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Corepoxylone, a Possible Precursor of Mono-Tetrahydrofuran γ-Lactone Acetogenins : Biomimetic Synthesis of Corossolone[1]

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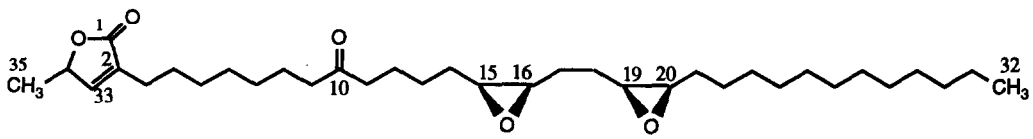
Abstract: *A new diepoxy acetogenin with a carbonyl group, corepoxylone (1), has been isolated from the non-polar fractions of* Annona muricata *seeds. Its structure was elucidated on the basis of the spectral data, NMR and MS in particular. Biomimetic synthesis of corossolone (2) from (1) suggests its role in the biogenesis of mono-tetrahydrofuran acetogenins bearing a carbonyl group at C-10.*

Our previous bioactivity-directed phytochemical studies on *Annona muricata* (Annonaceae) led to isolation of four new cytotoxic acetogenins.[3-6] In continuation of our investigation of this plant, we have succeded in isolation of corepoxylone (**1**), a new cytotoxic diepoxy acetogenin possesing a carbonyl group at C-10. This product may be considered as a precursor of corossolone (**2**), mono-tetrahydrofuran acetogenin only present in this plant. The biomimetic synthesis in acidic medium of corossolone (**2**) from corepoxylone (**1**) is proposed (Figure 1).

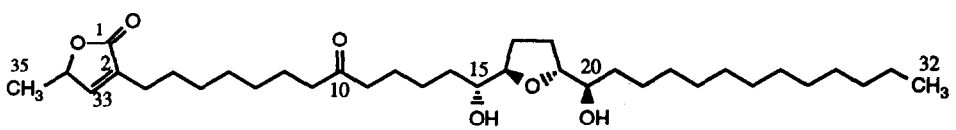
In this paper, in addition to structure elucidation of corepoxylone (**1**), some biosynthetic and stereochemical implications are discussed.

Figure 1. Chemical structures of compounds (**1**) and (**2**)



corepoxylone (1)

(absolute configurations might be inverted)



corossolone (2)

(absolute configurations might be inverted)

RESULTS AND DISCUSSION

Corepoxylone (1), obtained after chromatographic separation from the less polar fractions of methanolic extract from the seeds of *A. muricata*, showed positive response to Kedde reagent which was predictive of α,β-unsaturated γ-lactone. It was confirmed by IR (absorption at 1750 cm^{-1}) and ^1H NMR spectrum, doublet at δ 6.99 (H-33), quartet of doublets at δ 4.98 (H-34) and three-proton doublet at δ 1.40 (Me-35).[7] The molecular formula $C_{35}H_{60}O_5$ was deduced from EIMS $[M]^+$ *m/z* 560, as well as L-SIMS $[M+H]^+$ *m/z* 561 and L-SIMS + LiCl $[M+Li]^+$ *m/z* 567.

Beside the γ-lactone moiety, the ^1H NMR spectrum displayed a multiplet of four protons centred at δ 2.96, due to two groups of *cis*-disubstituted epoxides.[6,8] In addition, a system of four protons adjacent to the carbonyl was observed at δ 2.38. A two-proton triplet at δ 2.26 due to two equivalent protons at C-3 indicated a non-hydroxylated C-4 for (1).[4] Characteristic chemical shifts for a long hydrocarbon chain (δ, 1.20-1.70) with a terminal methyl group (*t*, δ 0.89), have also been observed. Most of the characteristic protons was assigned by analysis of the 2D homodecoupling experiments (COSY 45 and COSY-RELAY, see Table 1). The ^1H-^{13}C heteronuclear correlation (XH CORR) was carried out to obtain the relationships between the carbon atoms and their respective protons. Therefore, we were able to characterise the carbon signals of the unsaturated methyl γ-lactone moiety (C1/C2/C33/C34 and C35), as well as the carbon signals corresponding to the carbonyl (δ 210.89) and diepoxy groups (δ 56-57), see Table 1.

Table 1. ^1H NMR and ^{13}C NMR spectral data of corepoxylone (1) ($CDCl_3$, 200 MHz and 50 MHz, respectively).

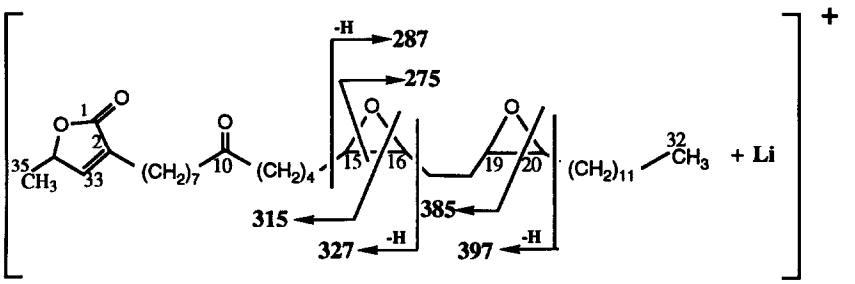
	δ (ppm)*	Correlation in COSY 45 and RELAY-COSY spectra	Correlation in ^1H-^{13}C spectra (multiplicity SPIN ECHO)
2	-		134.24 (C)
3	2.26 *td*	H-4 (1.55), H-33 (6.99), H-34 (4.98) H-35 (1.40)	25.14 (CH_2)
4	1.55 *m*	H-3 (2.26), H-33 (6.99)	27.65 (CH_2)
5-6	1.20-1.70 *m*		29.62-29.06 (CH_2)
7	1.30 *m*	H-8 (1.60)	29.62-29.06 (CH_2)
8	1.60 *m*	H-7 (1.30), H-9 (2.38)	23.73 (CH_2)
9	2.38 *t*	H-8 (1.60), H-7 (1.30)	42.77 (CH_2)**
10	-		210.89 (C)
11	2.38 *t*	H-12 (1.60), H-13 (1.50)	42.53 (CH_2)**
12	1.60 *m*	H-11 (2.38), H-13 (1.50)	23.62 (CH_2)
13	1.50 *m*	H-12 (1.60), H-14 (1.60-1.70), H-15 (2.96)	29.62-29.06 (CH_2)
14	1.60-1.70 *m*	H-13 (1.50), H-15 (2.96)	25.00-27.80 (CH_2)
15	2.96 *m*	H-14 (1.60-1.70), H-13 (1.50)	57.33 (CH)***
16	2.96 *m*	H-17 (1.60-1.70)	56.70 (CH)***
17-18	1.60-1.70 *m*	H-16, H-19 (2.96)	25.00-27.80 (CH_2)
19	2.96 *m*	H-18 (1.60-1.70)	56.40 (CH)***
20	2.96 *m*	H-21 (1.60-1.70)	57.01 (CH)***
21	1.60-1.70 *m*	H-20 (2.96)	25.00-27.80 (CH_2)
22-29	1.20-1.70 *m*		29.62-29.06 (CH_2)
30	1.25 *m*	H-31 (1.34)	31.89 (CH_2)
31	1.34 *m*	H-30 (1.25), H-32 (0.89)	22.6 (CH_2)
32	0.89 *t*	H-31 (1.34)	14.09 (CH_3)
33	6.99 *d*	H-34 (4.98), H-3 (2.26), H-4 (1.55)	148.90 (CH)
34	4.98 *dq*	H-33 (6.99), CH_3-35 (1.40), H-3 (2.26)	77.38 (CH)
35	1.40 *d*	H-34 (4.98), H-3 (2.26)	19.20 (CH_3)

* $J_{3\text{-}4}$ = 7 Hz; $J_{3\text{-}35}$ = 1.44 Hz; $J_{8\text{-}9}$ = $J_{11\text{-}12}$ = 7 Hz; $J_{31\text{-}32}$ = 7 Hz; $J_{33\text{-}34}$ = 1.6 Hz; $J_{34\text{-}35}$ = 6.8 Hz.
,* Chemical shifts may be inverted.

The position of the carbonyl and epoxide groups in the alkyl chain of corepoxylone (1) was deduced from the mass spectra analysis. Conventional electron impact mass spectrometry (EIMS) showed intense ion at *m/z* 223 resulting from an α-cleavage to the carbonyl $[CO\text{-}(CH_2)_7\text{-lactone}]^+$, typical of the corossolone type.[4] In addition, ion at *m/z* 238 formed by Mc Lafferty fragmentation with the initial charge on the oxygene atom of the carbonyl group, including the γ-lactone moiety, was present. Loss of one molecule of water gave another peak at *m/z* 220 which was confirmed by the presence of a metastable ion.

The EI mass spectrum lacked fragment ions allowing to determine the position of epoxy rings in the alkyl chain. So, Liquid - Secondary Ion Mass Spectrometry (L-SIMS) and collision-induced dissociations (CID) ions, using linked scan at constant B/E were performed.[6] For location of the epoxy rings at C-15 , C-16 and C-19, C-20 the presence of two pairs of peak at *m/z* 385, 397 and 315, 327 was crucial. Comparison to the CID spectrum of diepomuricanin, the first diepoxy acetogenin reported so far,[6] showed the same series of ions, shifted by 14 mass units, which confirmed the presence of the carbonyl group and the two epoxy rings placed between 15- and 20-positions, separated by two methylenes. Two peaks of weak intensity at *m/z* 287 and 275 formed by fragmentation of the epoxide ring placed at C-15, C-16, and containing the methyl terminal, have also been observed (Figure 2).

Figure 2. Fragment ions in the CID, constant B/E linked-scan spectrum of corepoxylone (1)



Corepoxylone (1) is the first example of acetogenin in which tetrahydrofuran ring is replaced by two epoxide groups, and possessing a carbonyl group in the aliphatic chain. As a proof of the previously proposed biogenetical pathway, these epoxy units might play a key role in the biosynthesis of mono-tetrahydrofuran acetogenins. Indeed, such a biogenetical hypothesis has been proposed for a related compound by Ito *et al.*[9] and by Jolad for the biogenesis of uvaricine.[10] So, corepoxylone (1) bearing a carbonyl group at C-10 might serve as precursor of corossolone (2) previously isolated from *A. muricata*.[4] To confirm this hypothesis, biomimetic synthesis of corossolone (2) was carried out. Our previous essays of the base catalyzed transformation of (1) into (2) by either KOH or NaOH[11] did not give the expected product. Furthermore, addition of MeOH in the reaction medium has only resulted in the opening of γ-unsatured lactone, as expected,[12] without formation of corossolone. So, the method of acid catalyzed reaction with perchloric acid was performed.[13] The opening of the epoxide system of (1) can either occur at C-15 or C-20 position. In this way we have obtained the expected compounds bearing a tetrahydrofuran ring and giving only one spot by TLC. ^1H NMR spectrum of reaction products displayed the characteristic *threo/trans/threo* (and not *threo/trans/erythro*) relative configuration for the chiral centres of THF system[4,14] which confirmed the *cis* relationship of the two epoxyde rings (a *trans* relationship would have afforded a THF system with *erythro/trans/erythro* relative configuration). The CIMS

spectral data were in agreement with those of corossolone (**2**).[4] The HPLC analysis of the obtained mixture showed the presence of two peaks (in 1:1 ratio) which were attributed to corossolone (**2**) and its tetraepimer. The peak corresponding to more polar compound was identified as natural corossolone (**2**) by the method of internal standard (Figure 3). The less polar compound is a tetraepimer of (**2**) with the same relative configuration across the tetrahydrofuran ring (*threo/trans/threo*) but of opposite absolute configuration.[15]

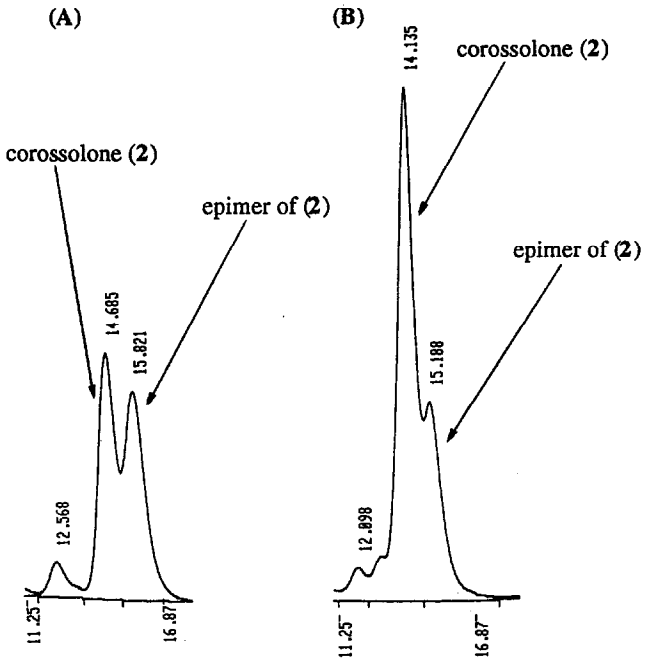


Figure 3 . HPLC analysis of : **A**) synthetic mixture (12.5 μg) of corossolone (**2**) and its tetraepimer obtained from corepoxylone (**1**) in acidic medium; and **B**) the same synthetic mixture (8.9 μg) and natural corossolone (**2**) (5 μg).

In the present work we demonstrate the possible separation of two compounds having the same absolute configuration for the chiral centre in the lactone ring but with opposite absolute configurations for the stereogenic centres across the THF skeleton.[16] In addition, this separation confirms the diastereoisomeric purity of natural corossolone (**2**).

It is noteworthy that corepoxylone (**1**), considered as a precursor of corossolone (**2**), showed higher cytotoxicity on KB and VERO cell culture systems (LD_{50} = $1.6x10^{-3}$ μg/ml and 2.5 μg/ml, respectively) that (**2**) showing LD_{50} = 10^{-1} and $3x10^{-1}$ μg/ml in the above mentioned tests, respectively.

The results presented in this paper confirm the biosynthesis of mono-tetrahydrofuran acetogenins from polyunsaturated fatty acids derivatives *via* epoxidation of the olefinic functions followed by ring opening and ring closure to afford the tetrahydrofuran system.[17]

EXPERIMENTAL

General methods. UV spectra were measured in MeOH and IR spectra as films. ^1H NMR and ^{13}C NMR ($CDCl_3$) spectra were recorded on a AC 200 Bruker at 200 and 50 MHz, respectively.

Plant material. Seeds of *A. muricata* were collected on the cultivated trees of this species by Marçal de Queiros Paulo in João Pessoa in Brazil.

Extraction and isolation. Extraction and fractionation were evaluated for lethality to brine shrimp larvae *Artemia salina.*[18] Dried and powdered seeds (8 kg) were extracted by MeOH (100 l), the resultant extract was concentrated *in vacuo* to give 833 g of dry extract (**E1**) (LD_{50} = 5 ppm). 100 g of **E1** was partitioned between MeOH-H_2O and hexane to yield 25 g of hexanic extract (**E2**) (LD_{50} = 20 ppm) and MeOH-H_2O extract (**E3**) (LD_{50} < 5 ppm). After evaporation of methanol, a liquid/liquid partition between H_2O and CH_2Cl_2 of extract **E3** gave 60 g of the dichloromethane extract **E4** (LD_{50} = 0.9 ppm). Aqueous extract (**E5**) showed LD_{50} > 100 ppm. Dichloromethane extract (**E4**) was applied to a column of Silica gel (70-230 mesh) packed in a hexane slurry, and developped with hexane containing gradually increasing amounts of EtOAc, finally with MeOH. Elution with hexane-EtOAc (3:2) yielded fractions 70-85 containing (**1**) which was repurified by column chromatography (Silica gel 60H) using toluene-EtOAc (3:1) as eluant, to afford 50 mg of pure (**1**).

Corepoxylone (**1**). Amorphous powder. $[\alpha]_D$ = +36.8 (c 0.08, $CHCl_3$). UV λ max. nm (log ε): 228 (3.09). IR ν max. cm^{-1}: 1750, 1705, 1465, 1270, 1080, 750. L-SIMS (NBA + LiCl), *m/z*: 567 $[M+Li]^+$, 537, 482, 455, 413, 397, 385, 327, 315, 287, 257. EIMS (70 eV), *m/z* 560 $[M]^+$, 238, 223 $[CO\text{-}(CH_2)_7\text{-lactone}]^+$, 220, 195 $[(CH_2)_7\text{-lactone}]^+$, see Figure 2. ^1H and ^{13}C NMR spectra, see Table 1.

Preparation of corossolone (**2**) *from corepoxylone* (**1**) : (**1**) (3.9 mg) in acetone (40 µl) was treated with 70 % perchloric acid (5 µl). The reaction mixture was stirred 8 h at room temperature, then evaporated to dryness under vacuum. The crude material was purified by TLC (hexane-EtOAc 2:3) to afford 2 mg (ca. 49%) of a mixture of two compounds which were identified as corossolone (**2**) and its tetracepimer on the basis of HPLC, ^1H NMR and CIMS analysis. ^1H NMR of mixture of (**2**) and its epimer identical to ^1H NMR spectrum of (**2**).[4,16] CIMS (*iso*-butane), *m/z*: 579 $[M+H]^+$, 561 $[M+H\text{-}H_2O]^+$, 309, 269, 199, 195, 181, EIMS (70 eV), *m/z* 361, 309, 269, 251, 223, 199, 195, 181.

The HPLC analysis was performed on a Waters system, consisting of an 6000-A pump, U6K injector, a UV detector Model 455 LC Spectrophotometer and a 740 Data Module recorder/integrator. The separation was carried out isocratically at 25°C on a µBondapak C18 300 x 3.9 mm I.D. column. The mobile phase (MeCN-H_2O-THF 80:20:1) was degassed by vacuum filtration through a membrane filter (fluoropore, 0.5 µm, Millipore) and the flow rate was 1ml/min. The standard and sample solutions were filtered through a Millex-HV 0.45 µm filter units, Millipore.

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15. ^{1}H NMR spectra of (**2**) and its tetraepimer are identical because of the same relative configuration for the chiral centres of THF system. The presence of chiral centre at C-34 do not allow to differentiate these two compounds by NMR (**2** and its tetraepimer are pseudoenantiomers).
16. Recently, we have realized the separation of isomeric acetogenins with different relative configurations for the tetrahydrofuran system: rolliniastatin-1 (*threo/cis/threo/cis/erythro*) and rolliniastatin-2 (*threo/trans/threo/trans/erythro*), by the means of HPLC.[17]
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