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GLAUCABELLIN AND GLAUCAFLORIN, TWO ACETOGENINS FROM *ANNONA GLAUCA**

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Key Word Index—*Annona glauca*; Annonaceae; acetogenins; glaucabellin; glaucaflorin.

Abstract—The seeds of *Annona glauca* have yielded four Annonaceous acetogenins. Two of them, glaucabellin and glaucaflorin, whose chemical structure was deduced by spectral and chemical methods, are new.

INTRODUCTION

Annona glauca, namely *dugor mer*, is a spontaneous arborescent shrub or a shrubby tree growing in the sandy soil along the coast of Senegal. The roots of which are used in traditional medicine [1]. Previous phytochemical studies on this plant resulted in the isolation of five new acetogenins, annoglaucin [2], glaucanisin [3], glaucafilin [4], glaucanetin [5] and 10-hydroxy-glaucanetin [5]. We now report on the isolation of four acetogenins from the seeds of *A. glauca*, two of which are new monotetrahydrofuran acetogenins and which we have named glaucabellin (**1**) and glaucaflorin (**2**). The other two are the already known muricatetrocin B (**3**) [6] and squamocin B (**4**) [7]. Their occurrence in this plant is reported here for the first time.

RESULTS AND DISCUSSION

Mr of glaucabellin (**1**) was indicated by a peak at *m/z* 609 $[MH]^+$ in its CI mass spectrum corresponding to the molecular formula $C_{37}H_{69}O_6$. The existence of an α,β-unsaturated γ-lactone was suggested by an IR carbonyl absorption at 1750 cm^{-1}, a UV λ_{max} at 208 nm, six NMR resonances at δ_H 7.18 (H-35), 5.05 (H-36), 2.52 (H-3a), 2.39 (H-3b), 3.81 (H-4) and 1.43 (CH_3-37), and six at δ_C 174.6 (C-1), 151.7 (C-35), 131.1 (C-2), 77.9 (C-36), 70.0 (C-4) and 19.1 (C-37) (Table 1). These are all characteristic spectral features for the methyl α,β-unsaturated γ-lactone fragment of an Annonaceous acetogenins bearing a 4-hydroxyl group [8].

The existence of three hydroxyl groups in **1** was obvious by an IR absorption at 3473 cm^{-1} and three successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the CI mass spectrum. Furthermore, the ^{13}C NMR spectrum of **1** showed three resonances due to oxygen-bearing carbons at δ 74.3, 71.6 and 70.0, indicating the presence of three secondary hydroxyls, the chemical shift at δ 70 being the typical carbon resonance for 4-OH in reported acetogenins.

The monotetrahydrofuran (THF) ring was indicated by signals at δ_H 3.84 and 3.38 integrating, respectively, for four and one protons at δ_C 83.2 (C-18) and 82.1 (C-21). This suggested that there were two hydroxyl groups adjacent to the THF ring.

To establish the position of the THF ring and the hydroxyl groups adjacent to the THF ring along the hydrocarbon chain, mass spectral studies were undertaken (Scheme 1). Fragments in the EI mass spectrum of **1** at *m/z* 339 clearly placed the THF ring at C-18 and allowed the assignment of the hydroxyl groups adjacent to the THF ring at C-17 and C-22.

The relative stereochemistry of the α,α'-dihydroxylated THF system between carbons 17/18 and 21/22 of **1** was determined by comparing the ^{13}C NMR signals for the oxygenated carbons at C-18 and C-21 with those of model compounds of known relative stereochemistry synthesized by Fujimoto *et al.* [9], as well as by comparisons with the reported data for annonacin A [10] and murisolin A [11]. The comparison suggested that the relative stereochemistry was *threo/trans/erythro*.

An enzymatic method [12] was used to determine the absolute configuration of the lactic acid formed

* Part 65 in the series Acetogenins of Annonaceae. For Part 64, see Peyrat J.-F., Mahuteau J., Figadère B., Cavé A., (1996) *The Journal of organic Chemistry* (in press).

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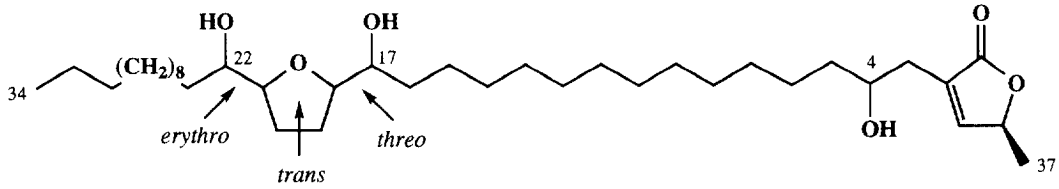
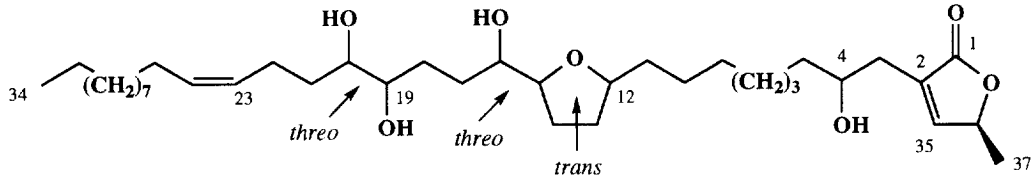
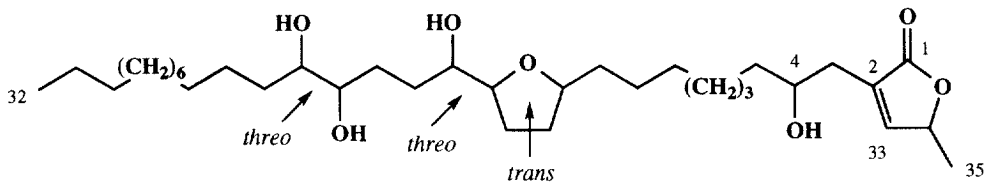


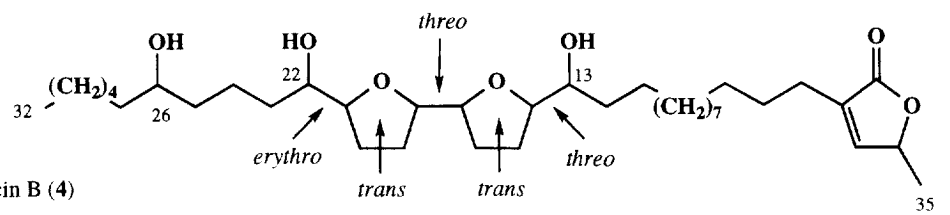
Glaucabellin (**1**)



Glaucaflorin (**2**)



Muricatetrocin B (**3**)



Squamocin B (**4**)

after oxidative degradation of **1**. The presence of only the *L*-isomer proved that **1** had the *S* configuration. Thus, the structure of **1** was deduced to be as illustrated and was named glaucabellin.

The Mr of glaucaflorin (**2**) was indicated by a peak at *m/z* 623 $[MH]^+$ in its CI mass spectrum corresponding to the molecular formula $C_{37}H_{67}O_7$. The existence of an α,β-unsaturated γ-lactone was suggested by an IR carbonyl absorption at 1742 cm^{-1}, a UV λ_{max} at 209 nm, six NMR resonances at δ_H 7.19 (H-35), 5.05 (H-36), 2.53 (H-3a), 2.40 (H-3b), 3.85 (H-4) and 1.42 (CH_3-37), and six at δ_C 174.6 (C-1), 151.8 (C-35), 131.1 (C-2), 78.0 (C-36), 69.8 (C-4) and 19.1 (C-37) (Table 2). These are all characteristic spectral features for a methyl α,β-unsaturated γ-lactone fragment of an Annonaceous acetogenins bearing a 4-hydroxyl group [8].

The existence of four hydroxyl groups in **2** was obvious by an IR absorption at 3367 cm^{-1} and four successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the CI mass spectrum. Moreover, the EI mass spectrum of the tetra-trimethylsilyl (TMSi) derivative **2a** showed four successive losses of TMSiOH (*m/z* 90) from the $[M]^+$. Furthermore, the ^{13}C NMR spectrum of **2** showed four resonances due to oxygen-bearing carbons at δ 74.4, 74.2, 74.0 and 69.8, indicating the presence of four secondary hydroxyls, the chemical shift at δ 69 being the typical carbon resonance for 4-OH in reported acetogenins.

The monotetrahydrofuran ring with one OH group adjacent to the ring was indicated by the signals at δ_H 3.89 (H-12), 3.82 (H-15) and 3.45 (H-16) and at δ_C 81.7 (C-15) and 79.3 (C-12). The signal at δ 3.45 was observed to have correlation cross peaks with one of the THF methine protons at δ 3.82 in the ^1H–^1H COSY spectrum. Moreover, the carbon chemical shift δ 79.3 is characteristic for the oxygenated carbons of THF rings that lack adjacent hydroxyl groups, such as glaucafilin [4]. The presence of an isolated *cis* double bond was suggested by a signal integrated for two protons at δ 5.37 (H-23, H-24) and two carbon peaks at δ 130.7 and 129.0. The configuration was established by the *J* values measured by double resonance selective decoupling experiment (J = 10.9, 5.3 Hz, H-23 and J = 10.8, 5.8 Hz, H-24).

To establish the position of the THF ring and the hydroxyl groups adjacent to the THF ring along the hydrocarbon chain, mass spectral studies were under-

Table 1. ^1H and ^{13}C NMR data (δ, $CDCl_3$) of glaucabellin **1***

Position	H	*J* (Hz)	C
1	–		174.7
2	–		131.1
3a	2.52 *ddd*	15.5; 4.8; 1.4	33.3
3b	2.39 *dd*	8.3; 15.5	33.3
4	3.81 *m*		70.0
5	1.48 *m*		37.4
6–15	1.25–1.76		25.3–29.6
16	1.45 *m*		33.4
17	3.38 *m*‡		74.3‡
18	3.84 *m*		83.2§
19, 20	1.98–1.69 *m*		28.6–32.6
21	3.84 *m*		82.1§
22	3.84 *m*‡		71.6‡
23	1.45 *m*		33.4
24–33	1.25–1.76		25.3–29.6†
32	1.25–1.76		31.9
33	1.25–1.76		22.7
34	0.38 *t*	6.6	14.1
35	7.18 *d*	1.1	151.7
36	5.05 *dq*	1.1; 6.6	77.9
37	1.43 *d*	6.6	19.1

* The assignments were confirmed by comparison with spectral data of annonacin A [10], murisolin A [11] and by 2D experiments (COSY 45 and HMQC).

† δ_C: 25.3, 25.6, 26.0, 28.6, 29.3, 29.6.

‡,§ The assignments are interchangeable with the columns suggesting a *threo/trans/erythro* or *erythro/trans/threo* relative configuration of the THF system.

Table 2. ^1H and ^{13}C NMR data (δ, $CDCl_3$) of glaucaflorin **2***

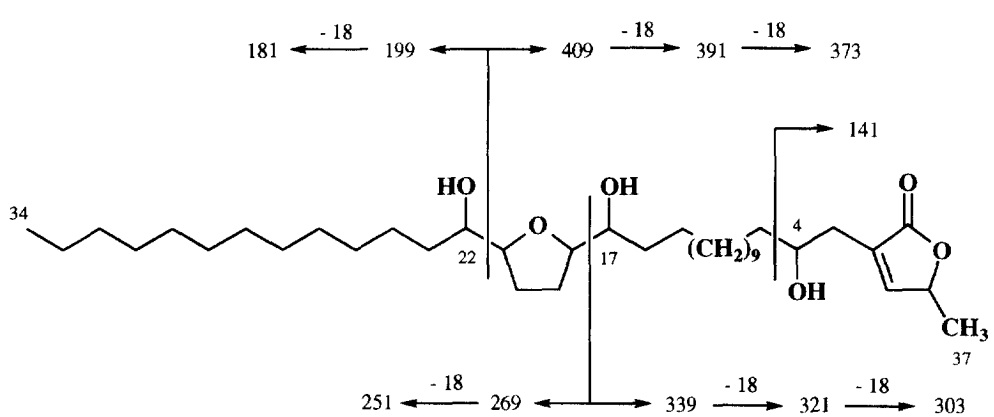
Position	H	*J* (Hz)	C
1	–		174.7
2	–		131.1
3a	2.53 *ddd*	15.1; 4.9; 1.5	33.3
3b	2.40 *dd*	8.2; 15.1	33.3
4	3.85 *m*		69.9
5	1.48 *m*		37.3
6–11	1.25–1.76		25.5–29.6
12	3.89 *m*		79.3
13, 14	2.00–1.68 *m*		28.4–32.4
15	3.82 *m*		81.7
16	3.45 *q*		74.4
17	1.52 *m*		35.5
18	1.69 *m*		33.3
19	3.45 *m*		74.2
20	3.45 *m*		74.0
21	1.45 *m*		31.9
22	2.20 *m*		23.5
23	5.37 *m*	10.9; 5.3	129.0
24	5.37 *m*	10.8; 5.8	130.7
25	2.02 *m*		32.3
26–33	1.25–1.76		22.6–31.9
34	0.88 *t*	6.8	14.1
35	7.19 *d*	1.5	151.9
36	5.05 *dq*	1.5; 6.8	77.6
37	1.42 *d*	6.8	19.1

* The assignments were confirmed by 2D experiments (COSY 45 and HMQC).

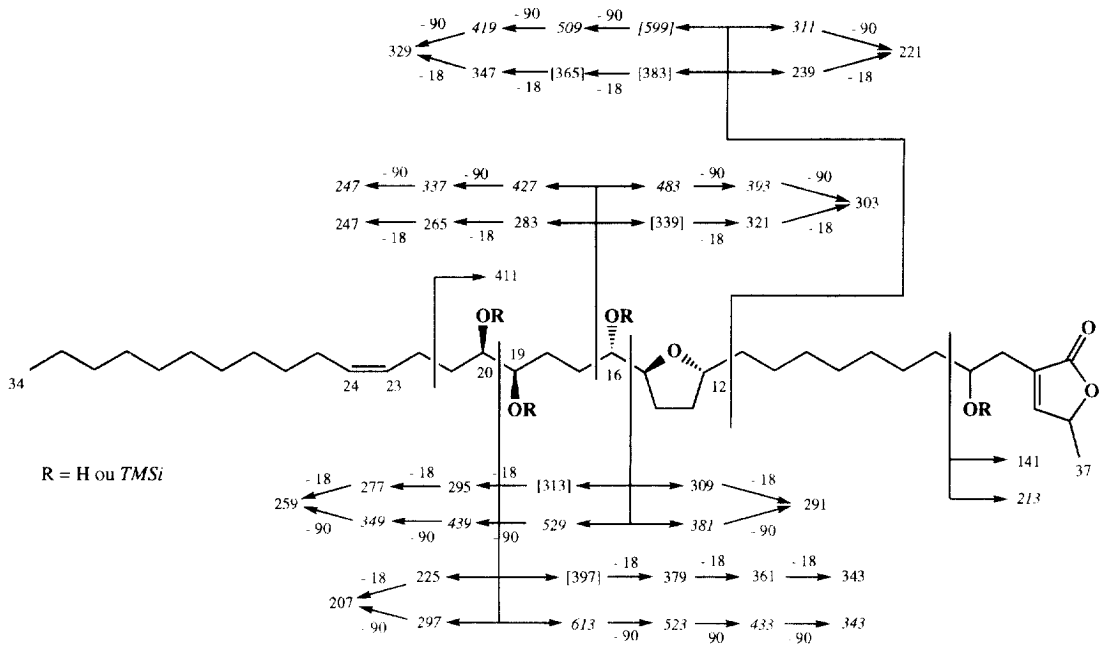
taken (Scheme 2). Fragments in the EIMS of **2** at *m/z* 239 clearly placed the tetrahydrofuran ring at C-12 and allowed the assignment of the hydroxyl group adjacent to the tetrahydrofuran ring at C-16. The structure of the carbon skeleton of **2** was confirmed by EIMS fragmentation of the TMS derivative (**2a**, Scheme 2).

The relative stereochemistry of the α-monohydroxylated THF system between C-12 and C-15 of **2** was determined by comparing the ^{13}C NMR signals for the oxygenated carbons at C-12 and C-16 with those of model compounds of known relative stereochemistry [9]. The comparison suggested that the relative stereochemistry was *threo/trans*.

In the ^1H NMR spectrum of **2**, the other two methine protons on the hydroxylated carbons were overlapped at δ 3.45. In the ^1H–^1H COSY spectrum, the methine proton at δ 3.45 (H-16) had cross peaks with the methylene protons at δ 1.52 (H-17). The methylene



Scheme 1. EI fragmentations of glaucabellin (**1**).



Scheme 2. EI fragmentations of glaucaflorin (**2**) and its TMSi derivative (**2a**). The absolute stereochemistry has been chosen arbitrarily and could be inverted.

protons at δ 1.52 had cross peaks with the methylene protons at δ 1.69 (H-18), which correlated with one of the methine protons at δ 3.45. The other methine proton at δ 3.45 had correlation cross peaks with methylene protons at δ 1.45 (H-21). These data suggested the presence of a vicinal diol group. The ^{13}C NMR signals for these two oxygenated carbons at δ 74.2 and 74.0 supported this conclusion. The placement of this vicinal diol at C-19 and C-20 was clearly confirmed by EIMS fragmentations analysis of **2** and **2a**. The relative configuration between C-19 and C-20 of **2** was suggested as *threo* by comparing the ^1H NMR signals for H-19 and H-20 at δ 3.45 with those of the *threo* group in muricatetrocins A and B [6] and the *erythro* group in glaucafilin diols [4].

This structure was confirmed by chemical oxidation of **2** with $H_5IO_6/RuCl_3$ [12]. GC analysis of the silylated degradation products showed a peak with the same R_t as that of an authentic sample of silylated undecanoic acid. Thus, the location of the double bond was unambiguously confirmed to be between the C-23 and C-24 positions. Enzymatic oxidation of the lactic acid formed on chemical degradation [12] of **2** showed the presence of only the *L*-isomer and proved that **2** had *S* configuration.

Thus, the structure of **2** was deduced to be as illustrated and was named glaucaflorin.

Muricatetrocin B and squamocin B were also isolated and showed identical spectral data to those previously reported for these compounds [6, 7].

EXPERIMENTAL

NMR: 200 and 400 MHz (^1H) and 50 and 100 MHz (^{13}C), $CDCl_3$; EIMS and CIMS (CH_4): Nermag R10-10C spectrometer; HPLC: μBondapak C_{18} prepacked column (10 μm, 8 × 100 mm), elution with various gradients of MeOH–H_2O, flow rate 1 ml/min, detection 214 nm; Prep HPLC: μBondapak C_{18} prepacked column (10 μm, 25 × 100 mm), elution with various gradients of MeOH–H_2O, flow rate 10 ml/min, detection 214 nm; GC: HT5 capillary column (0.22 mm × 25 m, 0.1 μm), FID detector, N_2 carrier gas at flow rate 1.4 ml/min and temp. 100–200° at 5°/min.

Plant material

Seeds of *Annona glauca* were collected in September 1994 in Senegal by D. Fall and authenticated by Prof. A. Le Thomas, Museum National d'Histoire Naturelle, Paris.

Extraction and isolation

Dried pulverized seeds (680 g) were macerated with MeOH. The MeOH extract was diluted with 0.1 vol. water and partitioned with hexane, leading to 24 g of a concentrated extract. The aq. MeOH phase was extracted with CH_2Cl_2, and the concentrated CH_2Cl_2 extract (14 g), containing acetogenins (Kedde+), was fractionated by flash chromatography on silica gel 60 eluted with solvents of increasing polarity leading to several fractions, one of which was purified by HPLC to give glaucabellin (**1**). Another fraction was chromatographed over a silica-gel 60 H column eluted with CH_2Cl_2–AcOEt–MeOH (90:8:3) to give two acetogenins, squamocin B (**4**) and glaucaflorin (**2**), which were further purified by HPLC. Another fraction was chromatographed over a silica-gel 60 H column which

on elution with CH_2Cl_2–AcOEt MeOH (80:15:5) afforded one acetogenin, muricatetrocin B (**3**), which was further purified by HPLC.

Glaucebellin (**1**)

White solid (4 mg) by prep. HPLC: (μBondapak C_{18}; MeOH–H_2O 22:3), R_t 26.1 min; $C_{37}H_{68}O_6$; $[\alpha]_D^{20}$ +18 ($CHCl_3$; *c* 0.2); UV λ_{max}^{EtOH} nm (log ε) 208 (3.9); IR_{max}^{NaCl} cm^{-1}: 3473, 2933, 2859, 1750, 1463, 1377, 1080, 1025, 953; ^1H NMR: Table 1; ^{13}C NMR: Table 1; CIMS (CH_4) *m/z*: 609 $[MH]^+$ (100%), 591 $[M\text{-}H_2O]^+$, 573 $[M\text{-}2H_2O]^+$, 551 $[M\text{-}3H_2O]^+$; EIMS 40 eV *m/z*: 409, 391, 373, 339 (100%), 321, 303, 269, 251, 199, 181, 141.

Degradative oxidation and enzymatic incubation of **1**

Periodic acid, H_5IO_6, (15 eq.) and a catalytic amount of $RuCl_3$ were added to a biphasic soln of glaucabellin (1.3 mg, 2.1 μmol.) in ternary medium CCl_4–MeCN–H_2O (57:57:86). The mixture was stirred for 20 h at room temp. Water (1 ml) was then added and the soln. was extracted with Et_2O (4 × 0.5 ml). The organic layer was concentrated and the residue was dissolved in 100 μl 0.1 M TRIS, pH 9. 50 μl aliquots of this solution were then separately incubated for 20 min at 40° with *L*- or *D*-LDH (10 μl) and a 1% aq. soln of NAD (50 μl), and NADH formation measured by HPLC [Sup-Rs Spherisorb S50DS2 column (4.6 × 250 nm), Prolabo, France; mobile phase: EDTA (76 mg/l) 0.5 M TRIS (pH 8) MeOH (95:5); flow rate: 1 ml/min; UV detection of NADH at 340 nm; sample vol injected: 50 μl].

Glaucaflorin (**2**)

White solid (4 mg) by prep. HPLC: (μBondapak C_{18}; MeOH–H_2O 17.L 3) R_t 22.5 min; $C_{37}H_{66}O_7$; $[\alpha]_D^{20}$ +15° ($CHCl_3$; *c* 0.2); UV λ_{max}^{EtOH} nm (log ε) 209 (3.9); IR_{max}^{NaCl} cm^{-1}: 3367, 2934, 2860, 1742, 1460, 1354, 1098, 977; ^1H NMR and ^{13}C NMR: Table 2; CIMS (CH_4) *m/z*: 623 $[MH]^+$ (100%), 605 $[M\text{-}H_2O]^+$, 587 $[M\text{-}2H_2O]^+$, 569 $[M\text{-}3H_2O]^+$, 551 $[M=4H_2O]^+$; EIMS 40 eV *m/z*: 379, 361, 347, 343, 329, 321, 309, 303, 295, 291, 283, 277, 265, 259, 251, 247, 239 (100%), 225, 221, 207.

TMS derivatization

Compound **2** (0.3 mg) was mixed with *N,O*-bis(trimethylsilyl)-acetamide (20 μl) and pyridine (2 μl) and heated at 70° for 30 min to yield the tetra-TMS derivative **2a**.

Oxidation of **2**

Periodic acid, H_5IO_6 (15 eq.), and a catalytic amount of $RuCl_3$ were added to a biphasic soln of glaucaflorin (1.3 mg, 2.1 μmol) in ternary medium CCl_4–CH_3CN–H_2O (57:57:86). The mixture was stirred for 20 h at room temp. Water (1 ml) was then added and the soln was extracted with Et_2O (4 × 0.5 ml). The organic phases were evaporated and then analysed by GC. Undecanoïc acid was identified at R_t 8.5 min by comparison with an authentic sample.

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