CORIACYCLODIENIN AND CORIACYCLOENIN: TWO NEW ANNONACEOUS ACETOGENINS FROM *ANNONA CORIACEA* [1]

Edna Lucia Meneses Da Silva, François Roblot, and André Cavé*

Laboratoire de Pharmacognosie, URA 1843 CNRS (BIOCIS),
Faculté de Pharmacie, 92296 Châtenay-Malabry, France

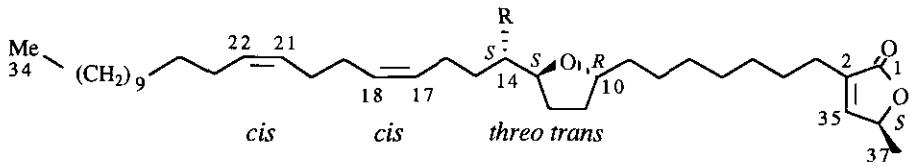
Abstract - Two new Annonaceous acetogenins, coriacyclodienin (**1**) and coriacycloenin (**2**), have been isolated from the roots of *Annona coriacea* Mart. (Annonaceae). **1** is the first mono-THF acetogenin which bears two double bonds in the aliphatic chain, while **2** has the same mono-THF ring and only one double bond. Both compounds (**1**) and (**2**) were oxidized and cyclized, in a one pot reaction, to give the mixtures of the four possible stereoisomer derivatives (**3**, **4**, **5** and **6**) of **1** and the two possible stereoisomer derivatives (**7** and **8**) of **2**. The absolute stereochemistries of **1** and **2** were assigned using NMR analysis of their Mosher esters and enzymatic method.

In our continuous search of acetogenins from Annonaceae, we have reported the first example of an acetogenin with two double bonds on the aliphatic chain, coriadienin,[2] without any THF ring, which supported the polyketide biogenetic pathway.[3] In this paper, we report the structure elucidation of two acetogenins with an α-hydroxylated mono-THF system, coriacyclodienin (**1**), possessing two double bonds and coriacycloenin (**2**), that possesses only one double bond. Both compounds can be proposed as potent intermediates in the biogenesis of bis- or tri-THF acetogenins.

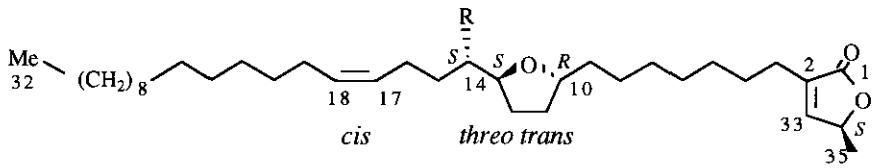
The absolute stereochemistry of the carbinol center at C-14 in both compounds was determined by Mosher esters methodology,[4] while the carbinol centers at C-36 in **1** and C-34 in **2** were determined by the recent enzymatic method followed by the HPLC detection of NADH issued from the enzymatic oxidation of L- or D-lactic acid.[5]

Coriacyclodienin (**1**) and coriacycloenin (**2**) have been isolated by HPLC from a hexane extract of the roots of *Annona coriacea*. Spectral characteristics, including ^{1}H and ^{13}C NMR and EIMS data, suggested that both compounds have an α-hydroxylated mono-THF ring in the aliphatic chain, along with two double bonds in **1** and one double bond in **2**.

Compound (**1**) was isolated as a white waxy solid, $[\alpha]_D$ + 8.0° (*c* 1.2, $CHCl_3$). The molecular formula of **1** was established to be $C_{37}H_{64}O_4$ by HRCIMS (NH_4^+), which gave a [MH^+] ion at *m/z* 573.4878 (calcd 573.4882).



1 R = OH
1s R = O-(*S*)-MTPA
1r R = O-(*R*)-MTPA



2 R = OH
2s R = O-(*S*)-MTPA
2r R = O-(*R*)-MTPA

The existence of a γ-methyl-α,β-unsaturated γ-lactone in **1** was indicated by a strong IR absorption at 1762 cm^{-1}, the ^1H NMR resonances at δ 6.97 (d, J =1.6 Hz, H-35), 4.97 (qd, J = 6.7 and 1.7 Hz, H-36), 2.26 (td, J = 7.2 and 1.6 Hz, H-3) and 1.40 (d, J = 6.7 Hz, H-37) and by the ^{13}C NMR signals at δ 173.7 (C-1), 148.8 (C-35), 134.2 (C-2), 77.3 (C-36), 25.1 (C-3) and 19.2 (C-37) (Table 1).[6]

The presence of an α-hydroxylated mono-THF ring (IR absorption at 3552 cm^{-1}) was indicated by the proton signals at δ 3.86 (m, H-10), 3.78 (td, J = 7.6 and 6.9 Hz, H-13), 3.38 (m, H-14), 2.04 (m, H-11), 1.95 and 1.62 (m, H-12) in the ^1H NMR spectrum and by the signals at δ 79.2 (C-10), 81.8 (C-13), 73.5 (C-14), 32.4 (C-11) and 28.3 (C-12) in the ^{13}C NMR spectrum (Table 1). These NMR data also indicated that the relative stereochemistry between C-13 and C-14 was *threo*[7] and that the configuration across the THF ring (C-10/C-13) was *trans*, by comparisons with compounds of known relative stereochemistry.[8]

The presence of two isolated double bonds in **1** was indicated by proton NMR signals at δ 5.40-5.34 (m, 4H), and by ^{13}C NMR signals at δ 130.4, 129.5, 129.8 and 129.0. The configurations of both double bonds was determined as *cis* by selective irradiation of the allylic protons over 2.00 ppm region, and observation of the coupling constants (J = 10.7 and 9.6 Hz) between H-17/18 and H-21/22.

The placement of the α-hydroxylated THF moiety at C-10/14 and of one double bond at C-17/18 were achieved by analysis of the EIMS fragments of **1** (Figure 1).

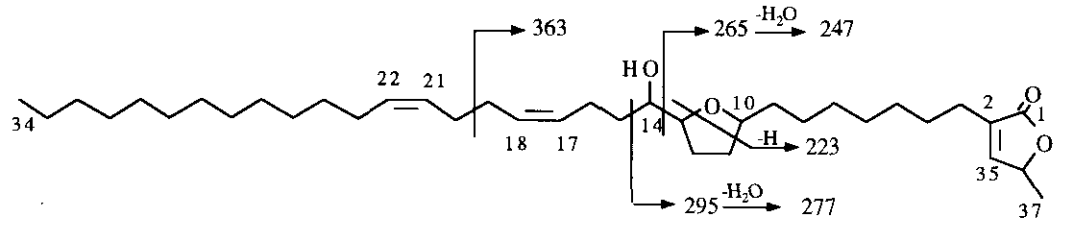


Figure 1. Diagnostic fragment ions in the EIMS of coriacyclodienin (**1**).

Table 1. NMR data of **1**, **1s** and **1r**.

Carbon	^{1}H of **1**[a]	^{13}C of **1**[b]	^{1}H of **1s**[a] *	^{1}H of **1r**[a] *	$\Delta\delta_H$ (δ_S-δ_R)
1	-----	173.7	-----	-----	----
2	-----	134.2	-----	-----	----
3	2.26 td (7.2; 1.6)	25.1	2.25 td (7.2; 1.6)	2.25 td (7.2; 1.6)	0
4	1.56 m	29.0	1.55 m	1.54 m	pos
8	1.32 m		1.31 m	1.29 m	pos
9	1.42 m	35.6	1.44 m	1.39 m	pos
10	3.86 m	79.2	3.88 m	3.79 m	pos
11	2.04 m	32.4	2.03 m	1.86 m	pos
12	1.95 m; 1.62 m	28.3	2.03 m; 1.56 m	1.93 m; 1.54 m	pos
13	3.78 td (7.6; 6.9)	81.8	4.02 td (7.2; 6.8)	4.02 td (7.6; 6.8)	0
14	3.38 m	73.5	5.07 m	5.06 m	*S*[d]
15	1.45 m	33.2	1.55 m	1.69 m	neg
16	2.22 m	23.4	2.04 m	2.12 m	neg
17	5.40-5.34 m[c]	130.4	5.40-5.22 m	5.44-5.28 m	neg
18	5.40-5.34 m[c]	129.5	5.40-5.22 m	5.44-5.28 m	neg
19	2.10 m	27.3	2.03 m	2.07 m	neg
20	2.10 m	27.3	2.03 m	2.05 m	neg
21	5.40-5.34 m[c]	129.8	5.40-5.22 m	5.44-5.28 m	neg
22	5.40-5.34 m[c]	129.0	5.40-5.22 m	5.44-5.28 m	neg
23	2.02 m	27.2	1.96 m	2.02 m	neg
24	1.33 m		1.30 m	1.33 m	neg
33	1.32 m	22.3	1.30 m	1.30 m	0
34	0.87 t (7.0)	14.0	0.87 t (6.4)	0.87 t (6.8)	0
35	6.97 d (1.6)	148.8	6.97 d (1.6)	6.97 d (1.6)	0
36	4.97 qd (6.7; 1.7)	77.3	4.98 qd (6.8; 1.6)	4.98 qd (6.8; 1.6)	0
37	1.40 d (6.7)	19.2	1.39 d (6.8)	1.39 d (6.8)	0
MeO			3.63 s	3.56 s	
Ar			7.66 (2H)	7.63 (2H)	
			7.37 (3H)	7.38 (3H)	

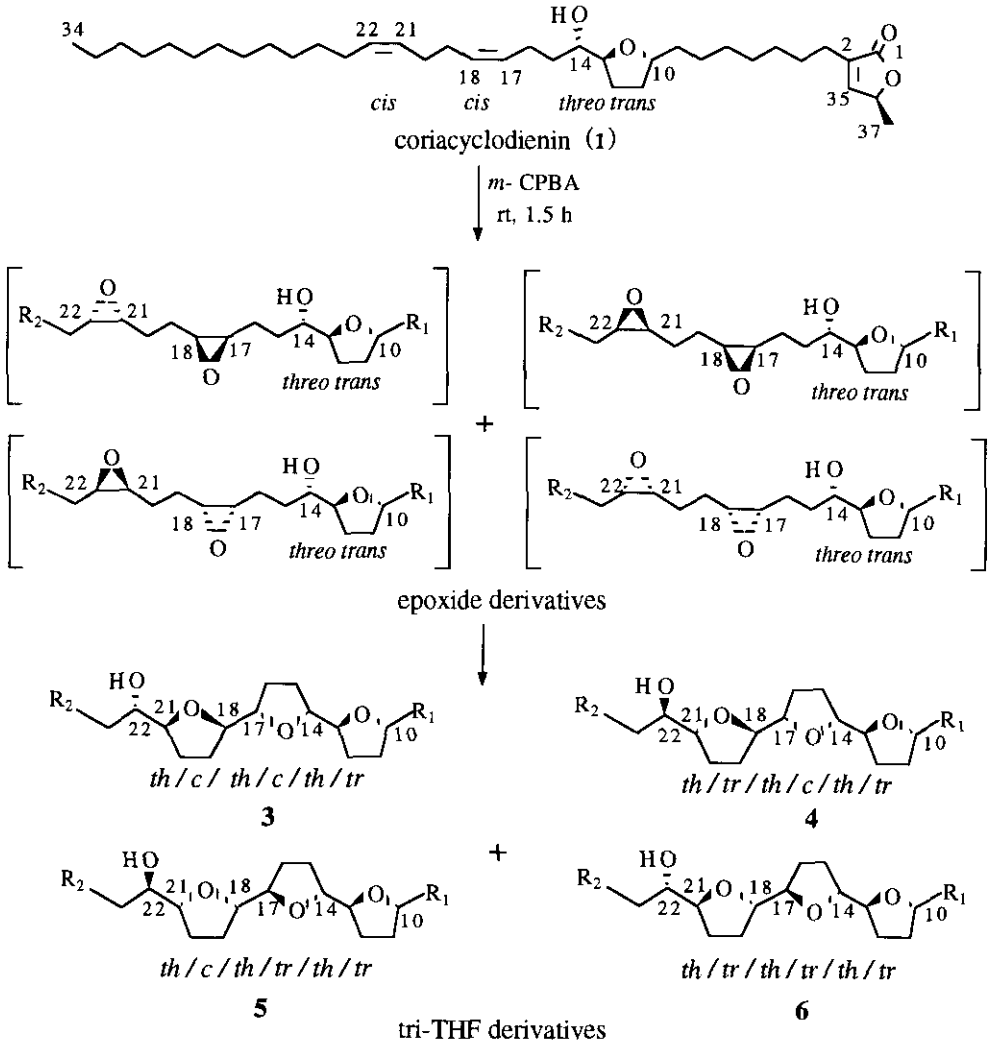
[a]: $CDCl_3$, 400 MHz (J Hz); [b]: $CDCl_3$, 50 MHz; [c]: $^3J_{17,18;\ 21,22}$ = 10.7; 9.6 Hz; [d]: Absolute configuration of the carbinol center; * : ^{1}H NMR attributions were made according to COSY and HOHAHA spectra.

The position of the double bond at C-17/18, two methylenes away of the OH group, was confirmed by the HOHAHA spectrum, which showed magnetization transfers from H-14 (δ 3.38) to H-15 (δ 1.45), H-16 (δ 2.22), and H-17,18 (δ 5.40-5.34). Magnetization transfers were also observed from H-19,20 (δ 2.10) to H-16, H-15 and H-14 via H-17,18 and to H-23 via H-21,22. The absence of magnetization transfer of the four allylic protons H-19 and H-20 to any other methylene group indicated the position of the second double bond at C-21/22. Thus, the structure of **1** was determined as shown in Figure 1. Coriacyclodienin (**1**) is the first example of a mono-THF acetogenin bearing two double bonds in the aliphatic chain.

In order to confirm the position of the two double bonds, **1** was oxidized with *m*-CPBA. The epoxide derivatives obtained were directly cyclized, in a one-pot reaction, into a mixture of tri-THF acetogenins (Scheme 1). The formation of the four possible stereoisomers (**3**-**6**) was evidenced by the presence of four peaks in the HPLC chromatogram. The very close retention times observed between these products did not allow their separation.

The presence of the signals at δ 4.00-3.75 (m, 6H) and 3.40-3.30 (m, 1H), and the absence of epoxy groups and double bonds in the oxidation products of **1**, indicated the formation of the tri-THF acetogenins.

The ^{13}C NMR spectrum demonstrated the signals of a complicated mixture between δ 84.0 and 79.5, and four signals at δ 74.6, 74.4, 74.2 and 74.1, corresponding to the OH-bearing carbon (C-22) for each stereoisomer compound.



Scheme 1. Obtention of **3**, **4**, **5**, **6** from **1**. R_1= $C_{12}H_{19}O_2$; R_2 = $C_{11}H_{23}$.

The molecular formula $C_{37}H_{64}O_6$ of these tri-THF acetogenins was determined by CIMS (NH_4^+) of the mixture, which demonstrated a single ion peak at *m/z* 622 [M+NH_4^+], without any protonated ion peak at *m/z* 605. The location of the three THF rings was confirmed by EIMS fragmentations (Figure 2). The conversion of **1** to **3-6** subsequently confirmed the position of the double bonds at C17/18 and C21/22 in **1**.

Only two tri-THF acetogenins, goniocin, isolated from *Goniothalamus giganteus*[9] and cyclogoniodenin C, obtained by epoxidation and cyclization from goniodenin,[9] have been previously reported. The tri-THF derivatives (**3-6**) synthesised from **1** represent new semi-synthesized tri-THF acetogenins.

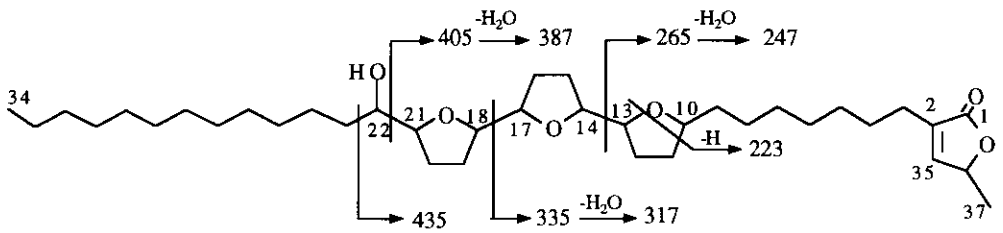


Figure 2. EIMS fragmentations of the tri-THF derivatives mixture (**3-6**) of **1**.

Compound (**2**) was obtained as a waxy solid, $[\alpha]_D$ + 6.0° (*c* 0.5, $CHCl_3$) and its molecular formula was determined as $C_{35}H_{62}O_4$ on the basis of the HRCIMS (CH_4^+) which gave a [MH^+] ion at *m/z* 547.4734 (calcd 547.4695).

The ^1H and ^{13}C NMR data of **2** (Table 2) were very similar to those of **1** (Table 1). The only difference observed was the presence of two ethylenic protons and two allylic methylene groups in **2**, indicating the presence of only one double bond.

Table 2. NMR data of **2**, **2s** and **2r**.

Carbon	^1H of **2**[a]	^{13}C of **1**[b] *	^1H of **2s**[a] *	^1H of **2r**[a] *	$\Delta\delta_H$ (δ_S-δ_R)
1	-----	173.7	-----	-----	-----
2	-----	134.2	-----	-----	-----
3	2.26 td (7.2; 1.6)	25.2	2.25 td (7.2; 1.6)	2.25 td (7.6; 1.6)	0
4	1.55 m	29.1	1.54 m	1.53 m	pos
8	1.32 m	26.2	1.35 m	1.34 m	pos
9	1.42m	35.6	1.31 m	1.30 m	pos
10	3.87 m	79.2	3.88 m	3.79 m	pos
11	2.02 m	32.4	1.98 m	1.85 m	pos
12	1.95 m; 1.62 m	28.3	2.04 m; 1.58 m	1.92 m; 1.51 m	pos
13	3.78 td (7.2;6.8)	81.8	4.02 td (7.6; 6.8)	4.02 td (7.6; 6.8)	0
14	3.38 m	73.6	5.08 m	5.06 m	S[d]
15	1.45 m	33.3	1.54 m	1.69 m	neg
16	2.20 m	23.3	1.97 m	2.12 m	neg
17	5.35 m[c]	130.6	5.25 m	5.32 m	neg
18	5.38 m[c]	129.0	5.35 m	5.42 m	neg
19	2.05 m	27.2	1.90 m	1.99 m	neg
20	1.33 m		1.26 m	1.30 m	neg
30	1.41 m	31.9	1.52 m	1.55 m	neg
31	1.28 m	22.6	1.26 m	1.26 m	0
32	0.87 t (6.7)	14.1	0.87 t (6.8)	0.87 t (6.8)	0
33	6.97 d (1.5)	148.8	6.97 d (1.6)	6.97 d (1.6)	0
34	4.98 qd (6.7; 1.7)	77.3	4.98 qd (7.2; 1.6)	4.98 qd (7.2; 1.6)	0
35	1.40 d (6.7)	19.2	1.40 d (6.8)	1.39 d (6.8)	pos
MeO			3.63 s	3.56 s	
Ar			7.66 (2H)	7.62 (2H)	
			7.37 (3H)	7.38 (3H)	

[a]: $CDCl_3$, 400 MHz (*J* Hz); [b]: $CDCl_3$, 50 MHz; [c]: $^3J_{17,18}$ = 10.5 Hz; [d]: Absolute configuration of the carbinol center;

*: ^1H NMR attributions were made according to COSY and HOHAHA spectra.

The γ-methyl-α,β-unsaturated γ-lactone in **2** was evidenced by IR absorption at 1761 cm^{-1} and characteristic ^1H and ^{13}C NMR data. The absence of hydroxy group at C-4 in **2** was substantiated by the presence of a 2H signal (td, *J* = 7.2 and 1.6 Hz) at δ 2.26 for H-3.[5]

The presence of a mono-THF ring flanked by one OH group was indicated by the proton signals at δ 3.87 (H-10), 3.78 (H-13), 3.38 (H-14), 2.02 (H-11), 1.95 and 1.62 (H-12) in the ^{1}H NMR spectrum and by the carbon signals at δ 79.2 (C-10), 81.8 (C-13), 73.6 (C-14), 32.4 (C-11) and 28.3 (C-12) in the ^{13}C NMR spectrum (Table 2). The existence of one OH group was substantiated by a *m/z* 528 ion generated by loss of H_2O from the molecular ion at *m/z* 546 in the EIMS of **2**.

The NMR data also indicated that the relative stereochemistry between C-13 and C-14 was *threo*[7] and that the configuration across the THF ring (C-10/13) was *trans*, by comparison with compounds of known relative stereochemistry.[8]

The existence of an isolated double bond in **2** was indicated by two proton resonances at δ 5.35 (H-17) and 5.38 (H-18), correlated to carbon peaks at δ 130.6 (C-17) and 129.0 (C-18). The configuration of the double bond was determined as *cis* by selective irradiations of the allylic protons at δ 2.20 and 2.05 and observation of the coupling constant (J = 10.5 Hz) between H-17 and H-18.

EIMS analysis of **2** (Figure 3) demonstrated that the position of the α-hydroxylated THF ring was the same as in **1**. However, the absence of significant ions did not allow the determination of the double bond position.

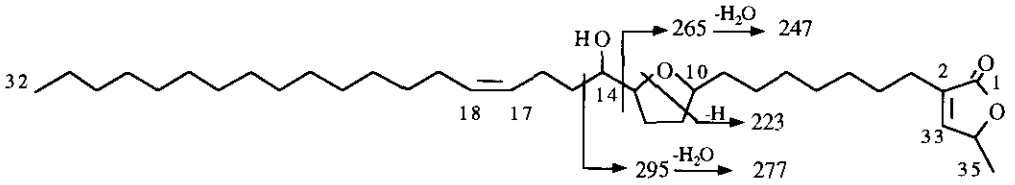


Figure 3. Diagnostic fragment ions in the EIMS of coriacycloenin (**2**).

The position of the double bond was demonstrated by the HOHAHA correlation spectrum of **2** (Figure 4). This spectrum showed magnetization transfers from H-14 (δ 3.38) to H-15 (δ 1.45), H-16 (δ 2.20), H-17 (δ 5.35), H-18 (δ 5.38), H-19 (δ 2.05) and to the THF protons H-13 (δ 3.78), H-12 (δ 1.95 and 1.62), H-11 (δ 2.02) and H-10 (δ 3.87). Thus, the double bond was located at C-17/18, two methylenes away from the carbinolic proton H-14.

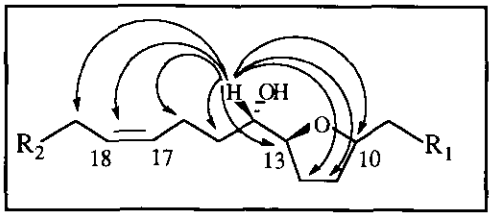
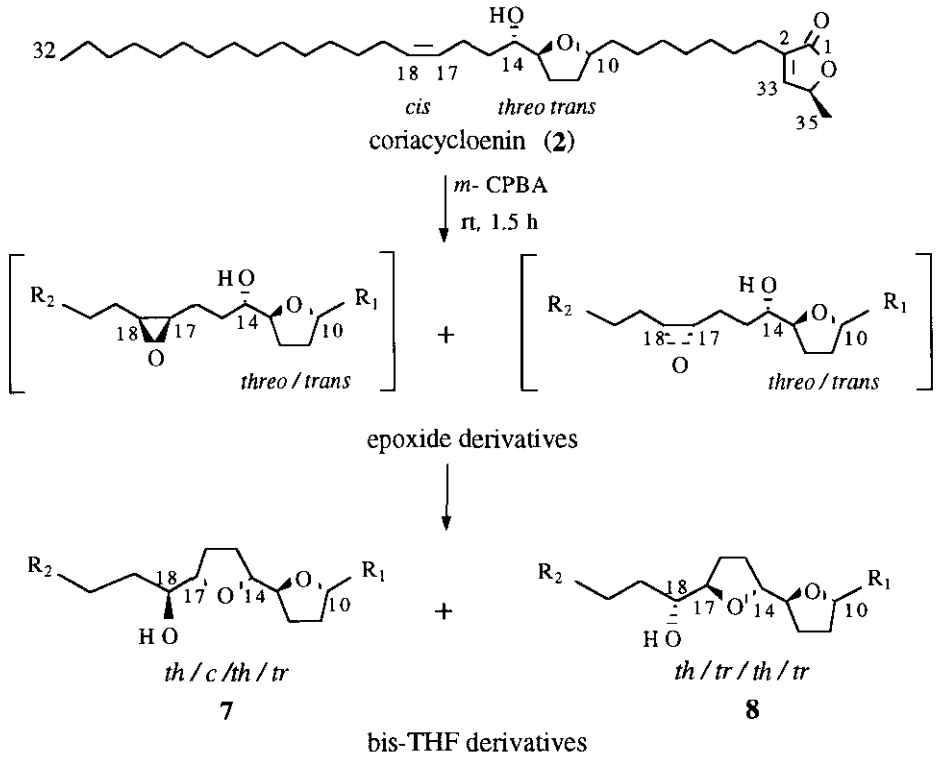


Figure 4. ^{1}H-^{1}H Magnetization transfers in the HOHAHA spectrum of coriacycloenin (**2**).
($R_1 = C_{11}H_{17}O_2$; $R_2 = C_{13}H_{27}$).

Compound (**2**) was also submitted to oxidation and subsequent cyclization with *m*-CPBA, giving the mixture of the two possible bis-THF stereoisomer derivatives (**7**) and (**8**) (Scheme 2).

This mixture was obtained as a colorless oil and the molecular formula was determined as $C_{35}H_{62}O_5$ by CIMS (NH_4^+), which presented a [MH^+] ion at m/z 563.



Scheme 2. Obtention of **7** and **8** from **2**. (R_1= $C_{12}H_{19}O_2$; R_2 = $C_{12}H_{25}$).

The ^{1}H NMR spectrum of the mixture of **7** and **8** showed signals at δ 3.39 (1H, H-18) and at δ 3.96-3.83 (4H, H-10, H-13, H-14 and H-17), suggesting the presence of bis-THF acetogenins. The absence of the ethylenic protons at δ 5.35 and 5.38 and of any epoxide protons evidenced the formation of bis-THF derivatives. The position of the substituents in the aliphatic chain of both **7** and **8** was confirmed by EIMS (Figure 5).

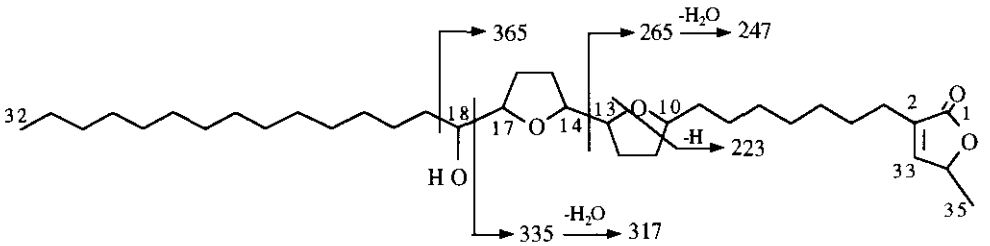


Figure 5. EIMS fragmentations of the bis-THF derivatives (**7** and **8**) of **2**.

Since both compounds (**1**) and (**2**) have only one carbinol center at C-14, the absolute configuration at this position was established using the Mosher esters methodology.[3]

With the relative stereochemistries around the THF rings already in hand, the absolute stereochemistries of the stereocenters could be concluded by analysis of the ^{1}H NMR, ^{1}H-^{1}H-COSY and HOHAHA spectral data of the (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl) phenylacetic ester derivatives (**1s**), (**1r**) (Table 1), and (**2s**), (**2r**) (Table 2).

The analysis of the $\Delta\delta_H(\delta_S-\delta_R)$ data of **1s** and **1r** around the substituted THF ring showed positive values for H-12 to H-4, and negative ones for H-15 to H-24, on the double bond side (Table 1), indicating an *S* configuration at C-14. Similar results were observed for the Mosher esters (**2s**) and (**2r**) (Table 2). Although the reasons for a $\Delta\delta_H = 0$ for H-13, in the derivatives of both **1** and **2**, are not clear, this outcome has been already reported in other acetogenins.[10,11]

According to Mosher's configurational correlation model, we can assign the *S* configuration for C-14 in **1** and **2**. For both compounds, the relative stereochemistries were established as *trans* between C-10/13 and *threo* between C-13/14, permitting to conclude their absolute configurations as 10*R*, 13*S*, and 14*S*.

The absolute configuration of the carbinol center at C-36 in **1** and at C-34 in **2** was determined by oxidative cleavage of their α,β-unsaturated γ-methyl-γ-lactones with periodic acid, which gave lactic acid as degradative product. The latter were incubated with *L*-(+) and *D*-(-)-lactate dehydrogenase (LDH) and nicotinamide-adenine-dinucleotide (NAD) as coenzyme. The subsequent HPLC analysis, against blanks, demonstrated the presence of NADH for the *L*-(+)-LDH incubated media obtained from both **1** and **2**.

This stereospecific formation of NADH using the *L*-LDH indicated the presence of *L*-lactic acid in the degradative products of **1** and **2**, showing the C-36*S* and C-34*S* absolute configuration for **1** and **2**, respectively.

Thus, the relative and absolute stereochemistries of **1** and **2** were fully and unambiguously determined.

EXPERIMENTAL

Optical rotations were determined on a Schmidt-Haensch Polartronic E polarimeter at 25°C. IR spectra (film) were measured on a Perkin-Elmer 257 spectrophotometer. UV spectra were obtained in EtOH for **1** and **2** on a Philips PU 8720 spectrophotometer. Mono and bidimentional ^{1}H and ^{13}C NMR spectra were obtained on Bruker ARX-400 and AC-200P instruments, at 400 and 50 MHz, respectively. EIMS and CIMS (NH_4^+) were performed on a Nermag R10-10 C spectrometer. HPLC purifications were performed 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, under the auspices of Dr. Afranio Fernandes, Department of Biology, Federal University of Ceará, where voucher specimens are maintained.

Extraction and isolation. - The dried and pulverized roots (2.4 kg) were extracted with EtOH (5 L) for 8 h in a Soxhlet apparatus. The EtOH extract (320 g) was partitioned between H_2O-MeOH (90:10) and hexane. The hexane extract (4.5 g) was submitted to fractionation by column chromatography (Si gel, 230-400 mesh), eluting with cyclohexane-EtOAc (95:5 to 50:50) gradients, which yielded eight principal fractions.

The partially purified fractions 7-8 (0.39 g) containing **1** and **2** were submitted to HPLC purification using a μ-Bondapack C-18 prepacked column [10 μm, 25 x 100 mm], eluted with MeOH-H_2O (92:8), flow rate 10 mL/min, UV detection at 214 nm, affording **1** (0.04 g, t_R 45 min) and **2** (0.02 g, t_R 40 min) as white waxes.

Coriacyclodienin (1) = 5*S*-3-[(8*R*,11*S*,12*S*)-(15*Z*,19*Z*)-(8,11-epoxy-12-hydroxydotriaconta-15,19-dienyl)]-5-methylfuran-2-(5*H*)-one. - White oil, $[\alpha]_D$ +8.0° (*c* 1.2, $CHCl_3$); UV λ_{max} (EtOH) 208 nm (log ε, 3.87); IR ν_{max} (film) 3522, 2929, 2856, 1762, 1076 cm^{-1}; ^1H and ^{13}C NMR: see Table 1.

Coriacycloenin (2) = 5*S*-3-[(8*R*,11*S*,12*S*)-(15*Z*)-(8,11-epoxy-12-hydroxytriaconta-15-enyl)]-5-methylfuran-2-(5*H*)-one. - White waxy solid, $[\alpha]_D$ +6.0° (*c* 0.5, $CHCl_3$); UV λ_{max} (EtOH) 207 nm (log ε, 3.40); IR ν_{max} (film) 3488, 2929, 2856, 1761, 1465, 1075 cm^{-1}; ^1H and ^{13}C NMR: see Table 2.

Preparation and purification of Mosher Esters. - To **1** (3 mg) dissolved in CH_2Cl_2 (1 mL) were sequentially added pyridine (0.1 mL), 4-dimethylaminopyridine (1 mg), and 49 mg of (*R*)-(-)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (Aldrich chemicals). The mixture was stirred at rt for 12 h, then washed using 1% $NaHCO_3$ (3 mL) and H_2O (3 x 3 mL), and the solvent evaporated *in vacuo*, affording the crude ester (**1s**). A similar process using (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride afforded (**1r**). The crude esters were purified by HPLC using a μ–Bondapack C-18 prepacked column [5μm, 8 x 100 mm], eluted with MeOH-H_2O (97:3), flow rate 0.8 ml/min, UV detection at 214 nm, affording **1s** (2.4 mg, t_R 36.4 min) and **1r** (2.2 mg, t_R 31.8 min).
2s and **2r** were prepared in the same way from **2** (2.5 mg for each). Further HPLC purification using MeOH-H_2O (95:5) as eluent, flow rate 1.0 ml/min, UV detection at 214 nm, afforded **2s** (1,8 mg, t_R 48.8 min) and **2r** (1.7 mg, t_R 44.2 min). The overall yields were typically over 70% for **1s**, **1r**, **2s** and **2r**.

(*S*)- and (*R*)-MTPA Esters (1s) and (1r). - Colorless oils. ^1H NMR data: see Table 1.

(*S*)- and (*R*)-MTPA Esters (2s) and (2r). - Colorless oils. ^1H NMR data: see Table 2.

Degradative oxidation and enzymatic incubation. - Periodic acid H_5IO_6 (14 mg, 15 eq.) and a catalytic amount of $RuCl_3$ were added to a biphasic solution of **1** (2.4 mg) in a ternary mixture (200 μL) of CCl_4 / CH_3CN / H_2O [57 : 57 : 86]. The mixture was stirred at rt for 15 h, then the complete solution was filtered and the solvents evaporated *in vacuo*. Water (0.5 mL) was added to the residue and the solution was extracted with EtOAc (3 x 0.5 mL). The organic layer was concentrated and the residue was dissolved in 100 μL of a 0.1 M buffered solution (pH = 9) of TRIS [tris (hydroxymethyl) aminomethane, Trizma base, SIGMA], prepared from 0.2 M TRIS (50 mL), hydrazine (5 mL), 1N HCl (15 mL), and water (30 mL). 50 μL of this alkaline solution were separately incubated for 20 min at 40°C with *L*- or *D*-LDH (10 μL) and a 1% aqueous solution of NAD (50 μL). A similar process was achieved for **2** (2.7 mg).

NADH formation was evidenced by HPLC using a Sup-Rs Spherisorb S50DS2 column [4.6 x 250 mm], Prolabo, France; mobile phase: buffered solution of TRIS (pH = 8) and MeOH [95:5] v/v; flow rate: 1 mL/min; specific UV detection of NADH at 340 nm; sample volume injected: 50 µL; the buffered solution of TRIS (pH = 8) is prepared from 0.2 M TRIS (250 mL), 0.1 N HCl (280 mL), EDTA (tetrasodium salt, SIGMA, 76 mg) and water (470 mL).

Tri-THF acetogenins (3), (4), (5) and (6). - To **1** (15.5 mg) dissolved in $CHCl_3$ (2 mL) was added *m*-CPBA (29 mg) and the mixture was stirred for 1.5 h at rt, then washed with saturated $NaHCO_3$ (3 mL) and H_2O (3 x 5 mL) [Procedure a]. The $CHCl_3$ layer was evaporated *in vacuo* giving the mixture of **3**, **4**, **5** and **6** (13 mg). The normal and reversed phase HPLC analysis demonstrated the presence of four compounds, but did not allow their separation. CIMS (NH_4^+) m/z = 605 [MH^+] (7%), 622 [$M+NH_4^+$] (100%); EIMS: see Figure 4; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.97 (1H, d, J= 1.6 Hz), 4.97 (1H, qd, J = 6.7 and 1.7 Hz), 4.00-3.75 (6H, m), 3.40-3.30 (1H, m), 2.25 (2H, t, J = 7.2 Hz), 1.40 (3H, d, J = 6.7 Hz), 0.87 (3H, t, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 148.8 (C-35), 134.2 (C-2), 83.0-79.8 (C-10, C-13, C-14, C-17, C-18, C-21), 77.4 (C-36), 74.6, 74.4, 74. 2 and 74.0 (C-22), 19.2 (C-37) and 14.1 (C-34).

Bis-THF acetogenins (7) and (8). - **2** (5.9 mg) was submitted to procedure a. The $CHCl_3$ layer afforded the mixture of **7** and **8** (5.8 mg). CIMS (NH_4^+) m/z = 563 [MH^+] (87%), 580 [$M+NH_4^+$] (100%); EIMS: see Figure 5; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, d, J = 1.5 Hz), 4.98 (1H, qd, J = 6.7 and 1.7 Hz), 3.96-3.80 m (4H, m), 3.40-3.33 m (1H, m), 2.25 (2H, t, J = 7.2 Hz), 1.40 (3H, d, J = 6.7 Hz), 0.87 (3H, t, J = 6.8 Hz).

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