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BULLADECIN AND ATEMOTETROLIN, TWO BIS-TETRAHYDROFURAN ACETOGENINS FROM *ANNONA ATEMOYA* SEEDS†

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Key Word Index—*Annona atemoya*; Annonaceae; seeds; acetogenins; bulladecin; atemoterolin; γ-epimerization.

Abstract—Two new C_{37} tetrahydroxy adjacent bis-tetrahydrofuran acetogenins with a vicinal diol, bulladecin and atemotetrolin, were isolated from the methanol extract of *Annona atemoya* seeds and characterized by spectroscopic techniques. In addition the known compounds, cherimolin-2 and almunequin, were isolated and identified.

INTRODUCTION

Acetogenins from Annonaceae are known to exhibit a variety of pharmacological activities such as parasiticide, insecticide, cytotoxic, antitumoral, and immunosuppressive [2]. Continuing our studies on this family, we have investigated the acetogenins from the seeds of *Annona atemoya* (cv African Pride), hybrid between *Annona squamosa* L. and *Annona cherimolia* Mill., and cultivated for its edible fruits in Africa, Israel and Australia. We have recently described one new C_{35} bis-tetrahydrofuran acetogenin from the seeds that we named annonisin [3]. In the present paper, we report the isolation and structure elucidation of bulladecin (**1**) and atemotetrolin (**2**), two new adjacent bis-tetrahydrofuran acetogenins from the methanol extract of *Annona atemoya* seeds. Their structures were determined by ^{1}H NMR and ^{13}C NMR (COSY, HOHAHA, HMQC and HMBC) and mass spectrometry on the native compounds and confirmed by chemical transformations. Artificial γ-epimerization of the α,β-unsaturated γ-lactone is also discussed.

RESULTS AND DISCUSSION

Bulladecin (**1**) was isolated as a transparent oil by the usual chromatographic methods followed by semi-preparative reversed phase HPLC. Its M_r was determined by mass spectrometry using fast-atom bombardment ionization and *m*-nitrobenzyl alcohol doped with LiCl as a liquid matrix (FAB-Li) [4]. Compound **1** showed $[M+Li]^+$ at *m/z* 645 indicating a M_r of 638. The CI-mass spectrum gave $[M+H]^+$ at *m/z* 639 corresponding to the molecular formula $C_{37}H_{67}O_8$. The presence of four hydroxyl groups was indicated by the losses of four molecules of water from $[M+H]^+$ in the CI-mass spectrum. This was confirmed by preparation of the tetraacetate derivative (**1a**), the high resolution CI-mass spectrum of which showed the $[M+H]^+$ ion at *m/z* 807.5282 confirming the molecular formula of **1**.

A positive reaction to Kedde's reagent suggested the presence of an α,β-unsaturated γ-lactone. Resonances in the ^{1}H NMR spectrum at δ 7.18 (H-35), 5.04 (H-36) and 1.42 (H-37), corresponding in the ^{13}C NMR spectrum to resonances at δ 151.7 (C-35), 77.9 (C-36) and 19.1 (C-37), respectively, supported this assignment. An ABX system in the ^{1}H NMR spectrum was observed with two protons on C-3 at δ 2.51 (H-3a) and 2.37 (H-3b) and a single proton at δ 3.83 (H-4), establishing the presence of an hydroxyl group at C-4 characteristic for acetogenins of sub-type 1b [2].

An adjacent bis-tetrahydrofuranic system was indicated by the signals at δ 3.93 (H-12) and 3.87 (H-15, H-16, H-19) in the ^{1}H NMR spectrum assigned to four oxymethine protons and by the signals for carbons at δ 79.9 (C-12), 81.2 (C-15), 82.1 (C-16) and 82.7 (C-19) in the ^{13}C NMR spectrum (Table 1). Three protons attached to hydroxylated carbons were observed at δ 3.47 (1H) and 3.60 (2H) in the ^{1}H NMR spectrum of **1**. Only the hydroxymethine proton at δ 3.47 showed correlation crosspeaks with one of the THF methine

† Part 66 in the series "Acetogenins from Annonaceae". For part 65 see Ref [1].

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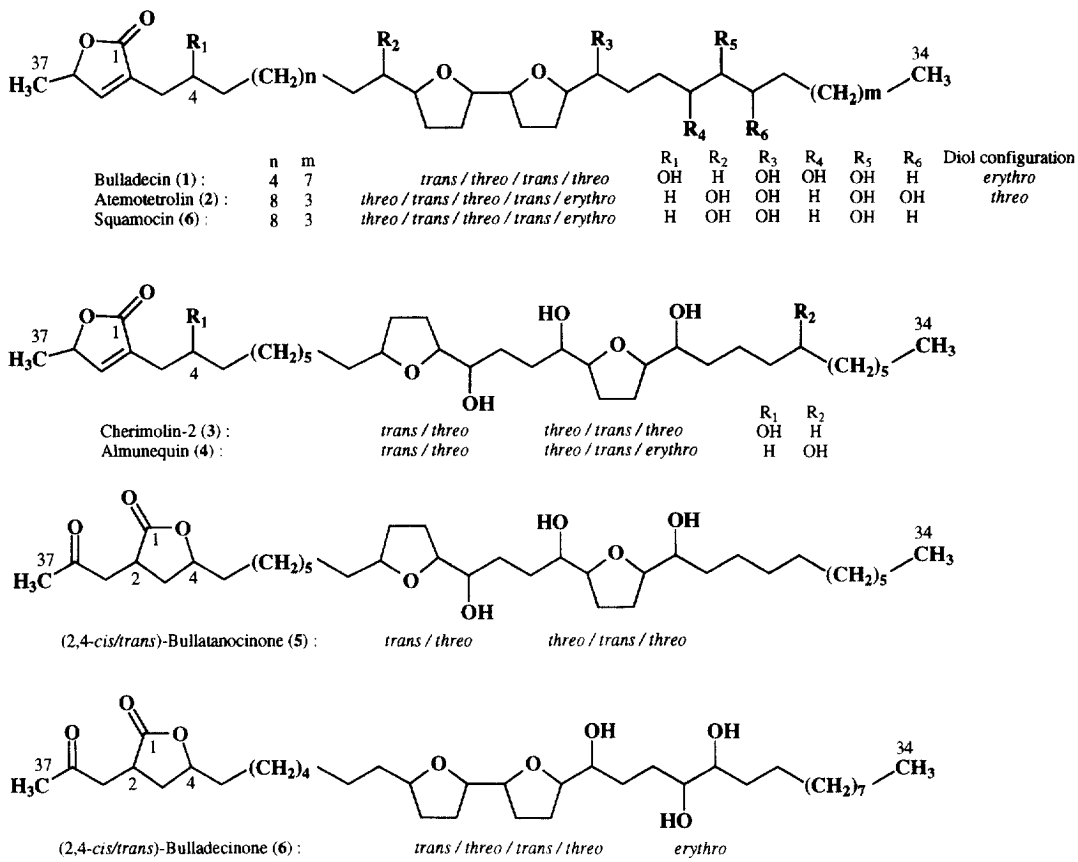

protons at δ 3.87 in the ^{1}H-^{1}H COSY spectrum. This suggested that there was only one hydroxyl group adjacent to one of the THF rings. It was further confirmed by the carbon chemical shift at δ 79.9 characteristic for the oxygenated carbons of THF rings that lack adjacent hydroxyl groups as in cherimolin-2 (**4**) [5] (-bullatanocine) [6] and almunequin (**5**) [5] (-squamostatin-A) [7]. The other two oxymethine protons at δ 3.60 were observed to have correlation crosspeaks with two oxygenated carbons at ca δ 74 in the HMQC spectrum. The data was strongly indicative of a vicinal diol, as the carbons having a single isolated hydroxyl group are always at ca δ 71 in other acetogenins [2].

The location of the bis-THF system was established by analysis of the EI and CI mass spectra of **1** and **1a** (Fig. 1). The fragmentation pattern observed in the mass spectra showed that the two THF rings of **1** were located between C-11 and C-20 as in bulladecinone [8]. The relative stereochemistry around the bis-THF rings in bulladecin (**1**) was determined by comparing the ^{1}H NMR and ^{13}C NMR signals of **1** and the ^{1}H NMR spectrum of its tetraacetate **1a** with those of model compounds of known relative stereochemistry [9, 10]. The proton signal for H-20 of **1** at δ 3.47 which was correlated on ^{1}H-^{13}C correlation spectra (HMQC and HMBC) with carbon atoms at δ 74.2 (C-20) and 82.7 (C-19), respectively, suggested that the relative configuration of C-19/C-20 was *threo* [11]. For **1a** the signals for the protons of the acetyl methyl of C-20 at δ 2.09 and H-20 at δ 4.87 corroborated this assumption [10].

The determination of the complete stereochemistry between C-12 and C-20 was established by the close examination of the homonuclear and heteronuclear correlations observed in the 2D NMR spectra of **1** (COSY-DQF, HMBC, HMQC). The methylene protons at C-17 and C-18 were assigned to signals at δ 1.98/1.61 from their correlations with H-19 and H-20, indicating a *trans*-stereochemistry for the C-16/C-19 THF ring [12, 13]. In the same way, the methylene protons at C-13 and C-14 were assigned to signals at δ 2.03/148 and 1.98/1.61, respectively, and suggested a *trans*-stereochemistry across the C-12/C-15 THF ring [8, 13]. The relative configuration between C-15 and C-16 was then determined as *threo* by the ^{1}H NMR signals for H-15 and H-16 at δ 3.87 in **1** and at δ 3.89 in **1a**. These two adjacent protons should resonate between δ 3.9 and 4.0 for an *erythro*-relationship between the two THF rings [12]. Thus, the relative configuration around the bis-THF system from C-12 to C-20 of **1** was concluded to be *trans/threo/trans/threo*. Besides the hydroxyl group at C-4, one of the other three hydroxyl groups was identified to be adjacent to one of the THF rings; the last two being attributed to a vicinal diol. Its placement at C-23 and C-24 was deduced from the EI and CI mass spectral

Table 1. ^{1}H NMR and ^{13}C NMR data ($CDCl_3$, δ, J in Hz) for bulladecin (**1**) and the derivatives **1a** and **1b**

Position	^{1}H NMR*			^{13}C NMR*
	1	**1a**	**1b**	**1**
1	—	—	—	174.7
2	—	—	—	131.2
3a	2.51 *ddd* (15.2; 3.6; <1)	2.54 *m*	2.51 *d* (15.1)	33.3
3b	2.37 *dd* (15.2; 8.0)		2.37 *dd* (15.0; 7.9)	
4	3.83 *m*	5.09 *m*	3.87–3.93 *m*	69.9
5	1.48 *m*	1.20–1.70 *m*	1.20–2.00 *m*	37.4
6–11	1.20–1.70 *m*	1.20–1.70 *m*	1.20–2.00 *m*	26.1–35.7†
12	3.93 *m*	3.89 *m*	3.87–3.93 *m*	79.9
13a,b	2.03–1.48 *m*	2.00–1.45 *m*	1.60–2.00 *m*	32.0–28.9†
14a,b	1.98–1.61 *m*	1.93–1.68 *m*	1.60–2.00 *m*	32.0–28.9†
15	3.87 *m*	3.89 *m*	3.87–3.93 *m*	81.2‡
16	3.87 *m*	3.89 *m*	3.87–3.93 *m*	82.1‡
17a,b	1.98–1.61 *m*	1.93–1.68 *m*	1.60–2.00 *m*	32.0–28.9†
18a,b	1.98–1.61 *m*	1.93–1.68 *m*	1.60–2.00 *m*	32.0–28.9†
19	3.87 *m*	4.02 *m*	3.87–3.93 *m*	82.7
20	3.47 *m*	4.87 *m*	3.45 *m*	74.2
21	1.55 *m*	1.20–1.70 *m*	1.20–2.00 *m*	35.7–31.9†
22	1.62 *m*	1.20–1.70 *m*	1.20–2.00 *m*	35.7–31.9†
23	3.60 *m*	4.92 *m*§	4.01 *m*	74.5§
24	3.60 *m*	4.98 *m*§	4.01 *m*	74.7§
25	1.43 *m*	1.20–1.70 *m*	1.20–2.00 *m*	25.5–27.2†
26–31	1.20–1.70 *m*	1.20–1.70 *m*	1.20–2.00 *m*	25.5–31.9†
32	1.20–1.70 *m*	1.20–1.70 *m*	1.20–2.00 *m*	31.8
33	1.20–1.70 *m*	1.20–1.70 *m*	1.20–2.00 *m*	22.7
34	0.87 *t* (6.7)	0.88 *t* (6.6)	0.87 *t* (6.8)	14.1
35	7.18 *d* (1.2)	7.08 *d* (1.5)	7.18 *d* (1.5)	151.7
36	5.04 *dq* (6.7; 1.2)	5.01 *m*	5.04 *dq* (7.0; 1.5)	77.9
37	1.42 *d* (6.7)	1.39 *d* (6.7)	1.42 *d* (6.7)	19.1
4-OAc	—	2.02 *s*	—	—
20-OAc	—	2.09 *s*	—	—
23-OAc	—	2.04 *s*	—	—
24-OAc	—	2.04 *s*	—	—
Acetonyl	—	—	1.32 *s*	—
Methyls			1.43 *s*	

* The assignments were confirmed by comparison with spectral data of (2,4-*cis*/*trans*)-bulladecinone [8] and asimilobin [13].

† δ_C 25.5; 26.1; 27.2; 28.4; 28.9; 29.4; 29.6; 31.9; 32.0; 35.7.

‡,§ Interchangeable assignments within the columns.

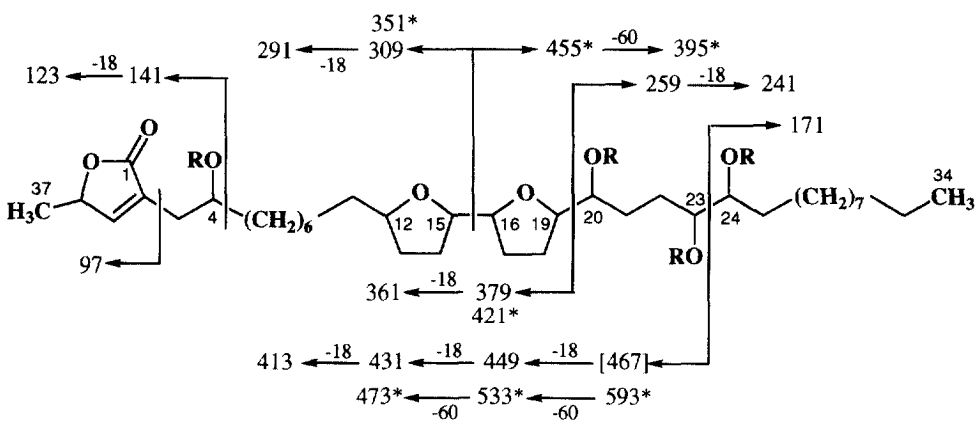

Fig. 1. Mass spectral fragmentations of bulladecin (**1**) and its tetraacetyl derivative **1a** (R = AcO*). Value in brackets was not observed.

fragmentation analyses of **1** and **1a**. This placement was confirmed by a chemical method using oxidation of **1** with lead tetraacetate [14]. GC analysis of the degradation product showed a peak possessing the same retention time as that of an authentic sample of undecanal. Thus, the location of the vicinal diol was unambiguously determined to be at C-23/C-24 positions.

In order to determine the relative configuration at C-23/C-24, the acetonide derivative **1b** was prepared. The ^{1}H NMR signals for H-23 and H-24 at δ 4.01 in **1b** and the signals for the acetonyl methyl groups, showing two separate singlet peaks at δ 1.32 and 1.43, suggested the *cis*-configuration for the dioxolane ring [8, 15]. The *erythro*-configuration for the vicinal diol was consequently established. Spectral data of **1** were very close to those of (2,4-*cis*/*trans*)-bulladecinone (**6**) isolated from the bark of *Annona bullata* except for the γ-lactone group [8]. (2,4-*cis*/*trans*)-bulladecinone has an α-acetonyl-butyrolactone characteristic of isoacetogenins, artifacts easily issued from translactonization of the corresponding 4-OH α,β-unsaturated γ-lactone acetogenins during their extraction or purification [16]. Therefore, compound **1** was considered as parent acetogenin of (2,4-*cis*/*trans*)-bulladecinone and named bulladecin for homogeneous nomenclatural grounds.

Atemotetrolin (**2**), cherimolin-2 (**3**) [5, 6] and almunequin (**4**) [5, 7] were isolated as a mixture in amorphous waxy form by successive open column chromatographies on silica gel. Compounds **2**, **3** and **4** were very difficult to separate. A series of experiments was carried out to find a high performance liquid chromatographic (HPLC) system which would resolve this mixture. Our attempts to achieve reasonable resolution of the three compounds were unsuccessful. In order to separate the major compounds, atemotetrolin (**2**) and almunequin (**4**), we have quantitatively transformed the minor product, cherimolin-2 (**3**) into the corresponding isoacetogenin (**5**) by translocatonization (diethylamine/methanol) [16]. (2,4-*cis*/*trans*)-Bullatanocinone (**5**) [6] (-isocherimolin-2) [16] was then easily separated from **2** and **4** by flash chromatography on silica gel and identified by spectroscopic techniques (NMR, MS). Analytical HPLC of the residual mixture after translactonization showed a base line separation allowing us to purify atemotetrolin (**2**) and almunequin (**4**) by semi-preparative HPLC. Thus, compounds **2** and **4** were obtained as transparent oils. The molecular weight of **2** was indicated by peak at *m/z* 639 $[M+H]^+$ in the CI-mass spectrum. The HRCI-mass spectrum gave *m/z* 639.4843 for the $[M+H]^+$ corresponding to the molecular formula $C_{37}H_{67}O_8$. A strong IR absorption at 1756 cm^{-1} and a positive reaction to Kedde's reagent suggested the presence of an α,β-unsaturated γ-lactone subunit for **2**. The close inspection of the ^{1}H NMR spectrum revealed four resonances at δ 6.98 (H-35), 4.99 (H-36), 2.25 (H-3), and 1.40 (H-37) which were correlated on ^{1}H-^{13}C correlation spectra (HMQC, HMBC) with carbon atoms at δ 176.2 (C-1), 148.9 (C-35), 134.1 (C-2), 77.4 (C-36), 25.1 (C-3) and 19.2 (C-37 (Table 2). These spectral data substantiated the presence of an α,β-unsaturated γ-lactone. The absence of an ABX system corresponding to the 4-hydroxyl acetogenins class suggested that the 4-OH was non-existent in **2** as in almunequin (**4**).

The presence of four hydroxyl groups in **2** was suggested by an IR absorption at 3447 cm^{-1}, four successive losses of water from the $[M+H]^+$ in the CI-mass spectrum, and the preparation of the tetraacetate derivative (**2a**). The presence of an adjacent bis-THF system was certified by the ^{1}H NMR signals at δ 3.82 (1H) and 3.92 (3H) assigned to four oxymethine protons (H-16, H-19, H-20, H-23) in agreement with their ^{13}C NMR signals at δ 83.2, 82.6, 82.1 and 82.8. Proton resonances at δ 3.40 (3H) and 3.87 (1H) were assignable to four protons attached to secondary hydroxylated carbons observed at δ 74.2 and 71.4 in the ^{13}C NMR spectrum of **2**. 2D NMR experiments (COSY-DQF, HMQC, HMBC) confirmed that two hydroxyl groups were on each side of the bis-THF ring system whereas the other two were attributed to a vicinal diol.

The location of the α,α'-dihydroxylated bis-THF system as well as the vicinal diol were unambiguously established by the close analysis of mass spectra of atemotetrolin (**2**) and its tetraacetate (**2a**). The peaks at *m/z* 295, 365, 435 and *m/z* 407, 477 in the EI-mass spectra of **2** and **2a** respectively, allowed the placement of the bis-THF part between C-15 and C-24, whereas the fragment ions at *m/z* 519, 501, 483 for **2** and *m/z* 663, 603 for **2a** (C-38/C-29 cleavage) in the EI-mass spectra, suggested that the diol was at C-28/C-29 (Fig. 2). In the same manner as for bulladecin (**1**), the location of the vicinal diol at C-28/C-29 positions was confirmed by GC identification of hexanal in the lead tetraacetate oxidation product of atemotetrolin (**2**).

The relative stereochemistry of the bis-THF moiety of **2** was fixed as *threo*/*trans*/*threo*/*trans*/*erythro* or *erythro*/*trans*/*threo*/*trans*/*threo* by careful comparison of the ^{1}H NMR and ^{13}C NMR signals of **2** and ^{1}H NMR spectrum of **2a** with those of model compounds of known relative sterochemistry [9, 10]. To determine the relative configuration at C-28/C-29, the acetonide derivative of **2** (**2b**) was prepared. The configuration of the diol was fixed as *threo* based on the ^{1}H NMR signals for H-28 and H-29 of **2b** at δ 3.58 and one singlet peak at δ 1.37 for the acetonyl methyls suggesting the *trans*-configuration for the dioxolane ring (Table 2) [8, 15].

Magnetization transfers observed from H-28 (δ 3.58) to H-24 (δ 3.86) in the HOHAHA correlation spectrum of **2b** proved that the relative configuration between the vicinal hydroxylated carbon of the THF system were *erythro* at C-23/C-24 and *threo* at C-15/C-16 (Fig. 3). thus, the complete stereochemistry of atemotetrolin (**2**) was determined as *threo*/*trans*/*threo*/*trans*/*erythro* from C-15 to C-24 with a *threo*-stereochemistry for the vicinal diol at C-28/C-29.

Table 2. ^{1}H NMR and ^{13}C NMR data ($CDCl_3$, δ, J in Hz) for atemotetrolin (**2**) and the derivatives **2a** and **2b**

Position	^{1}H NMR			^{13}C NMR
	2	**2a**	**2b**	**2**
1	—	—	—	176.2
2	—	—	—	134.1
3	2.25 *t* (7.6)	2.26 *t* (7.0)	2.26 *t* (7.0)	25.1
4	1.52 *m*	1.52 *m*	1.53 *m*	27.4
5–13	1.20–1.50 *m*	1.20–1.65 *m*	1.20–1.60 *m*	25.1–29.6*
14	1.48 *m*	1.20–1.65 *m*	1.20–1.60 *m*	33.1
15	3.40 *m*	4.86 *m*	3.40 *m*	74.2
16	3.82 *m*	3.96 *m*	3.86 *m*	83.2
17, 18	1.57–1.95 *m*	1.60–2.00 *m*	1.60–2.00 *m*	25.1–29.6*
19	3.92 *m*	3.88 *m*	3.86 *m*	82.6†
20	3.92 *m*	3.88 *m*	3.86 *m*	82.1†
21, 22	1.57–1.95 *m*	1.60–2.00 *m*	1.60–2.00 *m*	25.1–29.6*
23	3.92 *m*	3.96 *m*	3.86 *m*	82.8
24	3.87 *m*	4.91 *m*	3.86 *m*	71.4
25	1.37 *m*	1.20–1.65 *m*	1.20–1.60 *m*	33.2
26, 27	1.20–1.50 *m*	1.20–1.65 *m*	1.20–1.60 *m*	25.1–29.6*
28, 29	3.40 *m*	4.97 *m*	3.58 *m*	74.2
30, 31	1.20–1.50 *m*	1.20–1.65 *m*	1.20–1.60 *m*	25.1–29.6*
32	1.20–1.50 *m*	1.20–1.65 *m*	1.20–1.60 *m*	31.9
33	1.20–1.50 *m*	1.20–1.65 *m*	1.20–1.60 *m*	22.6
34	0.88 *t* (6.4)	0.86 *t* (6.8)	0.87 *t* (6.8)	14.0
35	6.98 *d* (1.3)	6.98 *d* (1.5)	6.99 *d* (1.5)	148.9
36	4.99 *dq* (6.8; 1.4)	4.99 *m*	4.99 *dq* (6.7; 1.5)	77.4
37	1.40 *d* (6.8)	1.40 *d* (6.9)	1.40 *d* (6.8)	19.2
15-OAc	—	2.07 *s*	—	—
24-OAc	—	2.04 *s*	—	—
28-OAc	—	2.07 *s*	—	—
29-OAc	—	2.07 *s*	—	—
Acetonyl	—	—	1.37 *s*	—
Methyls			1.37 *s*	

* δ_C: 25.1; 25.6; 29.0; 29.3; 29.6.

† Interchangeable assignments within the columns.

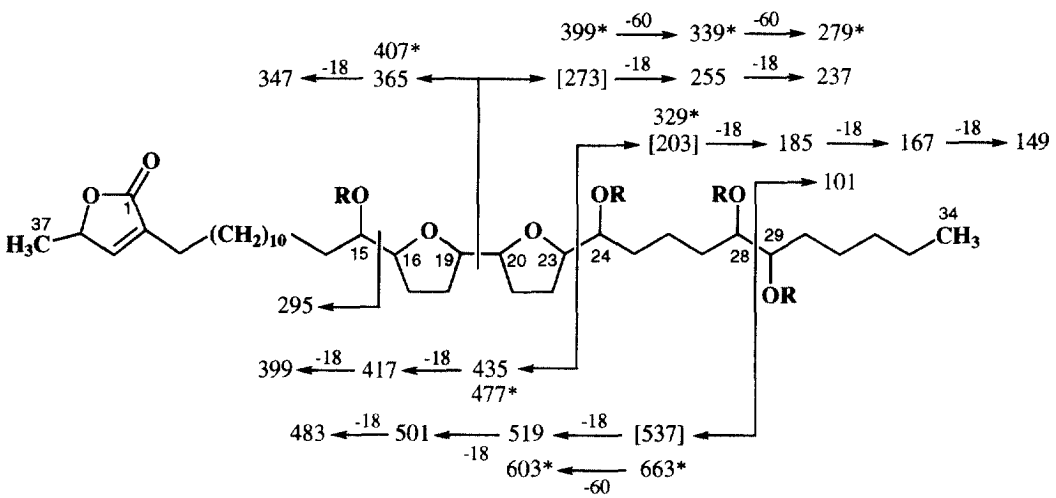

Fig. 2. Mass spectral fragmentations of atemotetrolin (**2**) and its tetraacetyl derivative **2a** (R = AcO*). Value in brackets was not observed.

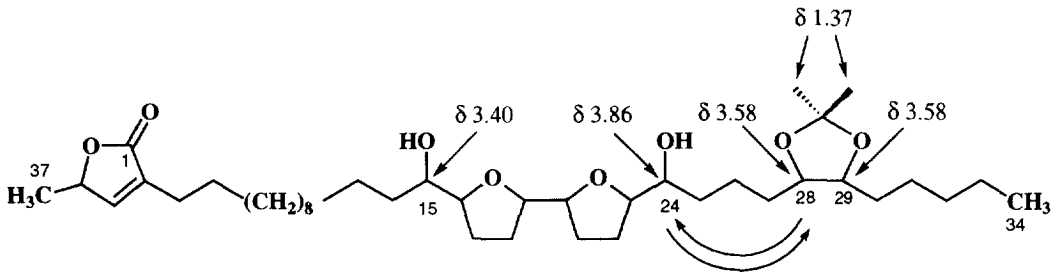

Fig. 3. Magnetization transfers observed from H-28 to H-24 in the HOHAHA spectrum of acetonide derivative of atemotetrolin (**2a**).

Because of the very limited amounts of samples available after chemical transformations, no study of the absolute configurations of the chiral centres of **1** and **2** was possible by Mosher esters methodology [17]. Nevertheless the absolute configuration at C-36 of **2** was established by an enzymatic method recently developed in our laboratory [18]. Oxidative cleavage of the α,β-unsaturated γ-methyl γ-lactone of atemotetrolin (**2**) was first performed with $RuCl_3$-H_5IO_6. The so formed lactic acid was then separately incubated in turn with stereospecific L(+)- and D(−)-lactate dehydrogenase (L-LDH and D-LDH, respectively) and NADH as coenzyme. The absolute configuration of the stereogenic center of the parent molecule was finally determined by HPLC detection of NADH in the L- or D-LDH incubation medium which was directly correlated to the presence of either L- or D-lactic acid in the degradation product. All acetogenins examined up to now possess the same C-34 (36) *S*-absolute configuration [18]. Unexpectedly L- and D-lactic acids were both detected in the degradative product of **2** suggesting the presence of two C-36 epimers for **2**. γ-Epimerization during translactonization process was suspected. To verify the absence of the two epimers in the plant, the LDH method was directly performed on the initial mixture of **2**, **3** and **4** before translactonization of cherimolin-2 (**3**). NADH was only detected in the L-LDH incubation medium suggesting the same 36*S*-absolute configuration for **2**, **3** and **4**. This suggested that a γ-epimerization occurred during the translactonization procedure in alkaline medium. Such γ-epimerization of acetogenins under basic conditions was recently studied and demonstrated in our laboratory [19].

In summary, we have isolated two new bis-tetrahydrofuranic acetogenins with a vicinal diol: bulladecin (**1**) identified as the parent acetogenin of (2,4-*cis*/*trans*)-bulladecinone (**6**) issued from translactonization, and atemotetrolin (**2**) isomer of the recently related rollitacin [20].

Alkaline translactonization was used to separate **2** from the two known acetogenins, cherimolin-2 (**3**) and almunequin (**4**). Using the LDH method to determine the C-36 absolute stereochemistry of **2**, we demonstrated the γ-epimerization of α,β-unsaturated γ-lactone of subtype 1 illustrating how extraction and purification processes may interact to alter the chemical integrity of those products and allowing us to state that treatment with base should be avoided in order to exclude γ-epimerization of the terminal butenolide of acetogenins.

EXPERIMENTAL

General

Optical rotations were determined on a Schmidt-Haensch Polartronic I polarimeter. UV spectra were obtained in MeOH on a Philips PU 8720 spectrometer. IR spectra were measured on a Perkin–Elmer 257 spectrometer. ^1H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, on a Bruker AC-200P spectrometer, and the 2D NMR spectra (COSY-DQF, HOHAHA, HMQC, HMBC) were obtained with a Bruker ARX-400 spectrometer. EIMS and CIMS were performed on a Nermag R10-10C spectrometer. HPLC analytic analyses were performed with a Beckman 112 pump, a Varian 9050 spectrophotometer (214 nm) and a U6K Waters injector on a μBondapak C_{18} prepacked column (10 μm, 8 × 100 mm), elution with 15–18% H_2O–MeOH at flow rate 0.8 ml min^{-1}. Prep. HPLC was carried out with a Millipore-Waters system equipped with a 590 pump, a SSV injector, and a 484 UV detector (214 nm), on a μBondapack C_{18} prepacked column (10 μm, 25 × 100 mm), elution with 15–18% H_2O–MeOH at flow rate 8 ml min^{-1}. HPLC detection of NADH in the LDH incubation media was performed on a Spherisorb S5-ODS2 (5 μm, 4.6 × 250 mm), elution with 5% MeOH in aq. soln of 0.2 M Tris/0.1 N HCl/EDTA/H_2O [250 ml:279 ml:79 mg:471 ml] at flow rate 1 ml min^{-1}, UV detection of NADH (R_t = 5.2 min) at 340 nm [18]. GC analyses were performed on Hewlett-Packard HP5890A with HP1 capillary column (0.2 mm × 25 m, 0.33 μm), FID detector, N_2 carrier gas at flow rate 0.7 ml min^{-1}, temp. programmed 100° to 200° at 4° min^{-1} for undecanal and 50° (5 min isothermal) to 150° at 4° min^{-1} for hexanal. Undecanal and hexanal were identified by comparison with authentical samples at retention times 5.4 min and 2.6 min, respectively.

Plant material

Seeds of *Annona atemoya* were collected in September, 1993 in Australia and authenticated by Dr D. Batten, Tropical Fruit Research Station, Alstonville, New South Wales, Australia.

Extraction and isolation

The dried and pulverized seeds (930 g) were macerated with MeOH. The MeOH extract (79.7 g; $LC_{50} < 0.01$ μg ml^{-1} in BST [21]) was partitioned between H_2O and hexane to yield 6 g of hexane extract ($LC_{50} = 0.12$ μg ml^{-1} in BST). The aq. MeOH fraction was partially evaporated and extracted with CH_2Cl_2. 10.1 g of the CH_2Cl_2-soluble extract ($LC_{50} < 0.01$ μg ml^{-1} in BST), was fractionated by flash chromatography (Si gel, elution with toluene–AcOEt–EtOH [30:70:5]) leading to several fractions. Bulladecin (**1**) and atemotetrolin (**2**) were obtained as impure fractions and were further purified by HPLC.

Bulladecin (**1**). Obtained as a white solid (8 mg) by prep. HPLC μBondapak C_{18}; MeOH–H_2O [82:18]; flow rate 8 ml min^{-1}; R_t 27.0 min. $C_{37}H_{66}O_8$. $[\alpha]_D^{20}$ +11° (MeOH; *c* 0.20); UV λ_{max}^{MeOH} nm (log ε): 214 (4.85); FAB-Li (*m*-NBA + LiCl) *m/z*: 645 $[M+Li]^+$ (100%), 627, 601, 533, 515, 503, 487, 473, 461, 443, 415, 385, 343, 289, 273; CIMS (CH_4) *m/z*: 639 $[M+H]^+$ (100%), 621 $[MH-H_2O]^+$, 603 $[MH-2H_2O]^+$, 585 $[MH-3H_2O]^+$, 567 $[MH-4H_2O]^+$, 449, 431, 393, 379, 361, 309, 241, 171, 141, 111, 97; EIMS 40 eV *m/z*: 449, 431, 413, 379, 361, 291, 141, 123, 97 (Fig. 1). ^{1}H NMR (Table 1); ^{13}C NMR (Table 1).

Bulladecin tetraacetate (**1a**). Treatment of **1** (2 mg) with acetic anhydride/pyridine (room temp. overnight) and subsequent work-up gave compound **1a** (quantitative yield) as an oil. HRCIMS (CH_4) *m/z*: 807.5282 $[M+H]^+$ ($C_{45}H_{75}O_{12}$ requires 807.5228); CIMS (CH_4) *m/z*: 807 $[M+H]^+$, 747 $[MH-AcOH]^+$, 705, 687 $[MH-2AcOH]^+$, 627 $[MH-3AcOH]^+$, 593, 431, 351, 171, 111, 97; EIMS 40 eV *m/z*: 747, 687, 627, 593, 533, 473, 455, 421, 413, 395, 361, 351, 309, 291, 273, 111, 97 (Fig. 1); ^{1}H NMR (Table 1).

23,24-*Acetonide of bulladecin* (**1b**). To **1** (4 mg) dissolved in C_6H_6 (1.5 ml) was added 2,2-dimethoxypropane (50 μl) and traces of *p*-toluenesulfonic acid. The mixture was stirred under reflux for 1 h. K_2CO_3 (0.2 mg) was added, and the mixture stirred for 3 h at room temp., then extracted with Et_2O to give **1b** (3.2 mg, 75%) ^{1}H NMR (Table 1).

(2,4-*cis/trans*)-*Bullatanocinone* (**3a**). A mixture of **2**, **3** and **4** was dissolved in MeOH (10 ml) and 1 ml of diethylamine was added. The soln was stirred at room temp. and the translactonization was monitored by TLC. After 20 h, solvent was evaporated under red. pres. and the residue was submitted to purification by Si gel CC, eluting with CH_2Cl_2–*iso*-PrOH [100:8]. Compound **3a** was separated from atemotetrolin (**2**) and almunequin (**4**). Bullatanocinone was identified by spectroscopic techniques [6]. CIMS (CH_4) *m/z*: 639 $[M+H]^+$, 621 $[MH-H_2O]^+$, 603 $[MH-2H_2O]^+$, 585 $[MH-3H_2O]^+$, 567 $[MH-4H_2O]^+$, 527, 491, 449, 431, 413, 379, 361, 335, 311, 309, 293, 291, 241, 223, 171, 141, 123, 111, 97; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.53 (*m*, H-4 *trans*), 4.39 (*m*, H-4 *cis*), 3.85 (*m*, H-12, H-20, H-23), 3.40 (*m*, H-16, H-19, H-24), 3.10–2.90 (*m*, H-2 *cis/trans*, H-35b *cis/trans*), 2.72–2.50 (*m*, H-3a, *cis*, H-35a *cis/trans*), 2.20 (*s*, CH_3-37), 2.10–1.20 (*m*, CH_2, H-3a,3b *trans*, H-3b *cis*), 0.87 (*t*, *J* = 6.7 Hz, CH_3-34); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 205.5 (C-36), 178.8/178.3 (C-1), 82.7 (C-20, C-23), 82.0 (C-15), 79.3/78.9 (C-4), 79.3 (C-12), 74.4 (C-16), 74.3 (C-19, C-24), 44.2/43.8 (C-35), 36.7/34.4 (C-2), 35.3 (C-3), 33.5–22.7 (CH_2), 29.9 (C-37), 14.1 (C-34); for MS, ^{1}H and ^{13}C NMR data, see also Ref. 6.

Atemotetrolin (**2**). Obtained as a transparent oil (14 mg) by prep. HPLC μBondapak C_{18}; MeOH–H_2O [80:15]; flow rate 8 ml min^{-1}; R_t 17.4 min. $C_{37}H_{66}O_8$. $[\alpha]_D^{20}$ +23° (MeOH; *c* 0.20); UV λ_{max}^{MeOH} nm (log ε): 219 (3.54); IR (film) ν_{max} cm^{-1}: 3447, 2927, 2851, 1756; HRCIMS (CH_4) *m/z*: 639.4843 $[M+H]^+$ ($C_{37}H_{67}O_8$ requires 639.4836); CIMS (CH_4) *m/z*: 639 $[M+H]^+$, 621 $[MH-H_2O]^+$, 603 $[MH-2H_2O]^-$, 585 $[MH-3H_2O]^+$, 567 $[MH-4H_2O]^+$, 543, 519, 501, 347, 295, 185, 167, 101; EIMS *m/z*: 567, 533, 519, 501, 495, 483, 465, 435, 417, 399, 365, 347 (100%), 255, 237, 185, 167, 149, 101 (Fig. 2); ^{1}H NMR (Table 1); ^{13}C NMR (Table 1).

Atemotetrolin tetraacetate (**2a**). Treatment of **2** (4 mg) with acetic anhydride/pyridine (room temp. overnight) and subsequent work-up gave compound **1a** (quantitative yield) as an oil. CIMS (CH_4) *m/z*: 807 $[M+H]^+$, 747 $[MH-AcOH]^+$, 705, 687 $[MH-2AcOH]^+$, 663, 645, 627 $[MH-3AcOH]^+$, 603, 567 $[MH-4AcOH]^+$, 477, 407, 399, 347, 339, 329, 295, (Fig. 2); ^{1}H NMR (Table 2).

28,29-*Acetonide of atemotetrolin* (**2b**). To **2** (4 mg) dissolved in C_6H_6 (1.5 ml) was added 2,2-dimethoxypropane (20 μl) and traces of *p*-toluenesulfonic acid. The mixture was stirred under reflux for 1 h. K_2CO_3 (0.3 mg) was added, and the mixture stirred for 3 h at room temp., then extracted with Et_2O to give **2b** (2.6 mg, 59%). CIMS (CH_4) *m/z*: 679 $[M+H]^+$, 661 $[MH-H_2O]^+$, 621 $[MH-(CH_3)_2CO]^+$, 603 $[MH-(CH_3)_2CO-H_2O]^+$, 585 $[MH-(CH_3)_2CO-2H_2O]^+$, 567 $[MH-(CH_3)_2CO-3H_2O]^+$, 429, 347; ^{1}H NMR (Table 2).

Almunequin (**4**). Separated from atemotetrolin (**2**) after translactonization process, by prep. HPLC μBondapak C_{18}; MeOH–H_2O [80:15]; flow rate 8 ml min^{-1}; R_t 13.0 min. $C_{37}H_{66}O_8$. $[\alpha]_D^{20}$ +22° (MeOH *c* 0.3); UV λ_{max}^{MeOH} nm (log ε): 212 (5.92); CIMS (NH_4) *m/z*: 639 $[M+H]^+$, 621 $[MH-H_2O]^+$, 603 $[MH-2H_2O]^-$, 585 $[MH-3H_2O]^+$, 567 $[MH-4H_2O]^+$; EIMS *m/z*: 638, 620, 602, 584, 566, 553, 535, 517, 499, 433, 415, 397, 363, 345 (100%), 327, 309, 293, 291, 275, 257, 251; ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.99 (1H, *d*, *J* = 1.2 Hz, H-35), 4.99 (1H, *dq*, *J* = 7.0; 1.2 Hz, H-36), 3.84 (5H, *m*, H-12, H-15,

H-20, H-23, H-24), 3.60 (1H, *m*, H-28), 3.41 (2H, *m*, H-16, H-19), 2.25 (2H, *t*, $J = 6.8$ Hz, H-3), 2.00–1.60 (4H, *m*, H-13, H-14, H-21, H-22), 1.60–1.20 (*m*, CH_2), 1.40 (3H, *d*, $J = 6.9$ Hz, CH_3-37), 0.87 (3H, *t*, $J = 6.8$ Hz, CH_3-34); ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.4 (C-1), 148.8 (C-35), 134.2 (C-2), 83.2 (C-19), 82.1 (C-15), 81.9 (C-23), 79.3 (C-12), 77.3 (C-36), 74.4 (C-16, C-19), 72.0 (C-24), 71.8 (C-28), 37.3 (C-27), 37.0 (C-29), 35.6, 32.3, 29.5, 29.3, 29.1, 28.6, 28.34, 26.1, 25.6 (C-5–C-11, C-13, C-14, C-18, C-19, C-21, C-22, C-25, C-30, C-31), 31.8 (C-32), 27.3 (C-4), 25.1 (C-3), 22.5 (C-33), 22.0 (C-26), 19.1 (C-37), 14.0 (C-34); for MS, ^1H and ^{13}C NMR data, see also Refs 5 and 7.

Lead tetraacetate oxidation of **1** *and* **2**

To a soln of $Pb(OAc)_4$ (5 mg) in 500 μl of toluene was added 1–2 mg of acetogenin and the reaction mixture was stirred at room temp. overnight. 1% $NaHCO_3$ soln. (5 ml) was added and aq. soln. was extracted with EtOAc. The organic phase was washed, dried, concentrated and then analysed by GC.

LDH method

H_5IO_6 (15 eq.) and a catalytic amount of $RuCl_3$ were added to a biphasic soln of acetogenin (1–4 mg, 1.6–6.4 μmol) in ternary mixture CCl_4–CH_3CN–H_2O [57:57:86]. The mixture was stirred at room temp. overnight before being filtered and concentrated. H_2O (0.5–1 ml) was then added to the residue and the soln was extracted with Et_2O. The organic phase was evaporated and the residue was solubilized in (100–500 μl) buffered soln of 0.1 M Tris–1N HCl–hydrazine [80:15:5]. 20–50 μl of this alkaline soln were separately incubated in turn with D- or L-LDH (10 μl) and 20–50 μl of 1% aq. soln of NAD. Enzymatic preparations were incubated during 20 min at 40° before HPLC analysis.

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REFERENCES

1. Waechter, A.-I., Szlosek, M., Hocquemiller, R., Laurens, A. and Cavé, A., *Phytochemistry*, 1998, in press.
2. Cavé, A., Figadère, B., Laurens, A. and Cortes, D., *Progress in the Chemistry of Organic Natural Produces: Acetogenins from Annonaceae*, Vol. 70, ed. Hertz W., Springler-Verlag; Wien, New-York, 1997, pp. 81–288.
3. Duret, P., Hocquemiller, R. and Cavé, A., *Phytochemistry*, 1997, **45**, 1423.
4. Laprévote, O., Girard, C., Das, B., Cortes, D. and Cavé, A., *Tetrahedron Letters*, 1992, **33**, 5237.
5. Cortes, D., Figadère, B. and Cavé, A., *Phytochemistry*, 1993, **32**, 1467.
6. Gu, Z.-M., Fang, X.-P., Rieser, M. J., Miesbauer, L., Smith, D. L., Wood, K. V. and McLaughlin, J. L., *Tetrahedron*, 1993, **49**, 747.
7. Fujimoto, Y., Murasaki, C., Shimada, H., Nishioka, S., Kakinuma, K., Singh, S., Singh, M. and Gupta, Y. K., *Chemical and Pharmaceutical Bulletin*, 1994, **36**, 4802.
8. Gu, Z.-M., Fang, X.-P., Zeng, L., Kozlowski, J. F. and McLaughlin, J. L., *Bioorganic & Medicinal Chemistry Letters*, 1994, **4**, 473.
9. Born, L., Lieb, F., Lorentzen, J. P., Moescher, H., Nonfon, M., Sollner, R. and Wendisch, D., *Planta Medica*, 1990, **56**, 312.
10. Hoye, T. R. and Zhuang, Z.-P., *Journal of Organic Chemistry*, 1988, **53**, 5578.
11. Harmange, J.-C., Figadère, B. and Cavé, A., *Tetrahedron Letters*, 1992, **34**, 5749.
12. Woo, M. H., Cho, K. Y., Zhang, Y., Zeng, L., Gu, Z.-M. and McLaughlin, J. L., *Journal of Natural Products*, 1995, **58**, 1533.
13. Zhao, G.-X., Gu, Z.-M., Zeng, L., Chao, J.-F., Kozlowski, J. F., Wood, K. V. and McLaughlin, J. L., *Tetrahedron*, 1995, **51**, 7149.
14. Duret, P., Hocquemiller, R., Laurens, A. and Cavé, A., *Natural Product Letters*, 1995, **5**, 295.
15. Kurosawa, E., Fukusawa, A. and Irie, T., *Tetrahedron Letters*, 1972, **21**, 2121.
16. Duret, P., Laurens, A., Hocquemiller, R., Cortes, D. and Cavé, A., *Heterocycles*, 1994, **39**, 741.
17. Rieser, M. J., Hui, Y.-H., Rupprecht, J. K., Kozlowski, J. F., Wood, K. V., McLaughlin, J. L., Hanson, P. R., Zhuang, Z. and Hoye, T. R., *Journal of American Chemical Society*, 1992, **114**, 10203.
18. Duret, P., Waechter, A.-I., Figadère, B., Hocquemiller, R., Cavé, A., Piérard, C. and Pérès, M., *Tetrahedron Letters*, 1996, **37**, 7043.
19. Duret, P., Figadère, B., Hocquemiller, R. and Cavé, A., *Tetrahedron Letters*, 1997, **38**, 8849.
20. Shi, G., McDougal, J. M. and McLaughlin, J. L., *Phytochemistry*, 1997, **45**, 719.
21. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L., *Planta Medica*, 1982, **45**, 31.