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NEW BIOACTIVE ADJACENT BIS-THF ANNONACEOUS ACETOGENINS FROM *ANNONA BULLATA*

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Key Word Index—*Annona bullata*; Annonaceae; annonaceous acetogenins; adjacent bis-THF rings; 32-hydroxybullatacin; 31-hydroxybullatacin; 30-hydroxybullatacin; (2,4-*cis* and *trans*)-28-hydroxybullatacinones; cytotoxicities.

Abstract—Five new adjacent bis-THF annonaceous acetogenins, 32-hydroxybullatacin, 31-hydroxybullatacin, 30-hydroxybullatacin, and (2,4-*cis* and *trans*)-28-hydroxybullatacinones, were isolated from the ethanolic extract of the bark of *Annona bullata* Rich. (Annonaceae). The absolute configurations of the above five compounds, as well as those of (2,4-*cis* and *trans*)-32-, 31-, and 30-hydroxybullatacinones and (2,4-*cis* and *trans*)-bulladecinones, previously isolated from the same extract, were defined by the application of the advanced Mosher ester [methoxy(trifluoromethyl)phenyl acetate or MTPA] methodology. The determination of the absolute configuration of C-20 of (2,4-*cis* and *trans*)-bulladecinones to be *S* supports our hypothesis that the cyclization of the THF rings of (2,4-*cis* and *trans*)-bulladecinones starts from C-12 (the right side). The first five compounds listed above showed potent bioactivities in the brine shrimp lethality test (BST) and among six human solid tumour cell lines.

INTRODUCTION

The ethanolic extract of the bark of *Annona bullata* Rich. (Annonaceae) has, so far, yielded 40 annonaceous acetogenins [1–3]. Most of them belong to the adjacent bis-THF subclass and show potent activities in the brine strimp lethality test (BST) and against human solid tumour cell lines. Bullatacin (**14**), the first acetogenin isolated from this plant [4], is still among the most potent of all of the acetogenins and shows promising *in vivo* antitumour effects [3]. Our continuing BST-directed isolated has now yielded three new hydroxylated bullatacins, 32-hydroxybullatacin (**1**), 31-hydroxybullatacin (**2**), and 30-hydroxybullatacin (**3**), and a pair of new hydroxylated bullatacinones, (2,4-*cis* and *trans*)-28-hydroxybullatacinones (**4** and **5**).

Repeated open and flash silica gel column chromatography of F005, from the ethanolic extract of the bark [4], yielded a mixture of **1**–**3**, which was resolved to the pure compounds as white waxes, respectively, by normal phase HPLC over a silica gel column. The sequence of mobilility was **3** > **2** > **1**. The HRFABMS of **1**–**3** (glycerol) gave [MH^+] ions at *m/z* 639.4823 for each compound (**1**–**3**) (calcd 639.4829), consistent with molecular formulae of $C_{37}H_{66}O_8$. The EIMS fragmentations (Fig. 1) of the TMSi derivatives of **1**–**3** and the ^{1}H (Table 1) and ^{13}C NMR (Table 2) data of **1**–**3** indicated that they are adjacent bis-THF α,β-unsaturated γ-lactone acetogenins [1–3].

The α,β-unsaturated γ-lactone with a 4-OH group in **1**–**3** was indicated by six ^{1}H NMR signals at δ7.19 (*q*, H-35), 5.06 (*qq*, H-36), 3.84 (*m*, H-4), 2.53 (*ddd*, H-3a), 2.40 (*ddd*, H-3b), and 1.42 (*d*, H-37), and seven ^{13}C NMR resonances at δ174.6 (C-1), 151.8, (C-35), 131.2, (C-2), 78.0 (C-36), 70.0 (C-4), 33.3 (C-3), and 19.1 (C-37); typical NMR data exist in the ^{1}H and ^{13}C NMR spectra of all of the acetogenins having such a subunit [1–3]. The absolute configuration of C-4 in **1**–**3** was determined to be *R* by the use of advanced Mosher ester [methoxy(trifluoromethyl)phenyl acetate or MTPA] methodology [5]. The absolute configuration of C-36 was assigned to be *S* based on the C-4*R*/C-36*S* relationship found in all of the 4-OH α,β-unsaturated γ-lactone acetogenins [5].

The presence of the adjacent bis-THF rings with a flanking hydroxyl group on both sides in **1**–**3** was indicated by the ^{1}H NMR signals at δ3.96–3.90 (*m*, 2H, H-19, 20), 3.88–3.82 (*m*, 3H, H-16, 23, 24), 3.40 (*m*, H-15), 1.98 (*m*, 3H, H-17a, 18a, 21a), 1.88 (*m*, H-22a), 1.81 (*m*, H-21a), and 1.63 (*m*, 3H, H-17b, 18b, 21b), and ^{13}C NMR resonances at δ83.2 (C-16), 82.8–82.3 (3C, C-19, 20, 23), 74.1 (C-15), 71.3 (C-24), 28.9–28.3 (3C, C-17, 18, 21), and 24.5 (C-22). These NMR data also suggested the relative stereochemistry of this bis-THF subunit (C-15 to C-24) to be *threo-trans-threo-trans-erythro*, by comparison with the ^{1}H and ^{13}C NMR data of model compounds [6–9]. The close similarity of these ^{1}H and ^{13}C NMR data with

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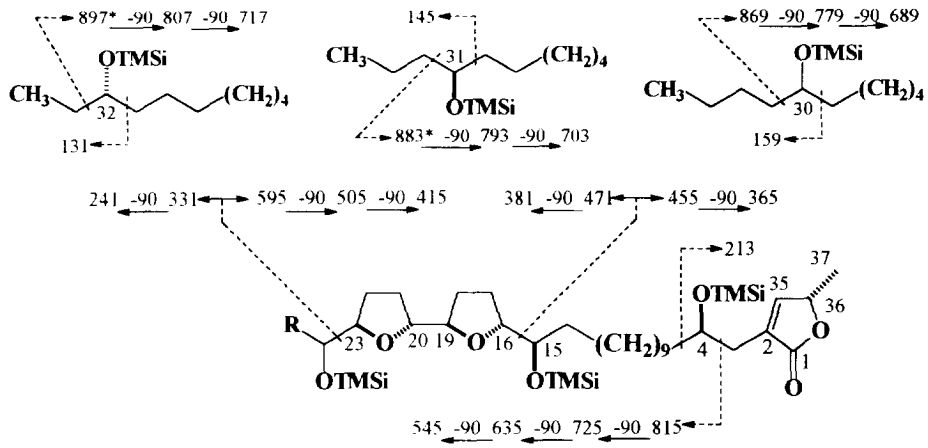


Fig. 1. Diagnostic EIMS fragment ions of the tetra-trimethylsilyl (TMSi) derivatives of 32-hydroxybullatacin (**1**), 31-hydroxybullatacin (**2**), and 30-hydroxybullatacin (**3**). Ions with an asterisk were not observed.

Table 1. ^{1}H NMR (500 MHz) data of **1**–**3** and **14** ($CDCl_3$)

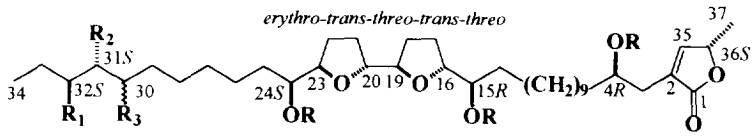
	Compounds δ (ppm)			
Protons	**1**	**2**	**3**	**14**
3a	2.54 *dddd*	2.53 *dddd*	2.54 *dddd*	2.54 *dddd*
3b	2.40 *dddd*	2.40 *dddd*	2.40 *dddd*	2.40 *dddd*
4	3.84 *m*	3.84 *m*	3.84 *m*	3.84 *m*
5	1.45 *m*	1.45 *m*	1.45 *m*	1.45 *m*
6–13	1.60–1.20 *m*	1.60–1.20 *m*	1.60–1.20 *m*	1.60–1.20 *m*
14	1.40 *m*	1.40 *m*	1.40 *m*	1.40 *m*
15	3.40 *m*	3.40 *m*	3.40 *m*	3.40 *m*
16	3.85 *m*	3.85 *m*	3.85 *m*	3.85 *m*
17a, 18a	1.98 *m*	1.98 *m*	1.98 *m*	1.98 *m*
17b, 18b	1.62 *m*	1.62 *m*	1.62 *m*	1.62 *m*
19	3.93 *m*	3.93 *m*	3.93 *m*	3.93 *m*
20	3.85 *m*	3.85 *m*	3.85 *m*	3.85 *m*
21	1.98, 1.62	1.98, 1.62	1.98, 1.62	1.98, 1.62
22a	1.89 *m*	1.89 *m*	1.89 *m*	1.89 *m*
22b	1.81 *m*	1.81 *m*	1.81 *m*	1.81 *m*
23	3.93 *m*	3.93 *m*	3.93 *m*	3.93 *m*
24	3.87 *m*	3.87 *m*	3.87 *m*	3.87 *m*
25	1.35 *m*	1.35 *m*	1.35 *m*	1.35 *m*
26–29	1.60–1.20 *m*	1.60–1.20 *m*	1.60–1.20 *m*	1.60–1.20 *m*
30	1.60–1.20 *m*	1.60–1.20 *m*	3.58 *m*	1.60–1.20 *m*
31	1.60–1.20 *m*	3.60 *m*	1.60–1.20 *m*	1.60–1.20 *m*
32	3.52 *m*	1.60–1.20 *m*	1.60–1.20 *m*	1.60–1.20 *m*
33	1.60–1.20 *m*	1.60–1.20 *m*	1.60–1.20 *m*	1.60–1.20 *m*
34	0.939 *t*	0.927 *t*	0.907 *t*	0.878 *t*
35	7.19 *q*	7.19 *q*	7.19 *q*	7.19 *q*
36	5.06 *qq*	5.06 *qq*	5.06 *qq*	5.06 *qq*
37	1.44 *d*	1.44 *d*	1.44 *d*	1.44 *d*

those of bullatacin suggested that **1**–**3** are bullatacin type acetogenins [3, 4]. The EI mass spectra of the TMSi derivatives of **1**–**3** showed intense ion peaks at *m/z* 455 (fission at C-15/16) and 595 (fission at C-23/24) and placed the bis-THF rings at C-16 to C-23 (Fig. 1).

The position of the *erythro* relative stereochemistry cannot be simply solved by analyses of the ^{1}H and ^{13}C NMR data, since its placement at C-15/16 or C-23/24 will give very similar NMR signals [10]. The *erythro* arrangement is usually found at C-23/24 (or C-21/22 in the C_{35} acetogenins) in the bis-THF compounds, except in the rare trilobacin type acetogenins (where the *erythro* configuration is at C-19/20). Sahai *et al.* [10] suggested that the *erythro* configuration might also exist

Table 2. ^{13}C NMR (125 MHz) data of **1**–**3** and **14** ($CDCl_3$)

Carbons	Compounds δ (ppm)				
	1	**2**	**3**		**14**
1	174.6	174.6	174.6		174.6
2	131.2	131.2	131.2		131.2
3	33.3	33.3	33.3		33.3
4	70.0	70.0	70.0		70.0
5	37.4	37.4	37.3		37.4
6	25.5	25.5	25.5		25.5
7–12	29.7–29.4	29.7–29.5	29.6–29.4		29.7–29.3
13	25.6	25.6	25.6		25.6
14	33.3	33.3	33.1		33.4
15	74.1	74.1	74.1		74.1
16	83.2	83.2	83.2		83.2
17	28.9	28.9	28.9		28.9
18	28.9	28.9	28.9		28.9
19	82.3	82.3	82.3		82.3
20	82.5	82.5	82.5		82.5
21	28.3	28.3	28.3		28.3
22	24.5	24.5	24.5		24.5
23	82.8	82.8	82.8		82.8
24	71.3	71.3	71.3		71.3
			major (30*S*)	minor (30*R*)	
25	32.4	32.9	32.18	32.20	32.4
26	26.0	25.9	25.97	25.91	2.60
27	29.7–29.4	29.7–29.5	29.7–29.4	29.7–29.4	29.7–29.3
28	29.7–29.4	29.7–29.5	25.38	25.44	29.7–29.3
29	29.7–29.4	25.5	37.07	37.12	29.7–29.3
30	25.6	37.4	71.77	71.84	29.7–29.3
31	36.9	71.7	37.28	37.30	29.7–29.3
32	73.3	39.6	27.8		31.9
33	30.1	18.8	22.7		22.7
34	9.87	14.1	14.0		14.1
35	151.8	151.8	151.8		151.8
36	78.0	78.0	78.0		78.0
37	19.1	19.1	19.1		19.1



	R	R_1	R_2	R_3
32-hydroxybullatacin (**1**)	H	OH	H	H
31-hydroxybullatacin (**2**)	H	H	OH	H
30-hydroxybullatacin (**3**)	H	H	H	OH
bullatacin (**14**)	H	H	H	H

at C-15/16 (C-13/14 in the C_{35} acetogenins); nevertheless, they did not provide experimental evidence to substantiate this conclusion [10]. The position of the *erythro* configuration in bullatacin (**14**) was unambiguously determined to be at C-23/24 by the analyses of the ^{1}H NMR and EI mass spectra of C-4, 15- and C-4, 24-bis-Mosher ester derivatives of **14** [4, 11], and that of squamocin [12], a bullatacin type acetogenin, was unambiguously determined to be also at C-23/24 by X-ray crystallographic analyses of its saturated γ-lactone derivative [7] and by the analysis of the ^{1}H NMR data of its C-24, 28-formaldehyde acetal derivative [13]. The *erythro* configuration of bullatalicin, a non-adjacent bis-THF acetogenin, was unambiguously determined to be also at C-23/24 by preparation of its C-16,19-formaldehyde acetal derivative [13]. The position of the *erythro* configuration in **3** was assigned to be at C-23/24 by analysis of the ^{13}C NMR data of **3**, which is a mixture of C-30*S* and C-30*R* (see details below). The C-25 to C-31 of **3** all showed double signals (Table 1), and the signals at *ca*

δ32.2 (C-25), corresponding to the methylene carbons next to the THF-flanking carbinol center having an *erythro* configuration with the THF ring [3, 6], also presented double signals. This could only be explained if the *erythro* configuration were located at C-23/24. Since **1–3** are analogous and all of their ^{1}H and ^{13}C NMR data are extremely similar, the *erythro* configuration was also assigned to be at C-23/24 for **2** and **3**. The absolute configurations of C-15 and C-24 in **1–3** were determined to be *R* and *S*, respectively, the same as those of bullatacin (**14**), by analyses of the ^{1}H NMR data of the (*S*)- and (*R*)-Mosher ester derivatives of **1–3** [5].

FABMS showing the M_r of **1–3** to be 638 (16 amu more than that of **14**) indicated that **1–3** had one more OH group than bullatacin (**14**). The NMR spectra of **1–3** showed an extra proton signal at *ca* δ3.60 corresponding to the methine proton of this additional aliphatic chain OH. The examination of the proton chemical shifts of the terminal methyls (C-34), at δ0.939 in **1**, 0.927 in **2**, and 0.907 in **3**, are reminiscent of the terminally hydroxylated bullatacinones [14]. The proton chemical shifts of the terminal methyls of these compounds gradually shift downfield as the position of the hydroxyl substitution approaches the terminal methyl [3, 15]. The proton chemical shifts of the terminal methyls of **1–3** matched very well (differences of only 0.001 or 0.002 ppm) with those of the known compounds having a 32-, 31-, or 30-hydroxyl substitution, respectively. Thus **1–3** were determined to be 32-, 31-, and 30-hydroxylated bullatacins. The 32-hydroxyl substitution in **1** was also indicated by the single-relayed COSY spectrum of **1**, showing correlation cross peaks between the signals at δ0.939 (H-34) and 3.52 (H-32), and the 31-hydroxyl group in **2** was also indicated by the double-relayed COSY spectrum of **2**, showing correlation cross peaks between the signals at δ0.927 (H-34) and 3.60 (H-31). In addition, the placements of the fourth hydroxyl group of **1–3** at C-32, C-31, or C-30 were clearly confirmed by the EIMS fragmentation of the TMSi derivatives of **1–3** (Fig. 1).

The absolute configurations of the aliphatic carbinol centers on the terminal hydrocarbon chain can be determined by simply examining the chemical shift differences of the terminal methyls of the (*S*)- and (*R*)-Mosher ester derivatives of such acetogenins [13]. Fujimoto's group also reached the same conclusion [16]. In this way, the absolute configurations of C-32 in **1** and C-31 in **2** were determined to be both *R* (Table 3). Although **1** and **2** were shown to be optically quite pure at C-32 or C-31, respectively, the C-30 of **3** appeared to be a mixture of *S* and *R* isomers, which could be easily recognized by two triplets for the terminal methyls (C-34) in the ^{1}H NMR spectra of its (*S*)- and (*R*)-Mosher ester derivatives (Table 3). The proton signals of the terminal methyls (H-34) appeared at much higher intensity at δ0.820 than at δ0.878 in the ^{1}H NMR spectrum of the (*S*)-Mosher ester of **3**, while they appeared at much higher intensity at δ0.880 than at δ0.817 in the ^{1}H NMR spectrum of the (*R*)-Mosher ester of **3**. These results also indicated that the 30*S* isomer of **3** was more abundant than the 30*R* isomer of **3** (ratio *ca* 4:1 in the NMR integrations). The C-25 to C-31 positions of **3**, as mentioned above, showed double signals in the ^{13}C NMR spectrum with different intensities in the ratio of *ca* 4:1. Based on the above results, we assigned the higher intensity signals to the C-30*S* isomer and the lower intensity signals to the C-30*R* isomer (Table 2).

Thus, the structures and the absolute configurations of **1–3** were concluded to be as illustrated and named 32-hydroxybullatacin (**1**), 31-hydroxybullatacin (**2**), and 30-hydroxybullatacin (**3**), respectively.

Compounds **4** and **5** were first obtained in a mixture as a whitish wax. The molecular M_r at 638 was established by HRFABMS (glycerol) for the $[MH^+]$ at *m/z* 639.4823, corresponding to $C_{37}H_{67}O_8$ (calcd 639.4836). The NMR spectra of **4** and **5** and the EIMS fragmentation of their TMSi derivatives indicated that they are 2,4-*cis* and *trans* isomers of the bullatacin type of ketolactone acetogenins. Finally, **4** and **5** were separated in small amounts by normal phase HPLC.

Actually, the entire ^{1}H and ^{13}C NMR spectra of **4** and **5** (Table 4) were very similar to those of (2,4-*cis* and *trans*)-bullatacinones (**15** and **16**) [4] except for an extra proton signal at δ3.62 with a corresponding carbon resonance at δ71.4; these data indicated the existence of one more hydroxyl group [1–3]. The M_r at 638 (16 amu more than those of **15** and **16**) confirmed this conclusion. The position of this hydroxyl group was suggested to be at C-28 by the proton chemical shift of the terminal

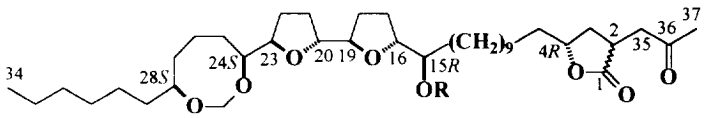
Table 3. ^{1}H NMR data (500 MHz, $CDCl_3$) of the terminal methyls of the (*S*)- and (*R*)-Mosher esters of **1–11**

	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta_H(\delta_S - \delta_R)$
1 (32*R*)	0.919	0.804	+ 0.115
6 and **7** (32*R*)	0.922	0.804	+ 0.118
2 (31*R*)	0.917	0.846	+ 0.071
8 and **9** (31*R*)	0.918	0.845	+ 0.073
3 (major-30*S*)	0.820	0.880	− 0.060
(major-30*R*)	0.878	0.817	+ 0.061
10 and **11**			
(major-30*S*)	0.822	0.881	− 0.059
(minor-30*R*)	0.884	0.818	+ 0.066
4 and **5** (28*S*)	0.860	0.875	− 0.015

methyl (C-34) at δ0.882 and by a carbon signal at *ca* δ22 (C-26), since these data were consistent with those for the terminal methyl and C-26 of other acetogenins having a 28-OH, e.g. squamocin, squamostatin A, and asiminacin [17]. This placement was subsequently demonstrated to be correct by the EIMS of the TMSi derivatives of **4** and **5** from the fragment ions at *m/z* 187 (cleavage at C-27/28) and 769 (cleavage at C-28/29).

In order to confirm the position of the *erythro* configuration in **4** and **5** and to determine the absolute configurations of stereogenic carbinol centers including that at C-28, the 24,28-formaldehyde acetal derivatives (**4a** and **5a**) of **4** and **5** were prepared (the only difference between **4** and **5** is their respective 2*S* or 2*R* configuration, and it was extremely difficult to separate the 2,4-*cis*- and *trans*-isomers; thus, a mixture of **4** and **5** was used). The remaining proton signal at δ3.39 and the disappearance of the proton signal at δ3.87 unquestionably placed the *erythro* configurations at C-23/24 (Table 4). The two well separated doublets of the acetal proton signals at δ5.21 and 4.64 indicated the *cis* configuration for the acetal ring [13]. The analyses of the $\Delta\delta_H$ (δ_S–δ_R) data of the (*S*)- and

cis-erythro-trans-threo-trans-threo



	R
24,28-formaldehyde acetal derivatives (**4a** and **5a**)	H
(*S*)-Mosher esters of **4a** and **5a** (**4b** and **5b**)	(*S*)-MTPA
(*R*)-Mosher esters of **4a** and **5a** (**4c** and **5c**)	(*R*)-MTPA

Table 4. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **4** and **5**, $CDCl_3$

	^{1}H of **4**	^{13}C of **4**	^{1}H of **5**	^{13}C of **5**
1	—	178.2	—	178.8
2	3.02 *m*	36.9	3.03 *m*	34.5
3a	1.48	35.6	2.23 *dddd*	33.3
3b	2.61 *m*		1.99 *m*	
4	4.39 *m*	79.4	4.54 *m*	78.9
5a	1.76	35.4	1.71	35.5
5b	1.60		1.58	
6	1.65–1.21	25.2	1.65–1.21	25.3
7–12	1.65–1.21	29.7–29.3	1.65–1.21	29.7–29.4
13	1.65–1.21	25.7	1.65–1.21	25.7
14	1.42 *m*	33.3	1.42 *m*	33.3
15	3.40 *m*	74.1	3.40 *m*	74.1
16	3.86 *m*	83.3	3.86 *m*	83.3
17	1.97, 1.63	28.9	1.97, 1.63	29.0
18	1.97, 1.63	28.9	1.97, 1.63	28.9
19	3.93 *m*	82.3	3.93 *m*	82.2
20	3.86 *m*	82.5	3.86 *m*	82.5
21	1.97, 1.63	28.4	1.97, 1.63	28.4
22	1.88, 1.82	24.9	1.88, 1.82	24.9
23	3.93 *m*	82.8	3.93 *m*	82.8
24	3.87 *m*	71.8	3.87 *m*	71.8
25	1.37	32.5	1.37	32.5
26	1.65–1.21	22.1	1.65–1.21	22.1
27	1.40	37.3	1.40	37.3
28	3.62	71.4	3.62	71.4
29	1.40	37.5	1.40	37.5
30	1.65–1.21	25.7	1.65–1.21	25.7
31	1.65–1.21	29.7–29.4	1.65–1.21	29.7–29.4
32	1.65–1.21	31.9	1.65–1.21	31.9
33	1.25	22.7	1.25	22.7
34	0.882 *t*	14.1	0.882 *t*	14.1
35a	2.61 *dd*	43.9	2.67 *dd*	44.3
35b	3.11 *dd*		3.04 *dd*	
36	—	205.5	—	205.4
37	2.20 *s*	30.05	2.20 *s*	30.00

(*R*)-Mosher esters of **4a** and **5a** (Table 5) determined the absolute configuration at C-15 to be *R* [5]. As all of the relative stereochemistry from C-15 to C-28 was already determined, the absolute configurations of this subunit were then readily deduced to be 16*R*, 19*R*, 20*R*, 23*R*, 24*S*, and 28*S*.

When we examined the difference of the proton chemical shifts of the (*S*)- and (*R*)-per-Mosher esters of **4** and **5**, a negative value was obtained for the H-34 (Table 3), indicating that this methyl was more highly shielded in the (*S*)-Mosher ester and less highly shielded in the (*R*)-Mosher ester. This result also suggested the *S* absolute configuration at C-28, and it furthermore proved that the absolute configuration of the carbinol centers on the terminal hydrocarbon chain can be reliably determined by simply observing the chemical shift changes of the terminal methyl in the (*S*)- and (*R*)-Mosher ester derivatives of the acetogenins [13, 16].

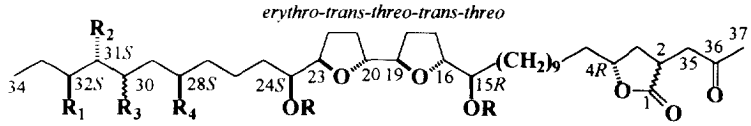
We have previously isolated and reported (2,4-*cis* and *trans*)-31-hydroxybullatacinones (**6** and **7**), (2,4-*cis* and *trans*)-31-hydroxybullatacinones (**8** and **9**), and (2,4-*cis* and *trans*)-30-hydroxybullatacinones (**10** and **11**), but we did not report their absolute configurations [14]. The analyses of the ^{1}H NMR data (Table 6) of their (*S*)- and (*R*)-Mosher ester derivatives now indicate that **6–11** share the same absolute configurations, as illustrated, with their respective α,β-unsaturated γ-lactone acetogenins (**1–3**). It is very interesting to note that the C-30 of **10** and **11** is also a mixture of *S* and *R* isomers (ratio *ca* 4:1), as indicated by the two triplet signals for the terminal methyls (C-34) in their ^{1}H NMR spectra of its (*S*)- and (*R*)-Mosher ester derivatives and by double signals of the C-25 to C-31 positions in their ^{13}C NMR spectra. Duret *et al.* [18] have experimentally demonstrated the ketolactone acetogenins were derived from the 4-OH-α,β-unsaturated γ-lactone acetogenins through translactonization, and this transformation likely occurs in the process of extraction and isolation. Our finding that the same absolute configurations exist in **6–11** and in **1–3** supports their conclusion.

Table 5. Partial ^{1}H NMR data of **4a** and **5a**, **4b** and **5b**, and **4c** and **5c**

Proton	**4a** and **5a**	**4b** and **5b**	**4c** and **5c**	$\Delta\delta_H(\delta_S - \delta_R)$
13	1.60–1.20	1.33 *m*	1.14 *m*	+ 0.19
14	14.0	1.63 *m*	1.50 *m*	+ 0.13
15	3.39	5.06 *m*	5.05 *m*	***R****
16	3.84	4.05 *m*	4.045 *m*	0
17a	1.96	1.89 *m*	2.02 *m*	− 0.13
17b	1.60	1.50 *m*	1.57 *m*	− 0.07
18a	1.96	1.72 *m*	1.92 *m*	− 0.20
18b	1.62	1.61 *m*	1.70 *m*	− 0.09
19	3.88	3.79 *m*	3.89 *m*	− 0.10
20	3.88	3.84 *m*	3.89 *m*	− 0.05
21a	1.96	1.87 *m*	1.92 *m*	− 0.05
21b	1.60	1.59 *m*	1.70 *m*	− 0.11
22a	1.96	1.87 *m*	1.91 *m*	− 0.04
22b	1.80	1.71 *m*	1.73 *m*	− 0.02
23	3.90	3.80 *m*	3.82	− 0.02
24	3.68	3.61 *m*	3.61 *m*	—
25	1.74/1.64	1.40 *m*	1.42 *m*	—
26	1.88/1.54	1.40–1.20	1.40–1.20	—
27	1.62	1.62 *m*	1.60 *m*	—
28	3.55	3.54 *m*	3.55 *m*	—
29	1.54	1.57 *m*	1.57 *m*	—
34	0.878	0.877	0.878	—

*Absolute configuration of carbinol center.

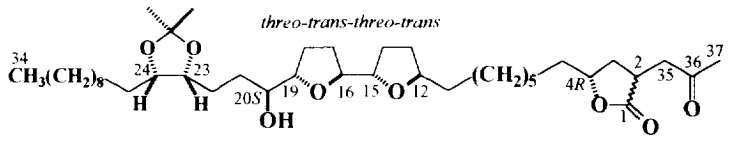
erythro-trans-threo-trans-threo



	R	R_1	R_2	R_3	R_4
(2,4-*cis* and *trans*)-28-hydroxybullatacinones (**4**, **5**)	H	H	H	H	OH
(2,4-*cis* and *trans*)-32-hydroxybullatacinones (**6**, **7**)	H	OH	H	H	H
(2,4-*cis* and *trans*)-31-hydroxybullatacinones (**8**, **9**)	H	H	OH	H	H
(2,4-*cis* and *trans*)-30-hydroxybullatacinones (**10**, **11**)	H	H	H	OH	H
(2,4-*cis* and *trans*)-bullatacinones (**15**, **16**)	H	H	H	H	H

Table 6. Partial ^{1}H NMR (500 MHz, $CDCl_3$) data of the (*S*)- and (*R*)-Mosher esters of **6**–**11**

	Compounds δ (ppm)					
	6 and **7**		**8** and **9**		**10** and **11**	
Protons	(*S*)-MTPA	(*R*)-MTPA	(*S*)-MTPA	(*R*)-MTPA	(*S*)-MTPA	(*R*)-MTPA
14	1.58 *m*	1.52 *m*	1.57 *m*	1.50 *m*	1.59 *m*	1.53 *m*
15	5.06 *m*	5.04 *m*	5.06 *m*	5.02 *m*	5.06 *m*	5.02 *m*
16	4.03 *q*	3.99 *q*	4.04 *q*	4.00 *q*	4.03 *q*	3.99 *q*
17a	1.94 *m*	2.00 *m*	1.94 *m*	2.00 *m*	1.93 *m*	2.00 *m*
17b	1.53 *m*	1.58 *m*	1.51 *m*	1.58 *m*	1.53 *m*	1.58 *m*
18a	1.82 *m*	1.88 *m*	1.80 *m*	1.87 *m*	1.80 *m*	1.87 *m*
18b	1.55 *m*	1.69 *m*	1.54 *m*	1.70 *m*	1.55 *m*	1.66 *m*
19	3.79 *m*	3.83 *m*	3.79 *m*	3.83 *m*	3.79 *m*	3.83 *m*
20	3.79 *m*	3.64 *m*	3.79 *m*	3.64 *m*	3.79 *m*	3.65 *m*
21a	1.82 *m*	1.72 *m*	1.80 *m*	1.73 *m*	1.80 *m*	1.74 *m*
21b	1.66 *m*	1.64 *m*	1.66 *m*	1.62 *m*	1.68 *m*	1.61 *m*
22a	1.83 *m*	1.75 *m*	1.82 *m*	1.75 *m*	1.82 *m*	1.75 *m*
22b	1.70 *m*	1.60 *m*	1.70 *m*	1.59 *m*	1.69 *m*	1.59 *m*
23	3.99 *m*	3.95 *m*	3.98 *m*	3.93 *m*	3.98 *m*	3.93 *m*
24	5.25 *m*	5.23 *m*	5.24 *m*	5.22 *m*	5.23 *m*	5.20 *m*
25	1.53 *m*	1.56 *m*	1.54 *m*	1.57 *m*	1.53 *m*	1.56 *m*
32/31/30	5.03 *m*	5.02 *m*	5.08 *m*	5.08 *m*	5.05 *m*	5.07 *m*
34	0.922 *t*	0.804 *t*	0.917 *t*	0.845 *t*	0.822 (major)	0.881 (major)
					0.884 (minor)	0.818 (minor)



23,24-acetonide derivatives of **12** and **13**

(2,4-*cis* and *trans*)-Bulladecinones (**12** and **13**) were also reported by us without definition of their absolute configuration [19]. The analyses of the $\Delta\delta_H$ ($\delta_S - \delta_R$) data of the (*S*)- and (*R*)-Mosher esters of the 23,24-acetonide derivatives of **12** and **13**, showing positive values of the THF ring side and negative values on the hydrocarbon chain side, determined the absolute configuration at C-20 to be *S* [5]. We have proposed that the bullatacin, bullatalicin and bulladecin type acetogenins are likely derived from the same precursor, but through different pathways of cyclization (Fig. 2) [3]. The 20*S* absolute configuration of **12** and **13** supports the hypothesis that the cyclization of the THF rings of **12** and **13** starts from C-12 (the right side). If the cyclization started from C-20 (the left side), the absolute configuration of the C-20 of **12** and **13** should be *R* rather than *S*.

Compounds **1**–**5** showed quite potent activities in the BST [20] and among six human solid tumour cell lines (Table 7) [21–25]. All of the acetogenins, tested so far, decrease oxygen uptake in mitochondrial tests [26, 27]. These results indicate that they act, at least in part, as potent inhibitors of ATP production via blocking at complex I in mitochondria. In addition, they act as potent inhibitors of the plasma membrane NADH oxidase of cancerous cells [28]; this action decreases cytosolic ATP production. The consequence of such ATP deprivations is apoptosis (programmed cell death) [29]. Recent results obtained by Sasaki *et al.* [30] suggested that the activity of the acetogenins may be attributed to the hydroxyl-flanked THF ring(s). We have observed that certain levels of polarities are very important to the bioactive potencies of the acetogenins [3]. This may explain why, although **1**–**3** have almost the same structures as bullatacin (**14**), they show somewhat less potent bioactivities in the BST than **14**; the extra hydroxyl of **1**–**3** would seem to make them a little too polar.

EXPERIMENTAL

Instrumentation. IR spectra (film) were measured on a Perkin-Elmer 1420 IR spectrometer. ^{1}H NMR (500 MHz), COSY (500 MHz), and ^{13}C NMR (125 MHz) spectra (all in $CDCl_3$) were obtained on a Varian VXR-500S spectrometer. Low-resolution FABMS data were collected on a Finnigan 4000 spectrometer. Low-resolution EIMS for TMSi derivatives was performed on a Kratos MS50. HRFABMS was obtained on the Kratos

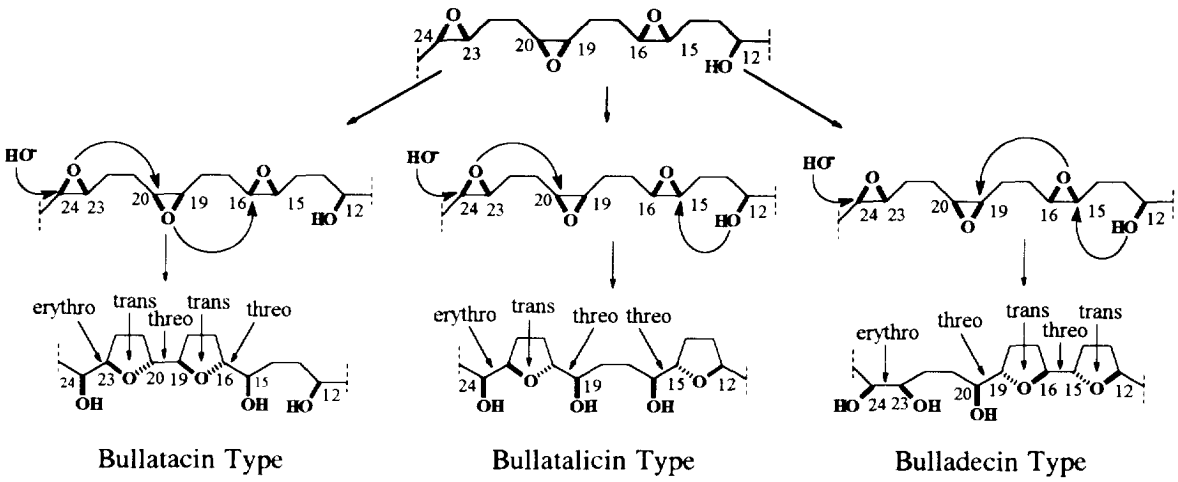


Fig. 2. Hypothesis for the biogenesis of the THF rings of bullatacin, bullatalicin, and bulladecin types of annonaceaous acetogenins.

Table 7. Bioactivities of compounds **1–5***

	BST† ED_{50} ($\mu g\,ml^{-1}$)	A-549‡ ED_{50} ($\mu g\,ml^{-1}$)	MCF-7§ ED_{50} ($\mu g\,ml^{-1}$)	HT-29¶ ED_{50} ($\mu g\,ml^{-1}$)	A-498** ED_{50} ($\mu g\,ml^{-1}$)	PC-3†† ED_{50} ($\mu g\,ml^{-1}$)	MIA PaCa-2‡‡ ED_{50} ($\mu g\,ml^{-1}$)
1	8.00×10^{-2}	$<1.00\times10^{-8}$	$<1.00\times10^{-8}$	1.48	$<1.00\times10^{-8}$	1.62×10^{-2}	$<1.00\times10^{-8}$
2	5.72×10^{-2}	$<1.00\times10^{-8}$	$<1.00\times10^{-8}$	1.21	$<1.00\times10^{-8}$	3.16×10^{-1}	$<1.00\times10^{-8}$
3	6.55×10^{-2}	$<1.00\times10^{-8}$	$<1.00\times10^{-8}$	1.17	$<1.00\times10^{-8}$	4.33×10^{-3}	$<1.00\times10^{-8}$
4	7.98×10^{-2}	4.38×10^{-4}	4.46×10^{-4}	6.90×10^{-4}	4.35	2.33	1.02×10^{-8}
5	7.98×10^{-3}	3.18×10^{-2}	1.71	1.45	1.00×10^{-3}	9.04	1.19×10^{-5}
14	1.59×10^{-3}	$<1.00\times10^{-8}$	$<1.00\times10^{-8}$	1.06	$<1.00\times10^{-8}$	1.62×10^{-2}	$<1.00\times10^{-8}$
Adr.§§	8.00×10^{-2}	4.00×10^{-2}	7.21×10^{-1}	8.92×10^{-2}	3.60×10^{-2}	1.17×10^{-1}	2.70×10^{-2}

*To permit optimal comparisons, samples of **1–3** and **14** were tested in the same run in each bioassay.
†Brine shrimp lethality test [20].
‡Human lung carcinoma [21].
§Human breast carcinoma [22].
¶Human colon adenocarcinoma [23].
**Human kidney carcinoma [21].
††Human prostate adenocarcinoma [24].
‡‡Human pancreatic carcinoma [25].
§§Adriamycin—standard positive control.

MS50 spectrometer through peak matching. HPLC was carried out using a Dynamax software system and a silica gel (8 μm) column (250 × 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 [1, 2].

Bioassays. The extracts, fractions, and isolated compounds were routinely evaluated for lethality in the BST [20]. Cytotoxicities against human solid tumour cells were measured in 7-day MTT assays at the Purdue Cell Culture Laboratory, Purdue Cancer Center [21–25].

Plant material. Bark of *A. bullata* Rich. (Annonaceae) (M-06983, PL-103509) was collected at the USA Subtropical Horticultural Research Station (Miami, FL, U.S.A.). The material was authenticated by Edward Garvey of the USDA. The dried bark was pulverized in a Wiley mill.

Extraction and isolation. The pulverized bark (3.9 kg) was extracted and partitioned, as previously described, to obtain F005 [4]. F005 (80 g) was subjected to open column chromatography (3 kg silica gel) eluted with a gradient of hexane–$CHCl_3$–MeOH. Fractions (F_1-1 to F_1-82) were collected, pooled according to their similar TLC patterns, and bioassayed by the BST. An active pool (F_1-51–F_1-60, 15 g, BST $LC_{50} = 2.58\times10^{-1}$ ppm) was further resolved on another silica gel column (230–400 mesh, 600 g), eluted with a gradient of $CHCl_3$–EtOAc–MeOH. Fractions (F_2-1 to F_2-135) were collected and pooled on the basis of similar TLC patterns, and again the BST of each pool was tested. The active pool (F_2-73–F_2-125, 3.5 g, BST LC_{50} =

3.25×10^{-1} ppm) was subjected to a flash silica gel column (600 g), eluted by a gradient of $CHCl_3$–MeOH. Fractions (F_3-1 to F_3-98) were collected and combined into eight pools on the basis of similar TLC patterns. P-2 (F_3-51–F_3-79, 0.96 g, BST $LC_{50} = 8.95 \times 10^{-2}$ ppm) was chromatographed on a column of silica gel in the HPLC eluted by hexane–MeOH–THF (80:18:2, flow rate 15 ml min^{-1} to afford the three white powders containing **1**, **2**, and **3** (retention times 55, 48, and 44 min, respectively). P-2 (F_3-21–F_3-34, 0.78 g, BST $LC_{50} = 8.9 \times 10^{-2}$ ppm) was chromatographed on a column of silica gel in the HPLC eluted by $CHCl_3$–MeOH (90:1, flow rate 10 ml min^{-1}) to afford the two white powders containing **4** and **5** (retention times 95 and 96 min, respectively).

TMSi derivatizations. Small amounts (*ca* 0.3 mg) of **1**–**3** and the mixture of **4** and **5** were treated with 20 μl of *N,O*-bis-(trimethylsilyl)-acetamide and 2 μl of pyridine (10:1) and heated at 70° for 30 min to yield the respective TMSi derivatives.

Preparation and purification of Mosher esters. To an acetogenin (0.5–1 mg, in 0.3 ml of CH_2Cl_2) were sequentially added pyridine (0.2 ml), 4-(dimethylamino)pyridine (0.5 mg), and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride. The mixture was stirred at room temperature for 4 hr and passed through a disposable pipet (0.6 × 6 cm) containing silica gel (60–200 mesh) and eluted with 3 ml of CH_2Cl_2. The CH_2Cl_2 residue, dried *in vacuo*, was redissolved in CH_2Cl_2 and washed using 1% $NaHCO_3$ (5 ml) and water (2 × 5 ml); the CH_2Cl_2 layer was dried *in vacuo* to give the *S*-Mosher esters. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride gave the *R*-Mosher ester [5, 13].

32-*Hydroxybullatacin* (**1**). White powder (8 mg), $C_{37}H_{67}O_8$: $[\alpha]_D$ + 18° ($CHCl_3$, *c* 0.10); IR ν_{max} (film) cm^{-1}: 3619, 2930, 2854, 1751; HRFABMS (glycerol) *m/z* 639.4823 for $[MH^+]$ (calcd 639.4836); ^1H and ^{13}C NMR, Tables 1 and 2; COSY and single-relayed COSY (τ = 35 ms) spectra were recorded. Tetra-(*S*)-Mosher ester, ^1H NMR δ: 0.919 (3H, *t*, *J* = 7.0 Hz, H-34), 1.28 (3H, *t*, *J* = 7.0 Hz, H-37), 1.53 (1H, *m*, H-17b), 1.65 (4H, *m*, H-5, H-18b, H-21b), 1.70 (1H, *m*, H-22b), 1.82 (3H, *m*, H-18a, H-21a, H-22a), 1.94 (1H, *m*, H-17a), 2.56 (1H, H-3b), 2.60 (1H, H-3a), 3.80 (2H, *m*, H-19, H-20), 3.99 (1H, *m*, H-23), 4.04 (1H, *q*, H-16), 4.86 (1H, *qq*, H-36), 5.03 (1H, *m*, H-32), 5.06 (1H, *m*, H-15), 5.26 (1H, *m*, H-24), 5.32 (1H, *m*, H-4), 6.73 (1H, *q*, *J* = 1.5, 1 Hz, H-35). Tetra-(*R*)-Mosher ester, ^1H NMR δ: 0.804 (3H, *t*, *J* = 7.0 Hz, H-34), 1.31 (3H, *t*, *J* = 7.0 Hz, H-37), 1.58–1.64 (5H, *m*, H-5, H-17b, H-21b, H-22b), 1.72–1.75 (3H, *m*, H-18b, H-21a, H-22a), 1.88 (1H, *m*, H-18a), 2.00 (1H, *m*, H-17a), 2.60 (1H, H-3b), 2.68 (1H, H-3a), 3.65 (1H, *m*, H-20), 3.82 (1H, *m*, H-19), 3.95 (1H, *m*, H-23), 4.00 (1H, *q*, H-16), 4.91 (1H, *qq*, H-36), 5.03 (1H, *m*, H-15), 5.24 (1H, *m*, H-24), 5.38 (1H, *m*, H-4), 6.97 (1H, *q*, *J* = 1.5, 1 Hz, H-35). EIMS fragmentation of TMSi derivative (Fig. 1).

31-*Hydroxybullatacin* (**2**). White powder (15 mg), $C_{37}H_{67}O_8$: $[\alpha]_D$ + 19° ($CHCl_3$, *c* 0.08); IR ν_{max} (film) cm^{-1}; 3630, 2926, 2854, 1752; HRFABMS (glycerol) *m/z* 639.4823 for $[MH^+]$ (calcd 639.4836); ^1H and ^{13}C NMR, Tables 1 and 2; COSY and single- and double-relayed COSY (τ = 35 and 70 ms, respectively) spectra were recorded. Tetra-(*S*)-Mosher ester, ^1H NMR δ: 0.917 (3H, *t*, *J* = 7.0 Hz, H-34), 1.28 (3H, *t*, *J* = 7.0 Hz, H-37), 1.53 (1H, *m*, H-17b), 1.65 (4H, *m*, H-5, H-18b, H-21b), 1.70 (1H, *m*, H-22b), 1.82 (3H, *m*, H-18a, H-21a, H-22a), 1.94 (1H, *m*, H-17a), 2.56 (1H, H-3b), 2.60 (1H, H-3a), 3.80 (2H, *m*, H-19, H-20), 3.99 (1H, *m*, H-23), 4.04 (1H, *q*, H-16), 4.86 (1H, *qq*, H-36), 5.07 (1H, *m*, H-15), 5.08 (1H, *m*, H-31), 5.29 (1H, *m*, H-24), 5.33 (1H, *m*, H-4), 6.72 (1H, *q*, *J* = 1.5, 1 Hz, H-35), Tetra-(*R*)-Mosher ester, ^1H NMR: δ0.846 (3H, *t*, *J* = 7.0 Hz, H-34), 1.31 (3H, *t*, *J* = 7.0 Hz, H-37), 1.58–1.64 (5H, *m*, H-17b, H-21b, H-22b), 1.72–1.75 (3H, *m*, H-18b, H-21a, H-22a), 1.88 (1H, *m*, H-18a), 2.00 (1H, *m*, H-17a), 2.60 (1H, H-3b), 2.68 (1H, H-3a), 3.64 (1H, *m*, H-20), 3.83 (1H, *m*, H-19), 3.98 (1H, *m*, H-23), 4.00 (1H, *q*, H-16), 4.91 (1H, *qq*, H-36), 5.03 (1H, *m*, H-15), 5.09 (1H, *m*, H-31), 5.24 (1H, *m*, H-24), 5.38 (1H, *m*, H-4), 6.98 (1H, *q*, *J* = 1.5, 1 Hz, H-35). EIMS fragmentation of TMSi derivative (Fig. 1).

30-*Hydroxybullatacin* (**3**). White powder (31 mg), $C_{37}H_{67}O_8$: $[\alpha]_D$ + 14° ($CHCl_3$, *c* 0.50); IR ν_{max} (film) cm^{-1}; 3629, 2928, 1753; HRFABMS (glycerol) *m/z* 639.4823 for $[MH^+]$ (calcd 639.4836); ^1H and ^{13}C NMR (Tables 1 and 2); COSY and single- and double-relayed COSY (τ = 35 and 70 ms, respectively) spectra were recorded. Tetra-(*S*)-Mosher ester, ^1H NMR: δ0.820 (major) 0.878 (minor, 3H, *t*, *J* = 7.0 Hz, H-34), 1.28 (3H, *t*, *J* = 7.0 Hz, H-37), 1.53 (1H, *m*, H-17b), 1.65 (4H, *m*, H-5, H-18b, H-21b), 1.70 (1H, *m*, H-22b), 1.82 (3H, *m*, H-18a, H-21a, H-22a), 1.94 (1H, *m*, H-17a), 2.58 (1H, H-3b), 2.61 (1H, H-3a), 3.79 (2H, *m*, H-19, H-20), 4.00 (1H, *m*, H-23), 4.05 (1H, *q*, H-16), 4.87 (1H, *qq*, H-36), 5.05 (1H, *m*, H-30), 5.06 (1H, *m*, H-15), 5.29 (1H, *m*, H-24), 5.32 (1H, *m*, H-4), 6.74 (1H, *q*, *J* = 1.5, 1 Hz, H-35). Tetra-(*R*)-Mosher ester, ^1H NMR: δ0.817 (minor), 0.880 (major, 3H, *t*, *J* = 7.0 Hz, H-34), 1.31 (3H, *t*, *J* = 7.0 Hz, H-37), 1.58–1.64 (5H, *m*, H-5, H-17b, H-21b, H-22b), 1.74–1.77 (3H, *m*, H-18b, H-21a, H-22a), 1.88 (1H, *m*, H-18a), 2.00 (1H, *m*, H-17a), 2.60 (1H, H-3b), 2.68 (1H, H-3a), 3.64 (1H, *m*, H-20), 3.83 (1H, *m*, H-19), 3.92 (1H, *m*, H-23), 4.00 (1H, *q*, H-16), 4.91 (1H, *qq*, H-36), 5.03 (1H, *m*, H-15), 5.06 (1H, *m*, H-31), 5.21 (1H, *m*, H-24), 5.38 (1H, *m*, H-4), 6.97 (1H, *q*, *J* = 1.5, 1 Hz, H-35). EIMS fragmentation of TMSi derivative (Fig. 1).

(2,4-cis)-28-*Hydroxybullatacinone* (**4**) *and* (2,4-trans)-28-*hydroxybullatacinone* (**5**). $C_{37}H_{67}O_8$: IR ν_{max} (film) cm^{-1}; 3621, 1760, 1716; HRFABMS (glycerol) *m/z* 639.4823 for $[MH^+]$ (calcd 639.4836); ^1H and ^{13}C NMR (Table 4); COSY and single- and double-relayed COSY (τ = 35 and 70 ms, respectively) spectra were recorded. Tri-TMSi derivatives, EIMS *m/z* (%): 839 (2.0), 769 (3.3), 523 (22.0), 453 (25.2), 433 (26.3), 383 (99.5), 363 (14.6), 345 (14.6), 311 (21.1), 293 (19.2), 241 (40.2), 187 (23.9). Tri-(*S*)-Mosher ester, ^1H NMR: δ0.860 (3H, *t*, H-34), 1.30 (1H, *m*, H-13), 1.52–1.58 (3H, *m*, H-17b, H-25), 1.61–1.68 (5H, *m*, H-14, H-18b, H-21b, H-22b), 1.82 (3H, *m*, H-18a, H-21a, H-22a), 1.93 (1H, *m*, H-17a), 3.79 (2H, *m*, H-19, H-20), 3.96 (1H, *m*, H-23), 4.03 (1H, *q*, H-16), 4.99 (1H, *m*, H-28), 5.06 (1H, *q*, H-15), 5.21 (1H, *m*, H-24). Tri-(*R*)-

Mosher ester, ^{1}H NMR: δ0875 (3H, *t*, H-34), 1.13 (1H, *m*, H-13), 1.46 (2H, *m*, H-14), 1.56 (5H, *m*, H-17b, H-21b, H-22b, H-25), 1.68 (1H, *m*, H-21a), 1.75 (2H, H-18b, H-22a), 1.87 (1H, *m*, H-18a), 2.01 (1H, *m*, H-17a), 3.63 (1H, *m*, H-20), 3.82 (1H, *m*, H-19), 3.88 (1H, *m*, H-23), 4.00 (1H, *q*, H-16), 5.06 (1H, *m*, H-28), 5.06 (1H, *q*, H-15), 5.14 (1H, *m*, H-24).

Formaldehyde derivatives (**4a** *and* **5a**) *of* **4** *and* **5**. To Me_3SiCl (100 mg, in 3 ml of CH_2Cl_2) was added Me_2SO (100 mg in 2 ml CH_2Cl_2), and the mixture was allowed to stand at room temperature for about 1 hr until a white precipitate appeared. The CH_2Cl_2 was decanted, and the white precipitate was quickly washed with 1 ml of CH_2Cl_2. To this precipitate, **4** and **5** (50 mg, in 5 ml of CH_2Cl_2) were added with stirring at room teperature for 48 hr. The mixture was washed using 1% $NaHCO_3$ (5 ml) and water (2 × 5 ml), and the CH_2Cl_2 layer was dried *in vacuo*. The products were purified by normal phase open column chromatography (0.5% MeOH in $CHCl_3$) to give **4a** and **5a**; ^{1}H NMR Table 5. Mono-(*S*)-(*R*)-Mosher esters, ^{1}H NMR (Table 5).

(2,4-cis *and* trans)-32-*Hydroxybullatacinones* (**6** *and* **7**), (2,4-cis *and* trans)-31-*hydroxybullatacinones* (**8** *and* **9**), *and* (2,4-cis *and* trans)-30-*hydroxybullatacinones* (**9** *and* **11**). Previously isolated from *Annona bullata* [14]. Tris-(*S*)- and (*R*)-Mosher esters, ^{1}H NMR Table 6.

(2,4-cis *and* trans)-*Bulladecinones* (**12** *and* **13**). Previously isolated from *Annona bullata* [19]. 23,24-Acetonide derivatives of **12** and **13**, previously prepared [19]. Mono-(*S*)-Mosher esters, ^{1}H NMR: δ1.30 (3H, *s*, CH_3), 1.40 (3H, *s*, CH_3), 1.62 (4H, *m*, H-13b, H-14b, H-17b, H-18b), 1.95 (3H, *m*, H-13a, H-14a, H-17a), 2.06 (1H, *m*, H-18a), 3.84 (1H, *m*, H-12), 3.92 (2H, *m*, H-15, H-16), 4.06 (1H, *q*, H-19), 5.07 (1H, *m*, H-20). Mono-(*R*)-Mosher esters, ^{1}H NMR: δ1.33 (3H, *s*, CH_3), 1.42 (3H, *s*, CH_3), 1.52 (3H, *m*, H-13b, H-14b, H-17b), 1.57 (1H, *m*, H-18b), 1.88 (2H, *m*, H-13a, H-14a, H-17a), 1.96 (1H, *m*, H-18a), 3.84 (2H, *m*, H-12, H-16), 3.87 (1H, *m*, H-15), 3.95 (1H, *m*, H-24), 4.03 (1H, *m*, H-23), 4.06 (1H, *q*, H-19), 5.08 (1H, *m*, H-20).

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