Three New Bistetrahydrofuran Acetogenins from the Seeds of *Annona spinescens*[1]

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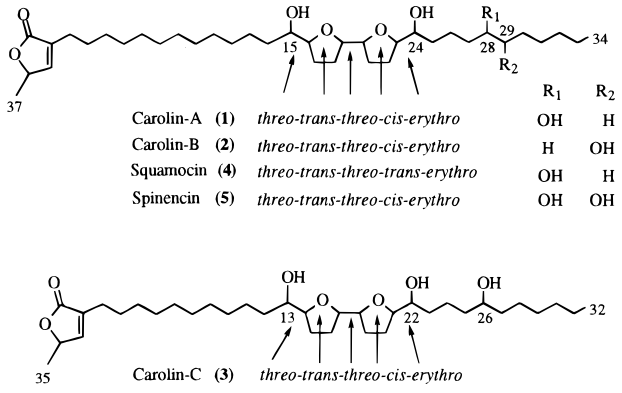
Three new bistetrahydrofuran acetogenins, carolins A−C (**1**−**3**), were isolated from the MeOH extract of *Annona spinescens* in addition to the known compound, squamocin (**4**). The structures of **1**, **2**, and **3** were elucidated by spectroscopic methods including LSIMS/MS technique and confirmed by a chemical transformation. The cytotoxic activity of the new compounds **1**−**3** is reported and discussed in comparison with **4** and the previously isolated spinencin **(5)**.

Acetogenins from Annonaceae are known to exhibit a variety of pharmacological effects such as antiparasitic, insecticide, cytotoxic, antitumor, and immunosuppressive activities.[3,4]

In our recent studies of the seeds of *Annona spinescens* Mart.,[5] we have described seven bis-tetrahydrofuran acetogenins.[6] Alkaloid components had been previously reported from this plant.[7]

The present study has led to the isolation and structure elucidation of three new bistetrahydrofuran (bis-THF) acetogenins, carolin A (**1**), carolin B **(2)**, and carolin C **(3),** together with the known squamocin **(4)**.[8] Their cytotoxic activity was investigated and compared with the first acetogenin presenting a *threo/trans/threo/cis/erythro* configuration, spinencin **(5)**.[6]



Results and Discussion

Carolins A, B**,** and C **(1**−**3)** were isolated as transparent oils from the MeOH extract of the seeds by usual chromatographic methods, followed by preparative HPLC. Their structures were determined by ^{1}H- and ^{13}C-NMR (COSY, HOHAHA, HMBC and HMQC) and MS (LSIMS and LSIMS/MS) on the native compounds. The molecular weights were established by LSIMS as 622 for **1** and **2**, corresponding to the molecular formula $C_{37}H_{66}O_7$, and 594 ($C_{35}H_{62}O_7$) for **3**; the $[M + Li]^+$ ions were observed at *m/z* 629 for **1** and **2,** and at *m/z* 601 for **3**.

Both **1** and **2** showed a weak UV absorption at 208.0 nm and a strong one at 1749 cm^{-1} for **1** and 1753 cm^{-1} for **2** in the IR spectrum, indicating the presence of an α,β-unsaturated γ-lactone moiety, characteristic for acetogenins of type 1.[3,4] This structural feature was confirmed by typical resonances in the ^{1}H-NMR (Table 1) and ^{13}C-NMR (Table 2) spectra, also indicating the absence of OH group at C-4 in **1** and **2**.[3,4] The presence of an adjacent bis-THF system was deduced from the ^{1}H-NMR signals at δ 3.87 (3H) and 3.93 (1H) for **1**, and δ 3.89 (3H) and 3.90 (1H) for **2,** assigned to four oxymethine protons, in agreement with their ^{13}C-NMR signals at δ 83.0, 82.6, 82.4, and 81.9.[9]

Two hydroxymethine groups flanking the bis-THF system were observed at δ 3.39 and 3.78 for **1** (δ 3.35 and 3.78 for **2**) in the ^{1}H−^{1}H COSY spectra, and at δ 73.9 and 71.6 or 71.5 in the ^{13}C-NMR spectra of **1** and **2** (Table 2). A further hydroxymethine proton appeared at δ 3.56 in the ^{1}H NMR of **1** and at δ 3.55 for **2**. Its ^{13}C-NMR resonance at δ 71.3 was indicative of an isolated hydroxy group in the aliphatic chain of **1** and **2**.[10,11] The position of the substituents in the aliphatic chain was subsequently determined by mass spectrometry.[3,4]

The high-energy collision-induced dissociation (CID) spectrum of the $[M + Li]^+$ ion displayed the typical fragmentation pattern of lithiated acetogenins.[12] Two pairs of fragment ion peaks at *m/z* 221/291 and 329/399 were assigned easily to fragmentations across two

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Table 1. ^{1}H-NMR Data ($CDCl_3$, 400 MHz) of **1**−**3** and Their Derivatives

position	**1**	**2**	**3**	**1a**	**1b**	**2a**	**3a**
1							
2							
3	2.26 t[a]	2.25 t[a]	2.26 t[a]	2.26 t[a]	2.26 t[a]	2.26 t[a]	2.26 t[a]
4	1.56 m	1.54 m	1.53 m	1.56 m	1.57 m	1.56 m	1.52 m
5−11	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m
12	1.20−1.30 m	1.20−1.30 m	1.39 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.35−1.45 m
13	1.20−1.30 m	1.20−1.30 m	3.43m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	4.84 m
14	1.40 m	1.39 m	3.85 m	1.35−1.45 m	1.40 m	1.35−1.45 m	3.98 m
15	3.39 m	3.35 m	1.65−1.97 m	4.86 m	3.39 m	4.86 m	1.50−2.00 m
16	3.87 m	3.89 m	1.65−1.97 m	3.95 m	3.87 m	3.95 m	1.50−2.00 m
17	1.52−1.91 m	1.50−1.92 m	3.90 m	1.50−2.00 m	1.50−1.95 m	1.50−2.00 m	3.89 m
18	1.52−1.91 m	1.50−1.92 m	3.90 m	1.50−2.00 m	1.50−1.95 m	1.50−2.00 m	3.89 m
19	3.87 m	3.89 m	1.82−1.97 m	3.88 m	3.86 m	3.88 m	1.80−2.00 m
20	3.87 m	3.89 m	1.82−1.97 m	3.88 m	3.86 m	3.88 m	1.80−2.00 m
21	1.81−1.89 m	1.82−1.90 m	3.91 m	1.80−2.00 m	1.81−1.90 m	1.80−2.00 m	3.98 m
22	1.81−1.89 m	1.82−1.90 m	3.79 m	1.80−2.00 m	1.81−1.90 m	1.80−2.00 m	4.96 m
23	3.93 m	3.90 m	1.40 m	3.95 m	3.90 m	3.96 m	1.35−1.45 m
24	3.78 m	3.78 m	1.20−1.30 m	4.93 m	3.68 m	4.93 m	1.20−1.30 m
25	1.40 m	1.40 m	1.45 m	1.35−1.45 m	1.44−1.50 m	1.35−1.45 m	1.20−1.45 m
26	1.20−1.30 m	1.20−1.30 m	3.61 m	1.20−1.30 m	1.20−1.40 m	1.20−1.30 m	4.84 m
27	1.46 m	1.20−1.30 m	1.45 m	1.20−1.45 m	1.64−1.69 m	1.20−1.45 m	1.35−1.45 m
28	3.56 m	1.44 m	1.20−1.30 m	4.86 m	3.57 m	4.85 m	1.20−1.30 m
29	1.46 m	3.55 m	1.20−1.30 m	1.20−1.45 m	1.64−1.69 m	1.20−1.45 m	1.20−1.30 m
30	1.20−1.30 m	1.44 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m
31	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m
32	1.20−1.30 m	1.20−1.30 m	0.89 t[b]	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	0.88 t[b]
33	1.20−1.30 m	1.20−1.30 m	6.97 d[c]	1.20−1.30 m	1.20−1.30 m	1.20−1.30 m	6.98 d[c]
34	0.88 t[b]	0.85 t[b]	4.99 qd[d]	0.88 t[b]	0.88 t[b]	0.88 t[b]	4.99 qd[d]
35	6.95 d[c]	6.97 d[c]	1.42 d[e]	6.98 d[c]	6.98 d[c]	6.98 d[c]	1.40 d[e]
36	4.99 qd[d]	4.99 qd[d]		4.97 qd[d]	4.98 qd[d]	4.97 qd[d]	
37	1.40 d[e]	1.40 d[e]		1.40 d[e]	1.40 d[e]	1.40 d[e]	
13/15-OAc				2.04 s		2.04 s	2.05 s
22/24-OAc				2.07 s		2.07 s	2.08 s
26-OAc							2.05 s
28/29-OAc				2.03 s		2.04 s	
38a					5.10 d[f]		
38b					4.57 d[f]		

[a] J = 6.5 Hz. [b] J = 6.5 Hz. [c] J = 1.5 Hz. [d] J = 6.7 Hz and J = 1.7 Hz. [e] J = 6.7 Hz. [f] J = 1.5 Hz.

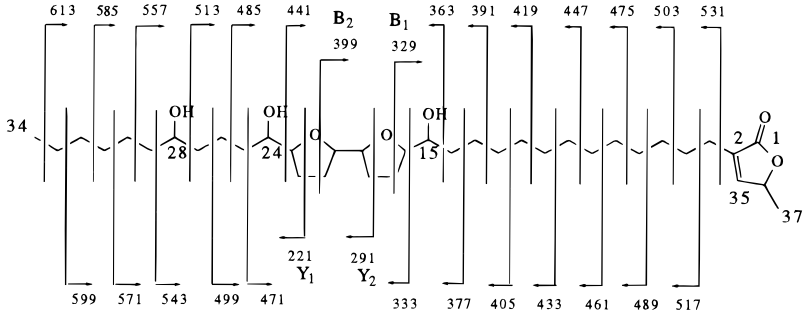

Figure 1. Collision-induced dissociations of the $[M + Li]^+$ ion (*m*/*z* 629) generated by LSIMS from carolin A **(1)**. Similar fragmentations were observed for **(2)**.

adjacent THF rings (ions Y_1−Y_2 and B_1−B_2, respectively, according to Laprévote and Das[12]), indicating the position of the bis-THF system along the alkyl chain (Figure 1). The *m*/*z* values of these fragments accounted for the presence of one hydroxy group between the THF and the terminal lactone ring and of two other hydroxy groups on the methyl-terminal side chain, their locations being deduced from careful scrutiny of the CID spectrum. Two series of fragment ion peaks were indeed attributed to charge−remote fragmentations of the alkyl chain from the $[M + Li]^+$ precursor ion (*m*/*z* 629) for **1** and **2**. Among them, the diagnostic fragment ions at *m*/*z* 543, 513 for **1** and *m*/*z* 557, 527 for **2** were indicative of the presence of a hydroxy group at position C-28 in **1**, and C-29 in **2**. The location of the two remaining OH groups at C-15 and C-24 positions was obtained by a similar way (Figure 1).

The relative stereochemistry around the bis-THF rings was determined by comparing the ^{1}H- and ^{13}C-NMR signals of **1** and **2,** and the ^{1}H-NMR data of their triacetates (**1a, 2a**) (Table 1), with those of model compounds of known relative stereochemistry.[13,14]

The comparison suggested that the relative configurations at C-15/C-16 and C-23/C-24 were different, according to the chemical shifts observed for H-15 and H-24 at δ 3.39 or 3.35 (*threo*) for **1** and **2,** respectively, and 3.78 (*erythro*) for both **1** and **2**. The signals of H-15/H-16 (δ 4.86/3.95) and H-23/H-24 (δ 3.95/4.93) in the acetoxy derivative **1a** and of H-15/H-16 (δ 4.86/3.95) and H-23/H-24 (δ 3.96/4.93) in **2a** were consistent with these configurations.[13,14]

In the HOHAHA correlation spectrum of **1** and **2**, magnetization transfers were observed from H-28 or H-29 to H-24 (δ 3.78). The chemical shift of the latter

Table 2. ^{13}C-NMR Data ($CDCl_3$, 50 MHz) of **1**, **2**, and **3**

position	1	2	3
1	174.1	174.1	174.3
2	134.0	134.0	134.0
3	25.0	25.0	25.0
4	24.8−29.3[a]	24.9−29.1[b]	24.5−29.4[c]
5−11	24.8−29.3[a]	24.9−29.1[b]	24.5−29.4[c]
12	24.8−29.3[a]	24.9−29.1[b]	33.0
13	24.8−29.3[a]	24.9−29.1[b]	73.9
14	32.7	32.9	83.2
15	73.9	73.9	24.5−29.4[c]
16	83.0	83.0	24.5−29.4[c]
17	24.8−29.3[a]	24.9−29.1[b]	82.6
18	24.8−29.3[a]	24.9−29.1[b]	82.0
19	82.6	82.6	24.5−29.4[c]
20	82.4	82.4	24.5−29.4[c]
21	24.8−29.3[a]	24.9−29.1[b]	82.7
22	24.8−29.3[a]	24.9−29.1[b]	71.2
23	81.9	81.9	32.0
24	71.6	71.5	25.8
25	32.2	32.0	37.2
26	25.5	25.5	71.5
27	37.0	25.8	37.2
28	71.3	37.1	25.8
29	37.0	71.3	30.5
30	25.5	37.1	31.5
31	24.8−29.3[a]	24.9−29.1[b]	22.6
32	31.7	31.8	14.0
33	22.5	22.6	148.8
34	13.8	14.0	77.2
35	149.1	148.9	19.0
36	77.6	77.6	
37	19.0	19.0	

[a] δ Values 24.8, 25.0, 25.4, 27.1, 28.1, 28.7, 28.9, 29.0, 29.1, 29.3. [b] δ Values 24.9, 25.1, 25.0, 25.4, 25.8, 27.2, 28.2, 28.8, 29.0, 29.1. [c] δ Values 24.5, 24.9, 25.5, 27.2, 28.3, 28.8, 29.0, 29.4.

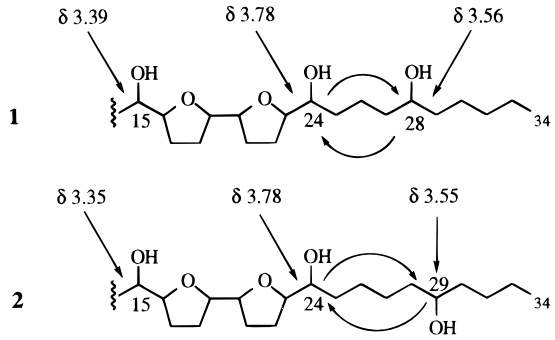

Figure 2. ^{1}H-^{1}H magnetization transfers in the HOHAHA NMR spectra of **1** and **2**.

proton indicated the *erythro* configuration at C-23/C-24. A *threo* configuration at C-15/C-16 was therefore deduced from the δ value of H-15 (δ 3.35 or 3.39) in both **1** and **2** (Figure 2).

To determine the relative configuration between C-24 and C-28, we attempted to prepare the formaldehyde acetal derivative **1b** from **1** according to the procedure already described for acetogenins.[15] Unfortunately, we obtained only minute amounts of the expected compound, so we decided to modify the described procedure, introducing Et_3N as a cosolvent and withdrawing CH_2Cl_2 in the preparation of the reagent obtained from Me_3SiCl and Me_2SO. These small but crucial modifications resulted in a quantitative transformation of **1** into **1b** in the crude reaction mixture. However, the overall yield of pure **1b** was only 49% after HPLC purification, but this represents a significant improvement with regard to the previously reported data. The downfield shifts (δ 3.68 and 3.57) of two hydroxymethine protons

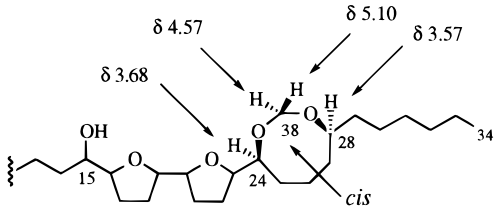

Figure 3. Relative configuration of the C-24 and C-28 OH groups in **1**, according to the ^{1}H NMR data of the acetal (**1b**).

(H-24 and H-28, respectively) and the appearance of two doublets at δ 5.10 and 4.57 (J = 7.5) in the ^{1}H NMR of **1b** confirmed the formation of a cyclic acetal of cis configuration (Figure 3), a trans configuration resulting in a unique multiplet near δ 4.70 as observed for dioxo-1,3-cycloheptanes.[16] The configuration between the two OH-groups at C-24 and C-28 was subsequently determined to be cis.

Using the same methodology, the preparation of the formaldehyde acetal derivative of **2** was attempted. According to the position of the OH-group at C-29, the cyclic acetal **2b** was not obtained, so the relative configuration between C-24 and C-29 remains unknown in **2**.

With the relative configurations at C-15/C-16 and C-23/C-24 already determined, the complete stereochemistry between C-15 and C-24 could be achieved with the help of the homonuclear correlations observed in the 2D NMR spectra (COSY DQF) of **1** and **2** (Figure 4).

From their correlations with H-24 and H-23, the protons at C-21 and C-22 were assigned to signals at δ 1.81−1.89 in **1** and δ 1.82−1.90 in **2**, indicating a cis stereochemistry for the C-20/C-23 THF ring.[17] In the same way, the protons at C-17 and C-18 were assigned to signals at δ 1.52−1.91 in **1** and δ 1.49−1.92 in **2**, diagnostic values for a C-16/C-19 THF ring of trans stereochemistry.[17]

The relative configuration at C-19/C-20 was then determined by the two overlapped NMR signals at δ 3.87 in **1** and δ 3.88 in **1a**, and δ 3.89 in **2** and δ 3.88 in **2a**. These data are in agreement with the *threo* configuration usually observed in the bis-THF acetogenins. If the relative stereochemistry was *erythro*, the two protons should be deshielded and resonate between δ 3.9 and 4.0, as observed for the two known acetogenins with such a configuration, trilobacin and trilobin.[18]

From these results, the relative configuration of carolin A **(1)** and carolin B **(2)** has been determined as *threo/trans/threo/cis/erythro* from C-15 to C-24, with a cis stereochemistry for the 1,5 diol at C-24/C-28 in **1**. Because of the small amount of **1** and **2** available after chemical transformations, the Mosher esters could not be prepared, so the absolute configurations of the carbinolic carbons remain unknown in the vicinity of the THF rings.

Using the same methodology as described above, the presence of an α,β-unsaturated γ-lactone ring, without hydroxy group at C-4, was established for carolin C **(3)** as well as an adjacent bis-THF system and three OH groups. The positions of the substituents in the aliphatic chain were subsequently determined by mass spectrometry.[3,4] The CID spectrum of the $[M + Li]^+$ ion displayed the typical fragmentation pattern of lithiated acetogenins.[12]

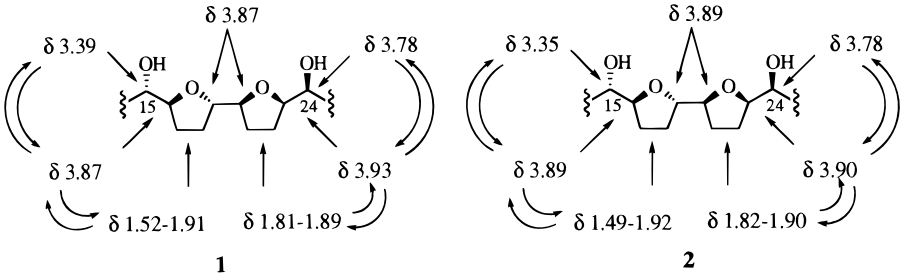

Figure 4. Relative configurations between C-15 and C-24 in **1** and **2**, according to ^{1}H-^{1}H NMR correlations.

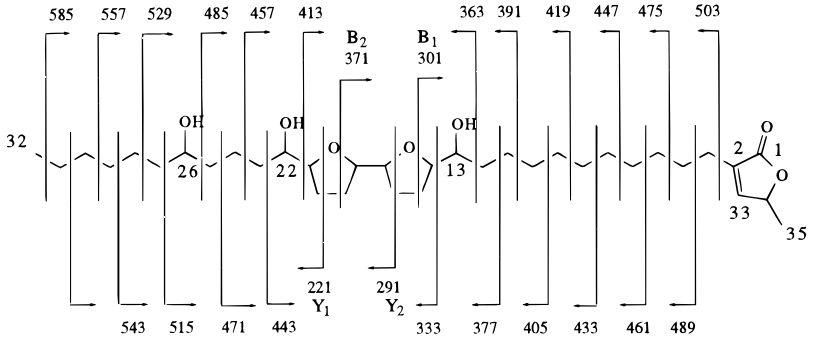

Figure 5. Collision-induced dissociations of the $[M + Li]^+$ ion (*m/z* 601) generated by LSIMS from carolin C **(3)**.

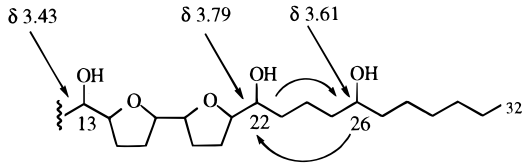

Figure 6. ^{1}H-^{1}H magnetization transfers in the HOHAHA NMR spectrum of **3**.

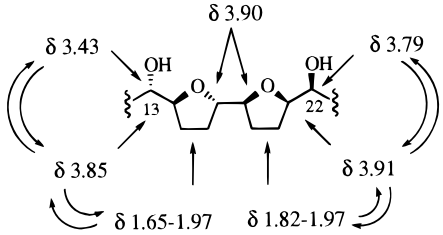

Figure 7. ^{1}H-^{1}H NMR correlations and relative configuration of **3**.

The presence of the four peaks at *m/z* 221 (Y_1), 301 (B_1), 291 (Y_2), and 371 (B_2) was sufficient for the localization of the THF rings in the alkyl chain. The location of the three OH groups at C-13, C-22, and C-26 was further deduced from the charge−remote fragmentations of the whole alkyl chain (Figure 5).

The relative stereochemistry around the THF rings was determined by comparing the ^{1}H- and ^{13}C-NMR signals of **3** and the ^{1}H-NMR data of its triacetate **3a** (Table 1) with those of model compounds of known relative stereochemistry.[13,14]

The comparison suggested that the relative configurations at C-13/C-14 and C-21/C-22 were different, according to the chemical shifts observed for H-13 and H-22 at δ 3.43 (*threo*) and 3.79 (*erythro*). The signals of H-13/H-14 (δ 4.84/3.98) and H-21/H-22 (δ 3.98/4.96) in the acetoxy derivative **3a** were consistent with these configurations.[13,14]

In the HOHAHA correlation spectrum of **3,** magnetization transfers were observed between H-26 (δ 3.61) and H-22 (δ 3.79), such a chemical shift being indicative of an *erythro* configuration at C-21/C-22; a *threo* configuration at C-13/C-14 was subsequently deduced from the δ value (3.43) of H-13 (Figure 6). The determination of the complete stereochemistry between C-13 and C-22 was then achieved with the help of the homonuclear correlations observed in the 2D NMR spectrum (COSY DQF) of **3** (Figure 7).

From their correlations with H-22 (δ 3.79) and H-21 (δ 3.91), the protons at C-19 and C-20 were assigned to signals at δ 1.82−1.97 in **3**, indicating a cis stereochemistry for the C-18/C-21 THF ring.[17] In the same way, the protons at C-15 and C-16 were assigned to signals at δ 1.65−1.97, diagnostic values for a C-14/C-17 THF ring of trans stereochemistry.[17]

The relative configuration at C-17/C-18 was then determined by the two overlapped NMR signals at δ 3.90 in **3** and δ 3.89 in **3a**, in agreement with a *threo* configuration.[18] From these results, the relative configuration of carolin C **(3)** has been determined as *threo/trans/threo/cis/erythro* from C-13 to C-22. The absolute configuration of the C-34 or C-36 chiral centers of **1**, **2**, and **3** has been determined by the simple enzymatic method developped by Duret et al.[19] The results are showing that the three acetogenins have the same 34*S* or 36*S* absolute configuration.

The cytotoxic activity of carolins A, B, and C (**1**−**3**) was investigated and compared with that of squamocin (**4**) and of the recently reported spinencin (**5**).[6] The five acetogenins display a significantly higher activity on cancer cell lines (KB) compared with normal cells (VERO) (Table 3). A modulation of the activity is observed between carolin A (**1**) and carolin B (**2**), correlated with the position of the OH group at C-28 or C-29, and between carolin A (**1**) and carolin C (**3**), in which the lactone ring and bis-THF moiety are separated by a smaller aliphatic chain. The comparison of carolin A (**1**) with squamocin (**4**), which differ only in the stereochemistry of the THF rings, reveals a strong

Table 3. Cytotoxic Activity of Compounds **1**−**5**

compound	KB[a] (ED_{50}, μg/mL)[c]	VERO[b] (ED_{50}, μg/mL)[c]
carolin A (**1**)	10^{-7}	2×10^{-3}
carolin B (**2**)	5×10^{-6}	3.7×10^{-3}
carolin C (**3**)	2×10^{-4}	5×10^{-2}
spinencin (**5**)	1×10^{-5}	6×10^{-3}
squamocin (**4**)	4×10^{-4}	10^{-2}
VLB[d]	10^{-3}	>1

[a] Human nasopharyngeal carcinoma cells. [b] Monkey epithelioid renal cells. [c] ED50 ≤ 10^{-1} μg/mL is considered active with a factor of selectivity of at least 2 $\log_{10}$ between KB and VERO cell lines. [d] Vincaleucoblastine, reference product.

enhancement of the cytotoxic activity when the configuration of one of the THF rings is cis.

Experimental Section

General Experimental Procedures. CIMS were obtained on a Nermag R1010 C spectrometer. UV spectra were recorded on a Philips PU 8700 series UV/vis spectrophotometer and IR spectra on a Perkin-Elmer 257 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 200 and 50 MHz, respectively, on a Bruker AC-200 P spectrometer; and the ^{1}H−^{1}H (COSY-DQF) and ^{1}H−^{13}C (HMQC and HMBC) correlation spectra and ^{1}H−^{1}H HOHAHA at 400 MHz, on a Bruker ARX-400 spectrometer. Optical rotations were determined using a Schmidt−Haensch Polartronic I polarimeter. HPLC was carried out with a Millipore-Waters (Milford, MA) system equipped with a Waters 484 spectrophotometer. MS/MS spectra were obtained from a Zabspec-T five-sector tandem spectrometer (Fisons Instruments, VG organic, Manchester, UK) using the experimental conditions described by Gleye et al.[20]

Plant Material. Seeds of *Annona spinescens* (Annonaceae) were collected in July 1994, along the Paraiba River, João Pessoa, Paraiba, Brazil. Voucher specimens are deposited at the "Prof. Lauro Pires Xavier" herbarium (JPB-no. 18.329) and identified by Prof. Carlos Alberto B. de Miranda of the Department of Natural Sciences, University of Paraiba, Brazil.

Extraction and Separation. The dried and pulverized seeds were macerated with MeOH. The MeOH extract was partitioned between H_2O and hexane to yield the hexane extract (45 g). The aqueous MeOH fraction was concentrated and extracted with CH_2Cl_2 to yield 25 g of extract, 10 g of which were submitted to a fractionation by column chromatography (Si gel 60 M, 230−400 mesh), eluting with a CH_2Cl_2−MeOH (99:1 to 60:40) gradient. Four bis-THF γ-lactone acetogenins were obtained: carolin A (**1**), carolin B (**2**), carolin C (**3**), and squamocin (**4**). HPLC purification, using a μBondapak C18 prepacked columm (10 μm, 25 × 100 mm), eluted with MeOH−H_2O (82:18) (flow rate 9 mL/min, UV detection at 214 nm), afforded **1** (28.6 mg, t_R = 50.5 min), **2** (5.2 mg, t_R = 45.2 min), **3** (5.3 mg, t_R = 28.6 min), and **4** (11.0 mg, t_R = 53.5 min)

Carolin A (1): transparent oil; $[\alpha]^{20}_D$ +22° (*c* 1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 208 (4.6) nm; IR ν_{max} (film) 3675, 3474, 1749, 1652, 1458 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 50 MHz), see Tables 1 and 2; CIMS (NH_4^+) *m/z* 623 $[M + H]^+$; LSIMS/MS of the $[M + Li]^+$ ion, see Figure 1.

Triacetate of Carolin A (1a). **1** (5 mg) was acetylated with Ac_2O + pyridine (1:1), affording **1a** (5.1 mg, 84.8%). For ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Table 1.

24,28-Formaldehyde Acetal Derivative of Carolin A (1b). To Me_3SiCl (1 mL) was added Me_2SO (1 mL), and the mixture was allowed to stand at room temperature for about 1 h until a white precipitate appeared. The excess of unreacted reagents was decanted, and the white precipitate was quickly washed with 1 mL of CH_2Cl_2. A solution of **1** (8 mg) in CH_2Cl_2 (2 mL) and an excess of $(C_2H_5)_3N$ was added to this precipitate and stirred at room temperature for 5 h. When the reaction was complete as estimated by TLC, the mixture was washed using 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL), and the CH_2Cl_2 layer was evaporated *in vacuo*. The crude product was purified by HPLC, using a μBondapak C18 prepacked columm (10 μm, 25 × 100 mm), eluted with MeOH−H_2O (95:5) (flow rate 10 mL/min, UV detection at 214 nm), affording **1b** (4.1 mg, 49%, t_R = 14.0 min): transparent oil; CIMS (NH_4^+), *m/z* 635 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz), see Table 1.

Carolin B (2): transparent oil; $[\alpha]^{20}_D$ + 5° (*c* 0.5, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 208 (3.6) nm; IR ν_{max} (film) 3677, 3575, 1753, 1619 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 50 MHz), see Tables 1 and 2; CIMS (NH_4^+), *m/z* 623 $[M + H]^+$; LSIMS/MS of the $[M + Li]^+$ ion, Series A (from the terminal methyl side) 613, 599, 585, 571, 557, 527, 513, 499, 485, 471, 441, 399 (B_2), 329 (B_1), series B (from the lactone side) 531, 517, 503, 489, 475, 461, 447, 433, 419, 405, 391, 377, 363, 333, 291 (Y_2), 221 (Y_1).

Triacetate of Carolin B (2a). Compound 2 (2 mg) was acetylated with Ac_2O + pyridine (1:1), affording **2a** (1.7 mg, 70.8%). For ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Table 1.

Carolin C (3): transparent oil; $[\alpha]^{20}_D$ + 8° (*c* 0.5, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 205 (4.0) nm; IR ν_{max} (film) 3657, 2479, 1751, 1625 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 50 MHz), see Tables 1 and 2; CIMS (NH_4^+) *m/z* 595 $[M + H]^+$; LSIMS/MS of the $[M + Li]^+$ ion: see Figure 5.

Triacetate of Carolin C (3a). Compound 3 (1.5 mg) was acetylated with Ac_2O + pyridine (1:1), affording **3a** (1.6 mg, 88.8%). For ^{1}H-NMR ($CDCl_3$, 400 MHz) data, see Table 1.

Squamocin (4): transparent oil; $[\alpha]^{20}_D$ + 10° (*c* 1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 209 (4.7) nm; IR ν_{max} (film) 3678, 2994, 1750, 1652 cm^{-1}; CIMS (NH_4^+) *m/z* 623 $[M + H]^+$; for EIMS, ^{1}H- and ^{13}C-NMR data, see Fujimoto et al.[8]

Biological Assays. The cytotoxicity experiments against human nasopharyngeal carcinoma cell lines (KB) and monkey epithelioid renal cells (VERO) were performed according to the methodology already discribed by Fleury et al.[21]

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References and Notes

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(2) At the occasion of Prof. André Cavé's retirement, the authors and all the members of the Pharmacognosy Department, together with those who spent a part of their life working in the laboratory, express their gratitude to Prof. André Cavé for his enduring interest in natural compounds chemistry and for his international leading position in the field of alkaloids and acetogenins from the Annonaceae.

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