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GLAUCAFILIN, AN ACETOGENIN FROM *ANNONA GLAUCA**

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Abstract—The seeds of *Annona glauca* yielded seven acetogenins. One of them, glaucafilin, whose chemical structure was deduced by spectral and chemical methods, is a new compound. Glaucafilin shares four hydroxyl groups, two of which are vicinal, with the rare *erythro* configuration. The six known acetogenins identified were gigantetrocin A, goniothalamicin, annonacin A, annonacin, gigantecin and squamocin. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Annona glauca (*dugor mer*) is a spontaneous arborescent shrub or a shrubby tree growing in the sandy soil along the coast of Senegal. Only the roots are used in traditional medicine [2]. Previous phytochemical studies on this species have resulted in the isolation of two new acetogenins, annoglaucin [3] and glaucanisin [4]. Our continuing search for other members of the same class in the seeds of the same species has led to the isolation of seven acetogenins, one of which is a novel monotetrahydrofuran acetogenin and was named glaucafilin (**1**). The other six are the already known gigantetrocin A (**2**) [5], goniothalamicin (**3**) [6], annonacin A (**4**) [7], annonacin (**5**) [8], gigantecin (**6**) [9] and squamocin (**7**) [10]. Their occurrence in *A. glauca* is reported here for the first time.

RESULTS AND DISCUSSION

The M_r of glaucafilin (**1**) was indicated by a peak at *m/z* 597 $[MH]^+$ in the CI-mass spectrum. The HRCI-mass spectrum gave *m/z* 597.4770 for the $[MH]^+$ (calcd 597.47303) corresponding to the molecular formula $C_{35}H_{65}O_7$. The existence of an α,β-unsaturated γ-lactone was suggested by IR carbonyl absorption at 1743 cm^{-1}, a UV λ_{max} at 209 nm, six resonances at δ 7.17 (H-33), 5.05 (H-34), 2.53 (H-3a), 2.40 (H-3b), 3.83 (H-4) and 1.42 (CH_3-35) in the ^1H NMR spectrum, and six peaks at δ 174.7 (C-1), 151.9 (C-33), 131.1 (C-2), 77.6 (C-34), 69.9 (C-4) and 19.1 (C-35) in the ^{13}C NMR spectrum (Table 1). These are all characteristic spectral features of the methyl α,β-unsaturated γ-lactone fragment of Annonaceous acetogenins bearing a 4-hydroxyl group [11].

The existence of four hydroxyl functionalities in **1** was obvious from IR absorption at 3367 cm^{-1} and four successive losses of water (*m/z* 18) from the $[MH]^+$ in the CI-mass spectrum. Furthermore, the ^{13}C NMR of **1** showed four resonances due to oxygen-bearing carbons at δ 74.7, 74.4, 74.3 and 69.9, indicating the existence of four secondary hydroxyls, the chemical shift at δ 69.9 being the typical carbon resonance for 4-OH in known acetogenins. The monotetrahydrofuran ring was indicated by the signals at δ 3.89 (H-12) and 3.82 (H-15) in the ^1H NMR spectrum and at δ 81.7 (C-15) and 79.3 (C-12) in the ^{13}C NMR spectrum. Three methine protons attached to hydroxylated carbons were evident at δ 3.60 (2H) and δ 3.45 (1H) in **1**, although only 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. This suggested that there was only one hydroxyl group adjacent to the THF ring. This was confirmed by the carbon chemical shift δ 79.3, which is characteristic of the oxygenated carbons of THF rings that lack adjacent hydroxyl groups, such as gigantetrocin A (**2**) and gigantecin (**6**).

To establish the position of the tetrahydrofuran ring, the hydroxyl group adjacent to the THF ring and the remaining two hydroxyl groups along the hydrocarbon chain, mass spectral studies were undertaken (Scheme 2). Fragments in the EI-mass spectrum

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* Part 50 in the series 'Acetogenins of Annonaceae'. For part 49, see ref. [1].

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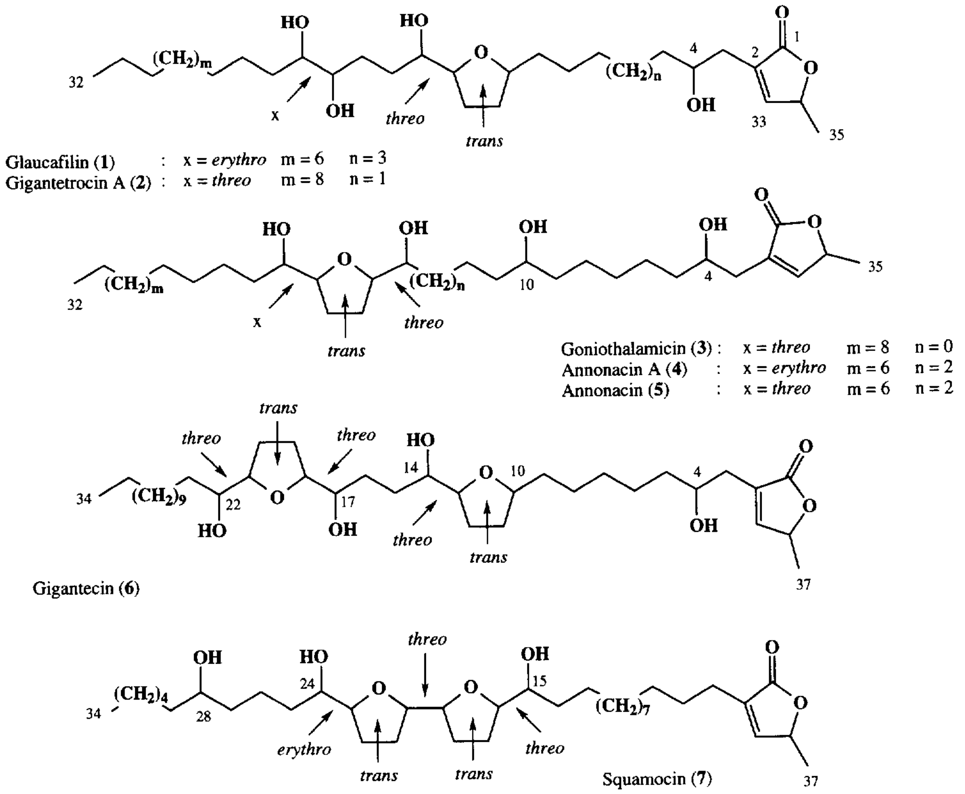


Scheme 1. Acetogenins isolated from seeds of *Annona glauca*.

Table 1. ^{1}H and ^{13}C NMR CDCl$_3$ data for glaucafilin (1)

Position	H	*J* (Hz)	C
1	–		174.7
2	–		131.1
3a	2.53 *ddd*	15.7; 4.8; 1.4	33.3
3b	2.40 *dd*	8.3; 15.7	33.3
4	3.83 *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 *q*		81.7
16	3.45 *q*		74.3
17	1.52 *m*		35.7
18	1.69 *m*		32.4
19	3.60 *m*		74.4
20	3.60 *m*		74.7
21	1.45 *m*		26.1
22–31	1.25–1.76		22.7–31.7
32	0.88 *t*	6.6	14.1
33	7.17 *d*	1.1	151.9
34	5.05 *dq*	1.1; 6.6	77.6
35	1.42 *d*	6.6	19.1

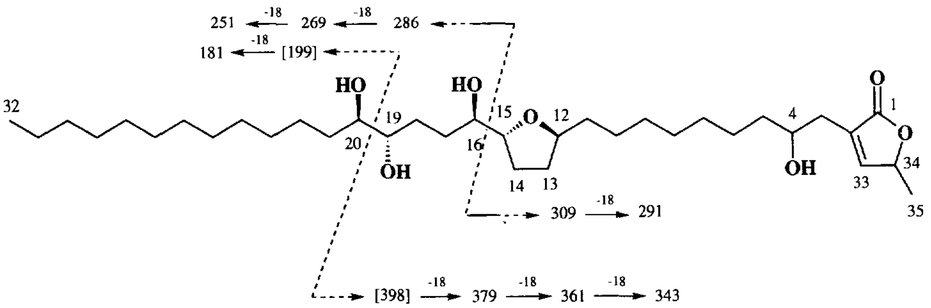
Assignments were confirmed by two-dimensional experiments (COSY 45 and HMQC).

of **1** at *m/z* 309 clearly positioned the tetrahydrofuran ring at C-12 and allowed the assignment of the hydroxyl group adjacent to the tetrahydrofuran ring at C-16.

The relative stereochemistry of the α-monohydroxylated THF system between C-15 and C-16 of **1** was determined by comparing the ^{13}C NMR signals for the oxygenated carbons at C-12, C-15 and C-16 with those of model compounds of known relative stereochemistry [12]. These suggested that the relative stereochemistry was *transthreo*. This assignment was further corroborated by resonances in the ^{1}H NMR of **1** at δ 3.82 (H-15) and δ 3.45 (H-16) indicative of a *threo* configuration.

In the ^{1}H NMR spectrum of **1**, the other two methine protons on the hydroxylated carbons were overlapped at δ 3.60. In the ^{1}H–^{1}H COSY spectrum, the methine proton at δ 3.45 (H-16) had cross-peaks with methylene protons at δ 1.52 (H-17). The methylene protons at δ 1.52 had cross-peaks with methylene protons at δ 1.69 (H-18), which correlated with one of the methine protons at δ 3.60. The other methine proton at δ 3.60 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 74.4 and δ 74.7 and the preparation of the acetonide derivative (**8**) of **1** strongly supported this conclusion. The CI-mass spectrum of **8** gave a [MH]$^+$ at *m/z* 637, confirming acetonide formation.

The placement of this vicinal diol at C-19 and C-20 was clearly confirmed by EI-mass spectral fragmentations analysis of **1**. The ^{1}H NMR signals for the acetonyl methyls of **8** showing two separate singlet peaks at δ 1.41 and δ 1.31, respectively, suggested an



Scheme 2. EI-mass spectral fragmentations of glaucafilin (**1**).

Table 2. ^1H NMR (400 MHz) signals for the protons of *threo* and *erythro* diols in Annonaceous acetogenins and their acetonide derivatives ($CDCl_3$, δ) [16]

	Methine protons		Acetonyl methyls	
	threo	*erythro*	*threo*	*erythro*
Diols	3.40(2H)	3.60(2H)	–	–
Acetonides	3.55(2H)	4.00(2H)	1.36(6H)	1.41, 1.31

erythro-configuration for the diol group, as in bulladecinone [13] and annomuricin B [14] (Table 2). Many *threo* vicinal diols have been previously found in Annonaceous acetogenins, e.g. muricatetrocins A and B [15], but compounds such as **1**, having an erythro vicinal diol moiety are uncommon [13, 14]. Because of the very limited amount of the sample, no study of the absolute stereochemistry of the chiral centres of **1** was possible. Thus, the structure of **1** was deduced to be as illustrated and was named glaucafilin.

Gigantetrocin A (**2**), goniothalamicin (**3**), annonacin A (**4**), annonacin (**5**), gigantecin (**6**) and squamocin (**7**) were also isolated and showed identical spectral data to those previously reported [5–10].

EXPERIMENTAL

Optical rotations were determined on a Schmidt-Haensch Polartronic I polarimeter. UV spectra were obtained on a Philips PU 8720 spectrometer. IR spectra were measured on a Perkin–Elmer 257 spectrometer. The ^1H-NMR and ^{13}C-NMR spectra ($CDCl_3$) were obtained with Bruker AC-200 or AC-400 instruments at 200 and 50 MHz or at 400 and 100 MHz, respectively. EIMS and CIMS (methane) were performed on a Nermag R10-10C spectrometer. HPLC analytic analyses were performed with a Waters 501 pump, a Waters 991 spectrophotometer (214 nm) and a Waters WISP automatic injector on a μBondapak C_{18} prepacked column (10 μm, 8 × 100 mm) and elution with a MeOH–H_2O gradient at a flow rate of 1 ml, min^{-1}, with detection at 214 nm. Prep. HPLC was carried out with a Millipore-Waters (Milford, MA, U.S.A.) system equipped with a 590 pump, a SSV injector and a 484 UV detector (214 nm) on a μBondapak C_{18} prepacked column (10 μm, 25 × 100 mm) and elution with a MeOH–H_2O gradient at a flow rate of 10 ml min^{-1}.

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

Extraction and isolation. Dried pulverized seeds (680 g) were macerated with MeOH. The MeOH extract was diluted with a 10% vol. of H_2O and submitted to liquid–liquid partition with hexane, leading to 24 g of a concd extract. The aq. MeOH phase was extracted with CH_2Cl_2. The concd CH_2Cl_2 extract (14 g) containing acetogenins (Keede+) was fractionated by flash CC on silica gel 60 and eluted with solvents of increasing polarity giving several frs. One of these was chromatographed over a silica-gel 60 H column and elution with CH_2Cl_2–EtOAc–MeOH (90:8:3) yielded two acetogenins. Squamocin (**7**) was obtained as a pure fr.; HPLC was used to verify its purity. Glaucafilin (**1**) was obtained as an almost pure fr. and was further purified by HPLC. Another fr. was chromatographed over a silica-gel 60 H column and elution with CH_2Cl_2–EtOAc–MeOH (16:3:1) yielded four acetogenins. Annonacin A (**4**), annonacin (**5**) and gigantecin (**6**) were obtained as pure frs (HPLC). Gigantetrocin A (**2**) was obtained as an almost pure fr. and was further purified by HPLC. The last fr. studied was chromatographed over a silica-gel 60 H column and eluted with CH_2Cl_2–EtOAc–MeOH (80:13:7), yielding goniothalamicin (**3**) as a pure fr. (HPLC).

Glaucafilin (**1**). Solid (4 mg) by prep. HPLC μBondapak C_{18}. MeOH–H_2O, 41:9, R_t 17.3 min. $C_{35}H_{64}O_7$. $[\alpha]_D^{20}+30^\circ$ ($CHCl_3$; c 0.2). UV λ_{max}^{EtOH} nm (log ε) 209 (3.9). IR_{max}^{NaCl} cm^{-1}: 3367, 2920, 2851, 1743, 1439, 1376, 1061, 1032, 952, 756. ^1H NMR (Table 1). ^{13}C NMR (Table 1). CI-MS (CH_4) (m/z, rel. int.): 597 $[MH]^+$ (100), 579 $[M-H_2O]^+$, 561 $[M-2H_2O]^+$, 543 $[M-3H_2O]^+$, 525 $[M-4H_2O]^+$. EI-MS 40 eV (m/z, rel. int.): 379, 361, 343, 309 (100), 291, 286, 269, 251.

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