Chamuvarinin, an Acetogenin Bearing a Tetrahydropyran Ring from the Roots of *Uvaria chamae*[1]

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A new cytotoxic acetogenin, chamuvarinin (**1**), containing a tetrahydropyran ring with an adjacent bis-tetrahydrofuran ring, which corresponds to a novel carbon skeleton in this series, was isolated from the roots of *Uvaria chamae*, together with the previously reported acetogenins squamocin (**2**), desacetyluvaricin (**3**), and neoannonin (**4**). The structure determination of chamuvarinin (**1**) was based on extensive NMR studies and high-resolution mass spectral measurements. This new compound shows significant cytotoxicity toward the KB 3-1 cell line ($IC_{50} = 8 \times 10^{-10}$ M). In addition, a biosynthetic relationship between **1** and **2** is briefly discussed.

Annonaceous acetogenins are a relatively new class of natural polyketides that have promising anticancer, antiparasitic, and pesticidal properties.[2] Structurally, most of these long-chain fatty acid derivatives may be classified into three major groups: mono-tetrahydrofuran (THF), adjacent bis-THF, and nonadjacent bis-THF classes. Among the 400 or so annonaceous acetogenins previously reported, only seven possessed a tetrahydropyran (THP) ring. The THP ring of these molecules is linked in a nonadjacent manner to the mono-THF ring. These compounds have been recently isolated from *Rollinia mucosa*,[3−5] *Goniothalamus giganteus*,[6] and *Annona montana*.[7]

A cyclohexane extract of the roots of *Uvaria chamae* P. Beauv. (Annonaceae) was cytotoxic in vitro against human tumor cell lines in our preliminary screening tests. From this plant, we previously isolated mono-THF annonaceous acetogenins, namely, *cis*-bullatencin and seven known acetogenins (bullatencin, annotemoyin-1, uvariamicin-I, -II, and -III, *cis*-reticulatacin, and *cis*-uvariamicin-I).[8] A close study of the cyclohexane extract of *U. chamae* led to the isolation of chamuvarinin (**1**), which is the first acetogenin with a THP ring adjacent to a bis-THF ring, in addition to three known compounds (squamocin (**2**), desacetyluvaricin (**3**), and neoannonin (**4**)). The cytotoxic activity of this compound was evaluated on the KB 3-1 cell line.

The cyclohexane extract of *U. chamae* roots was subjected to repeated column chromatography and preparative HPLC to yield four compounds; **1** was found to be new, and it was given the trivial name chamuvarinin (Figure 1).

Compound **1** has a molecular mass 604, determined by HRESIMS $[M + Na]^+$ at *m/z* 627.460160 (calc 627.460059), corresponding to the molecular formula $C_{37}H_{64}O_6Na$.

The presence of an α,β-unsaturated γ-methyl-γ-lactone moiety, the common feature of acetogenins of type 1a, was suggested by a strong IR absorption peak (carbonyl) at 1756 cm^{-1} and a weak UV λ_{max} at 219.1 nm. The ^1H NMR spectrum indicated seven protons at δ 6.98 (CH-35), 4.98 (CH-36), 2.26 (CH_2-3), and 1.42 (CH_3-37), and the ^{13}C NMR spectrum showed six carbon at δ 173.8 (C-1), 148.8 (C-35), 134.3 (C-2), 77.4 (C-36), 25.1 (C-3), and 19.2 (C-37), corresponding to the spectroscopic features of the lactone ring (Table 1).

The ^1H NMR spectrum of compound **1** exhibited six well-defined signals in the range δ_H 3.0−4.0. Those at δ 3.37 (H-15), 3.82 (H-16), 3.88 (H-23), and 3.93 (H-19, H-20) were assigned to a bis-THF ring with a flanking hydroxyl group.[2] The remaining signals at δ 3.23 (H-28) and 3.28 (H-24) were consistent with the presence of a THP ring in the molecule.[3−7] The HOHAHA correlation found between

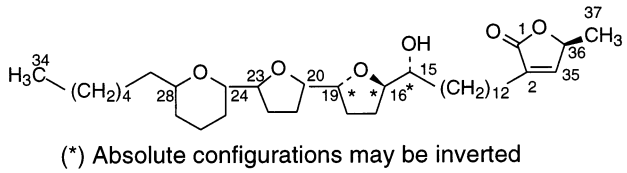

Figure 1. Chamuvarinin.

Table 1. NMR Spectroscopic Data of Chamuvarinin (**1**) in $CDCl_3$[a]

position	^1H	^{13}C
1		173.8
2		134.3
3	2.26 t (7.4)	25.1
4	1.55 m	26.9
5−13	1.25−1.29	25.5−32.4
14	1.38 m	25.7
15	3.37 m	74.1
16	3.82 m	83.0
17a, 18a	1.65 m	25.5−32.4
17b, 18b	1.97 m	25.5−32.4
19	3.93 m	81.4*
20	3.93 m	81.9*
21a, 22a	1.71 m	25.5−32.4
21b, 22b	1.92 m	25.5−32.4
23	3.88 m	82.0
24	3.28 m	79.9
25a	1.26 m	25.5−32.4
25b	1.48 m	25.5−32.4
26	1.82 m	23.4
27a	1.12 m	25.5−32.4
27b	1.33 m	25.5−32.4
28	3.23 m	77.8
29	1.52 m	25.5−32.4
30, 31	1.25−1.29	25.5−32.4
32	1.25 m	31.8
33	1.25 m	22.6
34	0.87 t (7.3)	14.1
35	6.98 d (1.5)	148.8
36	4.98 dq (6.7; 1.5)	77.4
37	1.42 d (6.7)	19.2

[a] Chemical shifts (δ) are in ppm relative to TMS, observed splittings *J* are in Hz, superscript * corresponds to interchangeable attributions.

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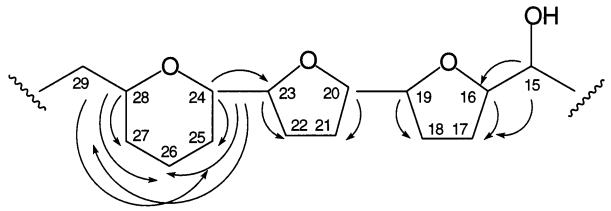

Figure 2. Structural subunit, assigned on the basis of $^1H-^1H$ HOHAHA NMR.

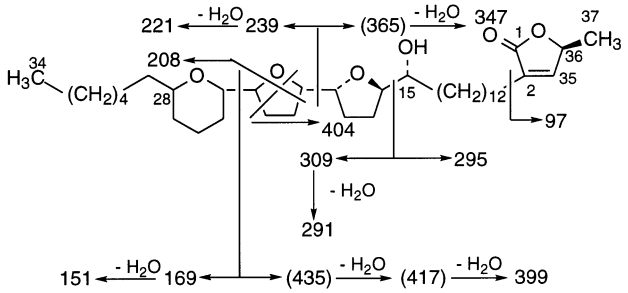

Figure 3. EIMS fragmentations of chamuvarinin (**1**).

H-28/H-27$_a$, H-28/H-27$_b$, H-28/H-26, H-28/H-25$_a$, H-28/H-25$_b$ and H-24/H-25$_a$, H-24/H-25$_b$, H-24/H-26, H-24/H-27$_a$, H-24/H-27$_b$, H-24/H-23 confirmed the presence of a THP moiety adjacent to the bis-THF ring (Figure 2).

The disposition of the adjacent THF-THP unit on the aliphatic chain was determined by the analysis of the fragmentation pattern displayed by **1** in EIMS (Figure 3). Thus, the fragment ion peaks at *m/z* 291, 295, 309 (cleavage at C-15/C-16), *m/z* 221, 239, 347 (cleavage at C-19/C-20), and *m/z* 169, 399 (cleavage at C-23/C-24) permitted location of the THF ring with a flanking hydroxyl between C-15 and C-23 and of the THP ring between C-24 and C-28.

The relative configuration at C-15/C-16 was assigned according to Born's rule.[12] Thus, the chemical shift values of both C-15 (δ 74.1) and H-15 (δ 3.37) indicated a *threo* relationship. On the other hand, the relative *trans* configuration of the chiral carbon centers of the first bis-THF moiety is demonstrated by a relatively large δ difference between the C-17 or C-18 methylene protons.[13] The absolute configuration of the C-36 stereocenter was established as *S* by Latypov's method using Pirkle reagent.[1]

Compounds **2**−**4** showed the characteristic NMR signals of bis-THF α,α′-dihydroxy acetogenins[2] and were identified by comparison of the physicochemical data of the previously isolated acetogenins squamocin[9] (**2**), desacetyluvaricin[10] (**3**), and neoannonin[11] (**4**, a C_{35} acetogenin with the configuration of desacetyluvaricin).

Chamuvarinin could biogenetically be derived from squamocin via desacetyluvaricin (**3**). The trisepoxidation of a triene such as $\Delta^{15,19,23}$-chatenaytrienin-4, previously isolated from roots of *Annona nutans*,[14] leads to tripoxyrollin, isolated from the seeds of *Rollinia membranacea*.[15] This triepoxide in turn converts to the bis-THF acetogenin desacetyluvaricin (**3**), which is presumably C-28 hydroxylated to squamocin (**2**). Therefore, a regioselective intramolecular nucleophilic substitution may lead to the THP ring of chamuvarinin (**1**).

Cytotoxic activity of chamuvarinin (**1**) was evaluated relative to squamocin (**2**) on KB 3-1 cells. With an IC_{50} of 8×10^{-10} M, chamuvarinin (**1**) appears to be less cytotoxic than squamocin (**2**) ($IC_{50} = 3 \times 10^{-12}$ M).

These results may rely on an insufficient amphiphilicity of chamuvarinin (**1**), in accordance with numerous examples.[15−17] On the other hand, it has to be noted that the C-24/C-28 absolute configurations of the THF nucleus of chamuvarinin remain unknown and that strong differences of cytotoxicities are frequently observed for diastereoisomeric acetogenins.[18−23]

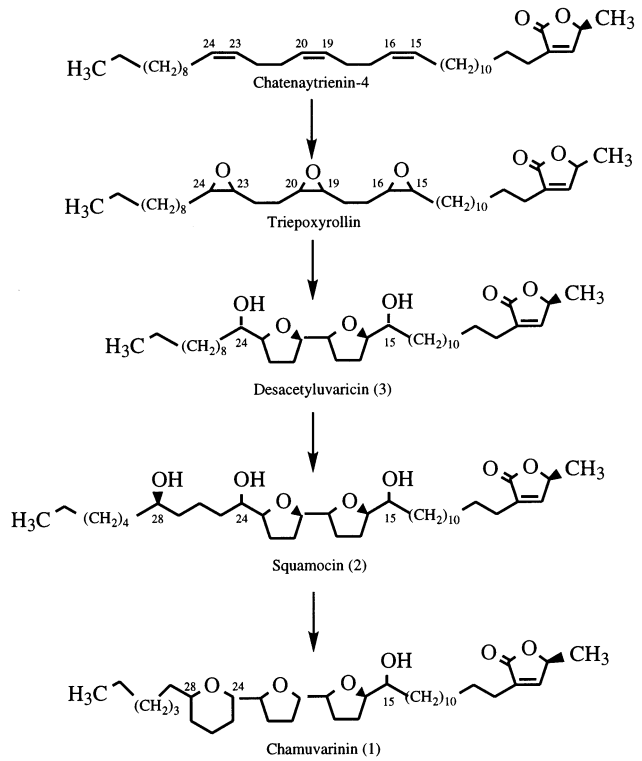

Figure 4. Biogenetic pathway proposed for chamuvarinin (**1**). (*) Absolute configurations may be inverted.

Experimental Section

General Experimental Procedures. UV spectra were determined in MeOH on a Philips PU 8720 spectrophotometer. IR spectra were recorded on a Bruker Vector 22 spectrophotometer. The ^1H NMR spectra were obtained with a Bruker AC-400 (at 400 MHz) and AC-200 (at 200 MHz). The ^{13}C NMR spectra were obtained with a Bruker AC-200 at 50 MHz. EIMS (70 eV) were recorded with an Automass multi spectrometer R10-10C, and HRESIMS were registered with a navigator spectrometer (Thermofinnigan, France). HPLC was performed with a pump (Waters 590), UV detector (Waters 84), and injector (Waters SSV).

Plant Material. Roots of *Uvaria chamae* P. Beauv. were collected in Casamance (Senegal) in August 1999. A voucher specimen (DF126) has been deposited at the Faculty of Medicine and Pharmacy of Dakar.

Extraction and Isolation. The dried and powdered roots (1.7 kg) were extracted by percolation (10 L of cyclohexane) during 48 h and evaporated to give a brown extract (66 g). Thirty grams of this extract was subjected to silica gel column chromatography (silica gel Merck 60 70−230 mesh) eluted with *n*-hexane/EtOAc in a gradient from 90:10 to 10:90. The eluate was collected in 16 fractions controlled by TLC (silica gel Merck 60 F 254). The solvent of the fraction 7 was evaporated under reduced pressure. The resulting residue (2.1 g) was subjected to silica gel column chromatography (silica gel Merck 60 H 230−400 mesh) eluted with *n*-hexane/CH_2Cl_2/EtOH, 70:30:2 v/v/v. The fraction corresponding to **1** was chromatographed on the same stationnary phase, eluted with *n*-hexane/CH_2Cl_2/EtOH, 70:30:1 v/v/v. A final purification by preparative HPLC using a reversed-phase Waters μBondapak C_{18} column (10 μm [250 × 20 mm] cartridge column, flow rate 9 mL/mn, 20 mg/injection, and eluant CH_3OH/H_2O/THF, 90:10:5 v/v/v) led to 14 mg of chamuvarinin (**1**) (t_R = 22.4 min), 73 mg of squamocin (**2**) (t_R = 38.0 min), 40 mg of desacetyluvaricin (**3**) (t_R = 31.2 min), and 12 mg of neoannonin (**4**) (t_R = 21.6 min).

Chamuvarinin (1): oil (14 mg); $[\alpha]_D$ +27.0° (*c* 0.026, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 219.1 (3.46) nm; IR ν_{max} 3474, 2924, 2854, 1756, 1464, 1373, 1318, 1199, 1074, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz), see Table 1; HRESIMS *m*/*z* 627.460160 (calc 627.460059 for $C_{37}H_{64}O_6Na$); EIMS, see Figure 3.

Determination of the Absolute C-36 Configuration. About 1 mg of acetogenin is dissolved in 1 mL of $CDCl_3$ and divided exactly in two tubes containing separately 15 equiv of the *R* and *S* alcohol of the Pirkle reagent. The ^1H NMR (400 MHz) is performed at low temperature (213−223 K). The difference of the resonance of H-36 ($\delta_R - \delta_S$) between the two complexes formed gives the absolute configuration of C-36. We assign the *S* absolute configuration when ($\delta_R - \delta_S$) > 0 and *R* when ($\delta_R - \delta_S$) < 0. With δ_R 5.021 and δ_S 4.955, the stereocenter was assigned as *S*.

Cytotoxic Assay. Cytotoxicities were colorimetrically evaluated through a 96-well plate assay after 72 h cell exposure to the acetogenins on the KB 3-1 cell line by the method of Fleury et al.[24]

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