Coriadienin, the First Annonaceous Acetogenin with Two Double Bonds Isolated from *Annona coriaceae*[1]

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A new Annonaceous acetogenin, coriadienin (**1**), has been isolated from the roots of *Annona coriacea* Mart. (Annonaceae). This structure is the first reported acetogenin containing two double bonds. Compound **1** showed potent cytotoxicity against VERO and KB cell lines. Compound **1** appears to play an important role in the biosynthetic pathway of mono- and bis-THF acetogenins, and it is proposed as a biogenetic precursor of coriacin. A known cytotoxic acetogenin, gigantetronenin, was also isolated from this plant.

In addition to the well-known mono- or bis-tetrahydrofuran (mono- or bis-THF) acetogenins of Annonaceae, bullatencin[2] and giganenin[3] were the first examples of acetogenins bearing a double bond, followed by about 12 orthers.[4] Isolated the same year was the first acetogenin bearing epoxy groups in place of the THF moiety, diepomuricanin,[5] considered to be a biogenetic precursor of the mono-THF acetogenin solamin. Acetogenins bearing an epoxy group and a double bond were further isolated,[6] but the hypothetic acetogenins with two double bonds had not been reported until now. In this paper we report the structure elucidation of coriadienin **1**, a new acetogenin lacking THF rings but bearing two double bonds and a diol system in the aliphatic chain, in addition to the known acetogenin gigantetronenin.[7] The isolation of **1** provides further evidence to support the polyketide biogenetic pathway.[8] In addition, **1** is proposed as the most likely precursor of coriacin, the major new acetogenin recently isolated from this plant.[9] The cytotoxic activity of coriadienin, coriadienin bis-epoxide, coriacin, 4-deoxycoriacin, and gigantetronenin is also reported (Table 1).

Coriadienin (**1**) was isolated from the roots of *Annona coriacea* Mart. (Annonaceae) as a white waxy solid by preparative HPLC. Its molecular formula, $C_{37}H_{66}O_6$, was deduced from the exact mass measurement (*m*/*z* 607.4895) of the $[MH]^+$ ion in the HRCIMS.

The existence in **1** of a γ-methyl α,β-unsaturated γ-lactone was suggested by an IR carbonyl absorption at 1750 cm^{-1}, a UV λ max at 203 nm (log ϵ 4.08), six resonances at δ 7.18 (H-35), 5.04 (H-36), 2.38 and 2.51 (H-3a,3b), 3.83 (H-4), and 1.42 (H-37) in the ^{1}H-NMR spectrum, and six peaks at δ 174.6 (C-1), 151.8 (C-35), 131.1 (C-2), 77.9 (C-36), 69.9 (C-4), and 19.1 (C-37) in the ^{13}C-NMR spectrum. These are all characteristic spectral features for the γ-methyl α,β-unsaturated γ-lactone fragment with a 4-OH group in the Annonaceous acetogenins.[10]

The existence of four OH groups in **1** was evidenced by an IR absorption at 3468 cm^{-1} and resonances due to oxygen-bearing carbons at δ 69.9 (C-4), 71.5, 74.0, and 74.5, correlated with proton signals at δ 3.83 (H-4), 3.58, 3.41, and 3.39, respectively.

Table 1. Bioactivities of **1**, **1b**, Coriacin, 4-Deoxycoriacin, and Gigantetronenin

compound	KB[a] ED_{50} (μg/mL)	VERO[b] ED_{50} (μg/mL)
coriadienin **1**	1.9×10^{-6}	1.5×10^{-1}
bisepoxycoriadienin **1b**	2.5×10^{-7}	1.3×10^{-2}
coriacin	$<3 \times 10^{-7}$	5.7×10^{-3}
4-deoxycoriacin	6.0×10^{-4}	5.0×10^{-2}
gigantetronenin	$<3 \times 10^{-7}$	5.7×10^{-3}
paclitaxel[c]	2.0×10^{-2}	>0.3

[a] Human nasopharyngeal carcinoma cells. [b] Monkey epitheloid renal cells. [c] Reference product.

The presence of two isolated double bonds in **1** was suggested by two IR absorptions at 3009 and 1600 cm^{-1}, a four-proton multiplet at δ 5.43−5.35, and four carbon resonances at δ 129.9, 129.8, 129.7, and 129.6 in the ^{1}H- and ^{13}C-NMR spectra.

The existence of two vicinal OH groups was confirmed by the preparation[11,12] of the acetonide derivative **1a**, which showed a protonated molecular ion at *m*/*z* 647 in the CIMS (NH_4^+). The ^{1}H-NMR signals for H-21 and H-22 of **1a** at δ 3.58 and the signals for the acetonyl methyl groups, showing a singlet peak at δ 1.38, suggest a trans stereochemistry for the dioxolane ring.[11] Thus, the configuration of the diol was determined as threo, since the trans configuration of C-21/22 could be derived only from a vicinal diol with a threo configuration.

The location of the hydroxyl groups was established by collision-induced dissociation (CID) of the $[M + Li]^+$ ion of **1**, generated by FABMS and observed by linked scanning at constant B/E[13] (Figure 1). Fragments at *m*/*z* 443, 413, and 501 clearly indicated the position of three hydroxy groups at C-22, C-21, and C-4 along the hydrocarbon chain.

The presence of two double bonds in **1** was confirmed by their oxidation with *m*-CPBA, affording the bis-epoxy derivative **1b**. Its formation was indicated by a $[MH]^+$ ion peak at *m*/*z* 639 in the CIMS (NH_4^+) spectrum. The position of the double bonds at C-13/14 and C-17/18 and the location of the fourth OH group at C-10 were determined by observation of the EIMS fragmentations of **1b** (Figure 2). The cis configurations of the two double bonds of **1** were indicated by measurement of the vicinal coupling constants (^3J = 10.5 and 9.4 Hz, respectively) between the ethylenic protons after selective irradiation of the allylic methylene groups in the ^{1}H NMR. The position of the two double bonds at C-13/

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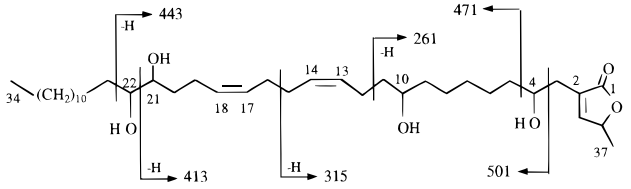


Figure 1. B/E linked scan spectrum of the $[M + Li]^+$ ion (*m/z* 613) generated by FAB from coriadienin **1**; FAB matrix: *m*-NBA + LiCl; all fragments retained the lithium cation.

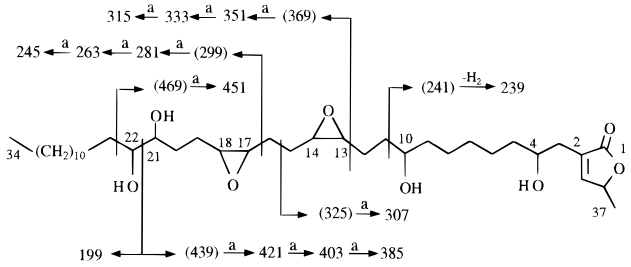


Figure 2. Diagnostic EIMS fragment ions of **1b**. Peaks in parentheses were not observed. a: loss of H_2O.

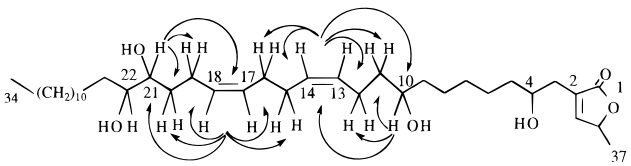
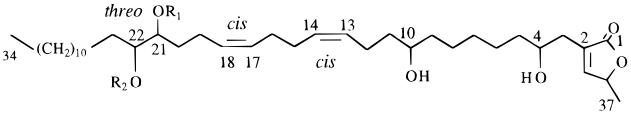


Figure 3. ^1H-^1H magnetization transfers in the HOHAHA NMR spectrum of coriadienin **1**.

14 and C-17/18, between the hydroxylated carbon at C-10 and the vicinal diol system at C-21/22, was confirmed by the HOHAHA correlation spectrum of **1** (Figure 3). This spectrum showed magnetization transfers from H-10 to H-11, -12, and -13 and from H-21 to H-20, -19, and -18, which proved the position of the diol system and of the isolated OH group two methylenes away from both double bonds. On the other hand, magnetization transfers were observed from the olefinic protons (H-13, -14, -17, -18) to the allylic (H-12, -15, -16, -19) and homoallylic (H-11, -20) methylenes and to the OH-bearing methine groups at C-10 and C-21. The absence of magnetization transfer from the olefinic protons to any highfield methylene group demonstrated the absence of any other methylene group between C-15 and C-16.



1	Coriadienin	R_1=R_2 = H
1a	Coriadienin acetonide	R_1,R_2 = $C(CH_3)_2$
1b	13,14,17,18 -Bisepoxy coriadienin	R_1=R_2 = H

Because of the small amount of **1** still available after chemical transformations and biological tests, Mosher esters could not be prepared, so the absolute configurations of the carbinolic carbons remain unknown.

The biological activity of **1** has been compared with its bisepoxy derivative (**1b**), coriacin, 4-deoxycoriacin, and gigantetronenin, and the results are summarized in Table 1. All of these products demonstrated a significant and promising cytotoxicity to the human nasopharyngeal carcinoma (9KB) cell line. The results

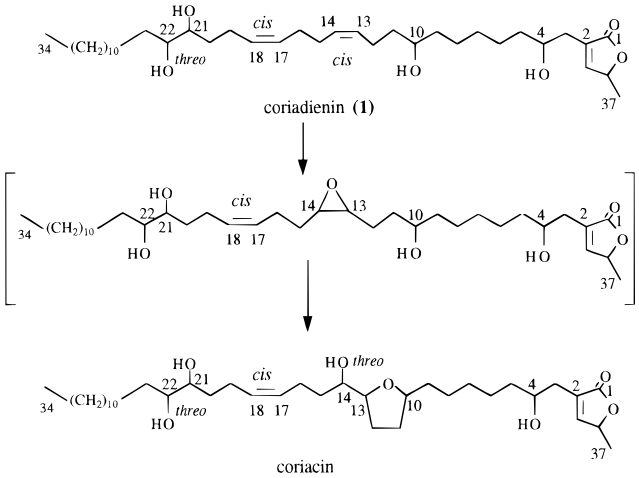


Figure 4. Hypothesis for the biogenetic pathway of coriacin from coriadienin **1**.

obtained help to confirm previous statements concerning structure−activity relationships of the Annonaceous acetogenins.[14] The non-THF compound coriadienin (**1**) is less potent than the mono-THF compounds coriacin and gigantetronenin. The bis-epoxy derivative (**1b**) is more potent than coriadienin against the KB and VERO cell lines. As previously reported,[11] the hydroxy group at the C-4 position in coriacin affords an enhancement of activity compared to 4-deoxycoriacin.

Although no experimental work has been reported on the biosynthesis of the Annonaceous acetogenins, a hypothesis has been reported[8] involving epoxidation of triene, diene, or triene ketone intermediates, followed by ring openings and closures, leading to different arrangements of the THF rings. Compound **1**, which contains two double bonds in the appropriate places in the hydrocarbon chain, provides evidence of its implication in the biosynthesis of coriacin (Figure 4). The threo configuration between the THF ring and the C-14 OH-group of coriacin is in agreement with the cis configuration of the C-13/14 double bond in coriadienin **1** and supports this biogenetic proposition.

According to the positions of the double bonds and OH groups, coriadienin **1** is also a potent intermediate in the generation of a tris-THF acetogenin such as goniocin, recently isolated from *Goniothalamus giganteus*.[15]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Schmidt-Haensch Polartronic E polarimeter. The UV spectrum was obtained in EtOH on a Philips PU 8720 spectrophotometer. The IR spectrum was measured (in CCl_4 solution) on a Perkin-Elmer 257 spectrophotometer. The ^1H- and ^{13}C-NMR spectra ($CDCl_3$) were obtained with a Bruker ARX-400 and a AC-200P instrument at 400 and 50 MHz, respectively. EIMS and CIMS (NH_4^+) were performed on a Nermag R 10-10 C spectrometer. FABMS (matrix: *m*-NBA + LiCl) were acquired with a Kratos NS 80 RF double focusing mass spectrometer, under conventional conditions.[5] Linked scan experiments (constant *B/E*) were performed under the control of a Kratos DS 90 data system using the following linked scan law: $B^2 (1\text{-}E)/E^2 = \text{constant}$.[14] HPLC was carried out with a Waters 590 pump system and a Millipore-Waters 484 (Milford, MA, USA) spectrophotometer.

Plant Material. Roots of *A. coriacea* Mart. (Annonaceae) were collected in July 1993, in Ceará, Brazil. The material was identified by Dr. Afranio Fernandes, Department of Biology, Federal University of Ceará. A voucher specimen is deposited in the herbarium of that university.

Extraction and Isolation. The dried and pulverized roots (2.4 kg) were extracted with EtOH. The EtOH extract (320 g) was partitioned between H_2O-MeOH (90:10) and hexane. The aqueous MeOH fraction was concentrated and extracted with CH_2Cl_2 to yield 55 g of CH_2Cl_2 extract. Of this extract, 25 g were submitted to one fractionation by column chromatography (Si gel S, 230−400 mesh), eluting with CH_2Cl_2−MeOH (99:1 to 80:20) gradients, which yielded 72 fractions (F001). The fractions 9−16 (3.7 g) of F001 were submitted to another column chromatography (Si gel S, 230−400 mesh), eluting with cyclohexane−EtOAc (80:20 to 40:60) and EtOAc−MeOH (98:2 to 50:50) gradients, yielding 68 fractions (F002). The fractions 41−47 (0.29 g) of F002 were eluted with CH_2Cl_2−MeOH (99:1 to 80:20) gradient, furnishing a partially purified fraction containing **1** (0.064g). HPLC purifications, using a μBondapack C18 prepacked column [10 μm, 25 × 100 mm], eluted with MeOH−H_2O (85:15) (flow rate 10 mL/min, UV detection at 214 nm) afforded **1** (16 mg). From the fractions 17−35 or F001 extract was isolated gigantetronenin (0.497 g) using column chromatography (Si gel S, 230−400 mesh), eluted with EtOAc−MeOH (99:1 to 92:8) gradient.

Coriadienin (1): white waxy solid (16 mg); mp 58−60 °C; $[\alpha]_D$ +6.2° (*c* 0.30, EtOH); IR ν max (solution in CCl_4): 3468 (OH), 3009, 2934, 2857, 1750, 1600, 1457 cm^{-1}; UV (EtOH) λ max (log ϵ) 203 (4.08) nm; HRCIMS (CH_4^+) *m/z* 607.4895 (MH^+) (calcd 607.9429 for $C_{37}H_{67}O_6$); CID *B/E* FABMS: see Figure 1; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.5 Hz, H-34), 1.20−1.30 (26H, m, H-6 to H-8, H-24 to H-33), 1.42 (3H, d, J = 6.8 Hz, H-37), 1.43 (2H, m, H-9), 1.44 (2H, m, H-23), 1.47 (2H, m, H-5), 1.48 (2H, m, H-20), 1.49 (2H, m, H-11), 2.08 (4H, m, H-15, -16), 2.12 (2H, m, H-12), 2.16 (2H, m, H-19), 2.38 (1H, dd, J = 15.0, 8.1 Hz, H-3a), 2.51 (1H, ddd, J = 15.0, 4.0, 1.8 Hz, H-3b), 3.39 (1H, m, H-22), 3.41 (1H, m, H-21), 3.58 (1H, m, H-10), 3.83 (1H, m, H-4), 5.04 (1H, qd, J = 6.8, 1.2 Hz, H-36), 5.35−5.43 (4H, m, H-13, -14, -17, -18), 7.18 (1H, d, J = 1.2 Hz, H-35); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1 (C-34), 19.1 (C-37), 22.6 (C-33), 23.5 (C-12, C-19), 25.5 and 25.6 (C-6, C-24), 27.4 (C-15, C-16), 31.8 (C-32), 33.3, 33.5 and 33.6 (C-3, C-20, C-23), 37.2 and 37.3 (C-5, C-9 and C-11), 69.9 (C-4), 71.5 (C-10), 74.0 (C-21), 74.5 (C-22), 77.9 (C-36), 129.6, 129.7, 129.8 and 129.9 (C-13, C-14, C-17, C-18), 131.1 (C-2), 151.8 (C-35), and 174.6 (C-1); HOHAHA correlations, see Figure 3.

Coriadienin Acetonide (1a). To **1** (2 mg) dissolved in C_6H_6 (1 mL) was 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 was stirred for 4 h at room temperature. The mixture was extracted with CH_2Cl_2 to give **1a** (1.3 mg): CIMS (NH_4^+) *m/z* 647 $[MH]^+$; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.87 (3H, t, J = 6.4 Hz, H-34), 1.20−1.60 (44H, m), 1.38 (6H, s, acetonide), 1.42 (3H, d, J = 6.8 Hz, H-37), 2.00−2.25 (4H, m, H-12, -15, -16, -19), 2.40 (1H, m, H-3b), 2.52 (1H, m, H-3a), 3.58 (3H, m, H-10, -21, -22), 3.85 (1H, m, H-4), 5.04 (1H, q, J = 6.7 Hz, H-36), 5.38 (4H, m, H-13, -14, -17, -18), 7.18 (1H, s, H-35).

13,14,17,18-Bisepoxycoriadienin (1b). Compound **1** (3 mg) was dissolved in $CHCl_3$ (1 mL) and added with *m*-CPBA (3 mg), and the mixture was stirred for 1.5 h at room temperature. The mixture was washed with a 1% $NaHCO_3$ solution and extracted with CH_2Cl_2, and the solvent was evaporated *in vacuo*. The ^{1}H-NMR spectrum of the bis-epoxy isomer mixture **1b** was recorded without further purification: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.5 Hz, H-34), 1.20−1.40 (38H, m), 1.43 (3H, d, J = 6.8 Hz, H-37), 1.46 (2H, m, H-5), 1.60 (4H, m, H-12, -19), 1.68 (4H, m, H-15, -16), 2.38 (1H, dd, J = 14.9, 8.2 Hz, H-3a), 2.50 (1H, d, J = 14.9 Hz, H-3b), 2.99 (4H, m, H-13, -14, -17, -18), 3.41 (2H, m, H-21, -22), 3.64 (1H, m, H-10), 3.84 (1H, m, H-4), 5.04 (1H, qd, J = 6.8, 1.2 Hz, H-36), 7.18 (1H, d, J = 1.2, H-35); CIMS (NH_4^+) *m/z* 639 $[MH]^+$; EIMS see Figure 2.

Gigantetronenin: white waxy solid (497 mg); $[\alpha]_D$ = +13° (*c* 1.0, $CHCl_3$); CIMS (NH_4^+) *m/z* 623 $[MH]^+$; for EIMS, ^{1}H and ^{13}C NMR data, see Fang *et al.*[7]

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References and Notes

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