Spinencin, a New Bis-tetrahydrofuran Acetogenin from the Seeds of *Annona spinescens*[1]

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A new bis-tetrahydrofuran acetogenin with a C-28/C-29 vicinal diol, spinencin (**1**), was isolated from the MeOH extract of *Annona spinescens*, in addition to the known compounds, almunequin (**2**), bullatanocin (**3**), isodesacetyluvaricin (**4**), squamocin K (**5**), desacetyluvaricin (**6**), and neoannonin (**7**). The structure of **1** was elucidated by spectroscopic methods including electrospray ionization and high-energy MS/MS and confirmed by chemical transformation. The ESI-MS/MS of **2** and **3** were also investigated, and it was found for **1**−**3** that very high levels of radical-cationized species were observed in the charge-remote fragmentation (CRF) processes.

Acetogenins from plants in the Annonaceae are known to exhibit a variety of biological effects, such as antiparasitic, insecticide, cytotoxic, antitumor, and immunosuppressive activities.[2,3] In a continuation of our studies on this family, we have investigated the acetogenins from the seeds of *Annona spinescens* Mart.,[4] a climber attaining 5 m in length that grows along the east coast of Brazil. Alkaloid constituents have been previously reported from this plant.[5]

The present study has led to the isolation and structure elucidation of spinencin (**1**) (Chart 1), a new C_{37} bis-tetrahydrofuran acetogenin, together with the known almunequin (**2**),[6] bullatanocin (**3**),[7] isodesacetyluvaricin (**4**),[8] atemoyin or squamocin K (**5**),[9,10] desacetyluvaricin (**6**),[11] and neoannonin (**7**).[12] All these acetogenins are reported for the first time from this plant. The structures were determined by ^{1}H- and ^{13}C-NMR (COSY, HOHAHA, HMBC, and HMQC) and MS (ESI-MS/MS) on the native compounds and on the acetonide derivative **1d** of spinencin (**1**). Electrospray ionization coupled with low-energy triple-quadrupole MS/MS has recently proved to be useful for detection of acetogenins in crude plant extracts.[13] In the present study, the gas-phase formation of alkali metal adducts on acetogenins under electrospray conditions has been used for generating precursor ions suitable for high-energy collision-induced dissociation MS/MS experiments. In such conditions, structurally informative high-energy processes such as charge-remote fragmentations are expected to occur.[14]

Results and Discussion

Spinencin (**1**) was isolated as a transparent oil from the MeOH extract of the seeds by the usual chromatographic methods followed by preparative HPLC. The electrospray-ionization (ESI) mass spectrum of compound **1** showed only two main ions, corresponding to the cationized species $[M + Li]^+$ and $[M + Na]^+$ (*m*/*z* 645.6 and 661.6, respectively). The molecular weight of 638.6 was also confirmed by the presence of two weak ion peaks appearing at *m*/*z* 1284.2 and 1300.2, which were attributed to the dimeric ion species $[2M + Li]^+$ and $[2M + Na]^+$. Such a molecular weight is in agreement with a C_{37} acetogenin ($C_{37}H_{66}O_8$) containing two tetrahydrofuran rings and four hydroxy groups.[2,3]

A weak UV absorption at 204.6 nm and a strong band at 1751 cm^{-1} in the IR spectrum of **1** indicated the presence of an α,β-unsaturated γ-lactone moiety, characteristic for acetogenins of type 1.[2,3] This structural feature was confirmed by resonances at δ 6.98 (H-35), 4.99 (H-36), 2.26 (H-3), 1.56 (H-4), and 1.37 (H-37) in the ^{1}H-NMR spectrum and resonances at δ 176.4 (C-1), 148.9 (C-35), 134.2 (C-2), 77.4 (C-36), 27.3 (C-4), 25.1 (C-3), and 19.1 (C-37) in the ^{13}C-NMR spectrum (Table 1), also indicating the absence of an OH group at C-4.[2,3] The presence of an adjacent bis-THF system was deduced from the ^{1}H-NMR signals at δ 3.84 (3H) and 3.91 (1H) assigned to four oxymethine protons, in agreement with their ^{13}C-NMR signals at δ 83.3, 82.5, 82.0, and 82.6.[15] Two hydroxymethine groups flanking the bis-THF system were observed at δ 3.36 and 3.82 in the ^{1}H−^{1}H COSY spectrum and at δ 73.9 and 71.4 in the ^{13}C-NMR spectrum of **1** (Table 1). Two further oxymethine protons appeared as an overlapped signal at δ 3.36. Their ^{13}C-NMR signals at δ 74.0 and 74.3 were indicative of a vicinal diol.[16,17]

The positions of the substituents in the aliphatic chain of **1** were subsequently determined by mass spectrometry.[2,3] The high-energy collision-induced dissociation (CID) mass spectrum of the $[M + Li]^+$ ion displayed the typical fragmentation pattern of a lithiated acetogenin.[18] Two pairs of fragment ion peaks at *m*/*z* 237.4/307.4 and *m*/*z* 329.4/399.5 were assigned to fragmentations across two adjacent THF rings (ions Y_1−Y_2 and B_1−B_2, respectively, according to Das and Laprévote),[18] 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 unit and the terminal lactone and for three other hydroxy groups on the methyl-terminal side chain, their locations being deduced from careful scrutiny of the CID mass 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* 645). Among them, the diagnostic fragment ions at *m*/*z* 573, 543, and 513 were indicative of the presence of a vicinal diol at positions C-28/C-29.

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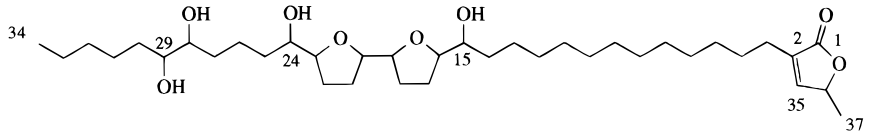
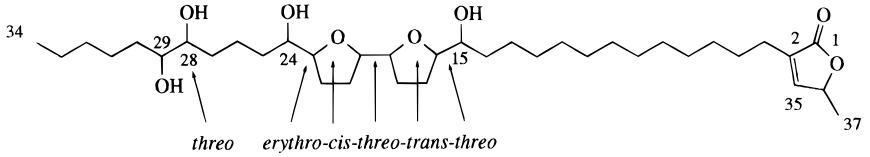
Chart 1



Spinencin (**1**)



Spinencin (**1**)

Table 1. NMR Data ($CDCl_3$, δ) for Spinencin (**1**) and the Derivatives **1a** and **1b**

	^{1}H-NMR (400 MHz, *J*, Hz)			^{13}C-NMR (50 MHz)
position	**1**	**1a**	**1b**	**1**
1				176.4
2				134.2
3	2.26 t (6.5 Hz)	2.26 t (6.5Hz)	2.26 t (6.5 Hz)	25.1
4	1.56 m	1.56 m	1.57 m	27.3
5−13	1.25−1.50 m	1.20−1.60 m	1.20−1.64 m	25.0−29.5[a]
14	1.40 m	1.40−1.53m	1.40 m	32.9
15	3.36 m	4.87 m	3.40 m	73.9
16	3.84 m	3.87 m	3.88 m	83.3
17−18	1.55−1.95 m	1.60−2.00 m	1.64−1.97 m	25.0−29.5[a]
19	3.84 m	3.90 m	3.86 m	82.5
20	3.84 m	3.90 m	3.86 m	82.0
21−22	1.83−1.94 m	1.60−2.00 m	1.83−1.96 m	25.0−29.5[a]
23	3.91 m	3.99 m	3.95 m	82.6
24	3.82 m	4.90 m	3.86 m	71.4
25	1.48 m	1.40−1.53	1.41 m	33.0
26	1.25−1.50 m	1.20−1.60 m	1.20−1.64	25.0−29.5[a]
27	1.48−1.60 m	1.20−1.60 m	1.50 m	25.0−29.5[a]
28	3.36 m	4.87 m	3.58 m	74.0
29	3.36 m	4.87 m	3.58 m	74.3
30	1.48−1.60 m	1.20−1.60 m	1.50 m	25.0−29.5[a]
31	1.25−1.50 m	1.20−1.60 m	1.20−1.64 m	25.0−29.5[a]
32	1.25−1.50 m	1.20−1.60 m	1.20−1.64 m	31.8
33	1.25 m	1.20−1.60 m	1.20−1.64 m	22.5
34	0.88 t (6.8 Hz)	0.86 t (6.8 Hz)	0.88 t (6.8 Hz)	14.0
35	6.98 d (1.4 Hz)	6.98 d (1.4 Hz)	6.98 d (1.5 Hz)	148.9
36	4.99 qd (6.7, 1.7 Hz)	4.99 m	4.98 qd (6.7, 1.7 Hz)	77.4
37	1.40 d (6.7 Hz)	1.40 d (6.7 Hz)	1.40 d (6.8 Hz)	19.1
15-OAc		2.08 s		
24-OAc		2.04 s		
28-OAc		2.08 s		
29-OAc		2.08 s		
$C(CH_3)_2$			1.37 s	

[a] δ values 25.0, 25.3, 26.0, 28.3, 28.7, 29.2, 29.3, 29.5.

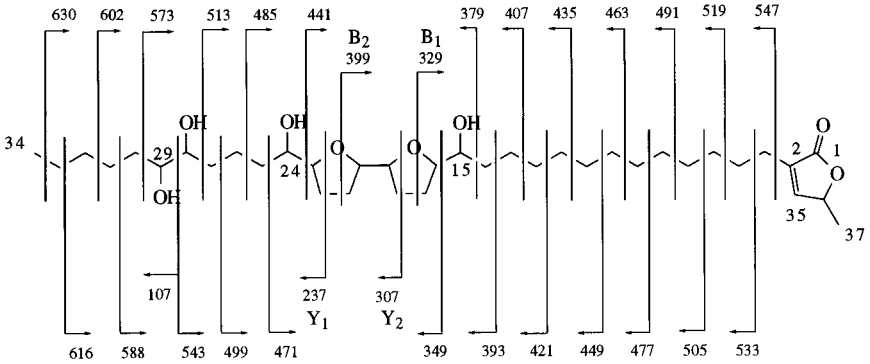


Figure 1. CID MS fragment ions (*m*/*z*) of the $[M + Li]^+$ ion (*m*/*z* 645) generated by ESI from spinencin (**1**).

This assignment was confirmed by the corresponding sodiated species at *m*/*z* 589, 559, and 529 produced by CID from the $[M + Na]^+$ ion species (*m*/*z* 661). The locations of the two remaining OH groups at the C-15 and C-24 positions were determined in a similar manner (Figure 1). The other spectral data were found to be in

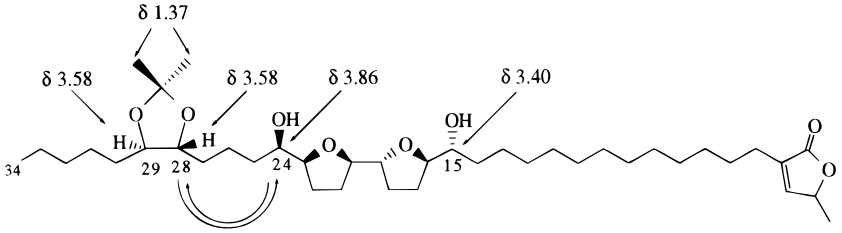


Figure 2. $^1H-^1H$ magnetization transfers in the HOHAHA NMR spectrum of **1b**.

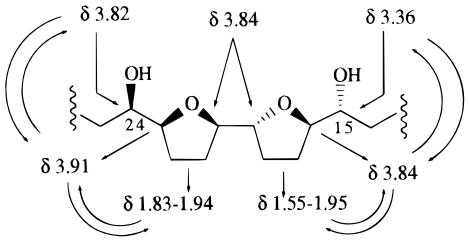


Figure 3. Correlations in the $^1H-^1H$ COSY NMR spectrum of **1**.

agreement with the structural features deduced from ESI-MS/MS.

The relative stereochemistry around the bis-THF rings in spinencin (**1**) was determined by comparing the ^1H- and ^{13}C-NMR signals of **1** and the ^1H-NMR data of its tetracetate (**1a**) (Table 1) with those of model compounds of known relative stereochemistry.[19,20] 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 3.36 (*threo*) and 3.82 (*erythro*) in **1**. The signals of H-15/H-16 (δ 4.87/3.87) and H-23/H-24 (δ 3.99/4.90) in the acetoxy derivative **1a** were consistent with these configurations.[19,20]

In order to determine the relative configuration at C-28/C-29, the acetonide derivative of **1** (**1b**) was prepared. The ^1H-NMR signals for H-28 and H-29 at δ 3.58 in **1b** and the signals for the acetonyl methyl groups, showing only one singlet peak at δ 1.37, suggested the *trans* configuration for the dioxolane ring (Figure 2). The configuration of the diol was subsequently determined to be *threo*.[21] In the HOHAHA correlation spectrum of **1b**, magnetization transfers were observed from H-28 (δ 3.58) to H-24 (δ 3.86), such a chemical shift being indicative of an *erythro* configuration at C-23/C-24.[3] The methine signal at δ 3.40 is therefore located at C-15, indicating a *threo* configuration at C-15/C-16 (Figure 2).

With the relative configurations at C-15/C-16 and C-23/C-24 already in hand, the determination of the complete stereochemistry between C-15 and C-24 was achieved with the help of the homonuclear correlations observed in the 2D NMR spectrum (COSY DQF) of **1** (Figure 3). From their correlations with H-24 (δ 3.82) and H-23 (δ 3.91), the protons at C-21 and C-22 were assigned to signals at δ 1.83−1.94, indicating a *cis* stereochemistry for the C-20/C-23 THF ring.[22] In the same way, the protons at C-17 and C-18 were assigned to signals at δ 1.55−1.95, diagnostic values for a C-16/C-19 THF ring of *trans* stereochemistry.[22] The relative configuration at C-19/C-20 was then determined by the two overlapped NMR signals at δ 3.84 in **1** and δ 3.90 in **1a**. These data are in agreement with the *threo* configuration usually observed in the bis-THF acetogenins. When the relative stereochemistry is *erythro*, the two protons are deshielded and resonate between δ 3.9 and 4.0, as observed for the two known acetogenins with such a configuration, trilobacin and trilobin.[22] From these results, the relative configuration of spinencin (**1**) was determined as *threo*/*trans*/*threo*/*cis*/*erythro* from C-15 to C-24, with a *threo* stereochemistry for the vicinal diol at C-28/C-29. Because of the small amount of **1** available after chemical transformations, Mosher esters could not be prepared, so the absolute configurations of the carbinolic carbons remain unknown.

The ESI mass spectrum of **2** was very similar to that of **1**, even though the sodium adduct at *m*/*z* 661.6 was significantly more abundant than the lithiated species at *m*/*z* 645.6; the same intensity ratio was observed in the case of the dimers $[2M + Li]^+$ and $[2M + Na]^+$. The MS/MS spectrum of the $[\mathbf{2} + Li]^+$ ion (Figure 4) displayed very different fragment ions with regard to the corresponding spectrum of the $[\mathbf{1} + Li]^+$ ion. The absence of fragment ion pairs differing by 70 mass units indicated the nonadjacent relationship of the two THF rings in compound **2**. Comparison of the CID spectrum of $[\mathbf{2} + Li]^+$ with the constant B/E-linked scan spectrum of the $[M + Li]^+$ ions produced by FAB from sylvaticin[18] suggested a structure of the type C,[2,3] in which the two THF units are separated by a four-carbon chain. The presence of the four peaks at *m*/*z* 221 (Y_1), 257 (B_1), 379 (Y_2), and 415 (B_2)[18] was thus sufficient for the localization of the tetrahydrofuran rings on the alkyl

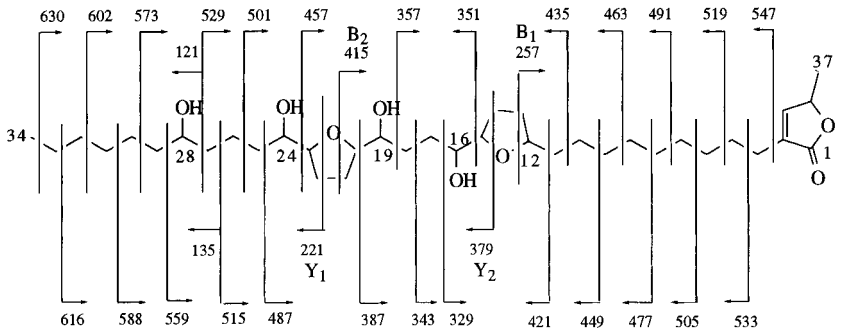


Figure 4. MS/MS fragment ions (*m*/*z*) of the $[M + Li]^+$ ion (*m*/*z* 645) of **2** generated by ESI.

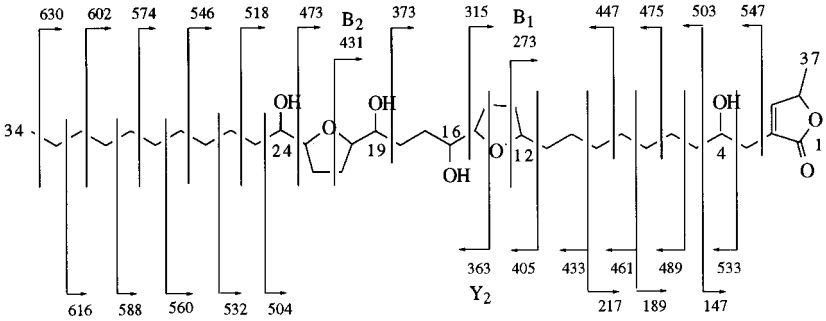


Figure 5. MS/MS fragment ions (m/z) of the $[M + Li]^+$ ion (m/z 645) of **3** generated by ESI.

Table 2. Relative Abundances of B and Y Ions in the ESI-MS/MS Spectra According to Structural Factors

compd	$B_1 + B_2$ (%)	$Y_1 + Y_2$ (%)
1 (type B, subtype 1a)[a]	44.5	55.5
2 (type C, subtype 1a)[b]	40.7	59.3
3 (type C, subtype 1b)[c]	65	35

[a] Adjacent bis-THF structure; no OH-group at C-4. [b] Nonadjacent bis-THF structure; no OH-group at C-4. [c] Nonadjacent bis-THF structure; OH-group at C-4.

chain (Figure 4). The location of four OH groups at C-16, C-19, C-24, and C-28 was further deduced from the charge-remote fragmentations of the whole alkyl chain. The ^{1}H- and ^{13}C-NMR data of **2** were similar to those of almunequin,[6] indicating the same relative configurations.

The ESI mass spectrum of **3** indicated, owing to the presence of both lithiated and sodiated species, a molecular weight of 638.6 Da, identical to that of compounds **1** and **2**. The MS/MS spectrum of the $[M + Li]^+$ ion of **3** was very simple, showing B and Y ion peaks identical to those previously observed in the case of sylvaticin[18] or bullatalicinone,[18] i.e., ions B_1, B_2, and Y_2 at m/z 273, 431, and 363, respectively, and absence of Y_1 (Figure 5). A complete series of charge-remote fragmentations along the alkyl side chain confirmed the nonadjacent bis-THF structure of compound **3**, including the location of the tetrahydrofuran rings and of three hydroxy groups at positions C-16, C-19, and C-24. The location of the fourth hydroxy group at C-4 was suggested by the loss of 112 and 142 amu from the lithiated precursor ions (m/z 533.7 and 503.7) and by the absence of the ion peaks at m/z 519.7 and 505.7 that should appear, as in compound **2**, in the absence of a substitution at C-4. The ^{1}H- and ^{13}C-NMR spectra of **3** were identical with those of bullatanocin,[7] indicating the same relative configurations around the THF rings.

The location of an alkali metal cation on the alkyl chain seems to be sensitive to the hydroxylation site with the relative abundances of B- and Y-type ions being likely influenced by this structural factor (Table 2). Thus, the presence of an OH group at C-4 in compound **3** resulted in a significantly higher abundance of B ions compared with the intensity of Y ions. By changing the hydroxylation site from C-4 to C-28 (compound **2**), the abundance ratio was reversed, in favor of fragments Y_1 and Y_2, containing the terminal methyl group. A similar effect was observed in the case of compound **1** (adjacent bis-THF moiety, type B), with the higher intensity of Y-type ions being accompanied by the presence of hydroxy groups at positions C-28 and C-29. On the other hand, the difference in the bis-THF arrangement between **1** (type B) and **2** (type C) did not result in a significant modification of the B/Y ratio. All these results suggest that the hydroxy groups are involved, at least partially, in cation coordination.

A second and unexpected result is related to the presence in very high amounts of radical-cationized species arising from the charge-remote fragmentation (CRF) process in the ESI mass spectra of compounds **1**−**3**. Besides the well-known [1,4]-rearrangement initially proposed by Gross and co-workers,[23,24] other fragmentation mechanisms involving radical intermediates have been published.[25,26] Among them, homolytic cleavage of the C−C bonds has been suggested as the first step of the CRF process, followed rapidly by losses of the H radical, thus leading to the even-electron species possessing the well-established terminal olefinic structure.[25] An alternative mechanism was recently published on the basis of isotope labeling,[26] in which the loss of hydrogen was presented as the initial fragmentation step, a β-cleavage leading consecutively to the even-electron fragment ions. The MS/MS spectra obtained in the case of lithium or sodium complexes of compounds **1**−**3** produced by electrospray displayed a significantly larger amount of odd-electron species. This effect, which was recently observed in the case of sulfated and sulfonated lipids,[27] is particularly surprising and reinforces the hypothesis of Wysocki and Ross,[25] involving an initial homolytic cleavage of C−C bonds during the dissociation pathways. The spectacular decrease of even-electron species in the case of ESI-generated precursor ions could be related to the thermal cooling in the ion source leading to vibrationally stabilized ions. Such an effect is expected to allow an initial energy deposition on the precursor ions significantly lower than in the case of FAB desorption. Although the loss of H radicals from odd-electron ions could be considered as a low-energy process, the excess of internal energy of the radical ions issued from the first homolytic cleavage step could be insufficient to induce a second fragmentation step. The comparison of the initial internal energy deposition and its effect on the high-energy CID processes under FAB or ESI ionization conditions is under further investigation in one of our laboratories.[28]

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. ^{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) and ^{1}H−^{1}H HOHAHA correlation spectra 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. A Zabspec/T five-sector tandem mass spectrometer was used in all experiments (Fisons Instruments, Micromass, Manchester, U.K.). ESI was performed by using an atmospheric pressure ion source fitted with a hexapolar ion guide. The samples were dissolved in methanol at a concentration of approximately 10^{-5} M. The solution was infused continuously in the ion source at a flow rate of 10 μL/min using a syringe pump (Model 11, Harvard Apparatus, South Natick, MA). The electrosprayed ions were introduced in the mass analyzer at 4 keV kinetic energy. Helium was used as collision gas for the MS/MS experiments (70% attenuation of the precursor ion beam), and the collision energy was 2 keV. MS/MS acquisitions were achieved by successive overlapping exposures of a multichannel array detector.[29]

Plant Material. Seeds of *A. 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 the plant was identified by Prof. Carlos Alberto B. de Miranda of the Department of Sciences of Nature, University of Paraiba, Brazil.

Extraction and Isolation. The dried and pulverized seeds were macerated with MeOH. The MeOH extract was partitioned between H_2O and hexane to yield a hexane extract (45 g). The aqueous MeOH fraction was concentrated and extracted with CH_2Cl_2 to yield 25 g of extract, of which 10 g was submitted to fractionation by column chromatography (Si gel 60 M, 230−400 mesh), eluting with a CH_2Cl_2−MeOH (99:1 to 60:40) gradient. Seven bis-THF γ-lactone acetogenins were obtained: spinencin (**1**), almunequin (**2**), bullatanocin (**3**), isodesacetyluvaricin (**4**), atemoyin (**5**), desacetyluvaricin (**6**), and neoannonin (**7**). HPLC purification, using a μBondapak C_{18} prepacked columm (10 μm, 25 × 100 mm), eluted with MeOH−H_2O (83:17) (flow rate 7 mL/min, UV detection at 214 nm), afforded **1** (10.1 mg, t_R = 24 min), **2** (6.0 mg, t_R = 31 min), and **3** (20.6 mg, t_R = 19 min). Elution with MeOH−H_2O (88:12) (flow rate 9 mL/min, UV detection at 214 nm) afforded **4** (7.5 mg, t_R = 50 min), **5** (5.5 mg, t_R = 28 min), **6** (5.0 mg, t_R = 36 min), and **7** (3.0 mg, t_R = 25 min).

Spinencin (1): transparent oil; $[\alpha]^{20}_D$ +24° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 204.6 (3.86) nm; IR ν_{max} (film) 3785, 3677, 2995, 2860, 1751 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 50 MHz), see Table 1; CIMS (NH_4^+) *m/z* 639 $[M + H]^+$; ESI-MS/MS of the $[M + Li]^+$ ion, see Figure 3; ESI-MS/MS of the $[M + Na]^+$ ion (*m/z* 661): lactone-containing fragment ions at *m/z* 646, 632, 618, 604, 589, 560, 529, 515, 501, 487, 457, 415 (B_2), 345 (B_1); terminal methyl-containing fragment ions at *m/z* 563, 549, 535, 521, 507, 493, 479, 465, 451, 437, 423, 409, 395, 365, 323 (Y_2), 253 (Y_1), 123.

Tetraacetate of Spinencin (1a). Compound **1** (4 mg) was acetylated with Ac_2O/pyridine (1:1), affording **1a** (4.0 mg, 79.2%): ^{1}H-NMR ($CDCl_3$, 400 MHz), see Table 1.

28,29-Acetonide of Spinencin (1b). To **1** (5 mg) dissolved in C_6H_6 (1 mL) were added 2,2-dimethoxypropane (10 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 4 h at room temperature and then extracted with CH_2Cl_2 to give **1b** (4.7 mg, 88%): ^{1}H-NMR ($CDCl_3$, 400 MHz) see Table 1.

Almunequin (2): white wax; $[\alpha]^{20}_D$ +18° (*c* 0.06, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 204.5 (4.6) nm; IR ν_{max} (film) 3867, 3677, 2995, 2859, 1751 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 50 MHz), see Cortes *et al.*;[6] CIMS (NH_4^+) *m/z* 639 $[M + H]^+$; ESI-MS/MS of the $[M + Li]^+$ ion, see Figure 4; ESI-MS/MS of the $[M + Na]^+$ ion: lactone-containing fragment ions at *m/z* 646, 632, 618, 604, 590, 576, 546, 531, 517, 503, 473, 431 (B_2), 403, 373, 359, 345, 315, 273 (B_1); terminal methyl-containing fragment ions at *m/z* 563, 549, 535, 521, 507, 493, 479, 465, 451, 437, 367, 395 (Y_2), 237 (Y_1), 151.

Bullatanocin (3): white wax; $[\alpha]^{20}_D$ +15° (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 209.3 (4.0) nm; IR ν_{max} (film) 3677, 2995, 2865, 1751 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 50 MHz), see Gu *et al.*;[7] CIMS (NH_4^+) *m/z* 639 $[M + H]^+$; ESI-MS/MS of the $[M + Li]^+$ ion, see Figure 5; ESI-MS/MS of the $[M + Na]^+$ ion: lactone-containing fragment ions at *m/z* 646, 632, 618, 604, 590, 576, 562, 548, 534, 520, 490, 447 (B_2), 389, 331, 289 (B_1); terminal methyl-containing fragment ions at *m/z* 563, 549, 519, 505, 491, 477, 463, 449, 435, 421, 379 (Y_2).

Isodesacetyluvaricin (4): transparent oil; $[\alpha]^{20}_D$ +24° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 203.2 (4.0) nm; IR ν_{max} (film) 3589, 3097, 2937, 1748, 1592 cm^{-1}; CIMS (NH_4^+) *m/z* 607 $[M + H]^+$; for EIMS and ^{1}H- and ^{13}C-NMR data, see Hisham *et al.*[8]

Squamocin K (5): transparent oil; $[\alpha]^{20}_D$ +28° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 203.8 (4.1) nm; IR ν_{max} (film) 3592, 3110, 2859, 1748, 1593 cm^{-1}; CIMS (NH_4^+) *m/z* 579 $[M + H]^+$; for EIMS and ^{1}H- and ^{13}C-NMR data, see Duret *et al.*[9]

Desacetyluvaricin (6): transparent oil; $[\alpha]^{20}_D$ +21° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 213.7 (3.4) nm; IR ν_{max} (film) 3677, 3099, 2934, 1744, 1591 cm^{-1}; CIMS (NH_4^+) *m/z* 607 $[M + H]^+$; for EIMS and ^{1}H- and ^{13}C-NMR data, see Jolad *et al.*[11]

Neoannonin (7): transparent oil; $[\alpha]^{20}_D$ +20° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211.1 (3.1) nm; IR ν_{max} (film) 3650, 3576, 2936, 1747, 1493 cm^{-1}; CIMS (NH_4^+) *m/z* 579 $[M + H]^+$; for EIMS and ^{1}H- and ^{13}C-NMR data, see Kawazu *et al.*[12]

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