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ANNOGLAUCIN, AN ACETOGENIN FROM *ANNONA GLAUCA**

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Abstract—Annoglaucin, a new bis-tetrahydrofuran acetogenin was isolated and characterized, in addition to the known compound, rolliniastatin-2, from the roots of *Annona glauca*.

INTRODUCTION

Annona glauca is a widespread small shrub growing in Senegal, which is used in traditional medicine for various pains [1]. Roots are used as a diuretic and against gonorrhoea. Until now, it has not been studied either for its chemical content or for its pharmacological properties. Among the Annonaceae, the genus *Annona* is represented by *ca* 120 species. Until 1980, species of this genus have been studied mainly for their alkaloidal content [2, 3], but, since 1985, the interest has been particularly related to the presence of neutral compounds of polyketide origin, the annonaceous acetogenins, characterized by their interesting biological activities, especially cytotoxic, antitumoral, antiparasitic and pesticidal properties. Acetogenins from *Annona bullata*, *A. cherimolia*, *A. densicoma*, *A. montana*, *A. muricata*, *A. reticulata* and *A. squamosa* have been studied [4] and, more recently, from *A. senegalensis* [5]. We decided to verify if acetogenins, largely distributed in the genus *Annona*, were present in *A. glauca* and could be responsible for its pharmacological activities in connection with its uses in traditional medicine.

RESULTS AND DISCUSSION

A methanol extract of *A. glauca* roots was partitioned between hexane and an aqueous solution of 95% methanol. The hydroalcoholic residue was extracted with dichloromethane. The dichloromethane extract containing acetogenins (Kedde-positive) [6] was fractionated by flash chromatography on silica gel 60 and purified by column chromatography (silica gel 60 H), leading to the isolation of **1** and **2**.

Annoglaucin (**1**) was isolated as a waxy solid. Its M_r was determined by mass spectrometry using a fast-atom beam as ionizing agent and *m*-nitrobenzyl alcohol doped with LiCl as a liquid matrix (FAB-Li) [7]. 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 $C_{37}H_{66}O_8$. The presence of four hydroxyl groups was indicated by the losses of four molecules of H_2O from $[M + H]^+$ in the CI-mass spectrum and by a broad absorption in the IR spectrum at 3430 cm^{-1}. This was confirmed by preparation of the tetraacetyl derivative (**1a**). A positive response to Kedde's reagent suggested the presence of an α,β-unsaturated γ-lactone. A strong IR absorption at 1740 cm^{-1} and an UV absorption maximum at 210 nm supported the presence of this function. Resonances in the ^1H NMR at δ7.17 (1H, *d*, J = 1.4 Hz, H-35), δ5.05 (1H, *dq*, J = 1.4 and 7 Hz, H-36) and δ1.44 (3H, *d*, J = 7 Hz, H-37), corresponding, in the ^{13}C NMR, to resonances at δ151.8 (C-35), δ77.8 (C-36) and δ18.9 (C-37), respectively, supported this assignment. The presence of an ABX system in the ^1H NMR was observed with coupling between two protons on C-3 at δ2.50 (H-3a) and δ2.38 (H-3b) and a single proton at δ3.86 (H-4), establishing the presence of an hydroxyl group at C-4, first recognized in asimicin [8]. In addition, there was a peak in the EI-mass spectrum at *m/z* 141; this peak corresponded to the fragment $C_7H_9O_3$, representing cleavage between C-4 and C-5. The EI-mass spectrum of the tetra-TMSi-derivative showed a peak at *m/z* 213, which was consistent with a trimethylsilyl group added to this fragment.

The structure of the bis-THF part of the molecule of **1** was elucidated by the same techniques. The ^{13}C NMR spectrum showed four resonances at δ83.1 (C-16), δ82.4 (C-19), δ82.1 (C-20), δ82.6 (C-23) due to methines bearing oxygen in THF rings, as well as δ74.0 (C-15) and δ71.3 (C-24) for the corresponding hydroxylated carbons. These

*Part 35 in the series Acetogenins of Annonaceae. For part 34, see Duret, P., Laurens, A., Hocquemiller, R., Cortes, D. and Cavé, A., (1994) *Heterocycles* (in press).

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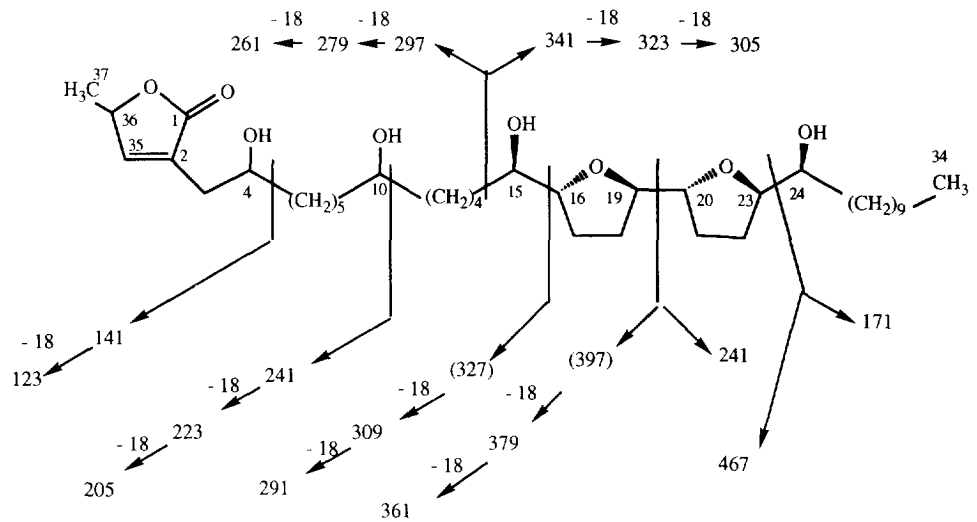
^{13}C NMR values are correlated (XH-CORR) to multiplets at δ3.80 (2H), 3.90 (2H), 3.39 (1H) and 3.85 (1H) in the ^{1}H NMR spectrum and were similar to signals of other acetogenins [8, 9], indicating the common bis-THF moiety.

These data and those of ^{1}H NMR of the acetyl derivative (**1a**), supported that the relative configuration of the chiral centres of the α,α'-*bis*-THF part of the molecule is *threo/trans/threo/trans/erythro* [10]. This relative stereochemistry has been found in several other acetogenins, like rolliniastatin-2 [11] (also isolated from this species), uvaricin [9], rioclarin [12] and others, always with the same shift pattern in the ^{13}C NMR spectrum [13, 14].

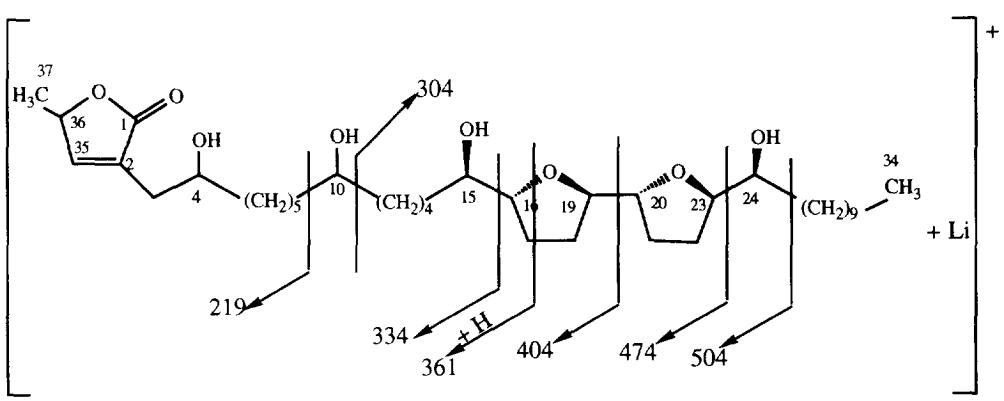
The fourth hydroxyl group was located on the alkyl chain. In the ^{1}H NMR spectrum, one more carbinol methine proton was present at δ3.59 (1H, *m*, H-10) and, in the ^{13}C NMR, the corresponding carbinol methine appeared at δ71.6 (C-10).

The position of the fourth hydroxyl group, as well as the total structure of the molecule, was established by close examination of the EI-mass spectral fragmentation pattern of annoglaucin (**1**) (Scheme 1) and its tetra-TMSi-derivative (**1b**). The peaks at *m/z* 467, 309, and 291 in the EI-mass spectrum of annoglaucin (**1**) allowed placement of the α,α'-bis THF system between C-15 and C-24, whereas the ion fragments at *m/z* 241, 223 and 205 suggested that the fourth hydroxyl group was on C-10. The fragmentation pattern obtained from FAB-Li (Scheme 2), was consistent with the proposed structure, with peaks at *m/z* 219 (C-9/C-10 cleavage) and *m/z* 361 (C–O/C-16 and C-17/C-18 cleavage). The EI-mass spectrum of the tetra-TMSi-derivative (**1b**), gave further confirmation, with peaks at *m/z* 385, (C-10/C-11 cleavage), as well as the intensive peak at *m/z* 283 (C-9/C-10 cleavage).

Annoglaucin (**1**) appears to be the first compound in the annonaceous acetogenins series with an hydroxyl group at C-10 and an adjacent bis-THF moiety.



Scheme 1. EI-mass spectral fragmentations of annoglaucin (**1**). Absolute configurations at C-15, C-16, C-19, C-20, C-23 and C-24 may be inverted.



Scheme 2. FAB-Li mass spectral fragmentation of annoglaucin (**1**). Absolute configurations at C-15, C-16, C-19, C-20, C-23 and C-24 may be inverted.

Table 1. ^{1}H NMR spectral data of compounds **1**, **1a** and **2** (200 MHz, $CDCl_3$)

C	1	1a	2
1	—	—	—
2	—	—	—
3a	2.50 *m*	2.53 *m*	2.50 *m*
3b	2.38 *m*		2.40 *m*
4	3.86 *m*	5.11 *m*	3.85 *m*
5	1.46 *m*	1.44–1.98	1.20–1.83
6–8, 12–13, 26–31	1.15–1.75	1.44–1.98	1.20–1.83
9, 11, 14, 25	1.42 *m*	1.44–1.98	1.20–1.83
10	3.59 *m*	5.84 *m*	1.20–1.83
15	3.39 *m*	5.84 *m*	3.37 *m*
16, 23	3.80 *m*	3.86–3.98	3.85 *m*
17, 22	1.85 *m*	1.44–1.98	1.57–1.83
18, 21	1.77 *m*	1.44–1.98	1.57–1.83
19, 20	3.90 *m*	3.86–3.98	3.85 *m*
24	3.85 *m*	5.84 *m*	3.85 *m*
32, 33	1.25 *m*	1.44–1.98	1.20–1.83
34	0.88 *t* (7)	0.90 *t* (7)	0.88 *t* (7)
35	7.17 *d* (1.4)	7.07 *d* (1.4)	7.17 *d* (1.4)
36	5.05 *dq* (7; 1.4)	5.02 *dq* (7; 1.4)	5.05 *dq* (7; 1.4)
37	1.44 *d* (7)	1.40 *d* (7)	1.40 *d* (7)
4-OAc	—	2.01 *s*	—
10-OAc	—	2.01 *s*	—
15-OAc	—	2.06 *s*	—
24-OAc	—	2.03 *s*	—

J (in Hz) in parentheses.

Rolliniastatin-2 (**2**), was also isolated as a waxy solid and its identity was established by IR, mass spectrometry, ^{1}H and ^{13}C NMR studies [9] and, finally, by TLC comparison with an authentic sample.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 200 MHz and ^{13}C NMR at 50 MHz in $CDCl_3$ using $CHCl_3$ as int. standard. CI-MS were recorded by a desorption chemical ionization technique using CH_4 gas. TLC was performed on 0.2 mm precoated plates and spots were detected by spraying with Kedde's reagent and H_2SO_4.

Plant material. Roots of *Annona glauca* were collected in September 1993 by one of us from 'Route des Niayes' near Keur Massar in Senegal and authenticated by Prof. A. Le Thomas (Muséum d'Histoire Naturelle, Paris). A voucher specimen (AL 301) is deposited at Jardin des Plantes Utiles, Faculté mixte de Médecine et Pharmacie, Dakar, Sénégal.

Isolation. Dried powdered roots (580 g) were extracted with MeOH. The concd extract (51 g) was solubilized in MeOH with an additional 5% of H_2O and fractionated by liquid–liquid partitioning with hexane, yielding 7 g of concd extract. The H_2O–MeOH phase was acidified with 1 M HCl and extracted with CH_2Cl_2. A part (12 g) of the CH_2Cl_2 extract (22 g) was subjected to flash CC over silica gel 60 and gradually eluted by mixts of increasing polarity containing hexane, EtOAc and MeOH. Rolliniastatin-2 (**2**) was obtained as an almost pure fr. and was further purified by CC on Sephadex LH 20 (MeOH). Another fr. was chromatographed on a silica gel 60 H column and eluted with CH_2Cl_2–MeOH (47:3), leading to the isolation of annoglaucin (**1**), which was further purified by CC on Sephadex LH 20 (MeOH).

Table 2. ^{13}C NMR spectral data of compounds **1** and **2** (50 MHz, $CDCl_3$)

C	1	2
1	174.5	174.5
2	131.0	130.9
3	33.0	33.1
4	69.5	69.7
5	37.3	36.8
6–8, 12–13, 27–31	24.2–29.4	25.3–29.5
9, 11	37.0	25.3–29.5
10	71.6	25.3–29.5
14, 25	32.7[a]	32.2[a]
15	74.0	74.0
16	83.1	83.2
17, 18, 21, 22	28.1–29.2	25.3 –29.5
19	82.4	82.4
20	82.1	82.1
23	82.6	82.7
24	71.3	71.2
32	31.7[a]	31.7[a]
33	22.5	22.5
34	13.9	14.0
35	151.8	151.7
36	77.8	77.8
37	18.9	18.9

[a]May be reversed within column.

Annoglaucin (**1**). Waxy solid (180 mg). $C_{37}H_{66}O_8$. $[\alpha]_D^{20}$ + 15.4° ($CHCl_3$; *c* 0.6). UV λ_{max}^{MeOH} nm (log ε) 210 (4.0). IR ν_{max}^{KBr} cm^{-1}: 3430, 2930, 2855, 1740, 1460, 1320, 1070. ^1H NMR (Table 1). ^{13}C NMR (Table 2). FAB-Li MS *m/z*: 645 $[M + Li]^+$, 504, 474, 404, 361, 334, 304, 219. CI-MS (CH_4) *m/z*: 639 $[M + H]^+$, 621 $[MH - H_2O]^+$, 603 $[MH - 3H_2O]^+$, 585 $[MH-4H_2O]^+$. EI-MS (probe) 70 eV *m/z*: 590, 570, 520, 471, 467, 379, 361, 341, 323, 309, 305, 297, 291, 279, 261, 241, 223, 205, 141, 123.

Annoglaucin tetraacetate (**1a**). Oil ^1H NMR (Table 1). CI-MS (CH_4) *m/z*: 807 $[M + H]^+$, 765, 747, 723, 705, 686, 680, 645, 627, 575, 532, 419, 299, 283. EI-MS (probe) 70 eV *m/z*: 482, 325, 311, 283, 241, 213, 183, 171.

Tetra-TMSi-annoglaucin (**1b**). EI-MS (probe) 70 eV *m/z*: 683, 441, 385, 313, 283, 243, 213.

Rolliniastatin-2 (**2**). Waxy solid. (85 mg). IR ν_{max}^{KBr} cm^{-1}: 3470, 2930, 2860, 1750, 1600, 1460, 1320, 1070. ^1H NMR (Table 1). ^{13}C NMR (Table 2). CI-MS (CH_4) *m/z*: 623 $[M + H]^+$, 605 $[MH - H_2O]^+$, 587 $[MH - 2H_2O]^+$, 569 $[MH - 3H_2O]^+$. EI-MS (probe) 70 eV *m/z*: 476, 453, 433, 416, 397, 381, 363, 345, 311, 293, 284, 241, 223, 171, 153, 141, 123.

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