20	**S**[d]
23	3.98	3.95	4.00	+0.05	3.96	3.94	4 00	+0.06
20	3.98	3.92	3.97	+0.05	3.97	3.84	3.85	+0.01
19	3.70	3.58	3.60	+0.02	3.69	3.61	3.64	+0.03
16	3.61	3.46	3.60	+0.14	3.63	3.46	3.59	+0.13
15	3.93	3.77	3.82	+0.05	3.82	3.77	3.81	+0.04
H_a	4.85 (d, 4.9)	4.83 (d, 5.2)	4.85 (d, 5.0)	+0.02	4.85 (d, 4.9)	4.83 (d, 5.2)	4.87 (d, 5.2)	+0.04
H_b	4.88 (d, 4.9)	4.84 (d, 5.2)	4.88 (d, 5.0)	+0.04	4.89 (d, 4.9)	4.84 (d, 5.2)	4.88 (d, 5.2)	+0.04

a) $\Delta\delta_{S-R} = \delta$(**1b$_S$**) - δ(**1b$_R$**); b) $\Delta\delta_{S-R} = \delta$(**2b$_S$**) - δ(**2b$_R$**); c) not assigned; d) the absolute configuration

It was suprising to find that, after the di-MTPA derivatives were made, the δ differences ($\Delta\delta$) of H_a and H_b in all cases (**1b$_R$**, **1b$_S$**, **2b$_R$**, **2b$_S$**) remained either unchanged (**1b$_S$**) or even diminished (**1b$_R$**, **2b$_R$**, **2b$_S$**), compared with the parent compounds (**1b** and **2b**). This was not expected because the chemical nature of the two side chain groups attached to the 1, 3-dioxacycloheptane ring, after the esterifications, had been made even more different from each other, at least in the nearby regions surrounding the ring, so that H_a and H_b should have been even more diastereotopic in their relationship. Nonetheless, we did not see them as a pair of more separated doublets. The data of the six compounds (**1b**, **2b**, **1b$_R$**, **1b$_S$**, **2b$_R$**, **2b$_S$**) which have *trans* 1, 3-dioxacycloheptane rings, plus those of many others which have the *cis* counterpart,[3b, 12] strongly suggests that the relative configuration of the 1, 3-dioxacycloheptane ring, and, hence, the 1, 4-diol, can be determined according to the chemical shift difference between the two acetal methylene protons, i.e., identical or almost identical δ values (e.g., $\Delta\delta$ = 0 - 0.04) indicate the *trans* configuration, and well separated δ values (e.g., $\Delta\delta$ = 0.3 - 0.7) indicate the *cis* configuration.

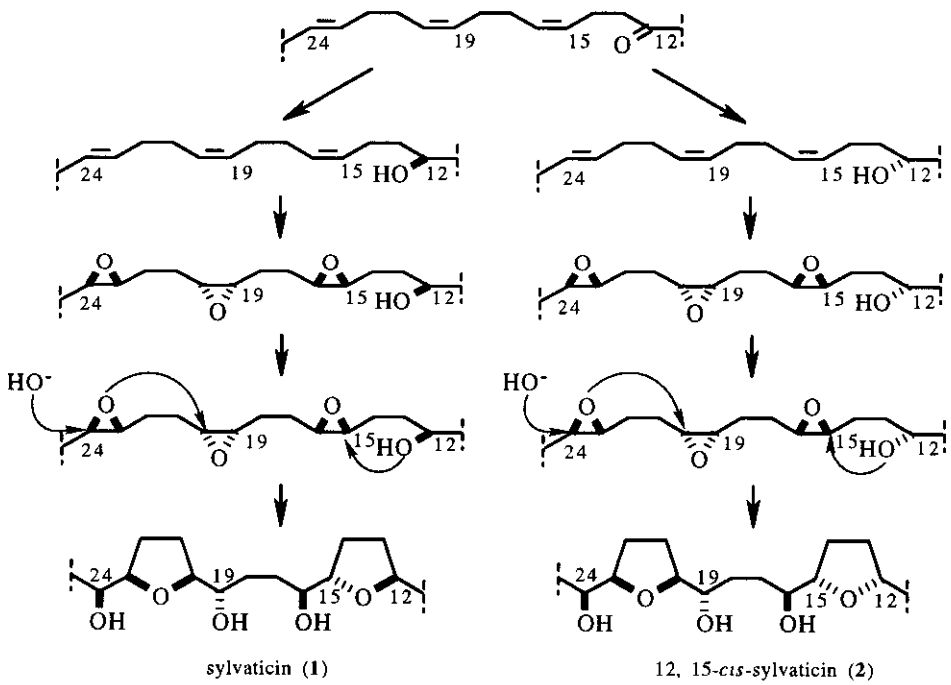
We previously contended that it was not feasible to assign all stereocenters in nonadjacent bis-THF acetogenins by directly applying Mosher methodology to their per-MTPA derivatives.[3b] This can now be illustrated with 12, 15-*cis*-sylvaticin (**2**) as a convenient example. The (R)- and (S)-tetra-MTPA deriva-

Table 3. ^{1}H Nmr data of (R)- and (S)-tetra-MTPA derivatives, **2R** and **2S**,respectively, of **2**.

	H-12	H-15	H-16	H-17	H-18	H-19	H-20	H-23	H-24	H-25
2R	3.74	3.81	5.01	1.60	1.64	5.05	3.84	3.90	5.08	1.70
2S	3.76	3.78	4.98	1.35	1.46	4.98	3.78	3.93	5.12	1.57
$\Delta\delta_{S-R}$ [a]	+0.02	- 0.03	- 0.03	- 0.25	- 0 18	- 0.07	- 0.06	+0.03	+0.04	- 0.13

a) $\Delta\delta_{S-R} = 2_S - 2_R$

tives (**2R**, **2S**) of **2** were prepared, and their proton resonances were assigned by ^{1}H-^{1}H COSY (Table 3). Surrounding the C-4 and C-36 stereocenters, all data were found being identical with those of **2bR** and **2bS** (upper part of Table 2). Apart from this part, the other usable data points were those of H-12 and H-25, where the $\Delta\delta_{S-R}$ effects ought be predominated by the C-16 and C-24 OMTPA groups, respectively, but lesser so by the other OMTPA groups. The positive $\Delta\delta_{S-R}$ of H-12 and the negative $\Delta\delta_{S-R}$ of H-25 suggested that the absolute configurations at C-16 and C-24 were both *S*. These were in accordance with the results already obtained from the formal derivatives. Using this method alone, however, we were unable to define unambiguously the configurations of C-16 and C-19 because of the abnormal $\Delta\delta_{S-R}$ values of H-15 and H-20 in the tetra-MTPA esters. These were not unexpected because of the close proximity and, hence, the strong interference between the C-16 and C-19 OMTPA groups.



Scheme 2. Proposed partial biogenetic pathway for sylvaticin (**1**) and 12, 15-*cis*-sylvaticin (**2**)

The stereochemical structures of **1** and **2** as defined above are also consistent with the proposed hypothesis for the biogenesis of Annonaceous acetogenins.[1,10,12] Shown in Scheme 2 is essentially the same biogenetic pathway which was proposed to explain the biogenesis of rolliniastatin 1.[10] An unique

epoxidase which makes the antisense epoxide at the C-19, 20 positions, seems to exist in *Rollinia* species and is ultimately responsible for the formation of the *cis*-C-20, 23 THF rings.

1, **2**, **1b** and **2b** showed very good bioactivities in the brine shrimp lethality test (BST)[18] and varying degrees of inhibitory effects among six human solid tumor cell lines (Table 4). In the cases of the A-549 (non-small cell lung cancer) and PACA-2 (pancreas cancer) cell lines, these compounds out performed adriamycin by wide margins and presented remarkable selectivities. **1** was generally more bioactive than **2** in the various cell lines. As previously reported,[3b] the formal acetals (**1b**, **2b**) retained bioactivities and, in certain cases, were either more or less active than **1** and **2**. The Annonaceous acetogenins act as potent inhibitors of ATP production *via* the blockage of mitochondrial complex I and as inhibitors of the plasma membrane NADH oxidase of cancerous cells.[2, 20]

Table 4. Bioactivities[a] of **1**, **1b**, **2** and **2b**.

	BST[b]	A-549[c]	MCF-7[d]	HT-29[e]	A-498[f]	PC-3[g]	PACA-2[h]
	LC_{50}(μg/ml)	ED_{50}(μg/ml)	ED_{50}(μg/ml)	ED_{50}(μg/ml)	ED_{50}(μg/ml)	ED_{50}(μg/ml)	ED_{50}(μg/ml)
1	8.0×10^{-1}	$< 10^{-8}$	3.79×10^{-5}	1.58×10^{-1}	2.78×10^{-2}	4.52×10^{-4}	$< 10^{-8}$
1b	9.7×10^{-2}	$< 10^{-8}$	1.50×10^{-1}	1.61×10^{-1}	1.55	1.20	$< 10^{-8}$
2	1.1	$< 10^{-8}$	1.15×10^{-1}	5.23×10^{-1}	1.41	1.80	$< 10^{-8}$
2b	3.4×10^{-2}	6.17×10^{-5}	5.18×10^{-1}	1.20	2.76	2.06	$< 10^{-8}$
Adriamycin[i]	8.0×10^{-2}	1.55×10^{-3}	7.99×10^{-2}	3.84×10^{-2}	3.12×10^{-2}	9.65×10^{-3}	2.53×10^{-2}

a)All samples were tested in the same run in each cytotoxicity bioassay[19]; b) brine shrimp lethality test[18]; c) human lung carcinoma; d) human breast carcinoma; e) human colon adenocarcinoma; f) human renal carcinoma; g) human prostate adenocarcinoma; h) human pancreas carcinoma; i) standard positive control.

EXPERIMENTAL

Instruments

Melting points were measured on a Fisher-Johns apparatus and the thermometer was not corrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. A Perkin-Elmer 1600 FTIR spectrophotometer and a Beckman DU-7 UV spectrophotometer were used for taking ir and uv spectra. All nmr experiments were carried out on our Varian-500S spectrometer (^1H at 500 MHz, ^{13}C at 125.75 MHz) using $CDCl_3$ as solvent and TMS as reference. Low resolution EIms experiments were taken on a Finnigan 4000 spectrometer; HRFABms and EIms of TMS derivatives were taken on a Kratos MS 50 spectrometer. A Rainin system equipped with Dynamax software and a Ranin UV-1 detector (set at 230 nm) was used for all normal phase (using a 250 x 21 mm silica gel column) and reverse phase (using a 250 x 21 mm C18 column) hplc separations.

Plant material

The leaves of *Rollinia mucosa* (Jacq.) Baill. were collected in the Conservatory of the Missouri Botanical Garden, St. Louis, Missouri; the associated plant identification numbers are: MBG #891568, voucher *Sherman* 285 (MO).

Extraction and purification procedures

The oven-dried (< 50°C) pulverized leaves (1630 g) were extracted exhaustively with 95% ethanol (8000 ml x 3) and CH_2Cl_2 (8000 ml x 2 then 5000 ml x 2) at room temperature and condensed under vacuum to yield a combined extract F001 (144 g; BST LC_{50} 2.1 μg/ml); F001 was partitioned between water (1500 ml) and $CHCl_3$ (3000 ml x 3) to yield the water soluble fraction (F002) (6 g; BST LC_{50} 25 μg/ml), the $CHCl_3$ soluble fraction (F003) (120 g; BST LC_{50} 0.9 μg/ml) and an insoluble interface (F004) (5 g). F003 was further partitioned between hexane (1000 ml) and 90% MeOH aq. solution (2000 ml x 2) to yield the MeOH soluble fraction (F005) (71 g; BST LC_{50} 0.7 μg/ml) and the hexane soluble fraction (F006) (33 g; BST LC_{50} > 1000 μg/ml). 68 g of F005 was fractionated on an open column (Ø = 9 cm, packed with 1.1 Kg of 60-200 mesh silica gel) using $CHCl_3$-MeOH gradient elution; 29 fractions were collected. Fractions F-13 to F-16 were pooled (3.9 g; BST LC_{50} 0.14 μg/ml) and further fractionated on a second open column (Ø = 5 cm, packed with 600 g of 60-200 mesh silica gel) using hexane-acetone gradient elution. Of the 71 fractions collected, the fractions F-(13, 16)-6 to F-(13, 16)-30 were subjected to repetitive reverse phase (H_2O-MeCN elution) and normal phase (hexane-THF-MeOH elution) hplc purifications to yield **1** (*ca.* 350 mg), **2**(*ca.* 25 mg) and muricatetrocin B[4] (*ca.* 150 mg); bullatalicin[3] (*ca.* 400 mg) was isolated from the fractions F-(13, 16)-35 to F-(13, 16)-70 by applying the same procedures.

Typical procedures of chemical derivatizations

(1) Intramolecular formal acetal: a mixture of DMSO and TMSCl (molar ratio 1.2 : 1) was mixed in 2 ml of benzene and placed in a refrigerator without stirring for 2 h to allow the formation of white crystals. The benzene was decanted and the crystals were washed twice with CH_2Cl_2. These crystals were added stepwise to a 0.5 ml $CHCl_3$ solution containing 5 mg of the acetogenin at room temperature (usually a large excess of the crystals were added). The reaction was monitored by tlc at intervals of 2 h and typically was quenched with H_2O after 12 h. After workup by extractions with 5% $NaHCO_3$ aq. solution, the reaction mixture was purified by normal phase hplc. The typical yield was 40-50%, with most of the unreacted starting material recovered.

(2) Per-MPTA esters. large excess of (R)- or (S)- (α)-methoxy-(α)-trifluromethylphenyl acetyl chloride was added to 1 mg of the starting material dissolved in 0.5 ml of CH_2Cl_2. The reaction vial was capped and kept in the refrigerator overnight. The reaction mixture was first purified over a small silica gel pipette column and then by 5% aq. $NaHCO_3$ workup. Quantitative conversions were usually achieved.

(3) TMS derivatives: appropriate starting materials (50 - 80 μg) were treated with *N,O*-bis(trimethylsilyl)-acetamide (20 μl) and pyridine (2 μl) and heated at 70 °C for 30 min to yield the respective per-TMS derivatives.

Bioassays

The brine shrimp (*Artemia salina* Leach) test (BST) was performed as modified to determine LC_{50} values in μg/ml for each partition fraction and chromatographic column pool.[18] Seven-day MTT *in vitro* cytotoxicity tests against human tumor cell lines were carried out on **1**, **2**, **1b**, **2b** at the Purdue Cancer Center, using standard protocols for A-549, MCF-7, HT-29, A-498, PC-3 and PACA-2 with adriamycin as the positive control[19] (Table 4).

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