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New Cytotoxic Annonaceous Acetogenins: Bullatanocin and cis- and trans-Bullatanocinone, from *Annona bullata* (Annonaceae)

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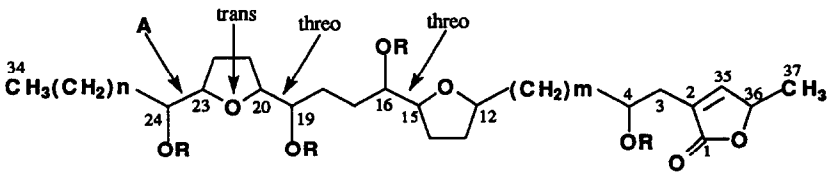
ABSTRACT Further bioactivity-directed fractionation of the ethanol extract of the bark of *Annona bullata* Rich (Annonaceae) has led to the isolation of the new nonadjacent bis-tetrahydrofuran acetogenins, bullatanocin (**1**), cis-bullatanocinone (**2**) and trans-bullatanocinone (**3**) A known adjacent bis-tetrahydrofuran acetogenin, desacetyluvaricin (**4**), which is new to this species, was also isolated Brine shrimp lethality test (BST) data and cytotoxicities against human solid tumor cell lines of **1-4** were compared with those of the diastereoisomers, bullatalicin (**5**), cis- and trans-bullatalicinone (**6** and **7**) and 4-deoxyasimicin (**8**) **1** shows cytotoxic potencies 10,000 times those of adriamycin in the lung and colon cancer cell lines

INTRODUCTION

Annona bullata Rich (Annonaceae) is a tropical tree native to Cuba In screening for bioactivities, the EtOH extract of the bark showed potent lethality to brine shrimp larvae and inhibited crown gall tumors on potato discs [1,2] By activity-directed fractionation and isolation, the Annonaceous acetogenins, bullatacin, bullatacinone,[2] bullatalicin,[3] bullatalicinone,[4] squamocin,[4] isodesacetyluvaricin[5] (syn 4-deoxyasimicin[6]), bullatencin[6] and a mixture of uvariamicins I-IV,[6] were isolated in our prior studies Further fractionation has now led to the identification of the new nonadjacent bis-tetrahydrofuran (THF) acetogenins, bullatanocin (**1**), cis-bullatanocinone (**2**) and trans-bullatanocinone (**3**), as well as a known adjacent bis-THF acetogenin, desacetyluvaricin (**4**) Bioactivities in the brine shrimp lethality test (BST) and against human solid tumor cells were determined and compared with bullatalicin (**5**), cis- and trans-bullatalicinone (**6** and **7**) and 4-deoxyasimicin (**8**), which are diastereomers of **1-4**

RESULTS AND DISCUSSION

Compound **1** was obtained as a whitish wax (chloroform) or white powder (hexane-chloroform), $[\alpha]^{22}_D$ = +14 4° (c 0 55, in $CHCl_3$) The molecular weight of **1** was suggested to be 638 by CIMS (isobutane) at m/z 639 (MH^+) HRCIMS (isobutane) gave m/z 639 4829 for MH^+ (calcd 639 4836) corresponding to the molecular formula $C_{37}H_{66}O_8$ Spectral characteristics of **1** and its derivatives, including ^1H NMR (Table 1), ^{13}C NMR (Table 2) and MS (Figure 1) data, suggested that **1** belongs to the relatively rare class of bioactive nonadjacent bis-tetrahydrofuran (THF) acetogenins This class of acetogenins includes bullatalicin (**5**),[3] cis- and trans-bullatalicinone (**6** and **7**),[4] gigantecin (**9**),[7] sylvaticin[8] and 4-deoxygigantecin [9]



Comp	1	1a	1b	1c	5[10]	5a	9
R	H	Ac	TMS	d-TMS	H	Ac	H
A	threo	threo	threo	threo	erythro	erythro	threo
n	9	9	9	9	9	9	11
m	7	7	7	7	7	7	5

The IR spectrum of **1** contained a prominent absorption peak at 3441 cm^{-1}, this peak and sequential losses of four molecules of H_2O from the MH^+ in CIMS indicated that **1** has four hydroxyl groups These were confirmed by the preparation of an acetyl derivative (**1a**). **1a** gave ^1H NMR peaks at δ 2 09 (3H, OAc), 2.07 (6H, 2-OAc), and 2 03 (3H, OAc), and two multiple proton resonances at δ 4 83 (3H) and 5 10 (1H) corresponding to the downfield shifts of four protons on secondary hydroxyl-bearing carbons as compared to **1**

An IR carbonyl absorption band in **1** at 1750 cm^{-1}, a UV (MeOH) λ_{max} at 208 5 nm (log ε 3 52), six proton resonances ($CDCl_3$) at δ 7 19 (q, H-35), 5 06 (qq, H-36), 3 87 (m, H-4), 2 53 (dddd, H-3a), 2 40 (dddd, H-3b), and 1 42 (d, H-37), and six carbon resonances at δ 174 44 (C-1), 151 68 (C-35), 130 95 (C-2), 77 88 (C-36), 69 74 (C-4), and 19 07 (C-37) provided characteristic spectral features for an α,β-unsaturated γ-lactone fragment with a 4-OH [7,11] The presence of two nonadjacent THF rings was indicated by proton resonances ($CDCl_3$) at δ 3 87 (H-12) and 3 80 (H-15, 20, 23) in **1**, and at δ 3 85 (H-12) and 3 96 (H-15, 20,23) in **1a**, and carbon resonances at δ 79 21 (C-12) and 82 67, 82 65, 81 97 (C-15, 20, 23) [7,11] The

Table 1 Comparisons of ^1H-NMR(500 MHz) Data of Bullatanocin (**1**), Bullatalicin (**5**) and Their Tetra-acetates (**1a** and **5a**)

Proton	Compounds δ [ppm, (J/Hz)]					
	1		1a	5		5a
	$CDCl_3$	C_6D_6	$CDCl_3$	$CDCl_3$	C_6D_6[a]	$CDCl_3$[a]
3a	2 53 dddd	2 31 dddd	2 57 dddd	2 53 dddd	2 30 dddd	2 57 dddd
3b	2 40 dddd	2 22 ddt	2 51 dddd	2 40 dddd	2 20 ddt	2 51 ddt
4	3 87 m	3 70 m	5 10 dddd	3 87 m	3 71 m	5 10 dddd
5-11	1 71-1 21	1 86-1 22	1 63-1 21	1 71-1 21	1 68-1 29	1 80-1 29
12	3 87 m	3 81 m	3 85 m	3 87 m	3 80 m	3 86 m
13-14	1 99-1 37	1 86-1 22	1 99-1 21	1 99-1 37	1 68-1 47	2 00-1 20
15	3 80 m	3 81m	3 96 m	3 80 m	3 82 m	3 97 m
16	3 41 m	3 43 m	4 83 m	3 41m	3 44 t	4 82 m
17-18	1 71-1 37	1 86, 1 62	1 63-1 44	1 71-1 37	1 85, 1 60	1 80-1 50
19	3 41 m	3 35m	4 83 m	3 41 m	3 39 t	4 82 m
20	3 80 m	3 70m	3 96 m	3 80 m	3 71 m	3 97 m
21	1 99-1 37	1 86-1 22	1 99-1 21	1 99-1 37	1 63, 1 44	2 00-1 20
22	1 99-1 37	1 86-1 22	1 99-1 21	1 90, 1 86	1 77, 1 53	2 00-1 20
23	3 80 m	3 70 m	3 96 m	3 87 m	3 64 m	3 97 m
24	3 41 m	3 43 m	4 83 m	3 87 m	3 74 m	4 91 ddd
25-33	1 71-1 21	1 86-1 22	1 63-1 21	1 71-1 21	1 38-1 29	1 60-1 29
34	0 89 t (7 0)	0 91 t (7 5)	0 89 t (7 0)	0 89 t (7 0)	0 91 t (7 1)	0 88 t (7 2)
35	7 19 q (1 5)	6 26 m	7 08 q (1 5)	7 19 q (1 5)	6 24 d (1 3)	7 09 d (1 6)
36	5 06 qq (6 9, 1 5)	4 25 qq (6 8, 1 2)	5 01 qq (7 0, 1 6)	5 06 qq (6 9, 1 5)	4 24 qq (6 7, 1 3)	5 01 qq (6 9, 1 6)
37	1 42 d (6 9)	0 81 d (6 5)	1 40 d (7 0)	1 42 d (6 9)	0 81 d (6 8)	1 40 d (6 9)
4-OAc	-	-	2 03s	-	-	2 03s
16-OAc	-	-	2 07s	-	-	2 08s
19-OAc	-	-	2 09s	-	-	2 09s
24-OAc	-	-	2 07s	-	-	2 05s

a data from Hui *et al* [3], with assignments as revised in a separate paper [10]

^{13}CNMR of **1** showed three more secondary hydroxy-bearing carbons at δ 74 33, 74 21 and 74 00, with corresponding ^{1}H NMR ($CDCl_3$) resonances all at δ 3 41 These data are characteristic of protons on secondary hydroxy-bearing carbons adjacent to a THF ring as are found in most Annonaceous acetogenins [11] Two of these hydroxyl groups were assigned as adjacent to one THF ring, and one was assigned as adjacent to the other ring. The upfield shift of C-12 to δ 79 21 indicated that there was no hydroxyl group adjacent to one side of one of the THF rings.[7] ^{1}H-^{1}H COSY spectra (C_6D_6) further confirmed the placement of the hydroxyl groups, showing cross peaks between H-15/H-16, H-19/H-20, and H-23/H-24

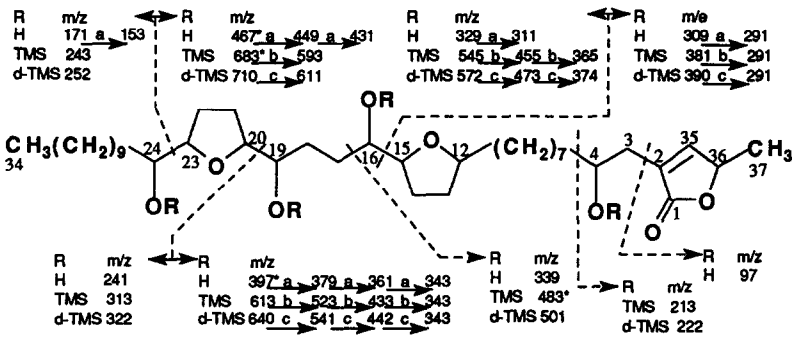


Figure 1 Diagnostic EIMS fragment ions of bullatanocin (**1**, **R=H**), tetra-trimethylsilyl derivative (**1b**, **R=TMS**), and tetra-perdeutero-trimethylsilyl derivative (**1c**, **R=d-TMS**) Peaks with an asterisk were not seen Letters above the arrows represent (a) loss of H_2O (m/z 18), (b) loss of TMSOH (m/z 90), and (c) loss of d-TMSOH (m/z 99)

The carbon skeleton and placement of the two THF rings of **1** were determined based on the EIMS analysis of **1** and its TMS (**1b**) and d-TMS derivatives (**1c**) Fragments in their EIMS (Figure 1) clearly located the THF rings at C-12 and C-20 along the hydrocarbon chain and also supported the placement of the four hydroxyl groups at C-4, C-16, C-19, and C-24, as suggested by the NMR data

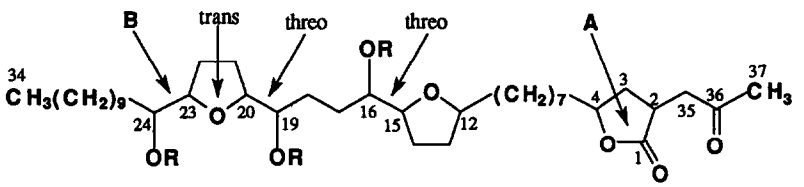
The relative stereochemistries within the carbon centers C-15/C-16, C-19/C-20 and C-23/C-24 were determined by comparing the ^{13}C NMR signals of **1** for the hydroxylated carbons C-16, C-19 and C-24 at δ 74 33, 74 21 and 74 00, respectively, as well as the ^{1}H NMR ($CDCl_3$) signals of **1** at δ 3 41 (H-15, 19, 24) and δ 3 80 (H-15, 19, 23), with those of model compounds of known relative stereochemistry [12] The comparisons showed that the relative configurations between the above couples of adjacent chiral carbons were all of the threo type By comparing with those of a group of diacetyl dibutylated bis-THFs of known relative stereochemistry,[13] the ^{1}H NMR signals of **1a** at δ 3 96 (H-15, 20, 23) and 4 83 (H-16, 19, 24) further substantiated these threo assignments, and the ^{1}H NMR signals of **1a** at δ 3 96 for H-20 and H-23 indicated the trans configuration of the substitution pattern of the C-20 to C-23 THF ring The configurations of the chiral centers at C-4, C-12 and C-36 remain undefined The structure of compound **1** was concluded to be as illustrated and was named bullatanocin

All of the spectral data of bullatanocin (**1**) were highly similar to those of both bullatalicin (**5**)[4] and giganteçin (**9**)[7] The difference between **1** and giganteçin (**9**) is that the two nonadjacent THF rings of **9** are at C-10 and C-18, respectively,[7] while they are at C-12 and C-20 in **1**.

Table 2 Comparisons of ^{13}C-NMR (125 MHz) Data of Bullatanocin (**1**), cis- and trans-Bullatanocinone(**2** and **3**), and Bullatalicin (**5**) ($CDCl_3$)

	Compounds δ [ppm, (J/Hz)]			
	1	**2**	**3**	**5**
1	174 44	178 21	178 73	174 48
2	130 95	43 6	44 13	131 08
3	37 31-25 52	35 52-25 12	35 52-25 12	37 40-25 20
4	69 74	79 26	78 81	69 91
5-11	37 31-25 52	35 52-25 12	35 52-25 12	37 40-25 20
12	79 21	79 20	79 20	79 28
13-14, 21-22	37 31-25 52	35 52-25 12	35 52-25 12	37 40-25 20
15, 20, 23	82 67, 82 65, 81 97	82 68, 82 57, 81 98	82 68, 82.57, 81 98	83.30, 82 17, 81 97
16, 19	74 33, 74 21	74.34, 74 19	74.34, 74 19	74 55, 74 42
17-18	37 31-25 52	35 52-25 12	35 52-25 12	37 40-25 20
24	74 00	73 98	73 98	71 46
25-32	37 31-25 52	35 52-25 12	35 52-25 12	37 40-25 20
33	22 64	22 60	22 60	22 72
34	14 10	14 05	14 05	14 17
35	151 68	35 52-25 12	35 52-25 12	151 70
36	77 88	205 52	205 46	77 95
37	19 07	36 60	36 60	19 16

Bullatanocin (**1**) and bullatalicin (**5**)[3] differ only in their stereochemistry ^{1}H and ^{13}C NMR assignments of **1** and **5** are compared in Tables 1 and 2 In bullatalicin (**5**), the ^{13}C NMR signals at δ 71 46, 74 42 and 74 55, with the corresponding ^{1}H NMR ($CDCl_3$) signals at δ 3 87 (1H), 3 41 (2H), indicated that one of the relative configurations between C-15/C-16, C-19/C-20 and C-23/C-24 was erythro, while the other two were threo [3,10,12] In **1**, as discussed above, all three assignments are threo



Comp	2	2a	2b	2c	3	3a	3b	3c	6[10]	6a	7[10]	7a
R	H	Ac	TMS	d-TMS	H	Ac	TMS	d-TMS	H	Ac	H	Ac
A	cis	cis	cis	cis	trans	trans	trans	trans	cis	cis	trans	trans
B	threo	threo	threo	threo	threo	threo	threo	threo	erythro	erythro	erythro	erythro

Compounds **2** and **3** were first obtained in a mixture. **2** and **3** showed a MH^+ ion at m/z 639 in CIMS (isobutane) indicating a molecular weight of 638 This was confirmed by HRCIMS (MH^+, 639 4825, calcd for $C_{37}H_{67}O_8$), suggesting that **2** and **3** are isomers of **1** and of cis- and trans-bullatalicinone (**6** and **7**)[4,10] The comparisons of the spectral characteristics of the mixture of **2** and **3**, and their derivatives, including ^{1}H and ^{13}C NMR (Table 2 and 3) and MS (Figure 2) data, with those of **1** and cis- and trans-bullatalicinone (**6** and **7**)[4,10] suggested that **2** and **3** are nonadjacent bis-THF ketolactone acetogenins with the same skeleton and relative stereochemistries in the C-5 to C-34 region as in **1**

The characteristic shifts in the ^{1}H NMR at δ 4 54 and 4 39 indicated that **2** and **3** are a mixture of C2/C4 diastereomers, according to the methods of Hoye and Hanson [14] So far, all of the other bis-THF ketolactone acetogenins, including bullatacinone,[2] rollinone[15] and bullatalicinone[4], were isolated and published as cis and trans mixtures Repeated open and flash column chromatography, eluted with several different solvent systems, failed to separate **2** and **3**. They were finally separated on silica gel HPLC eluted with $CHCl_3$-MeOH (99 1)

Table 3 ^{1}H-NMR (500 MHz) Data of cis-Bullatanocinone (2), trans- Bullatanocinone (3) and their Triacetates (2a and 3a)

Proton	Compounds δ [ppm, (J/Hz)]					
	2		2a	3		3a
	$CDCl_3$	C_6D_6	$CDCl_3$	$CDCl_3$	C_6D_6	$CDCl_3$
2	3 02 m	2 62 dddd (8 2, 8 2, 8 2, 3 3)	3 02 m	3 03 m	2 72 dddd (9 43, 9 36, 9 32, 3 3)	3 03
3a	1 48 m	0 91 m	1 48 m	2 23 dddd (12 9, 9 6, 3 4)	1 72 m	2 23 dddd (12 9, 9 6, 3 4)
3b	2 61 dddd (12 3, 9 4, 5 6)	2 01 ddd (12 1, 8 2, 5 6)	2 61 ddd (12 3, 9 4, 5 6)	1 99 m	1 40 m	1 99 m
4	4.39 dddd (10 7, 7 4, 5 4, 5 4)	3 70 m	4.39 dddd (10 7, 7 4, 5 4, 5 4)	4.54 dddd (8 3, 8 2, 5 7, 3 2)	4 05 dddd (9 8, 8 1, 4 7, 4 6)	4 54 dddd (8 3, 8 2, 5 7, 3 2)
5-11	1 68-1 21	1 80-1 10	1 64-1 21	1 68-1 21	1 80-1 05m	1 64-1 21
12	3 87 m	3 80 m	3 85 m	3 87 m	3 79 m	3 86 m
13-14, 21-22	1 99-1 35	1 87-1 10	2 00-1 34	1 99-1 35	1 87-1 10	2 00-1 34
15	3 80 m	3 82 m	3 96 m	3 80 m	3 82 m	3 96 m
16, 24	3 41 m	3 43 m	4 83 m	3 41 m	3 43 ddd	4 83 m
17, 18	1 68-1 35	1 80-1 10	1 64-1 34	1 68-1 35	1 80-1 10	1 64-1 34
19	3 41 m	3 35 m	4 83 m	3 41 m	3 35 ddd	4 83 m
20, 23	3 80 m	3 70 m	3 96 m	3 80 m	3 70 ddd	3 96 m
25-33	1 68-1 35	1 80-1 10	1 64-1 34	1 68-1 35	1 80-1 10	1 64-1 34
34	0 88 t (7 0)	0 91 t (7 0)	0 88 t (7 0)	0 88 t (7 0)	0 91 t (6 96)	0 88 t (6 97)
35a	2 61 dd (18 3, 9 2)	1 90 dd (18 1, 9 3)	2 61 dd (15 3, 8 6)	2 67 dd (18 5, 9 5)	1 93 dd (18 3, 9 2)	2 67 dd (19 8, 10 7)
35b	3 11 dd (18 5, 3 5)	2 64 dd (18 8, 3 5)	3 11 dd (18 5, 3 5)	3 02 dd (18 5, 3 4)	2 54 dd (18 3, 3 4)	3 05 dd (18 2, 3 4)
37	2 20 s	1 55 s	2 20 s	2 20 s	1 56 s	2 20 s
16-OAc	-	-	2 07 s	-	-	2 07 s
19-OAc	-	-	2 07 s	-	-	2 07 s
24-OAc	-	-	2 09 s	-	-	2 09 s

All of the ^{1}H NMR chemical shifts of **2** and **3** are the same except for those of H-2, 3_a, 3_b, 4, 35_a, and 35_b By comparisons with those of cis- and trans-substituted 2-acetonyl-4-butyl-γ-butyrolactone, Hoye and Hanson summarized the ^{1}H NMR chemical shifts of cis and trans C-2/C-4 diastereomers of Annonaceous ketolactone acetogenins [14] Our ^{1}H NMR data, including those of the mixture of **2** and **3** as well as those of pure **2** and **3**, exactly matched with the results of Hoye and Hanson Thus, compound **2**, with ^{1}H NMR signals at

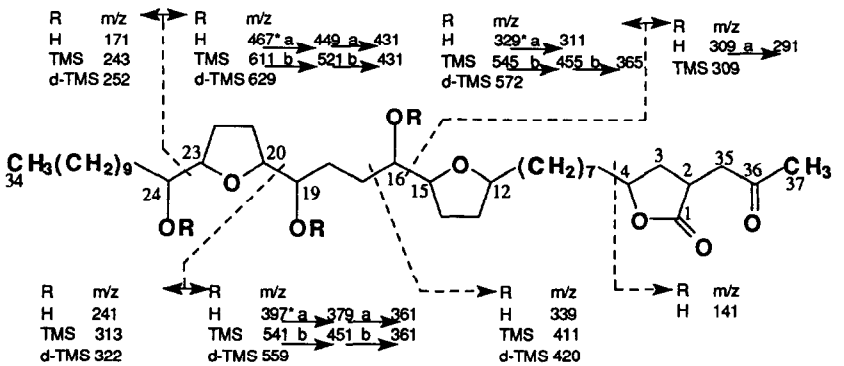
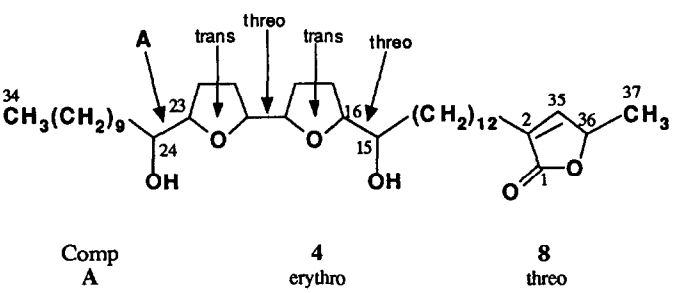


Figure 2 Diagnostic EIMS fragment ions of bullatanocinone (**2** and **3**, R=H), tetra-trimethylsilyl derivative (**2b** and **3b**, R=TMS), and tetra-perdeutero-trimethylsilyl derivative (**2c** and **3c**, R=d-TMS) Peaks with an asterisk were not seen Letters above the arrows represent (a) loss of H_2O (m/z 18), (b) loss of TMSOH (m/z 90), and (c) loss of d-TMSOH (m/z 99)

δ 3.02(H-2), 1 48(H-3_a), 2.61(H-3_b), 4 39 (H-4), 2 61 (H-35_a), and 3 11 (H-35_b) and ^{13}C NMR resonances at δ 178 21 (C-1), 43.69 (C-2), 79.26 (C-4) and 205 52 (C-36), has the cis configuration at C-2/C-4 While compound **3**, with ^{1}H NMR signals at δ 3.03 (H-2), 2 23 (H-3_a), 1 99 (H-3_b), 4 54 (H-4), 2.67 (H-35_a) and 3.05 (H-35_b) and ^{13}C NMR resonances at δ 178 73 (C-1), 44 13 (C-2), 78 81 (C-4) and 205.46 (C-36) has the trans configuration at C-2/C-4 All the above NMR data as well as the IR absorptions at 1715 cm^{-1} for a ketone and 1760 cm^{-1} for a γ-lactone also suggested the C-4 to C-37 ketolactone unit in both **2** and **3**. Thus, the structures of the compounds **2** and **3** were concluded to be as illustrated and were named cis-bullatanocinone and trans-bullatanocinone, respectively. This is the first time that the cis and trans C-2/C-4 diastereomers of Annonaceous ketolactone acetogenins have been separated and reported at the same time, and the above ^{1}H and ^{13}C NMR data also confirm the methodology of Hoye and Hanson [14]

As **1** differs from bullatalicin (**5**)[3,10], **2** and **3** differ from cis- and trans-bullatalicinone (**6** and **7**)[4,10] in the stereochemistry of C-23/C-24 which in **2** and **3** is threo, while in cis- and trans-bullatalicinone (**6** and **7**)[4,10] it is erythro The same reasoning applies as was discussed with bullatanocin (**1**)

Compound **4** was obtained as a whitish powder (hexane-chloroform) The molecular weight of **4** was determined to be 606 by CIMS (isobutane) at m/z 607(MH^+). The IR, ^{13}C NMR and ^{1}H NMR spectra were identical to the published values for desacetyluvaricin, which was previously reported only in *Uvaria acuminata* (Annonaceae)[16] and is a diastereomer of isodesacetyluvaricin[5] (**8**) (syn 4-deoxyasimicin[6]) Desacetyluvaricin (**4**)[16] is also identical to bullatacin[2] but without the 4-OH Both bullatacin and 4-deoxyasimicin[6] have been also isolated by our group from *Annona bullata* [2,6] By spectral comparisons with bullatacin and 4-deoxyasimicin (**8**), **4** was confirmed to be desacetyluvaricin, with the relative stereochemistry being threo, trans, threo, trans, erythro from C-15 to C-24, this stereochemistry agrees with that of uvaricin [17,18]



Comp A — **4** erythro — **8** threo

These new isolates (**1** - **4**) were isolated by activity-directed fractionation and are very active in the brine shrimp lethality test (BST)[1,19], as expected they are all significantly cytotoxic to human solid tumor cells in culture In order to access some structure activity relationships (SAR), the activities of their diastereomers, **5** - **8**, were also tested under the same conditions, the results are summarized in Table 4 The presence of the 4-OH appears to enhance activities, as **2** - **4**, and **6** - **8** appear to be about equivalent to or somewhat less than adriamycin, while **1** and **5** are over 10,000 times as cytotoxic as adriamycin both in the colon cell line (HT-29) and the lung cell line (A-549) The mechanism of action of the acetogenins has been determined,[20,21,22] they are powerful inhibitors of complex I in mitochondrial electron transport systems Thus, they selectively kill those types of cancer cells which have a higher energy demand than normal cells, the *in vivo* activities of bullatacin and bullatalicin, against human ovarian cell (A2780) xenografts in athymic mice, are comparable to the effects of

cisplatin but with higher potency (especially for bullatacin) and less weight loss in the treated mice.[22] New acetogenins, such as, **1** - **3**, give us the opportunity to select the isomeric structures which may be optimal for the treatment of various cancer types

Table 4 Bioactivities of compounds **1** - **8**

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	4.33×10^{-1}	$< 10^{-8}$	6.09×10^{-1}	$< 10^{-8}$
2	2.24×10^{-1}	1.26	4.41×10^{-2}	7.32×10^{-6}
3	3.31×10^{-1}	1.65	5.31×10^{-4}	5.67×10^{-4}
4	8.45×10^{-2}	6.96×10^{-4}	> 1.0	$< 10^{-4}$
5	1.24	$< 10^{-8}$	3.22×10^{-1}	$< 10^{-8}$
mixture of **6** and **7**	4.76×10^{-1}	1.6×10^{-2}*	8.5×10^{-4}*	5.0×10^{-5}*
8	2.01×10^{-1}	1.83×10^{-4}	> 1.0	$< 10^{-4}$
Adriamycin[e]	8×10^{-2}	4.02×10^{-4}	2.72×10^{-2}	5.13×10^{-4}

a) Brine shrimp lethality test, b) Human lung carcinoma, c) Human breast carcinoma, d) Human colon adenocarcinoma, e) Positive control standard * Data from Hui *et al*[4]

EXPERIMENTAL

Plant Material. Bark of *A bullata* Rich (Annonaceae) (M-06983, PL-103509) was collected at the USDA Subtropical Horticulture Research Station, Miami, Florida. The material was authenticated by Edward Garvey of the USDA

Bioassays. The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST) [1,19] Cytotoxicities against human solid tumor cells were measured at the Purdue Cell Culture Laboratory, Purdue Cancer Center for the A-549 lung carcinoma,[23] MCF-7 breast carcinoma,[24] and HT-29 colon adenocarcinoma [25]

Instrumentation. Optical rotations were determinated on a Perkin Elmer 241 polarimeter IR spectra (film) were measured on a Perkin-Elmer 1420 IR spectrometer UV spectra were taken on a Beckman DU-7 UV Spectrometer ^{1}H NMR, ^{1}H-^{1}H COSY, and ^{13}C NMR spectra were obtained on a Varian VXR-500S spectrometer Low resolution CIMS and EIMS data of isolates were collected on a Finnigan 4000 spectrometer Low resolution EIMS for TMS derivatives was done on MS50 Exact masses for MS measurements were obtained on a Kratos MS50 spectrometer through peak matching HPLC was carried out with a Rainin HPLC using Dynamax software system and a silica gel column (250 x 21 mm) equipped with a Rainin UV-1 detector Analytical TLC was performed on silica gel plates (0.25 mm) developed with $CHCl_3$-MeOH (9:1) and hexane-acetone (3:2) and visualized with 5% phosphomolybdic acid in EtOH [11]

Bullatanocin (1). White powder (hexane-chloroform) or whitish wax (chloroform), (90 mg), $[\alpha]_D^{22}$ = +14.4° (c 0.55, $CHCl_3$), CIMS (isobutane) m/z $[MH]^+$ 639 (100%), 621, 603, 585, 567, HRCIMS m/z 639.4829 for $C_{37}H_{67}O_8$ (calcd 639.4836), the key fragments in EIMS are shown in Figure 1, ^{1}H NMR ($CDCl_3$ and C_6D_6, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125.75 MHz) are shown in Tables 1 and 2, respectively (^{1}H NMR data were assigned based on the ^{1}H-^{1}H COSY), UV (MeOH) λ_{max} 208.5 nm (log ε = 3.52), IR (film) cm^{-1} 3430 (OH), 1750, 1728 (C=O)

Acetylation of 1. 3 mg of **1** was mixed with anhydrous pyridine/Ac_2O at room temperature overnight to give a tetra-acetate (**1a**, 2mg), CIMS (isobutane) m/z $[MH]^+$ 807, 747 (100%), 687, 627, 567, ^{1}H NMR data are shown in Table 1

TMS and d-TMS derivatization of 1. A small amount of **1** was treated with 20 μl of N,O-bis-(trimethylsilyl)-acetamide (BSA) or d_{18}-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and 2 μl of pyridine (10:1) and heated at 70° C for 30 min to yield the tetra-TMS derivative (**1b**) or tetra-d-TMS derivative (**1c**), EIMS data for the characteristic fragments are shown in Figure 1

cis- and trans-Bullatanocinone (2 and 3). White powder (hexane-chloroform) or whitish wax (chloroform), (75mg), $[\alpha]_D^{22}$ = +21.5° (c 0.20, $CHCl_3$), CIMS (isobutane) m/z $[MH]^+$ 639 (100%), 621, 603, 585, HR CIMS m/z 639.4825 for $C_{37}H_{67}O_8$ (calcd 639.4836), the key fragments in EIMS are shown in Figure 2, UV (MeOH) λ_{max} 203.5 nm (log ε = 1.34), IR (film) cm^{-1} 3420 (OH), 1760, 1715 (C=O)

Acetylation of 2 and 3. 4 mg of of **2** and **3** was mixed with anhydrous pyridine/Ac_2O at room temperature overnight to give a triacetate (**2a** and **3a**, 3 mg), CIMS (isobutane) m/z $[MH]^+$ 765, 705(100%), 645, 585, ^{1}H NMR data are shown in Table 3

TMS and d-TMS derivatization of 2 and 3. A small amount of 2 and 3 was treated with 20 µl of N,O-bis-(trimethylsilyl)-acetamide (BSA) or d_{18}-BSTFA and 2 µl of pyridine (10 1) and heated at 70° C for 30 min to yield the tri-TMS derivative (2b and 3b) or the tri-d-TMS derivative (2c and 3c), EIMS data for characteristic fragments are shown in Figure 2

cis-Bullatanocinone (2). White powder (hexane-chloroform) or whitish wax (chloroform), (5mg), $[\alpha]_D^{22}$ = +30 1° (c 0 20, $CHCl_3$), ^1H NMR ($CDCl_3$ and C_6D_6, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 75 MHz) are shown in Tables 3 and 2, respectively (^1H NMR data were assigned based on the ^1H-^1H COSY)

trans-Bullatanocinone (3). White powder (hexane-chloroform) or whitish wax (chloroform), (4mg), $[\alpha]_D^{22}$ = +14 4° (c 0 20, $CHCl_3$), ^1H NMR ($CDCl_3$ and C_6D_6, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 75 MHz) are shown in Tables 3 and 2, respectively (^1H NMR data were assigned based on the ^1H-^1H COSY)

Deacetyluvaricin (4). White powder (hexane-chloroform), (25 mg), $[\alpha]_D^{22}$ = +21 1° (c 0 50, $CHCl_3$), CIMS (isobutane) m/z 607 (30%) (MH^+), 589 (MH^+ - H_2O), 571 (MH^+ - $2H_2O$), ^1H NMR ($CDCl_3$, 500 MHz) 0 88 (3H, t, H-34), 1 26 (m, H-4-13, 26-33), 1 41 (3H, d, H-37), 1 54 (4H, m, H-14, 25), 1 60-2 0 (8H, m, H-17, 18, 21, 21), 2 26 (2H, m, H-3), 3 40 (1H, m, H-15), 3 82-3 96 (5H, m, H-16, 19, 20, 23, 24), 5 00 (1H, qq, H-36), 6 99 (1H, q, H-35), UV (MeOH) λ_{max} 208 5 nm (log ε = 3 52), IR (film) cm^{-1} 3430 (OH), 1748, 1726 (C=O)

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