Structural Revisions of Some Non-Adjacent Bis-Tetrahydrofuran Annonaceous Acetogenins

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STRUCTURAL REVISIONS OF SOME NON-ADJACENT BIS-TETRAHYDROFURAN ANNONACEOUS ACETOGENINS

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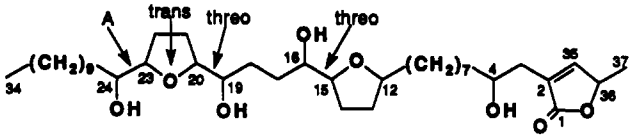
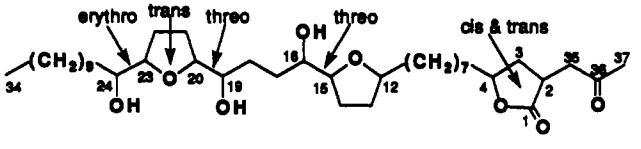
ABSTRACT.—The relative stereochemistries of bullatalicin [**1**] and bullatalicinone [**2**] were partially reassigned based on COSY and relayed COSY spectra. The structures of annonins VIII [**3**], IV [**4**], and XVI [**5**] were revised and concluded as bullatalicin [**1**], bullatanocin [**6**], and squamostatin A [**7**], respectively.

Among the structural subclasses of the Annonaceous acetogenins, non-adjacent bis-tetrahydrofuran (bis-THF) acetogenins are relatively new and have fewer known members (1,2). Because their spectral features are very similar to the adjacent bis-THF Annonaceous acetogenins, some incorrect structural elucidations and mis-assignments of the relative stereochemistries within this subclass exist in recent publications, namely with bullatalicin [**1**] (3), bullatalicinone [**2**] (4), and annonins VIII [**3**], IV [**4**], XVI [**5**], and XIV [**8**] (5). In work reported in this paper, we have partially reassigned the relative stereochemistries of bullatalicin [**1**] and bullatalicinone [**2**], by COSY and relayed COSY, and identified the structures of annonins VIII [**3**], IV [**4**], and XVI [**5**] as bullatalicin [**1**], bullatanocin [**6**] (6), and squamostatin A [**7**] (7), respectively, by comparisons of the redetermined nmr and ms spectra of **3–5** and the eims of their TMS derivatives with those of **1**, **6**, and **7**. The good activity of bullatalicin [**1**] against human ovarian cancer in athymic mice suggests the antitumor potential of this acetogenin subclass (8). The acetogenins act through their powerful inhibition of respiratory complex I in mitochondia (9,10); this action explains their potent pesticidal effects, and their antitumor effects are elicited when the aerobic energy demands of tumor cells exceed those of normal cells.

The relative stereochemistry of the bis-THF rings and three adjacent OH groups of bullatalicin [**1**] (3) was previously assigned as erythro (C-15/C-16), threo (C-19/C-20), and threo (C-23/C-24). A more detailed examination of the COSY spectrum (in C_6D_6) of bullatalicin [**1**] indicated that this assignment was incorrect. The coupling correla-



1 A=erythro
6 A=threo



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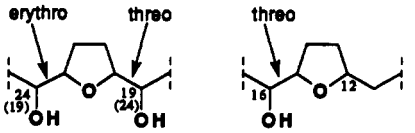
tions from H-12 to H-16 [δ 3.80 (H-12)–δ 1.68 and 1.50 (H-13)–δ 1.65 and 1.47 (H-14)–δ 3.82 (H-15)–δ 3.44 (H-16)] and from H-19 to H-24 [δ 3.39 (H-19)–δ 3.71 (H-20)–δ 1.63 and 1.44 (H-21)–δ 1.77 and 1.53 (H-22)–δ 3.64 (H-23)–δ 3.74 (H-24), or reversely from H-24 to H-19] were clearly shown by applying a trace function in a COSY program which can only display a specific group of coupling-correlated 1D proton signals to simplify crowded resonances. These connectivities were further confirmed by the single-relayed COSY of bullatalicin [**1**], which showed coupling correlations between H-12–H-14–H-16, H-13–H-15, H-19–H-21–H-23, and H-20–H-22–H-24. These indicated the existence of fragments **A** and **B**.



A **B**

Since we already knew that the proton signal at δ 3.74 should belong to an erythro hydroxylated methine and the other two resonances at δ 3.44 and 3.39 should belong to threo hydroxylated methines (1,2,11), the stereochemistry of C-15/C-16 was concluded to be threo rather than erythro, and the erythro configuration should be located on fragment A. The exact position of the erythro configuration was still uncertain based on the above information. This could be solved by correctly connecting fragments **A** and **B**. However, this connection could not be completed by the COSY and single-relayed COSY spectra, because the proton signals of H-17, H-18, and H-25 overlapped. To accomplish this connectivity, a double-relayed COSY experiment was applied. The double-relayed COSY of bullatalicin [**1**] showed correlation cross peaks between δ 3.44 (H-16) and δ 3.39 (H-19). Therefore, the configurations of C-15/C-16 and C-19/C-20 were assigned as threo, and the configuration of C-23/C-24 was assigned as erythro, as illustrated in structure **1**. The reassignments of the nmr data of bullatalicin [**1**] and its tetraacetate are reported in Table 1.

The ^{1}H-nmr signals around the bis-THF rings and the three adjacent OH groups of bullatalicinone [**2**] (4) were also reassigned by using the same nmr techniques as applied above for bullatalicin [**1**] (Table 2). Again, the double-relayed correlation cross peaks between δ 3.44 (H-16) and 3.38 (H-19) were observed in the double-relayed COSY spectrum of bullatalicinone [**2**]. As with bullatalicin [**1**], the configurations of C-15/C-16, C-19/C-20, and C-23/C-24 were reassigned as threo, threo, and erythro, respectively. Additionally, the ^{1}H- and ^{13}C-nmr resonances of the cis and trans C-2/C-4 diastereomers were clearly assigned by comparisons with the nmr signals of cis and trans substituted 2-acetonyl-4-butyl-γ-butyrolactones as reported by Hoye and Hanson (12). This assignment showed that the cis C-2/C-4 diastereomer was the major component of the diastereomeric mixture of **2**, and the trans C-2/C-4 diastereomer was the minor one. The reassigned nmr data of **2** and its triacetate are reported in Table 2.

Close examination of the published spectral data of annonin VIII [**3**], illustrated as previously reported (5), indicated that the carbon signal of C-16 could not be δ 79.51 if there were an OH group on its adjacent position, in which this THF ether carbon resonance should be around δ 81–83 (1,2). In fact, a carbon peak around δ 79–80 is a typical resonance of a THF ether carbon without an adjacent OH group, as seen in the Annonaceous acetogenins such as bullatalicin [**1**] (3), gigantecin (13), gigantetrocin, and gigantriocin (14). Comparisons of the spectral data of **1** and **3** revealed a considerable homogeneity. Remeasurements of the ^{1}H-nmr spectra of samples of both **1** and **3** under

TABLE 1. Reassignments of nmr Data[a] of Bullatalicin [**1**] and Its Tetraacetate.

Position	Compound			
	^{1}H			^{13}C
	1[b]	**1**[c]	**1**-tetraacetate[b]	**1**[b]
1	—	—	—	174.48
2	—	—	—	131.08
3a	2.53 dddd (15.0,4.0,1.5,1.5)	2.30 dddd (14.7,3.7,1.8,1.2)	2.57 dddd (18.5,3.3,2.9,1.3)	37.40–25.20
3b	2.40 dddd (15.1,8.0, 1.4,1.2)	2.20 ddt (14.7,8.2,1.2)	2.51 ddt (18.5,7.8,1.3)	
4	3.87 m	3.71 m	5.10 dddd (8.1, 7.8, 5.1, 3.3)	69.91
5	1.71–1.37	1.63, 1.39	1.80–1.50	37.40–25.20
6–10	1.36–1.21	1.29	1.29	37.40–25.20
11	1.71–1.37	1.68, 1.50	1.80–1.50	37.40–25.20
12	3.87 m	3.80 m	3.86 m	79.28
13	1.99–1.37	1.68, 1.50	2.00–1.20	37.40–25.20
14	1.99–1.37	1.65, 1.47	2.00–1.20	37.40–25.20
15	3.80 m	3.82 m	3.97 m	82.17[d]
16	3.41 m	3.44 t	4.82 m	74.55[e]
17–18	1.71–1.37	1.85, 1.60	1.80–1.50	37.40–25.20
19	3.41 m	3.39 t	4.82 m	74.42[e]
20	3.80 m	3.71 m	3.97 m	81.97[d]
21	1.99–1.37	1.63, 1.44	2.00–1.20	37.40–25.20
22	1.90, 1.86	1.77, 1.53	2.00–1.20	37.40–25.20
23	3.87 m	3.64 m	3.97 m	83.30
24	3.87 m	3.74 m	4.91 ddd	71.46
25	1.71–1.37	1.38 m	1.60 m	37.40–25.20
26–33	1.36–1.21	1.29	1.29	37.40–25.20, 22.72
34	0.89 t (7.0)	0.91 t (7.1)	0.88 t (7.2)	14.17
35	7.19 q (1.5)	6.24 d (1.3)	7.09 d (1.6)	151.70
36	5.06 qq (6.9,1.5)	4.24 qq (6.7,1.3)	5.01 qq (6.9,1.6)	77.95
37	1.42 d (6.8)	0.81 d (6.8)	1.40 d (6.9)	19.16
4-OAc	—	—	2.03 s	—
16-OAc	—	—	2.08 s	—
19-OAc	—	—	2.09 s	—
24-OAc	—	—	2.05 s	—

[a] ^{1}H nmr (500 MHz, J in Hz); ^{13}C nmr (125 MHz).
[b] In $CDCl_3$.
[c] In C_6D_6.
[d,e] Assignments may be interchangeable within each group.

identical conditions in $CDCl_3$ and C_6D_6, respectively, provided identical ^{1}H-nmr spectra. The eims of **3** and its TMS derivative also gave the same major ms fragments as those of **1** and its TMSi derivative. Co-tlc's developed in several different solvent systems, showed only one spot for mixed samples of **1** and **3**. Furthermore, the reported ^{1}H-nmr data of the tetraacetates of both **1** and **3** were essentially identical (3,5). Thus, we have concluded that annonin VIII [**3**] is equivalent to bullatalicin [**1**].

Annonin IV [**4**], illustrated as previously reported (5), differs from annonin VIII [**3**] in having three threo configurations of the THF rings adjacent to OH groups instead of two threo and one erythro, as in **3** (5). The spectral data of **4** were carefully compared with those of bullatanocin [**6**] (6), which is a single-chiral diastereomer of bullatalicin [**1**]. The homogeneity of the reported spectral data of **4** and **6**, as well as that of their tetraacetates (5,6), suggested that they possess the same structure. Finally, remeasurements

TABLE 2. Reassignments of nmr Data[a] of *cis*- and *trans*-Bullatalicinone [**2**] and Their Triacetates.

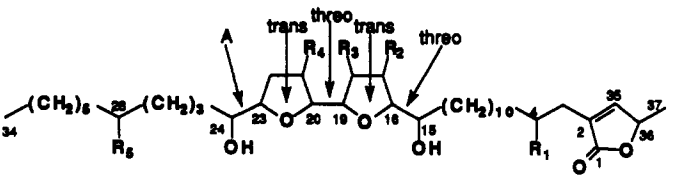
Position	Compound			
	^{1}H			^{13}C
	2[b]	**2**[c]	**2**-triacetate[b]	**2**[b]
1	—	—	—	cis 178.17
	—	—	—	trans 178.69
2 cis	3.02 m	2.61 m	3.02 m	cis 43.73
trans	3.03 m	2.71 dddd (9.43,9.37,9.33, 3.48)	3.028 m	trans 44.18
3a cis	1.48 m	0.90 m	1.50 m	35.52–25.12
trans	2.23 dddd (12.9,9.6,3.4)	1.71 m	1.96 m	
3b cis	2.61 dddd (12.3,9.4,5.6)	2.00 m	2.607 ddd	
trans	1.99 m	1.35 m	2.25 m	
4 cis	4.39 dddd (10.7,7.4,5.4,5.4)	3.71 m	4.39 dddd (10.56,7.28,5.58, 5.36)	cis 79.29
trans	4.54 dddd (8.3,8.2,5.7,3.2)	4.06 dddd (8.05,8.05,4.96, 3.48)	4.546 dddd (8.2,8.2,3.37, 2.93)	trans 78.85
5–11	1.68–1.21	1.70–1.20 m	2.00–1.20	35.55–25.14
12	3.87 m	3.79 m	3.86 m	79.22
13,14	1.99–1.35	1.70–1.20	2.00–1.20	35.55–25.14
15	3.81 m	3.82 m	3.96 m	81.98[d]
16	3.41 m	3.44 ddd	4.83 m	74.49[e]
17,18	1.68–1.35	1.85–1.60	2.00–1.20	35.55–25.14
19	3.41 m	3.38 ddd	4.83 m	74.39[e]
20	3.81 m	3.71 ddd	3.96 m	82.18[d]
21	1.99, 1.62	1.63, 1.44	2.00–1.20	35.55–25.14
22	1.90, 1.86	1.77, 1.52	2.00–1.20	35.55–25.14
23	3.87 m	3.62 m	3.96 m	83.28[d]
24	3.87 m	3.71 ddd	4.91 ddd	71.42[e]
25	1.68–1.35	1.85–1.20	2.00–1.20	35.55–25.14
26-33	1.40–1.21	1.70–1.20	2.00–1.20	35.55–25.14, 22.65
34	0.88 t (7.0)	0.91 t (6.96)	0.88 t (6.97)	14.10
35a cis	2.61 dd (15.3,8.6)	1.89 m	2.61 dd	35.55–25.14
trans	2.67 dd (19.9,10.6)	1.88 m	2.67	
35b cis	3.11 dd (18.5,3.4)	2.63 dd (19.14,3.5)	3.11 dd (18.5,3.48)	
trans	3.05 dd (18.2, 3.4)	2.52 dd (18.4,3.5)	3.05	
36	—	—	—	205.47
				205.41
37	2.20 s	1.53 s (cis) 1.55 s (trans)	2.200 s	36.66
16-OAc ...	—	—	2.08 s	—
19-OAc ...	—	—	2.09 s	—
24-OAc ...	—	—	2.05 s	—

[a] ^{1}H nmr (500 MHz, J in Hz); ^{13}C nmr (125 MHz).

[b] In $CDCl_3$.

[c] In C_6D_6.

[d,e] Assignments may be interchangeable within each group.

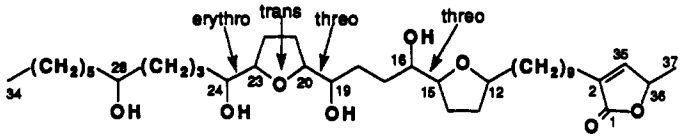


	R_1	R_2	R_3	R_4	R_5	A
3	OH	OH	H	H	H	erythro
4	OH	OH	H	H	H	threo
5	H	OH	H	H	OH	erythro
8	H	H	OH	OH	H	erythro

of the ^{1}H- and ^{13}C-nmr and ms spectra of **4** gave nmr and ms data identical to those of **6**. The eims spectrum of the tetra-TMSi derivative of **4** was also identical to that of the tetra-TMSi derivative of **6**. Based on the virtual identity of samples **4** and **6**, we concluded that annonin IV [**4**] is equivalent to bullatanocin [**6**].

The published nmr and ms data of annonin XVI [**5**], illustrated as previously reported (5), indicated that it possessed the same structural skeleton as annonins VIII [**3**] and IV [**4**] but with a 28-OH moiety instead of the 4-OH group. Comparison of all the spectral data of **5** and its tetraacetate with those of reported non-adjacent bis-THF acetogenins suggested that **5** was identical to squamostatin A [**7**] (7). The major diagnostic eims data of **5** and its tetra-TMSi derivative were analyzed and assigned as illustrated in Figure 1. Although a sample of **7** was not available for direct comparison, the spectral identities permitted the conclusion that annonin XVI [**5**] is identical to squamostatin A [**7**]. Evaluation of the ^{1}H-nmr data of the acetate derivatives of both **3** and **5** indicated that the relative stereochemistries of squamostatin A [**7**] between C-15/C-16, C-19/C-20, and C-23/C-24 should be threo, threo, and erythro; this structural refinement is new. Compound **5** was tested for its cytotoxicities against three human tumor cell lines, and it showed selective cytotoxicity to human lung carcinoma (A-549 $ED_{50}<10^{-8}$ μg/ml) and was less cytotoxic to human breast carcinoma (MCF-7 ED_{50} 1.46 μg.ml) and human colon adenocarcinoma (HT-29 ED_{50} 1.42 μg.ml).

The structure of annonin XIV [**8**] (5) also requires revision. The reported carbon



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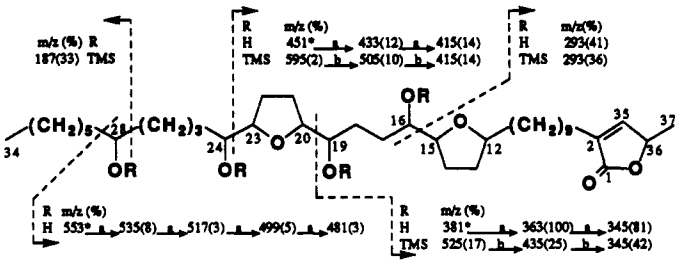

FIGURE 1. Diagnostic eims fragment ions of annonin XVI [**5**] and its tetra-TMSi derivative; the peaks with an asterisk were not seen, and letters above the arrows represent: (a) loss of H_2O (*m/z* 18) and (b) loss of TMSOH (*m/z* 90).

resonances of C-19 and C-20 would be more downfield than δ ca. 82 if there were OH groups at C-18 and C-21. The published nmr data indicate that **8** likely possesses the skeleton of an adjacent bis-THF acetogenin without a 4-OH moiety, although the correct structure of **8** could not be completely suggested due to lack of a sample for remeasurement of its ms fragments.

EXPERIMENTAL

INSTRUMENTATION.—Nmr spectra were obtained on a Varian VXR-500S spectrometer. Ms data were measured on a Finnigan 4000 spectrometer and a Kratos MS50 spectrometer.

SAMPLE SOURCES.—Bullatalicin [**1**] and bullatalicinone [**2**] were isolated from the bark of *Annona bullata* Rich. (3,4). Annonins VIII [**3**], IV [**4**], and XVI [**5**] were isolated from the seeds of *Annona squamosa* L. (5). Compounds **1** and **3** as well as **2** and **4** were indistinguishable in tlc systems on Si gel developed with $CHCl_3$-MeOH (9:1), hexane-EtOAc-MeOH (6:3:1), CH_2Cl_2-Me_2CO (8:2), and $CHCl_3$-Me_2CO (8.5:1.5) and visualized with phosphomolybdate spray and heat.

BIOASSAYS.—The cytotoxicity tests against A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), and HT-29 (human adenocarcinoma) cells were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols with adriamycin as a positive standard control (15). Adriamycin showed A-549 ED_{50} 2.08×10^{-4} μg/ml, MCF-7 ED_{50} 9.13×10^{-2} μg/ml, and HT-29 ED_{50} 1.30×10^{-3} μg/ml in the same run with annonin XVI [**5**].

RELAYED COSY.—The single- and double-relayed COSY spectra of bullatalicin [**1**] and bullatalicinone [**2**] (in C_6D_6) were obtained by using a relayed COSY program on the Varian VXR-500S spectrometer with 16 scans, 128 increments, 2K data points, 2K×2K data transfer, and 60 msec tau value.

TMSI DERIVATIZATION OF ANNONINS VIII [**3**], IV [**4**], AND XVI [**5**].—Small amounts of annonins VIII [**3**], IV [**4**], and XVI [**5**] (<0.2 mg) were treated with 20 ml of *N,O*-bis-(trimethylsilyl)-acetamide (BSA) and 2 ml of pyridine and heated at 70° for 30 min to yield the tetra-TMSi derivatives.

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