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NOVEL CYTOTOXIC ANNONACEOUS ACETOGENINS: (2,4-*CIS* AND *TRANS*)-BULLADECINONES FROM *ANNONA BULLATA* (ANNONACEAE)

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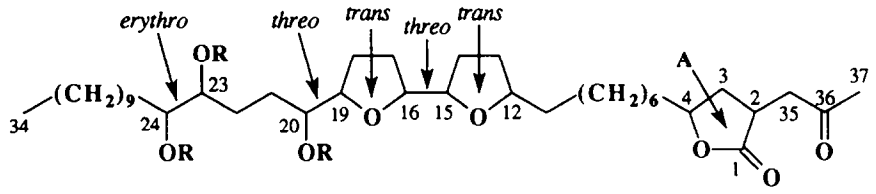
Abstract: A mixture of two novel cytotoxic adjacent bis-tetrahydrofuran (THF) ketolactone acetogenins, (2,4-*cis* and *trans*)-bulladecinones (**1** and **2**), was isolated from the bark of *Annona bullata* Rich. (Annonaceae). Adjacent bis-THF rings, located at C-12 and C-16 and having only one adjacent hydroxyl group, and the presence of an *erythro* vicinal diol in **1** and **2** are new features for the Annonaceous acetogenins.

Annona bullata Rich. is a tropical tree native to Cuba. F005, a partitioned fraction of the ethanol extract of its bark, was found to be highly toxic to brine shrimp larvae (BST).[1,2] Bullatacin and (2,4-*cis* and *trans*)-bullatacinones, which are very potent adjacent bis-tetrahydrofuran (THF) acetogenins, were reported in 1989,[1] and, to date, 31 Annonaceous acetogenins have been isolated from F005 by our group.[3] These acetogenins all show significant toxicities in the BST and, as well, are cytotoxic in human solid tumor cell lines; they explain the activity of F005 and show close relationships in their biogenesis.[3] Further BST-directed fractionation has now led us to a mixture of two novel ketolactone acetogenins named 2,4-*cis*-bulladecinone (**1**) and 2,4-*trans*-bulladecinone (**2**). The structures were determined by ^{1}H and ^{13}C NMR, COSY, NOESY, MS, and chemical derivations. Compounds **1** and **2** are the first acetogenins having two adjacent bis-THF rings located at C-12 and C-16, respectively, and having only one hydroxyl group adjacent to one side of the bis-THF rings. This suggests a new branch in the biogenetic pathway in the formation of the bis-THF rings. In addition, the *erythro* vicinal diol in **1** and **2** is a new feature to Annonaceous acetogenins; previously, only *threo* vicinal diols have been reported in this class of compounds.[4]

Like most of the previously reported ketolactone acetogenins, **1** and **2** were isolated as a mixture of *cis* and *trans* C-2/C-4 diastereomers. They are very difficult to separate, but distinctive NMR signals demonstrated



Comp	**1**	**1a**	**1b**	**1c**	**2**	**2a**	**2b**	**2c**
R	H	Ac	TMSi	*d*-TMSi	H	Ac	TMSi	*d*-TMSi
A	*cis*	*cis*	*cis*	*cis*	*trans*	*trans*	*trans*	*trans*

the presence of each component in the mixture. Their molecular weight of 638 was determined by HRFABMS of the mixture [MH^+ at m/z 639.4847 (calcd 639.4836), corresponding to the molecular formula $C_{37}H_{66}O_8$].

Adjacent bis-THF rings were indicated to exist in **1** and **2** by the signals for protons at δ 3.93 (H-12) and 3.87 (H-15, 16, 19) in the ^1H NMR spectrum (Table 1) and by the signals for carbons at δ 82.7 (C-19), 82.2 and 81.3 (C-15, 16), and 79.9 (C-12) in the ^{13}C NMR spectrum (Table 1).[4] There were three methine protons attached to hydroxylated carbons at δ 3.62, 3.58, and 3.47 in **1** and **2**, although, only the signal at δ 3.47 was observed to have correlation crosspeaks with one of the THF methine protons at δ 3.87 in the ^1H-^1H COSY spectrum. This suggested that there was only one hydroxyl group adjacent to one of the THF rings. The signals for the carbon at C-12 at δ 79.9 in the ^{13}C NMR spectrum supported this suggestion, since carbon chemical shifts at *ca.* δ 79 have been found to be characteristic for the oxygenated carbons of THF rings that lack adjacent hydroxyl groups; examples of such acetogenins are gigantecin, bullatalicin, and gigantetrocin.[4] This feature was confirmed by the EIMS fragmentation analysis of the TMSi (**1b** and **2b**) and perdeutero-TMSi (*d*-TMSi, **1c** and **2c**) derivatives, which also placed the bis-THF rings at C-12 and C-16, respectively (Figure 1).

The relative stereochemistry around the bis-THF rings was determined by comparing the ^1H and ^{13}C NMR signals of **1** and **2** and the acetates (**1a** and **2a**) with those of model compounds of known relative stereochemistry.[5,6,7] The comparison suggested that the relative stereochemistry of C-19/20 was *threo*. The proton signal for H-20 at δ 3.47 in **1** and **2** was shifted slightly downfield (normally *ca.* 3.39-3.41) compared with those of most other acetogenins having *threo* relative stereochemistries in such a moiety; this shift may be

Table 1. ^1H NMR of **1** and **2**, **1a** and **2a**, and **1d** and **2d**, and ^{13}C NMR Data of **1** and **2** ($CDCl_3$, δ).

	^1H NMR (500 MHz)						^{13}C NMR (125 MHz)	
	1	**2**	**1a**	**2a**	**1d**	**2d**	**1**	**2**
1	-	-	-	-	-	-	178.2	178.8
2	3.02 m	3.03 m	3.02 m	3.03 m	3.02 m	3.03 m	43.8	44.3
3a	2.61 dddd	2.23 m	2.61 dddd	2.23 m	2.61 dddd	2.23 m	34.5	35.4
3b	1.48 m	1.97 m	1.48 m	1.97 m	1.48 m	1.97 m		
4	4.39 dddd	4.55 dddd	4.39 dddd	4.55 dddd	4.39 dddd	4.54 dddd	79.4	78.9
5a	1.74 m	1.69 m	1.74 m	1.69 m	1.74 m	1.69 m	36.7	36.7
5b	1.57 m	1.60 m	1.57 m	1.60 m	1.57 m	1.60 m		
6 - 11	1.70 - 1.22	1.70 - 1.22	1.70 - 1.22	1.70 - 1.22	1.80 - 1.22	1.80 - 1.22	35.7 - 26.1	35.7 - 26.1
12	3.93 m	3.93 m	3.88	3.88	3.94	3.94	79.9	79.9
13a, b	2.02, 1.47	2.02, 1.47	2.00, 1.44	2.00, 1.44	2.00, 1.46	2.00, 1.46	29.3 - 28.4	29.3 - 28.4
14a,17a,18a	1.98	1.98,	1.92	1.92,	1.98,	1.98	29.3 - 28.4	29.3 - 28.4
14b,17b,18b	1.62	1.62	1.69	1.69	1.62	1.62	29.3 - 28.4	29.3 - 28.4
15, 16	3.87 m	3.87 m	3.90	3.90	3.88	3.88	81.3, 82.2	81.3, 82.2
19	3.87 m	3.87 m	4.02	4.02	3.83	3.83	82.7	82.7
20	3.47 m	3.47 m	4.86	4.86	3.40	3.40	74.1	74.1
21	1.54	1.54	1.57	1.57	1.39	1.39	35.7 - 31.7	35.7 - 31.7
22	1.60	1.60	1.57	1.57	1.49	1.49	35.7 - 31.7	35.7 - 31.7
23	3.58 m	3.58 m	4.92	4.92	4.00	4.00	74.4	74.4
24	3.62 m	3.62 m	4.97	4.97	4.03	4.03	74.7	74.7
25	1.45	1.45	1.50	1.50	1.49	1.49	27.1 - 26.1	27.1 - 26.1
26 - 33	1.70 - 1.22	1.70 - 1.22	1.70 - 1.22	1.70 - 1.22	1.80 - 1.22	1.80 - 1.22	31.9 - 22.7	31.9 - 22.7
34	0.88 t	0.88 t	0.88 t	0.88 t	0.88 t	0.88 t	14.2	14.2
35a	2.61 dd	2.67 dd	2.61 dd	2.67 dd	2.61 dd	2.67 dd	35.5	35.6
35b	3.10 m	3.06 m	3.10 m	3.06 m	3.10 m	3.06 m		
36	-	-	-	-	-	-	205.53	205.46
37	2.20 s	2.20 s	2.20 s	2.20 s	2.20 s	2.20 s	25.3	25.3
20-OAc	-	-	2.09 s	2.09 s	-	-	-	-
23-OAc	-	-	2.04 s	2.04 s	-	-	-	-
24-OAc	-	-	2.04 s	2.04 s	-	-	-	-
acetonyl methyls	-	-	-	-	1.43 s 1.33 s	1.43 s 1.33 s	-	-

caused by the effect of the hydroxyl group located only two carbons away at C-23; however, the proton signal at H-20 was much higher upfield when compared with the protons on the hydroxylated carbons having *erythro* relationships with the THF ring (at *ca.* δ 3.85), such as in bullatacin, squamocin and bullatalicin.[4] The carbon signal for C-20 at δ 74.1 in **1** and **2**, rather than at *ca.* δ 71 (for those with *erythro* relative stereochemistries),[6,7] confirmed this conclusion.[5] The signals for the protons of the acetyl methyl of C-20 at δ 2.09 and H-20 at δ 4.86 in **1a** and **2a** also supported the *threo* assignment.[5] The relative stereochemistry between C-15 and C-16 was determined as *threo* by the ^{1}H NMR signals for H-15 and H-16 at δ 3.87 in **1** and **2** and at δ 3.90 in **1a** and **2a**. So far, only one acetogenin, trilobacin, has been found having an *erythro* relationship between two THF rings; the signals for the adjacent protons of the two rings were at δ 4.01 and 3.93, respectively.[8] The relative stereochemistries of both THF rings were suggested as *trans* by the ^{1}H NMR and ^{13}C NMR signals for the protons attached to the oxygenated carbons of the THF rings in **1** and **2** and **1a** and **2a** as well as the ^{1}H NMR resonances for the methylene protons of the THF rings.[5-7] The methylene protons of H-13a, 14a, 17a, and 18a were at δ 2.02-1.98, shifted downfield, and those of H-13b, 14b, 17b, and 18b were at δ 1.62-1.47, shifted upfield, compared with those of the model compounds having *cis* configurations across the THF ring (at *ca.* δ 1.95-1.94 and 1.80-1.75, respectively).[7] Thus, the relative stereochemistries around the THF rings from C-12 to C-20 were concluded to be *trans/threo/trans/threo*.

Three successive losses of H_2O (m/z 18) from the MH^+ in both the CIMS and FABMS suggested the existence of three hydroxyl groups in **1** and **2**. This was confirmed by the preparation of the triacetates (**1a** and **2a**). **1a** and **2a** gave two singlet proton peaks at δ 2.09 (3H) and 2.04 (6H). From the discussion above, one of the hydroxyl groups was concluded to be adjacent to one of the THF rings. In the ^{1}H NMR spectrum of **1** and **2**, the other two methine protons on the hydroxylated carbons were overlapped at *ca.* δ 3.60. In the ^{1}H-^{1}H COSY spectrum, one of the methine protons had crosspeaks with methylene protons at *ca.* δ 1.60 (H-22), and the other had correlation crosspeaks with methylene protons at *ca.* δ 1.45 (H-25). When irradiating at δ 1.60 or 1.45 in 1D ^{1}H NMR experiments, the two methine proton signals split into two groups of peaks at δ 3.62 and 3.58, respectively. The methine proton at δ 3.62 became a doublet (J = 3.3 Hz) when irradiating at δ 1.45, while the proton at δ 3.58 remained as multiple peaks. Conversely, the proton at δ 3.58 became a doublet (J = 3.3 Hz) when irradiating at δ 1.60, while the proton at δ 3.62 remained as a multiplet. This coupling pattern could happen only if a vicinal diol were present. The ^{13}C NMR signals for these two oxygenated carbons at δ

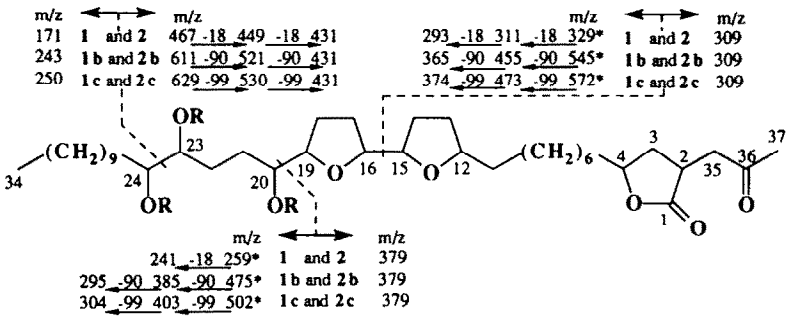


Figure 1. Diagnostic EIMS fragment ions of (2,4-*cis* and *trans*)-bulladecinones (**1** and **2**, R=H), their tri-TMSi derivatives (**1b** and **2b**, R=TMSi), and tri-perdeutero-TMSi derivatives (**1c** and **2c**, R=*d*-TMSi). Ions indicated with an asterisk (*) were not observed.

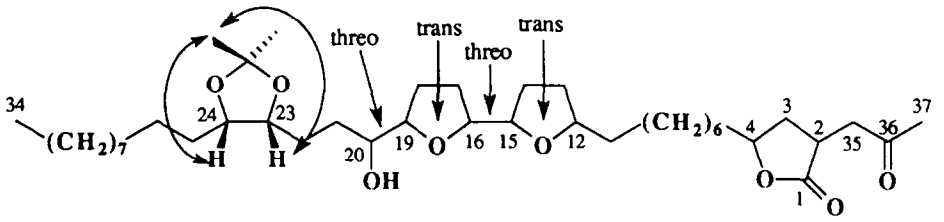


Figure 2. NOE effects in the acetonide derivatives of (2,4-*cis* and *trans*)-bulladecinones (**1d** and **2d**).

74.7 and 74.4 strongly supported this conclusion, as the carbons having a single isolated hydroxyl group are always at *ca.* δ 71 in other acetogenins.[4] The placement of this vicinal diol at C-23 and C-24 was made by EIMS fragmentation analysis of the tri-TMSi (**1b** and **2b**) and tri-*d*-TMSi (**1c** and **2c**) derivatives of **1** and **2** (Figure 1). This placement was confirmed by running double relayed COSY of **1** and **2**, which showed the correlation cross peaks between H-23 and H-20. To determine the relative configuration of C-23/24, the acetonide derivatives of **1** and **2** (**1d** and **2d**) were prepared (Figure 2).[9] The ^{1}H NMR signals for H-23 and H-24 of **1d** and **2d** at δ 4.03 and 4.00 and the signals for the acetonyl methyls, showing two separate singlet peaks at δ 1.43 and 1.33, respectively, suggested the *cis* configuration for the dioxolane ring (Figure 2).[9] The NOESY spectrum of **1d** and **2d** also suggested the *cis* assignment for the dioxolane ring, as one of the acetonyl methyls at δ 1.33 showed NOESY cross peaks with both of the methine protons (H-23, 24), while the other acetonyl methyl at δ 1.43 showed no cross peak with either H-23 or H-24. Thus, the configuration of the diol was determined to be *erythro*, since the *cis* configuration of C-23/24 in **1d** and **2d** could be derived only from a vicinal diol with an *erythro* configuration. Only *threo* vicinal diols have been previously found in the Annonaceous acetogenins,[4] and **1** and **2** are the first of these compounds having an *erythro* vicinal diol moiety in their structures. In Table 2 are summarized the proton chemical shifts for the *threo* and *erythro* diol group, that are now known to occur in Annonaceous acetogenins, and for their acetate and acetonide derivatives.

In the ^{1}H NMR spectrum of the mixture of **1** and **2**, H-2, H-3_a, H-3_b, H-4, H-35_a, and H-35_b showed double signals (Table 1) with unequal intensities. By comparisons with ^{1}H NMR signals of *cis* and *trans* substituted 2-acetonyl-4-butyl-γ-butyrolactone, Hoye and Hanson[10] found that these two groups of chemical shifts belong to C-2/4 *cis* and *trans* diastereomers, respectively. Thus, it is possible to determine the separate structures in such a mixture. The results of these comparisons indicated that **1**, in higher concentration, was the C-2/4 *cis* diastereomer, and **2**, in lower concentration, was the C-2/4 *trans* diastereomer. Thus, the structures of **1** and **2** were concluded to be as illustrated and were named (2,4-*cis*)-bulladecinone and (2,4-*trans*)-bulladecinone, respectively.

Table 2. The ^{1}H NMR (500 MHz) Signals for the Protons of *threo*[a] and *erythro* Diols in Annonaceous Acetogenins and their Acetate and Acetonide Derivatives ($CDCl_3$, δ).

	Methine Protons		Acetyl Methyls		Acetonyl Methyls	
	threo	*erythro*	*threo*	*erythro*	*threo*	*erythro*
Diols	3.45 (2H)	3.62, 3.58	-	-	-	-
Acetates	5.00 (2H)	4.97, 4.92	2.06, 2.05	2.04 (6H)	-	-
Acetonides	3.58 (2H)	4.03, 4.00	-	-	1.37 (6H)	1.43, 1.33

a) Data from Fang *et al.*[11]

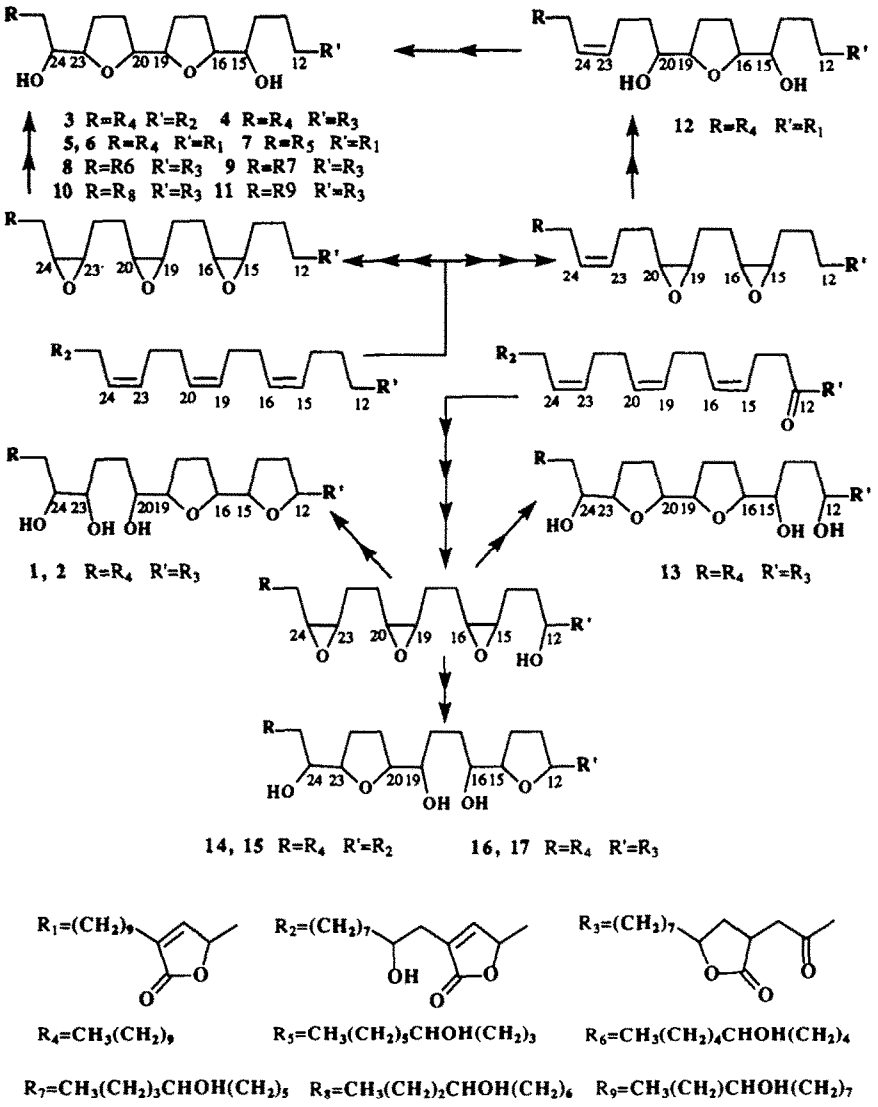


Figure 3. Hypothesis for the biogenesis of the tetrahydrofuran rings of the C_{37} Annonaceous acetogenins from *Annona bullata*. **1** and **2**: (2,4-*cis* and *trans*)-bulladecinones, **3**: bullatacin,[1] **4**: (2,4-*cis* and *trans*)-bullatacinones,[1] **5**: 4-deoxyasimicin,[3c] **6**: desacetyluvaricin,[3d] **7**: squamocin,[3c] **8**: (2,4-*cis* and *trans*)-29-hydroxybullatacinones,[3g] **9**: (2,4-*cis* and *trans*)-30-hydroxybullatacinones,[3e] **10**: (2,4-*cis* and *trans*)-31-hydroxybullatacinones,[3e] **11**: (2,4-*cis* and *trans*)-29-hydroxybullatacinones,[3e] **12**: bullatecin,[3c] **13**: (2,4-*cis* and *trans*)-12-hydroxybullatacinones,[3g] **14**: bullatalicin,[3a] **15**: bullatanocin,[3d] **16**: (2,4-*cis* and *trans*)-bullatalicinones,[3b] **17**: (2,4-*cis* and *trans*)-bullatanocinones.[3d]

The mixture of the new isolates (**1** and **2**) was very active in the BST,[2] and, as well, was significantly cytotoxic against human solid tumor cell lines in culture (Table 3).[12] Slight selectivity, with activity ten times greater than that of adriamycin, was exhibited for the lung cell line (A-549).

Table 3. Brine Shrimp Lethality and Cytotoxicities in Solid Tumor Cell Lines in **1** and **2**.

Compound	BST[a] LC_{50}(μg/ml)	A-549[b] ED_{50} (μg/ml)	MCF-7[c] ED_{50} (μg/ml)	HT-29[d] ED_{50} (μg/ml)
1 and **2**	1.37×10^{-1}	3.37×10^{-5}	1.07×10^{-3}	2.29×10^{-1}
Adriamycin[e]	8×10^{-2}	1.53×10^{-4}	1.38×10^{-2}	5.58×10^{-4}

a) Brine shrimp lethality test; b) Human lung carcinoma; c) Human breast carcinoma; d) Human colon adenocarcinoma; e) Positive control standard.

So far, 33 Annonaceous acetogenins have been identified from *A. bullata*. These natural products all show close structural relationships suggesting similar biogenesis. In Figure 3 are illustrated some possible biogenetic pathways for the formation of the THF rings leading to most of the C_{37} acetogenins from this plant. **1** and **2** suggest a new branch of the pathway, although they may have the same precursor as the non-adjacent bis-THF compounds such as bullatalicin and the 12-hydroxybullatacinones which are also oxygenated at C-12. The co-occurrence of these closely related structures strongly supports their formation through such polyketide precursors (Figure 3).

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