30-, 31-, and 32-Hydroxybullatacinones: Bioactive Terminally Hydroxylated Annonaceous Acetogenins from Annona bullata

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30-, 31-, and 32-HYDROXYBULLATACINONES: BIOACTIVE TERMINALLY HYDROXYLATED ANNONACEOUS ACETOGENINS FROM *ANNONA BULLATA*

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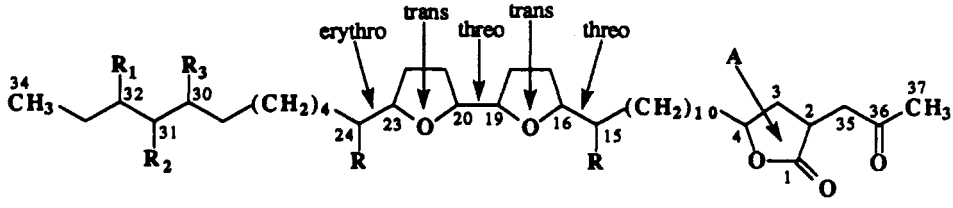
ABSTRACT.—From *Annona bullata*, three more pairs of new ketolactone Annonaceous acetogenins were isolated by bioactivity-directed isolation. They are hydroxylated adjacent bis-tetrahydrofuran (THF) acetogenins and are named (2,4-*cis* and *trans*)-32-hydroxybullatacinone (**1** and **2**), (2,4-*cis* and *trans*)-31-hydroxybullatacinone (**3** and **4**), and (2,4-*cis* and *trans*)-30-hydroxybullatacinone (**5** and **6**). The structures were elucidated by analysis of the ^{1}H- and ^{13}C-nmr spectra of **1–6** and their acetates and the ms of their tri-trimethylsilyl (TMSi) derivatives as compared with bullatacinone [**7**]. This is the first time that Annonaceous acetogenins with OH groups at successive positions near the end of the aliphatic chain have been reported. All of the new compounds showed potent activities in the brine shrimp lethality test and against human solid tumor cells in culture, with selectivities exhibited especially toward the colon cancer cell line (HT-29).

Annonaceous acetogenins (1,2) are a class of highly bioactive compounds that act via inhibition of complex I in mitochondrial electron transport systems (3–5). From the bioactive extract of the bark of *Annona bullata* Rich., seventeen Annonaceous acetogenins have been previously isolated and reported by our group (6–10). Among these, bullatacinone [**7**], an adjacent bis-tetrahydrofuran (THF) compound, was the first reported ketolactone acetogenin. It was published as a C-2/C-4 cis and trans configuration mixture. The work of Hoye and Hanson (11) helped to distinguish such stereoisomers. This mixture of (2,4-*cis* and *trans*)-bullatacinone [**7**] has also been reported in other Annonaceae, e.g., *Annona squamosa* (12) and *Asimina triloba* (13), and other ketolactone acetogenins are continually isolated and reported as such cis and trans mixtures.

In our continuing activity-directed isolation work, three more pairs of these ketolactone mixtures, (2,4-*cis* and *trans*)-32-hydroxybullatacinone (**1** and **2**), (2,4-*cis* and *trans*)-31-hydroxybullatacinone (**3** and **4**), and (2,4-*cis* and *trans*)-30-hydroxybullatacinone (**5** and **6**), were found in the bark extract of *An. bullata*. Compared with (2,4-*cis* and *trans*)-bullatacinone [**7**], these new compounds each have one more OH group near the end of the hydrocarbon chain. Downfield shifts of the ^{1}H-nmr signals of the terminal Me (C-



	1	1a	2	2a	3	3a	4	4a	5	5a	6	6a	7
R	OH	OAc	OH	OAc	OH	OAc	OH	OAc	OH	OAc	OH	OAc	OH
R_1	OH	OAc	OH	OAc	H	H	H	H	H	H	H	H	H
R_2	H	H	H	H	OH	OAc	OH	OAc	H	H	H	H	H
R_3	H	H	H	H	H	H	H	H	OH	OAc	OH	OAc	H
A	*cis*	*cis*	*trans*	*trans*	*cis*	*cis*	*trans*	*trans*	*cis*	*cis*	*trans*	*trans*	*cis* and *trans*

34) in each case indicated the proximity of the nearby OH group, and these distinct shifts were crucial in identifying these novel hydroxylations.

RESULTS AND DISCUSSION

Compounds **1** and **2** were obtained in a mixture as a whitish wax. The mol wt of 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 ms (Figure 1) and nmr spectra indicated that **1** and **2** are adjacent bis-THF ring ketolactone acetogenins (1,2).

The adjacent bis-THF rings, with the usual OH groups on each side, were indicated in **1** and **2** by the ^{1}H-nmr chemical shifts (Table 1) at δ 3.40 (H-15) and 3.86–3.93 (5H, H-16, -19, -20, -23, and -24) and ^{13}C-nmr signals (Table 2) at δ 74.08 (C-15), 82.76 (C-16), 82.50 (C-19), 82.28 (C-20), 83.22 (C-23), and 71.28 (C-24), (1,2,6). This structural unit was confirmed by COSY and single relayed COSY data in which the proton coupling correlations from H-15 to H-24 could be clearly seen. The placement of the adjacent bis-THF rings was determined at C-16 by the eims of the tri-trimethylsilyl (TMSi) derivatives (Figure 1).

The relative stereochemistries around the bis-THF rings of **1** and **2** were determined using the methodologies of Hoye and co-workers (14,15) and Born *et al.* (16), as well as by comparison with (2,4-*cis* and *trans*)-bullatacinone [**7**]. For these comparisons, the triacetates **1a** and **2a** were prepared, and their ^{1}H-nmr data are reported in Table 1. The ^{13}C-nmr signals of **1** and **2** for the hydroxylated carbons C-15 and C-24 at δ 74.08 and 71.28, with corresponding proton signals at δ 3.40 and 3.86, respectively, indicated that one of the stereochemistries within the carbon centers C-15/C-16 and C-23/C-24 was threo, while the other one was erythro. By comparison with the spectra of **7**, which was identical in this region, the stereochemistry at C-15/C-16 was assigned as threo and that at C-23/C-24 as erythro (17). The ^{1}H-nmr signals of **1a** and **2a** at δ 3.99 for H-16 and H-23 and 3.90 for H-19 and H-20 indicated the trans configuration for both THF rings.

In the ^{1}H nmr spectrum of the mixture of **1** and **2**, H-2, H_a-3, H_b-3, H-4, H_a-35, and H_b-35 showed double signals with unequal intensities. By comparisons with ^{1}H-nmr signals of *cis* and *trans* substituted 2-acetonyl-4-butyl-γ-butyrolactone, Hoye and Hanson (11) indicated that these two groups of chemical shifts belong to cis or trans C-

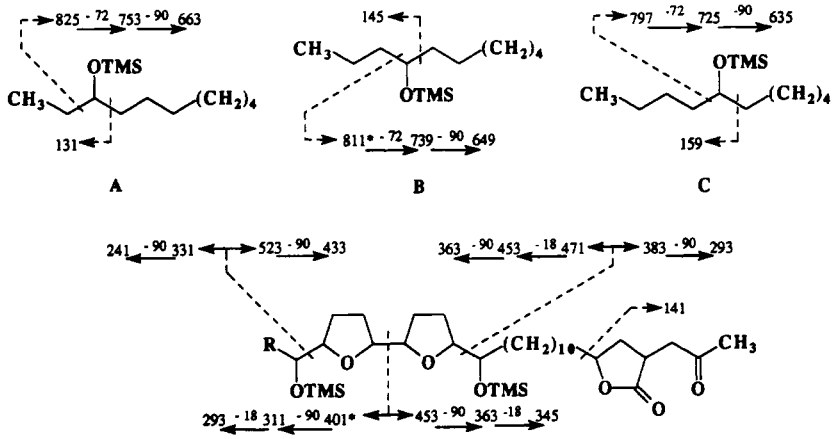


FIGURE 1. Diagnostic eims fragment ions of the tri-trimethylsilyl (TMSi) derivatized (2,4-*cis* and *trans*)-32-hydroxybullatacinone (**1** and **2**, R=**A**), (2,4-*cis* and *trans*)-31-hydroxybullatacinone (**3** and **4**, R=**B**) and (2,4-*cis* and *trans*)-30-hydroxybullatacinone (**5** and **6**, R=**C**). Ions with an asterisk were not observed.

TABLE 1. Comparisons of ^{1}H-nmr (500 MHz) Data of **1–6** and **1a–6a** ($CDCl_3$).

Proton	Compound δ [ppm, (J in Hz)]					
	1 and **2**	**1a** and **2a**	**3** and **4**[a]	**3a** and **4a**	**5** and **6**[b]	**5a** and **6a**
H-2 *cis*	3.02 m	3.02 m	3.02 m	3.02 m	3.02 m	3.02 m
trans	3.03 m	3.03 m	3.03 m	3.03 m	3.03 m	3.03 m
H_a-3 *cis*	1.48	1.48	1.48	1.48	1.48	1.48
trans	2.23 dddd (12.9,9.4,3.5)	2.23 dddd (12.9,9.4,3.5)	2.23 dddd (12.9,9.4,3.5)	2.23 dddd 12.9,9.4,3.5)	2.23 dddd (12.9,9.4,3.5)	2.23 dddd (12.9,9.4,3.5)
H_b-3 *cis*	2.61 m	2.61 m	2.61 m	2.61 m	2.61 m	2.61 m
trans	1.99 m	1.99 m	1.99 m	1.99 m	1.99 m	1.99 m
H-4 *cis*	4.393 m	4.393 m	4.392 m	4.392 m	4.394 m	4.394 m
trans	4.544 m	4.544 m	4.545 m	4.545 m	4.548 m	4.548 m
H_a-5 *cis*	1.76	1.76	1.76	1.76	1.76	1.76
trans	1.71	1.71	1.71	1.71	1.71	1.71
H_b-5 *cis*	1.60	1.60	1.60	1.60	1.60	1.60
trans	1.58	1.58	1.58	1.58	1.58	1.58
H-6–H-13	1.70–1.23	1.65–1.20	1.70–1.23	1.65–1.20	1.70–1.23	1.65–1.20
H-14	1.42	1.65–1.20	1.42	1.65–1.20	1.42	1.65–1.20
H-15	3.40 m	4.86 m	3.40 m	4.84 m	3.40 m	4.85 m
H-16	3.86 m	3.99 m	3.86 m	3.99 m	3.86 m	3.99 m
H-17	1.97, 1.63	2.00–1.60	1.97, 1.63	2.00–1.60	1.97, 1.63	2.00–1.60
H-18	1.97, 1.63	2.00–1.60	1.97, 1.63	2.00–1.60	1.97, 1.63	2.00–1.60
H-19	3.86 m	3.90 m	3.86 m	3.90 m	3.86 m	3.90 m
H-20	3.93 m	3.90 m	3.93 m	3.90 m	3.93 m	3.90 m
H-21	1.97, 1.63	2.00–1.60	1.97, 1.63	2.00–1.60	1.97, 1.63	2.00–1.60
H-22	1.88, 1.82	2.00–1.60	1.88, 1.82	2.00–1.60	1.88, 1.82	2.00–1.60
H-23	3.93 m	3.99 m	3.93 m	3.99 m	3.93 m	3.99 m
H-24	3.86 m	4.91 m	3.86 m	4.91 m	3.86 m	4.90 m
H-25	1.37	1.65–1.20	1.37	1.65–1.20	1.37	1.65–1.20
H-26–H-29	1.70–1.23	1.65–1.20	1.70–1.23	1.65–1.20	1.70–1.23	1.65–1.20
H-30	1.70–1.23	1.65–1.20	1.40	1.65–1.20	3.58 m	4.85 m
H-31	1.47	1.65–1.20	3.60 m	4.84 m	1.40	1.65–1.20
H-32	3.52 m	4.80 m	1.40	1.65–1.20	1.70–1.23	1.65–1.20
H-33	1.47	1.65–1.20	1.70–1.23	1.65–1.20	1.70–1.23	1.65–1.20
H-34	0.940 t (7.0)	0.876 t (7.0)	0.928 t (7.0)	0.903 t (7.0)	0.908 t (7.0)	0.888 t (7.0)
H_a-35 *cis*	2.61 dd (19.9,9.0)	2.61 dd (19.9,9.0)	2.61 dd (19.9,9.0)	2.61 dd (19.9,9.0)	2.61 dd (19.9,9.0)	2.61 dd (19.9,9.0)
trans	2.67 dd (19.9,9.8)	2.67 dd (19.9,9.8)	2.67 dd (19.9,9.8)	2.67 dd (19.9,9.8)	2.67 dd (19.9,9.8)	2.67 dd (19.9,9.8)
H_b-35 *cis*	3.11 dd (18.7,5.4)	3.11 dd (18.7,5.4)	3.11 dd (18.7,5.4)	3.11 dd (18.7,5.4)	3.11 dd (18.7,5.4)	3.11 dd (18.7,5.4)
trans	3.04 dd (19.6,5.4)	3.04 dd (19.6,5.4)	3.04 dd (19.6,5.4)	3.04 dd (19.6,5.4)	3.04 dd (19.6,5.4)	3.04 dd (19.6,5.4)
H-37	2.20 s	2.20 s	2.20 s	2.20 s	2.20 s	2.20 s
16-OAc	—	2.08 s	—	2.08 s	—	2.08 s
24-OAc	—	2.05 s	—	2.05 s	—	2.05 s
30/31/32-OAc	—	2.05 s	—	2.04 s	—	2.04 s

[a]Assigned based on the COSY and single- and double-relayed COSY.
[b]Assigned based on the COSY.

2/C-4 diastereomers, respectively. Thus, the structures could be separately determined in such a mixture. The results of these comparisons indicated that the higher intensity group of proton signals in the ^{1}H-nmr spectrum of **1** and **2** at δ 3.02 (H-2), 1.48 (H_a-3), 2.61 (H_b-3), 4.393 (H-4), 2.61 (H_a-35), and 3.11 (H_b-35) belong to the compound with the cis configuration at C-2/C-4, which here we assigned as **1**, while the lower intensity group of proton signals at δ 3.03 (H-2), 2.23 (H_a-3), 1.99 (H_b-3), 4.544 (H-4), 2.67 (H_a-35) and 3.04 (H_b-35) belong to the compound with the trans configuration at C-2/C-4, which here we assigned as **2**. In addition, the ^{13}C-nmr resonances for C-1, C-2, C-4, and C-36 also showed double peaks with different intensities. The higher intensity group of signals at δ 178.26 (C-1), 43.86 (C-2), 79.41 (C-4), and 205.54 (C-36) thus belong to the cis diastereomer **1**, while the lower intensity group of signals at δ 178.78 (C-1), 44.29 (C-2), 78.96 (C-4), and 205.46 (C-36) belong to the trans diastereomer **2**.

TABLE 2. ^{13}C-nmr (125 MHz) Data of **1–6** ($CDCl_3$).

Carbon	Compound δ (ppm)[a]		
	1 and **2**	**3** and **4**	**5** and **6**
C-1 *cis*	178.26	178.22	178.42
trans	178.78	178.74	178.94
C-2 *cis*	43.86	43.78	43.72
trans	44.29	44.22	44.15
C-3	36.95–25.25	37.42–25.20	37.24–25.09
C-4 *cis*	79.41	79.36	79.37
trans	78.96	78.91	78.92
C-5–C-14	36.95–25.25	37.42–25.20	37.24–25.09
C-15	74.08	74.09	74.09
C-16, -19, -20	82.76, 82.50, 82.28[b]	82.74, 82.48, 82.20[b]	82.75, 82.50, 82.21[b]
C-17, -18, -21, -22	36.95–25.25	37.42–25.20	37.24–25.09
C-23	83.22	83.25	83.27
C-24	71.28	71.23	71.25
C-25–C-29	36.95–25.25	37.42–25.20	37.24–25.09
C-30	36.95–25.25	37.42–25.20	71.77
C-31	36.95–25.25	71.57	37.24–25.09
C-32	73.30	39.64	37.24–25.09
C-33	30.05	18.84	22.66
C-34	9.946	14.15	13.98
C-35	36.95–25.25	37.42–25.20	37.24–25.09
C-36 *cis*	205.54	205.50	205.76
trans	205.46	205.43	205.69
C-37	24.54	24.49	24.40

[a]All of the assignments were made by comparison with (2,4-*cis* and *trans*)-bullatacinone (6,17).
[b]These data are interchangeable.

Both ^{1}H- and ^{13}C-nmr spectra of **1** and **2** were very similar to those of (2,4-*cis* and *trans*)-bullatacinone [**7**]. Fabms showing their mol wt 638 (16 more than that of **7**) indicated that **1** and **2** had one more OH group. The ^{1}H-nmr spectrum showed an extra proton signal at δ 3.52 corresponding to the methine proton of this additional aliphatic chain OH. In Annonaceous acetogenins, generally, the ^{1}H-nmr signals for such methine protons on chain-hydroxylated carbons are at ca. δ 3.60. This upfield shift indicated that the additional OH group was at an unusual place. The downfield shift of the proton of the terminal Me (H-34) to δ 0.940 (normally ca. δ 0.878) and the upfield shift of its carbon (C-34) to δ 9.946 (normally ca. δ 14.10) suggested that the new OH group was close to this terminal Me group. The COSY spectrum of **1** and **2** showed coupling correlation cross peaks between the signals at δ 0.940 (H-34) and 1.47. The latter signal was further coupled with the hydroxylated methine proton at δ 3.52. This indicated that the additional OH group should be at C-32. This placement was clearly confirmed by the eims fragmentation of TMSi derivatives of **1** and **2** (Figure 1). Thus, **1** and **2** were concluded to be hydroxylated at C-32 and were named (2,4-*cis*)-32-hydroxybullatacinone [**1**] and (2,4-*trans*)-32-hydroxybullatacinone [**2**].

Compounds **3** and **4** were also obtained as a mixture of cis and trans ketolactone acetogenins, as indicated by the characteristic proton signals at δ 4.392 and 4.545 (1,2,10). Their mol wt at 638 was suggested by fabms and confirmed by hrfabms (glycerol) with $[MH]^+$ at *m/z* 639.4849 for $C_{37}H_{67}O_8$ (calcd 639.4836). ^{1}H and ^{13}C nmr of **3** and **4** (Tables 1 and 2), ^{1}H nmr of the acetyl derivatives (**3a** and **4a**) (Table 1), and eims of the TMSi derivatives of **3** and **4** (Figure 1) were the same as those of **1** and **2**, **1a** and **2a**, and the TMSi derivatives of **1** and **2**, respectively, except for those derived from

the hydroxylated aliphatic chain. Thus, **3** and **4** were believed to have the same skeletons and the relative stereochemistries as **1** and **2** (i.e., a monohydroxylated bullatacinone) but with a different location of the terminal chain OH group.

The ^{1}H-nmr signal of **3** and **4** for the terminal Me (H-34) at δ 0.928 showed some downfield shift compared with most other Annonaceous acetogenins (1,2) but less than that of **1** and **2**. This indicated that the chain OH group was still close to the terminal Me but farther away than in **1** and **2**. In the single relayed COSY spectrum, there were no cross peaks between the protons at δ 0.928 (H-34) and 3.60, while the ^{1}H-^{1}H double relayed COSY spectrum showed the expected cross peaks between them. This result placed the chain OH group at C-31. The eims fragments of the TMSi derivatives of **3** and **4** (Figure 1) confirmed this placement. The compounds were named (2,4-*cis*)-31-hydroxybullatacinone [**3**] and (2,4-*trans*)-31-hydroxybullatacinone [**4**].

Like **3** and **4**, the mixture of **5** and **6** was also very similar to **1** and **2** and differed only in the location of the chain OH group. The ^{1}H-nmr signals of **5** and **6** at δ 0.907 for H-34 and at δ 3.58 for the chain oxymethine proton showed that the chain OH group was still farther from the terminal Me group (C-34) than that of **3** and **4**. In both single and double relayed COSY spectra, there were no correlation cross peaks between the protons as δ 0.908 (H-34) and 3.58, showing that the hydroxylation was upchain from C-31. The placement of the chain OH group at C-30 was convincingly determined by the eims fragmentation of the TMSi derivatives of **5** and **6** (Figure 1), which showed intense peaks at *m/z* 159 and 797. The mol wts of **5** and **6** were also 638 as determined by fabms and confirmed by hrfabms (glycerol), with the $[MH]^+$ at *m/z* 639.4830 for $C_{37}H_{67}O_8$ (calcd 639.4836). Thus, **5** and **6** were identified as (2,4-*cis*)-30-hydroxybullatacinone [**5**] and (2,4-*trans*)-30-hydroxybullatacinone [**6**].

This is the first time that a single OH group has been found in successive positions along the hydrocarbon chain of Annonaceous acetogenins close to the terminal Me group (C-34). The downfield shifts of the ^{1}H-nmr signals for the terminal methyls (H-34) showed interesting sequential changes and are quite characteristic for this series of compounds.

Like (2,4-*cis* and *trans*)-bullatacinone [**7**], all of these mixtures of the new isolates **1–6** were active in the brine shrimp lethality test (BST) (18,19) and also were highly cytotoxic to human solid tumor cells in culture. To permit more accurate comparisons, the cytotoxicities reported (Table 3), for all of these acetogenins and adriamycin, were determined in the same run. The addition of an OH group appears notably to enhance cytotoxicities, as all of the new isolates are more cytotoxic than bullatacinone [**7**]. This is especially evident in the human colon cell line (HT-29), in which all of the new

TABLE 3. Bioactivities of Compounds **1–7**.

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**	4.67×10^{-2}	1.25×10^{-3}	1.61×10^{-1}	$<10^{-12}$
3 and **4**	1.58×10^{-1}	3.29×10^{-11}	7.63×10^{-4}	1.09×10^{-12}
5 and **6**	4.05×10^{-2}	1.66×10^{-9}	1.05×10^{-1}	$<10^{-12}$
7	5.40×10^{-3}	2.18×10^{-3}	12.16	1.19×10^{-6}
Adriamycin[e]	8×10^{-2}	1.98×10^{-4}	2.02×10^{-3}	3.32×10^{-4}

[a]Brine shrimp lethality test.
[b]Human lung carcinoma.
[c]Human breast carcinoma.
[d]Human colon adenocarcinoma.
[e]Positive control standard. Cytotoxicity values reported were all determined in the same run.

compounds show one million or one hundred million times the activities of **7** and reference adriamycin, respectively.

EXPERIMENTAL

PLANT MATERIAL.—Bark of *An. bullata* (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. The dried bark was pulverized in a Wiley mill.

BIOASSAYS.—The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST) (18,19). Cytotoxicities against human solid tumor cells were measured at the Purdue Cell Culture Laboratory, Purdue Cancer Center for the A-549 lung carcinoma (20), MCF-7 breast carcinoma (21), and HT-29 colon adenocarcinoma (22).

INSTRUMENTATION.—Ir spectra (film) were measured on a Perkin-Elmer 1420 ir spectrometer. ^{1}H-nmr, COSY, and ^{13}C-nmr spectra 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 MS50 spectrometer through peak matching. Hplc was carried out using a Dynamax software system and a Si gel (8 μm) column (250×21 mm) equipped with a Rainin UV-1 detector. Analytical tlc was performed on Si gel plates (0.25 mm) developed with $CHCl_3$-MeOH (9:1) and hexane-Me_2CO (3:2) and visualized with 5% phosphomolybdic acid in EtOH (1,2).

EXTRACTION AND ISOLATION.—The pulverized bark (3.9 kg) was extracted and partitioned, as previously described, to obtain F005 (7). F005 (80 g) was subjected to open cc (3 kg Si 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 Si 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 $LC_{50}=3.25\times10^{-1}$ ppm) was subjected to flash Si gel cc (600 g), eluted by a gradient of $CHCl_3$/MeOH. Fractions (F_3-1 to F_3-98) were collected and combined into 8 pools on the basis of similar tlc patterns. P-2 (F_3-21–F_3-34, 0.78 g, BST $LC_{50}=8.9\times10^{-2}$ ppm) was chromatographed on a column of Si gel in the hplc eluted by $CHCl_3$-MeOH (90:1, flow rate 10 ml/min) to afford the three white powders containing **1** and **2**, **3** and **4**, and **5** and **6** (retention times 73, 90, and 110 min, respectively).

*(2,4-*cis *and* trans*)-32-Hydroxybullatacinone (**1** and **2**).*—White powder (7 mg): fabms (glycerol) *m/z* $[MH]^+$ 639 (99.6%); hrfabms (glycerol) *m/z* 639.4823 for $C_{37}H_{67}O_8$ (calcd 639.4836); ^{1}H nmr ($CDCl_3$, 500 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 125.75 MHz) see Table 2; COSY ($CDCl_3$, 500 MHz) and single-relayed COSY ($CDCl_3$, 500 MHz, tau=35 ms) spectra were obtained; ir (film) cm^{-1} 3426 (OH), 2919, 2361, 1763, 1715, 1062.

*(2,4-*cis-*and* trans*)-31-Hydroxybullatacinone (**3** and **4**).*—White powder (20 mg): fabms (glycerol) *m/z* $[MH]^+$ 639 (83.5%); hrfabms (glycerol) *m/z* 639.4849 for $C_{37}H_{67}O_8$ (calcd 639.4836); ^{1}H nmr ($CDCl_3$, 500 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 125.75 MHz) see Table 2; ir (film) cm^{-1} 3426 (OH), 2919, 2847, 1764, 1716, 1068.

*(2,4-*cis *and* trans*)-30-Hydroxybullatacinone (**5** and **6**).*—White powder (30 mg): fabms (glycerol) *m/z* $[MH]^+$ 639 (90.7%); hrfabms (glycerol) *m/z* 639.4830 for $C_{37}H_{67}O_8$ (calcd 639.4836); ^{1}H nmr ($CDCl_3$, 500 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 125.75 MHz) see Table 2; ir (film) cm^{-1} 3426 (OH), 2925, 2847, 2361, 1765, 1716, 1062.

ACETYLATIONS.—Compounds **1** and **2**, **3** and **4**, or **5** and **6** (3 mg of each) were mixed with anhydrous pyridine/Ac_2O at room temperature overnight and through the usual workup gave ca. 2 mg of the triacetates **1a** and **2a**, **3a** and **4a**, and **5a** and **6a**. ^{1}H-nmr data are shown in Table 1.

TMSI DERIVATIZATIONS.—Small amounts (< 1 mg) of **1** and **2**, **3** and **4**, and **5** and **6** were treated with 20 μl of *N,O*-bis-(trimethylsilyl)-acetamide and 2 μl of pyridine and heated at 70° for 30 min to yield the respective tri-TMSi derivatives. Eims of **1** and **2** *m/z* (rel. int.) 839 (3.0), 825 (5.3), 753 (3.1), 597 (11.6), 567 (4.7), 553 (13.1), 523 (20.0), 505 (6.1), 471 (12.7), 455 (22.4), 453 (25.2), 433 (24.3), 415 (21.3), 383 (99.4), 363 (14.6), 345 (16.6), 339 (10.9), 311 (20.1), 293 (29.2), 265 (16.4), 247 (11.1), 243 (16.9), 241 (40.2), 213 (11.0), 131 (61.9). Eims of **3** and **4** *m/z* (rel. int.) 739 (1.0), 649 (2.5), 597 (2.8), 567 (2.2), 553 (3.2), 523 (9.7), 505 (2.8), 471 (1.1), 453 (15.9), 433 (10.5), 415 (12.0), 383 (99.8), 367 (10.8), 363 (10.5), 345 (10.0), 311 (14.3), 293 (11.9), 265 (7.6), 241 (10.8), 145 (25.4). Eims of **5** and **6** *m/z* (rel. int.) 797 (3.2), 725 (1), 635 (2.9), 597 (3.1), 567 (2.8), 553 (3.7), 523 (9.9), 505 (3.6), 471 (2.0), 453 (15.7), 433 (10.1),

415 (11.9), 383 (99.8), 367 (11.9), 363 (13.4), 345 (11.0), 311 (19.9), 293 (15.3), 265 (9.5), 243 (10.0), 241 (14.4), 159 (22.2).

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