BULLATIN AND BULLANIN: TWO NOVEL, HIGHLY CYTOTOXIC ACETOGENINS FROM *ASIMINA TRILOBA*

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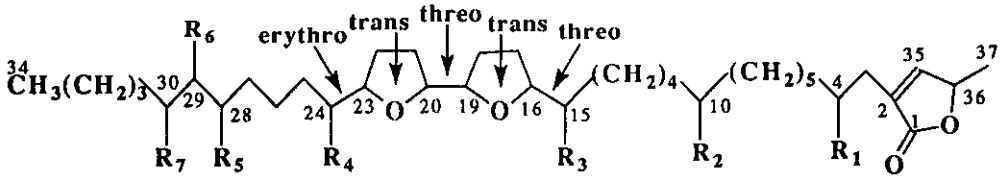
Abstract- Two new highly cytotoxic adjacent bis-tetrahydrofuran acetogenins, bullatin (**2**) (10-hydroxy-4-deoxybullatacin) and bullanin (**5**) (30-hydroxy-4-deoxybullatacin), have been isolated from the ethanolic extract of the stem bark of *Asimina triloba* by directing the fractionation with brine shrimp lethality. In addition to these two new compounds, two previously reported acetogenins, squamocin (**3**) (28-hydroxy-4-deoxybullatacin) and motrilin (**4**) (29-hydroxy-4-deoxybullatacin) which are new to this species, were obtained. Through spectral analysis these four acetogenins were structurally identified as hydroxylation isomers of bullatacin (**1**) which was recently patented as an antitumor agent. All of these acetogenins showed high potencies in the brine shrimp test (BST) and in cytotoxicity tests against human solid tumor cell lines in culture, with certain potencies quite comparable to those of bullatacin (**1**) and several orders of magnitude greater than those of adriamycin.

Bullatacin (**1**) (Figure 1) is one of the most potent antitumor and pesticidal Annonaceous acetogenins and was first reported from *Annona bullata* in 1989.[1] The correct absolute configurations of the stereogenic carbinol centers of **1** were recently established by ^{1}H and ^{19}F nmr spectral analysis of its (*S*)- and (*R*)-Mosher ester [methoxy(trifluoromethyl)phenylacetate or MTPA] derivatives.[2] Due to its remarkably potent cytotoxicities against a number of human tumor cell lines,[1] bullatacin has been recently patented as a chemotherapeutic

agent.[3] Like other adjacent bis-tetrahydrofuran (THF) Annonaceous acetogenins,[4-6] bullatacin (**1**) interfers with mitochondrial electron transport processes where it acts as a very potent inhibitor of NADH : ubiquinone reductase (complex I).[6] Bullatacin (**1**) also has shown potent in vivo antitumor activities, e.g., 67% tumor growth inhibition (TGI) at 50 μg/Kg in athymic mice bearing A2780 human ovarian cancer xenografts.[6] Bullatacin (**1**) likely inhibits cancer cell growth through inhibition of mitochondrial electron transport systems to reduce the ATP levels, and, if the cancer cells have higher energy demand than normal cells, then the antitumor activities can be elicited. Furthermore, the operation of the plasma membrane P-170 glycoprotein, which is believed to explain multidrug resistance (MDR), requires ATP,[7] and the acetogenins, by depleting intracellular ATP levels, offer an exciting potential for the inhibition of multidrug resistant cancer cells.



compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7
bullatacin (**1**)	OH	H	OH	OH	H	H	H
bullatin (**2**)	H	OH	OH	OH	H	H	H
bullatin triacetate (**2a**)	H	OAc	OAc	OAc	H	H	H
squamocin (**3**)	H	H	OH	OH	OH	H	H
motrilin (**4**)	H	H	OH	OH	H	OH	H
bullanin (**5**)	H	H	OH	OH	H	H	OH
bullanin triacetate (**5a**)	H	H	OAc	OAc	H	H	OAc
desacetyluvaricin (**6**)	H	H	OH	OH	H	H	H

Figure 1. Chemical Structures of Adjacent Bis-THF Acetogenins (**1-6** , **2a** and **5a**).

Eleven Annonaceous acetogenins have been previously reported from the EtOH extract of the stem bark of *Asimina triloba*.[8-10] The adjacent bis-THF acetogenins represent the most bioactive components in this plant and include three structural variations, bullatacin, trilobacin, and asimicin types, with regard to the stereochemistry of the six chiral centers between C-15 and C-24.[8] As well, a series of asimicin (threo/trans/threo/trans/threo from C-15 to C-24) isomers, asimin, asiminacin, and asiminecin,[10] have been found, which shift the 4-OH group in asimicin to the C-10, C-28, and C-29 positions, respectively. Directed by

the brine shrimp lethality test (BST),[11, 12] our continued investigation of these bark extracts has now yielded another series of isomeric acetogenins, the bullatacin (threo/trans/threo/trans/erythro from C-15 to C-24) isomers; these are bullatin (**2**), squamocin (**3**), motrilin (**4**) and bullanin (**5**), with the third hydroxyl group at C-10, C-28, C-29, and C-30, respectively, instead of at C-4. Bullatin (**2**) and bullanin (**5**) are new to the literature, while the other two acetogenins, squamocin (**3**) and motrilin (**4**), are reported here for the first time from this species.

Bullatin (**2**), a colorless wax, was obtained after hplc separation from the more polar fractions of the 90% methanolic partition extract.[8-10] The molecular formula was deduced as $C_{37}H_{66}O_7$ by the high resolution FAB mass spectrum which gave the $[M+H]^+$ ion at m/z 623.4865 corresponding to the calculated exact mass of 623.4887. The ir spectrum of **2** showed characteristic absorptions of the α,β-unsaturated γ-lactone (1748 cm^{-1}, C=O) and hydroxyl (3442 cm^{-1}, OH) functional groups. The presence of the α,β-unsaturated γ-lactone moiety was confirmed by the ^1H nmr resonance peaks (Table 1) at δ 6.99 (q, H-35), 5.00 (qq, H-36) and ^{13}C nmr resonances (Table 1) at δ 173.83 (C-1), 134.21 (C-2), 148.85 (C-35), 77.43 (C-36), and 19.27 (C-37). Spectral comparisons of **2** with bullatacin (**1**), the 4-hydroxylated isomer, indicated that the H-3 signals appeared as a multiplet peak at δ 2.26 (tt, 2H) in the ^1H nmr spectrum of **2** instead of an AB spin system corresponding to the H-3a and H-3b proton resonance in the ^1H nmr spectrum of **1**, and the upfield shift of proton signals for H-35 and H-36 of compound (**2**) suggested that the 4-OH group was absent in the structure of bullatin (**2**); this is similar to our recent observations with the series of 4-deoxyasimicin isomers. [10]

Careful examination of the ^1H nmr spectrum of **2** showed the typical proton resonances of the adjacent bis-THF acetogenins. The three multiplets at δ 3.41, 3.87, and 3.93 integrated for one proton, four protons and one proton, respectively, on oxygen-bearing carbons, and the ^{13}C nmr resonances of these methine carbons at δ 71.31, 73.99, 82.52, 82.33, 82.81 and 83.14 clearly indicated the presence of the structural unit of an adjacent bis-THF moiety with two flanking OH groups. ^1H and ^{13}C nmr spectral comparisons of **2** with bullatacin (**1**) suggested that the relative stereochemical relationship of **2** across the bis-THF skeleton from C-15 through C-24 is threo/trans/threo/trans/erythro, and, thus, **2** belongs to the bullatacin (**1**) type acetogenins.[1, 2] This conclusion was confirmed by the expected downfield shift of these methine proton resonances and their locations at δ 3.91, 3.98, 4.85, and 4.92 in the ^1H nmr spectrum of bullatin triacetate (**2a**) (Table 1).[13, 14]

The existence of three OH groups was determined by successive losses of three water molecules (m/z 18x3) in the EIms spectrum of **2** (Figure 2) and the three single acetate methyl proton signals, at δ 2.04, 2.05 and 2.08,

in the ^{1}H nmr spectrum of bullatin triacetate (**2a**) (Table 1). In addition to the two flanking OH groups on each side of the THF ring system there had to be a third hydroxyl in this structure and it could not be located at C-4. In the ^{1}H nmr spectrum of **2**, the methine proton on the carbon to which the third hydroxyl group was attached appeared at δ 3.59 and showed its only cross peak with the complex of methylene proton signals at δ 1.43 in the ^{1}H-^{1}H 2D homonuclear correlation (COSY) spectrum, indicating that this third OH group was located somewhere in the aliphatic chain.

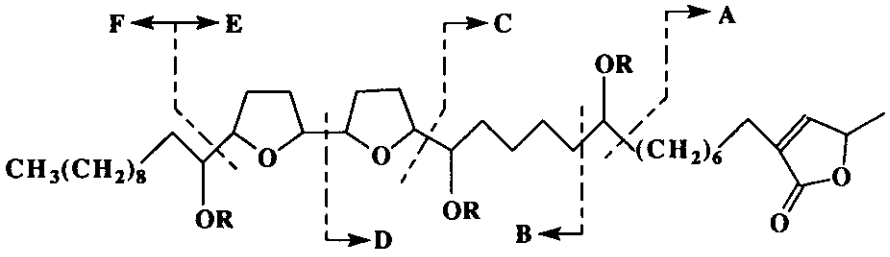
Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) nmr data of **2** and **2a**

H/C No.	^{1}H nmr of **2**	^{1}H nmr of **2a**	^{13}C nmr of **2**
1		-	173.83
2		-	134.21
3	2.26 tt (7.6, 1.5)	2.26 tt (7.6, 1.5)	22.73-31.94
4	1.55 m	1.40-1.80 m	22.73-31.94
9, 11	1.43 m	1.40-1.80 m	37.423 37.45
10	3.59 m	4.85 m	71.85*
14, 25	1.40 m	1.40-1.80 m	33.31, 33.46
15	3.41 m	4.85 m	73.99
16	3.87 m	3.98 m	83.14**
17, 18, 21	1.98 m, 1.64 m	1.98 m, 1.64 m	22.73-31.94
19, 20	3.87 m	3.91 m	82.52, 82.33
22	1.81 m, 1.89 m	1.80 m, 1.89 m	22.73-31.94
23	3.93 m	3.98 m	82.81**
24	3.87 m	4.92 m	71.31*
5-8, 12-13, 26-33	1.20-1.50 m	1.20-1.80 m	22.73-31.94
34	0.878 t (7.0)	0.878 t (7.0)	14.18
35	6.99 q (1.5)	6.99 q (1.5)	148.85
36	5.00 qq (6.8, 1.8)	5.00 qq (6.8, 1.7)	77.43
37	1.41 d (7.0)	1.41 d (7.0)	19.27
15-OAc		2.08 s	
24-OAc		2.05 s	
10-OAc		2.04 s	

*, ** Indicate assignments which may be interchangable.

The placement of the adjacent bis-THF ring at C-16 through C-23 and the three OH groups at C-10, C-15 and C-24 in the aliphatic chain was established by the analysis of the EIms spectra of the tri-trimethylsilyl (tri-TMS) and tri-deutero-trimethylsilyl (d_9-tri-TMS) derivatives of **2** (Figure 2). The crucial fragment peaks at m/z 297 in the EIms spectra of the tri-TMS derivative and m/z 306 in the EIms spectra of the d_9-tri-TMS derivative of **2**, due to the cleavage of the bond between C-10 and C-11, indicated that the third OH group was located at the C-10 position. This conclusion was further confirmed by the exact mass determination of these fragment ions of the tri-TMS and d_9-tri-TMS derivatives, i.e., for the m/z 297 ion peak the exact mass 297.1886 was found corresponding to the formula, $C_{16}H_{29}O_3Si$ (Calcd 297.1886), and for the m/z 306 ion the exact mass of

306.2451 was found corresponding to the formula of $C_{16}H_{20}D_9O_3Si$ (Calcd 306.2451). A detailed analysis of the EIms spectra of **2** and its tri-TMS and d9-tri-TMS derivatives is given in Figure 2. The full assignment of proton resonance signals (Table 1) was made by analysis of the ^1H-^1H 2D nmr (COSY) spectrum of **2**. Thus, the structure of bullatin (**2**) was concluded to be as indicated in Figure 1.



R	A	B	C	D	E	F
H	(427)	225	311	(381)		171
(EIMS)	409 (a)		293 (a)	363 (a)		
	391 (a)		275 (a)	345 (a)		
	373 (a)			327 (a)		
TMS	643	297	455	(525)	595	243
(EIMS)	553 (b)		365 (b)	435 (b)	505 (b)	
			275 (b)		415 (b)	
					397 (a)	
D_9-TMS	(670)	306	473	(543)	613	252
(EIMS)	571 (c)		374 (c)	444 (c)	514 (c)	
	472 (c)		275 (c)	345 (c)	415 (c)	
	373 (c)			327 (c)	397 (a)	

(a): loss of H_2O (m/z 18); (b): loss of TMSiOH (m/z 90); (c): loss of d_9-TMSiOH (m/z 99).

Figure 2. Diagnostic EIms fragmentation ions of **2** and its tri-TMS and tri-d9-TMS derivatives

Squamocin (**3**) and motrilin (**4**) (Figure 1) were, respectively, reported from *Annona squamosa* and *Annona cherimolia* in previous papers,[15, 16] and they are now identified herein in the *Asimina* genus. The structures of **3** and **4** were determined by the spectral analysis of their ^1H, ^{13}C nmr, CIms and EIms spectra, and by the spectral data comparisons with previously reported data.[15, 16] However, nmr data alone did not give sufficient information for the placement of THF rings and OH groups in the aliphatic chain, and these features can show a great deal of variation among acetogenin structures.[17, 18] For example, the asimicin series of isomers includes asimicin,[19] asimin, asiminacin, asiminecin,[10] parviflorin,[20] and bullacin.[21] Therefore, a key step in the structural elucidation of **3** and **4** was the determination of the exact position of the third OH group along the

aliphatic chain, and this relied on the EIms data analysis, especially the EIms spectral analysis of the tri-TMS and d_9-tri-TMS derivatives. Thus, the placement of the third OH group at C-28 for **3** and at C-29 for **4** were convincingly confirmed by the EIms spectral data of their tri-TMS and d_9-tri-TMS derivatives.

Bullanin (**5**) was also obtained in a waxy form. The ir, ^1H and ^{13}C nmr (Table 2), and ^1H-^1H correlation (COSY) spectra of **5** were very similar to those of **2-4.** The existence of three OH groups was again indicated by the broad ir absorption peak at 3442 cm^{-1} and the three acetate methyl resonances at δ 2.04, 2.05 and 2.08 in the ^1H nmr spectrum of bullanin triacetate (**5a**) (Table 2). The appropriate proton and carbon resonance signals (Table 2) were quickly attributed to the now familiar α,β-unsaturated γ-lactone moiety and the familiar adjacent bis-THF ring system, as the ^1H and ^{13}C nmr spectra of **5** showed little differences from those of **2-4.**

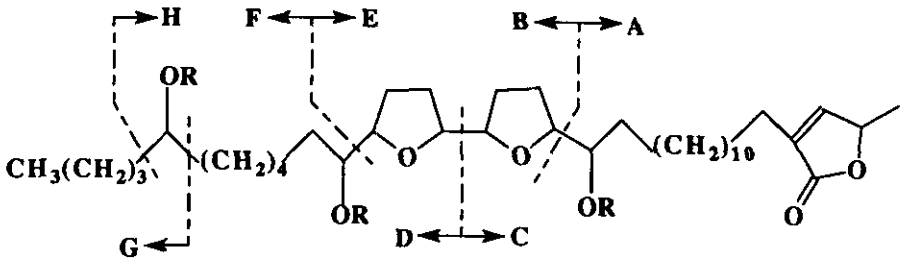
Table 2. ^1H (500 MHz) and ^{13}C (125 MHz) nmr data of **5** and **5a**

H/C No.	^1H nmr of **5**	^1H nmr of **5a**	^{13}C nmr of **5**
1	-	-	173.83
2	-	-	134.23
3	2.26 tt (7.5, 1.5)	2.26 tt (7.5, 1.5)	22.80-29.76
4	1.54 m	1.54 m	22.80-29.76
14, 25	1.39 m	1.46-1.64 m	33.33, 32.27
15	3.40 m	4.86 m	74.11
16	3.85 m	3.98 m	83.25**
17, 18, 21	1.98 m, 1.63 m	1.95 m, 1.78 m	22.80-29.76
19, 20	3.85 m	3.89 m	82.52, 82.26
22	1.90 m, 1.81 m	1.95 m, 1.78 m	22.80-29.76
23	3.93 m	3.98 m	82.75**
24	3.85 m	4.91 m	71.28*
29, 31	1.40 m	1.46-1.64 m	37.17, 37.34
30	3.58 m	4.86 m	71.87*
5-13, 26-27, 31-33	1.22-1.72 m	1.20-1.38 m	22.80-29.76
34	0.907 t (7.0)	0.907 t (7.0)	14.14
35	6.99 q (1.5)	6.99 q (1.5)	148.79
36	5.00 qq (7.0, 1.5)	5.00 qq (7.0, 1.5)	77.41
37	1.41 d (7.0)	1.41 d (7.0)	19.26
15-OAc		2.08 s	
24-OAc		2.05 s	
29-OAc		2.04 s	

*, ** Indicate assignments which may be interchangable.

The $[M+H]^+$ ion peak at m/z 623.4915 in the high resolution FAB mass spectrum indicated the molecular formula of $C_{37}H_{66}O_7$ (Calcd 623.4887). Thus, **5** was tentatively identified as another isomer of bullatacin (**1**), having the same carbon skeleton and relative stereochemistries as **2-4** but with a different location of the third OH group. Like compounds (**1-4**), the relative stereochemistry at the six chiral centers from C-15 through C-24 across the bis-THF ring of **5** was also confirmed as threo/trans/threo/trans/erythro by the typical downfield

shifts and placements of these oxygen-bearing methine proton signals in the bis-THF rings in the ^1H nmr spectrum of bullatacin triacetate (**5a**) (Table 2).[13, 14]



R	A	B	C	D	E	F	G	H
H	295	(327)		(257)	(435)	187		565
(EIMS)	277 (a)	(309)		239 (a)	417 (a)	169 (a)		(547)
		291 (a)		221 (a)		151 (a)		(529)
								511 (a)
TMS	367	(471)	437	(401)	507	331	159	781
(EIMS)		381 (b)	347 (b)	311 (b)	417 (b)	241 (b)		(691)
		291 (b)	329 (a)	293 (a)	399 (a)	151 (b)		601 (b)
d_9-TMS	376	(489)	(446)	419	516	349	168	(808)
(EIMS)	277 (c)	390 (c)	347 (c)	320 (c)	417 (c)	250 (c)		
		291 (c)	329 (a)		399 (a)	151 (c)		

(a): loss of H_2O (m/z 18); (b): loss of TMSiOH (m/z 90); (c): loss of d9-TMSiOH (m/z 99).

Figure 3. Diagnostic EIms fragmentation ions of **5** and its tri-TMS and tri-d_9-TMS derivatives

Again, the EIms diagnostic fragment peaks of the tri-TMS and d_9-tri-TMS derivatives of **5** (Figure 3) determined the positions of the adjacent bis-THF ring moiety and the third OH group. Careful analysis of the EIms fragmentation ions of the tri-TMS and d_9-tri-TMS derivatives led us to conclude that , like compounds (**1-4**), the bis-THF ring with two flanking OH groups was also located at C-15 through C-24 . The hypothesis that the third OH group was placed at C-30 was supported by the crucial diagnostic mass ion at m/z 159 in the EIms spectrum of the tri-TMS derivative (high resolution EIms found: m/z 159.1203, Calcd 159.1205 for $C_8H_{19}OSi$) due to cleavage between C-29 and C-30. Confirming support for the C-30 hydroxyl was made by the mass ion at m/z 168 in the EIms spectrum of the d_9-tri-TMS derivative (high resolution EIms found: m/z 168.1771, Calcd 168.1770 for $C_8H_{10}D_9OSi$). The proton signal assignments (Table 2) were made by detailed analysis of the ^1H-^1H correlated 2D spectrum (COSY). From these data, the structure of bullanin (**5**) was concluded to be as that shown in Figure 1.

The terminal methyl group of these acetogenins undergoes downfield chemical shifts when the third OH group approaches it along the aliphatic chain. Remeasurement of ^{1}H nmr spectra of **1** and deacetyluvaricin (**6**, Figure 1) along with compounds (**2-5**), on a Varian VXR-500S (at 500 MHz) spectrometer in $CDCl_3$ with reference to TMS, showed that the chemical shifts of the ^{1}H nmr signals for the terminal methyls appeared at δ 0.878 for compounds **1** (originally reported δ 0.85[1]), **2** (Table 1), and **6** (originally reported δ 0.88[22]) as the third hydroxyl is located either at C-4 or C-10 or is absent, respectively; and, more dramatically, the terminal methyl resonances sequentially changed to δ 0.882, 0.891 and 0.907 for compounds (**3-5**) as the third hydroxyl shifted to the C-28, C-29, and C-30 positions, respectively; this pattern is exactly the same as that observed with the similarly hydroxylated asimicin[10] and bullatacinone isomers.[23] Thus, the downfield shifts of proton signals of the terminal Me group (H-34), affected by the successive substitutions of a single OH group near the end of the hydrocarbon chain, are quite characteristic for the series of isomers of Annonaceous acetogenins. These proton chemical shifts and the proximity of the terminal methyl are , thus, quite diagnostic and useful in the prediction of the exact positions of the near terminal OH substitution. The absolute configuration of chiral centers of compounds (**2**) and (**5**) was not determined by Mosher esterification[2] due to the limited amount of the samples; however, the near identities of the pertinent nmr signals and biogenetic considerations suggest absolute stereochemistries identical to bullatacin (**1**).

Table 3. Bioactivity data of acetogenins (**1-6**) in the bullatacin series

Compounds	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 [e]	1.59×10^{-3}	1.25×10^{-13}	>10	1.0×10^{-12}
2	4.0×10^{-3}	9.39×10^{-6}	8.33×10^{-6}	3.78×10^{-6}
3	9.7×10^{-3}	2.94×10^{-9}	1.52×10^{-4}	3.79×10^{-11}
4	1.01×10^{-3}	8.52×10^{-12}	1.96×10^{-14}	4.46×10^{-6}
5	6.0×10^{-3}	3.11×10^{-14}	3.22×10^{-14}	4.77×10^{-12}
6 [f]	8.45×10^{-2}	6.96×10^{-4}	>1.0	$<10^{-4}$
Adriamycin [g]	2.57×10^{-1}	1.04×10^{-4}	1.76×10^{-2}	1.53×10^{-4}

a). Brine shrimp lethality test.[11, 12] b). Human lung carcinoma. c). Human breast carcinoma.
d). Human colon adenocarcinoma. e). Data taken from [1]. f). Data taken from [23].
g). Standard reference, variations between different runs were within one order of magnitude.

Bioactivity data obtained with **1-5** are summarized in Table 3. All of these acetogenins were highly toxic to the brine shrimp larvae, showed highly potent cytotoxicities against three solid tumor cell lines, A-549 (human

lung carcinoma), MCF-7 (human breast carcinoma) and HT-29 (human colon adenocarcinoma), and were several orders of magnitude more cytotoxic than the standard reference, adriamycin. By comparison, 4-deoxybullatacin (desacetyluvaricin) (**6**) is much less potent (Table 3).[22] The highly potent cytotoxicities of these bullatacin isomers (**2-5**), vs those of **6**, in all cytotoxicity tests, suggested that the presence of a third OH group at either C-4, C-10, C-28, C-29 or C-30 is responsible for the enhancement of activity. The general order of potencies of **1-5** suggests that hydroxylation at C-10 gives less activity, while hydroxylations at C-28, C-29, and C-30 give, respectively, much more activity as the position of hydroxylation nears the terminal methyl. Thus, bullatin (**5**) approaches the level of potencies of bullatacin (**1**). In mitochondrial inhibition assays, using rat liver mitochondria, bullatacin (**1**), bullatin (**2**) and bullanin (**5**) all show high activity with bullanin (**5**) showing slightly higher potencies than bullatacin (**1**). [6, 24]

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. The following instruments for spectra measurements were used; ir spectra: Perkin-Elmer 1600 FTIR spectrophotometer, uv spectra: Beckman DU-7 UV spectrophotometer, ^{1}H, ^{13}C nmr and ^{1}H-^{1}H COSY spectra: Varian VXR-500S (^{1}H at 500 MHz, ^{13}C at 125.75 MHz) spectrometer in $CDCl_3$ with TMS as reference, low resolution Clms and Elms: Finnigan 4000 spectrometer, FABms, Elms for TMSi and d9-TMSi derivatives and HRms: Kratos MS50 mass spectrometer. For tlc, silica gel 60 F-254 (EM5717) glass plates (0.25 mm) were used and visualized by spraying with 5% phosphomolybdic acid in EtOH and heating. Chromatotron plates (1 or 2 mm) were prepared with silica gel 60 PF 254 containing gypsum and dried at 70^{0}C overnight. Hplc was carried out with a Rainin HPLC instrument using the Dynamax software system and a silica gel column (250 x 21 mm) equipped with a Rainin UV-1 detector set at 220 nm.

Chemicals

For preparation of triTMSi and tri-d9-TMSi derivatives, N,O-bis-(trimethylsilyl)acetamide (BSA) and pyridine in silylation grade were purchased from Pierce Chemical Company (USA); d_{18}-bis-(trimethylsilyl)-trifluoroacetamide (d_{18}-BSTFA) was from Regis Chemical Company (USA) under the brand name Deutero Regisil-d_{18}.

Derivatization

TriTMSi and tri-d9-TMSi derivatives were prepared by treatment of the isolated acetogenins with BSA for the triTMSi derivatives or d_{18}-BSTFA for the tri-d9-TMSi derivatives in the presence of pyridine. Approximately

10-50 µg of pure compound was placed in a 100 µl conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 hrs. The sample was treated with 2 µl pyridine and 20 µl of BSA or d_{18}-BSTFA and heated at 70°C for 30 min. The EIms measurements of the derivatives were carried out at a resolution of 1500, scanning mass 900-100 at 30 sec/decade.

Bioassays

The brine shrimp (*Artemia salina* Leach) test (BST) was performed as modified to determine LC_{50} values in µg/ml.[11, 12] Seven-day in vitro cytotoxicity tests against human tumor cell lines were carried out at the Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma), MCF-7 (human breast carcinoma) and HT-29 (human colon carcinoma) with adriamycin as a positive control.[10] The reported ED_{50} values in µg/ml (Table 3) were tabulated from the same run in order to facilitate comparison for the SAR's.

Plant material

The bark of *Asimina triloba* (L.) Dunal was collected from stands growing wild at the Purdue Horticultural Research Farm, West Lafayette, Indiana, USA. The identification was confirmed by Dr. George R. Parker, Department of Forestry and Natural Resources, Purdue University. A voucher specimen of the bark is preserved in the pharmacognosy herbarium.

Extraction and purification of acetogenins

The air-dried pulverized stem bark (15 kg) was extracted exhaustively (12 days) at room temperature with 95% EtOH (45 l x 4) and vacuum evaporated to yield extract F001 (1645 g) which was partitioned between H_2O (4 l) and CH_2Cl_2 (4 l x 5), giving a water soluble fraction (F002, 5 g), CH_2Cl_2 soluble fraction (F003, 1560 g) and an insoluble interface (F004, 80 g). F003 was further partitioned between hexane (4 l) and 90% MeOH aq soln (4 l x 5) and yielded the MeOH fraction (F005, 650 g) and the hexane fraction (F006, 905 g).
Directed by the BST bioassay, the most bioactive fraction, F005 (BST LC_{50} 7.151×10^{-1} µg/ml) (200 g) was further fractionated by open column chromatography on silica gel (8 Kg, 60-200 mesh), eluting with hexane-EtOAc and EtOAc-MeOH gradients; 12 pools were made from the collected fractions according to their tlc patterns and evaluated by the BST bioassay. The most active pools (P_7-P_9) were combined (20 g) and subjected to further repeated separation by silica gel (1000 g, 230-400 mesh) column chromatography eluted with MeOH-$CHCl_3$ gradients. Further purifications of the most bioactive fractions F11-12 (1.2 g, BST LC_{50} 3.5×10^{-2} mg/ml) were carried out on Chromatotron plates (2 mm thick), eluted with hexane-CH_2Cl_2-MeOH (30 : 20 : 0-5). A white wax from the hexane-CH_2Cl_2-MeOH elutions was further resolved by HPLC eluted with 10%THF in MeOH - hexane gradients (5-15%), MeOH (0-1%) in the mixture of hexane and EtOAc (2:1) and acetone in $CHCl_3$ (0-10%) to yield acetogenins (2-5).

Bullatin (2)

Compound (**2**) was obtained as a colorless wax (12.5 mg). $[\alpha]_D$ $+7.5^0$ (c=0.4 mg / ml, EtOH). Uv λ_{max}^{EtOH} nm: 213 (log ε=2.34). Ir υ_{max}^{film} cm^{-1}: 3442 (OH), 2971, 2849, 1748 (C=O), 1458, 1186, 1051. FAB HR-ms (glycerol) *m/z*: 623.4865 ($[M+H]^+$, found), (required 623.4887, Calcd for $C_{37}H_{66}O_7$). EIms, EIms (triTMSi derivative), and EIms (tri-d9-TMSi derivative) *m/z* see Figure 2. ^1H Nmr (500 MHz, $CDCl_3$): see Table 1. ^{13}C Nmr (125.75 MHz, $CDCl_3$): see Table 1.

Bullatin triacetate (2a)

Treatment of compound (**2**) (2 mg) with Ac_2O-pyridine (at room temperature, overnight) and subsequent workup gave **2a** as a colorless wax. ^1H Nmr (500 MHz, $CDCl_3$): see Table 1.

Bullanin (5)

Compound (**5**) was obtained as a colorless wax (10 mg). $[\alpha]_D$ $+28^0$ (c=0.5 mg / ml, EtOH). Uv λ_{max}^{EtOH} nm: 220 (log ε=2.42). Ir υ_{max}^{film} cm^{-1}: 3442 (OH), 2927, 2852, 1747 (C=O), 1459, 1320, 1193, 1070. FAB HRms (glycerol) *m/z*: 623.4915 ($[M+H]^+$, found), (623.4887, Calcd for $C_{37}H_{66}O_7$). EIms, EIms (triTMSi derivative), and EIms (tri-d9-TMSi derivative) *m/z* see Figure 3. ^1H Nmr (500 MHz, $CDCl_3$): see Table 2. ^{13}C Nmr (125.75 MHz, $CDCl_3$): see Table 2.

Bullanin triacetate (5a)

Treatment of compound (**5**) (2 mg) with Ac_2O-pyridine (at room temperature, overnight) and subsequent workup gave **5a** as a colorless wax. ^1H Nmr (500 MHz, $CDCl_3$): see Table 2.

ACKNOWLEDGMENTS

This investigation was supported by grant no. R01 CA 30909 from the National Cancer Institute, NIH. Thanks are due to the Cell Culture Laboratory, Purdue Cancer Center, for the in-house cytotoxicity testing.

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Received, 7th April, 1994