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ANNOSENEGALIN AND ANNOGALENE: TWO CYTOTOXIC MONO-TETRAHYDROFURAN ACETOGENINS FROM *ANNONA SENEGALENSIS* AND *ANNONA CHERIMOLIA*

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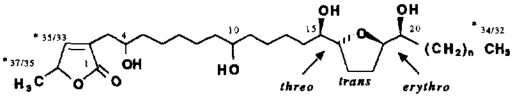
Key Word Index—*Annona senegalensis*; *A. cherimolia*; Annonaceae; acetogenins; mono-tetrahydrofuran γ-lactones; cytotoxicity.

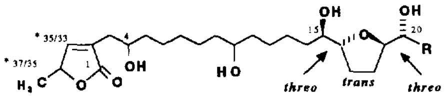
Abstract—Two new cytotoxic mono-tetrahydrofuran acetongenins, annosenegalin and annogalene, have been isolated from the cytotoxic methanolic extract of *Annona senegalensis* and *A. cherimolia* seeds. Their structures were established on the basis of 1D and 2D NMR spectroscopic techniques. Annosenegalin belongs to the rare type of C_{37} mono-tetrahydrofuran acetogenins, and annogalene is an olefinic acetogenin.

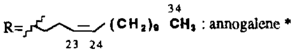
INTRODUCTION

Annona senegalensis Pers. is a shrub native to Senegal and is used in traditional medicine [1]. In our previous study of *A. senegalensis* seeds, we isolated a new mono-tetrahydrofuran (mono-THF) γ-lactone acetogenin (senegalene) and four known bis-tetrahydrofuran (bis-THF) γ-lactone acetogenins (molvizarin, asimicin, rolliniastatin-2 and squamocin) [2]. *Annona cherimolia* Mill. is a tropical tree native to Peru, which is used in traditional medicine as an insecticide and a parasiticide [3]. In our previous studies of *A. cherimolia* seeds, we isolated nine new bis- and mono-THF acetogenins (cherimolin-1, cherimolin-2, almunequin, itrabin, laherradurin, otivarin, jetein, molvizarin and motrilin), two known bis-THF acetogenins



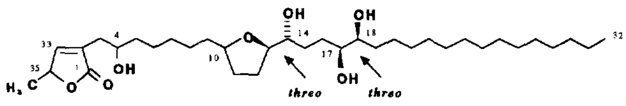
1: n=13: annosenegalin *
3: n=11: annonacin-A





2: R= $(CH_2)_8$ CH_3 : annogalene *

4: R= $(CH_2)_{11}$ CH_3 : annonacin



(rolliniastatin-2 and squamocin) [4–9], and three 'iso'-acetogenins (isocherimolin-1, isomolvizarin-1 and isomolvizarin-2) [10]. All these compounds showed antiparasitic and cytotoxic activities [11–14]. Some of these acetogenins are very potent inhibitors of the NADH: ubiquinone oxidoreductase (complex I) activity of mammalian mitochondria [15].

We report here on the structural elucidation, on the basis of 2D NMR and mass spectroscopic methods, of two novel cytotoxic mono-THF acetogenins, annosenegalin (**1**) and annogalene (**2**), as well as the isolation of three known mono-THF acetogenins, annonacin-A (**3**), annonacin (**4**) and gigantetrocin (**5**), from *A. senegalensis* seeds, and of **1**, **3** and **5** from *A. cherimolia* seeds.

RESULTS AND DISCUSSION

The methanolic extract from *A. senegalensis* seeds showed antiparasitic activity against *Leishmania major*, *L. donovani* and *Trypanosoma brucei brucei* [14]. This extract (with 10% H_2O) was partitioned with hexane, and the aqueous methanolic fraction extracted with methyl chloride. The methylene chloride soluble portion showed antiparasitic activity (against *Leishmania* and *Trypanosoma*), and an LC_{50} of 1.7×10^{-2} μg ml^{-1} in the brine shrimp larvae (*Artemia salina*) assay. On fractionation by flash chromatography, 60H silica gel column chromatography and HPLC it gave five bioactive mono-THF γ-lactone acetogenins, two of which are new, annosenegalin (**1**) and annogalene (**2**), and three others being the known compounds annonacin-A (**3**) [16], annonacin (**4**) [17] and gigantetrocin (**5**) [18].

Compound **1** was assigned the molecular formula $C_{37}H_{68}O_7$ {FAB mass spectrum (+LiCl), *m/z* 631 $[M+Li]^+$ and *m/z* 625 $[MH]^+$}. It gave a positive reaction with Kedde reagent and an absorption at 1745 cm^{-1} in the IR spectrum, characteristic of an α,β-unsaturated γ-lactone acetogenin [13]. The presence of four hydroxyl groups in **1** was suggested by a broad hydroxyl absorption at 3400 cm^{-1} in the IR spectrum, the sequential loss of four molecules of H_2O from the $[MLi]^+$ (FAB mass spectrum (+LiCl)), and the formation of a tetraacetyl derivative on treatment with acetic anhydride–pyridine.

The 1D (^{1}H and ^{13}C NMR and spin echo) and 2D homo- and hetero-nuclear correlations (COSY 45 and XH CORR) of **1** were typical of α,α'-dihydroxylated mono-THF γ-lactone acetogenins [19]. The signals for an α,β-unsaturated γ-methyl γ-lactone (4-pentanolide) system was observed in the ^{1}H NMR spectrum (δ 7.17, H-35; δ 5.05, H-36; δ 1.42, CH_3-37) and in the ^{13}C NMR spectrum (δ 151.83, CH-35; δ 78.00, CH-36; δ 19.13, CH_3-37) of **1**. Compound **1** showed an ABX spin system characteristic of the presence of a hydroxyl group at C-4 [20]: δ 3.82/69.96 (CH-4) and δ 2.41, 2.53/33.43 (CH_2-3).

The remaining five groups associated with oxygen-bearing carbon atoms belong to a hydroxyl group in the alkyl chain, and a mono-THF α,α'-dihydroxylated system, respectively. A multiplet (1H) at δ 3.57, which correlated with a methine carbon signal at δ 71.70, was characteristic of a hydroxyl group in the alkyl chain similar to that observed for **3** at C-10 [21]. Typical resonances in the ^{1}H NMR spectrum about δ 3.82 and 3.40, correlated with oxygenated carbons at δ_c 83.24, 82.26 and 71.70 and at δ 74.31, respectively, and correspond to the rare mono-THF α,α'-dihydroxylated system with the relative configuration *threo/trans/erythro* (Fig. 1). Other examples, **3** [16] and jetein [7], a β-hydroxy-γ-methyl γ-lactone acetogenin, belong to this class. In nature, the more frequent relative configuration shown in mono-THF α,α'-dihydroxylated acetogenins is *threo/trans/threo* i.e. of the annonacin type [19].

Analysis of the mass fragmentation of **1** (Fig. 2) showed that annosenegalin possessed a similar structure to **3**, with a hydroxyl group at C-10, and a mono-THF α,α'-dihydroxylated system between C-15 and C-20. However, **1** had two more methylene groups than **3** in the alkyl chain. Annosenegalin is the first tetrahydroxylated C_{37} mono-THF acetogenin with a *threo/trans/erythro* relative configuration.

Compound **2** was assigned the molecular formula $C_{37}H_{66}O_7$ {FAB mass spectrometry (+LiCl), *m/z* 629

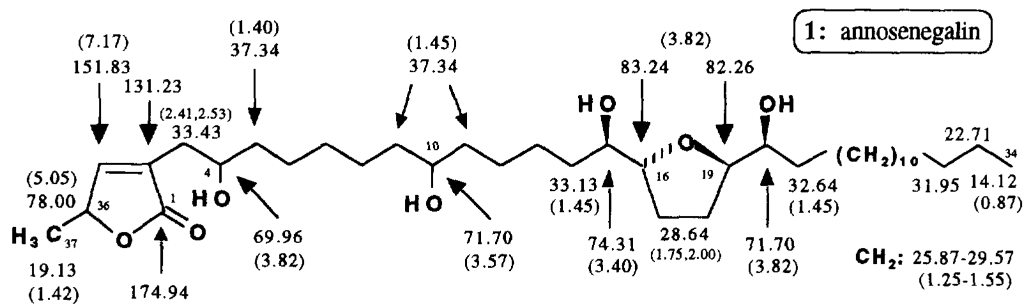
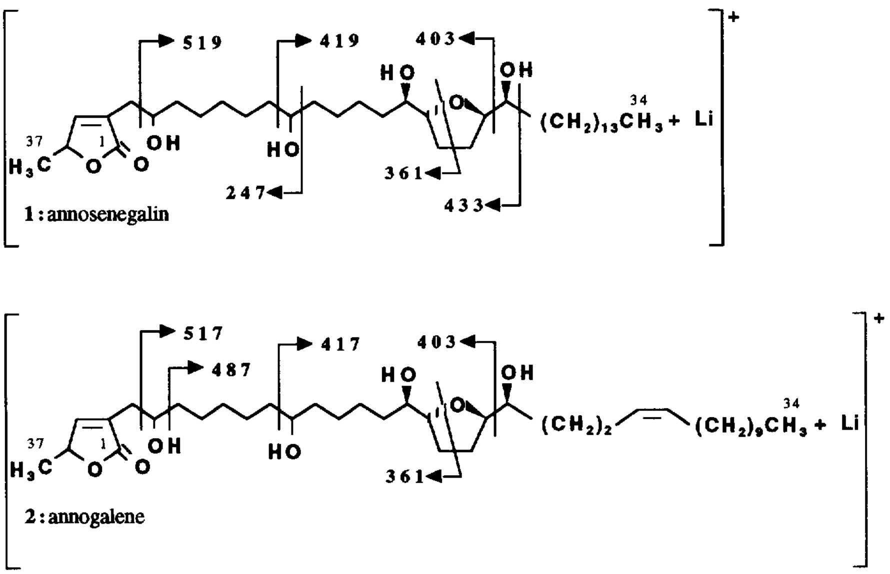


Fig. 1. Results of ^{13}C NMR (^{1}H NMR in parentheses) and 2D NMR experiments of annosenegalin (1). ^{1}H NMR



1 : annosenegalin

2 : annogalene

Fig. 2. Fragment ions in FABMS (+Li) of compounds **1** and **2**.

$[M+Li]^+$ and *m/z* 623 $[MH]^+$}. Its structure was elucidated by 2D NMR spectroscopy and FAB mass spectrometry compound. **2** showed four hydroxyl groups placed at C-4, 10, 15 and 20, identical to those of **1**. The mono-THF α,α'-dihydroxylated system of **2** was assigned the same *threo/trans/threo* relative configuration, based on $^1H/^{13}C$-NMR correlations, as **4** [19].

The remaining NMR signals for **2** were characteristic of a mono-THF γ-lactone acetogenin [19]; however, two sp^2 methine resonances in the ^{13}C NMR spectrum at δ 130.73 and 128.88, correlated with multiplets at δ 5.38 and 5.33 (1H each) in the ^1H NMR spectrum, and were consistent with an olefinic acetogenin with a double bond in the alkyl chain (giganenin type: the first mono-THF olefinic acetogenin, isolated from *Goniothalamus giganteus* [21]).

The position of the double bond was determined by mass spectrometry and 2D NMR (Figs 2 and 3). Fragment ions obtained by FAB mass spectrometry + Li at *m/z* 361 and 403 suggested the presence of a double bond between the mono-THF system and the

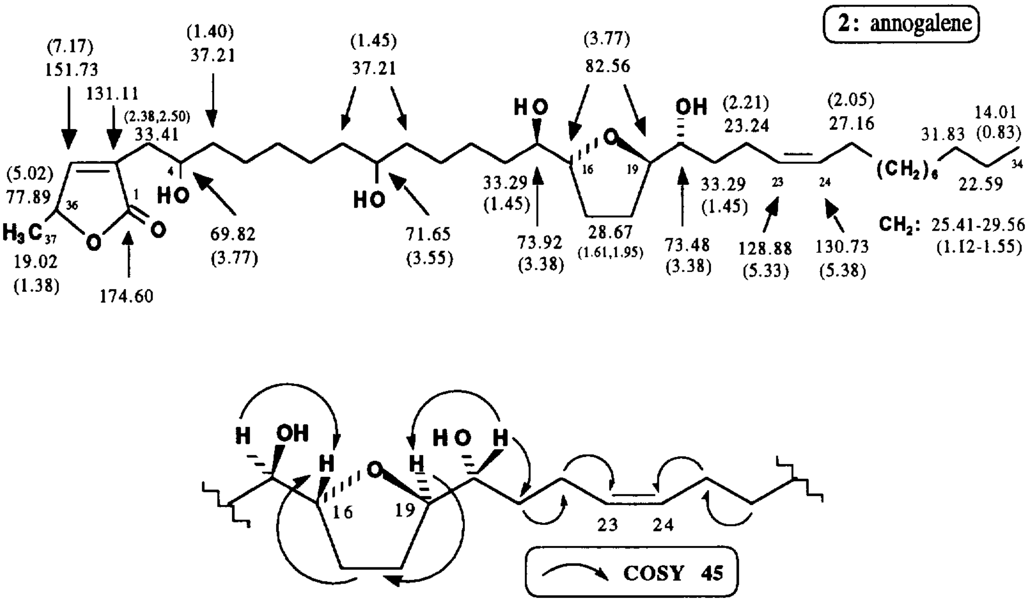


Fig. 3. ^{13}C NMR (^1H NMR in parentheses) and 2D NMR experiments of annogalene (2). ^1H NMR spectra were recorded at

terminal CH_3-34. The 400 MHz COSY spectrum of **2** revealed correlations between the olefinic protons (δ 5.33 and 5.38) and two methylenes (δ 2.21 and 2.05, respectively). A multiplet at δ 2.21 was correlated with resonances at δ 1.45, and this was correlated with the methine hydroxyl carbon belonging to the mono-THF α,α'-dihydroxylated system (δ 3.38 at position 20). The other methylene multiplet, α to olefinic group (δ 2.05), was correlated with the resonances of methylenes of the alkyl chain (δ 1.3).

Thus, the double bond of **2** was unambiguously determined to be at the C-23/24 position i.e. two carbons from the mono-THF α,α'-dihydroxylated system similar to that of bullatencin [22], squamosten-A [23] and gonionenin [24]. Selective decoupling of methylenes 22 and 25 indicated that the double bond has a *cis* configuration ($J_{23\text{-}24} = 10$ Hz). Compound **2** is a new C_{37} mono-THF acetogenin isomer to squamosten-A [23] and gonionenin [24]. Recently, an olefinic mono-THF acetogenin, xylomatenin, which is very similar to annogalene, has been reported from *Xylopia aromatica* [25].

Some of the mono-THF acetogenins isolated from *A. senegalensis* seeds (**1–5**) were isolated from *A. cherimolia* seeds: annosenegalin (**1**), annonacin-A (**3**) and gigantetrocin (**5**).

EXPERIMENTAL

Optical rotations: 589 nm; IR: film; ^1H NMR and ^{13}C NMR: $CDCl_3$ sol; EIMS: Nermag-Sidar; FAB MS: Kratos MS-80.

Plant material. Seeds of *A. senegalensis* Pers. were obtained from ripe fruits collected in Senegal in July 1990. The seeds of *A. cherimolia* Mill. were obtained from fruits ('cherimoya') collected in December 1989 in 'cherimoyo-vale', Granada Coast (Spain). Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy and Medicine of Dakar in Senegal (reference AL 282) and in the herbarium of the Department of Botany, University of Valencia, Spain (reference VF 10463), respectively.

Extraction and isolation. Dried and powdered seeds of *A. senegalensis* (2.3 kg) were macerated with MeOH at room temp. The conc MeOH extract (155 g, A) was partitioned between hexane and 10% aq MeOH. After removal of the hexane fr. (47 g, B), the aq. MeOH sol was partially evap under red. pres. and extracted with CH_2Cl_2. The CH_2Cl_2 soluble portion was dried and conc (56 g, C) and fractionated by silica gel flash chromatography (Merck 9385; elution with CH_2Cl_2–AcOEt–MeOH, 20:19:1) and by 60H silica gel CC (Merck 7736; elution with CH_2Cl_2–MeOH 24:1). Extraction and fractionation were monitored by Kedde reagent, and the brine shrimp (*Artemia salina*) test. Five bioactive mono-THF γ-lactone acetogenins were isolated. **1** (5 mg), **3** (15 mg) and **5** (12 mg) were isolated from *A. cherimolia* seeds (1 kg).

Annosenegalin (**1**). Obtained in an amorphous form by prep. HPLC: μ-Bondapak C_{18}; MeOH-H_2O 17:3, R_t 24.5 min.; $C_{37}H_{68}O_7$; $[\alpha]_D + 15°$ ($CHCl_3$; *c* 0.27); IR ν_{max} (film) cm^{-1}: 3400, 2910, 2845, 1745; EIMS, *m/z*: 397, 379, 361, 343, 327, 309 (100%), 297, 291, 273, 241, 227, 223, 141, 111; FABMS (NBa + LiCl), *m/z*: 631 $[M + Li]^+$ (100%), 625 $[MH]^+$, 613 $[MLi - H_2O]^+$, 595 $[MLi - 2\ H_2O]^+$, 577 $[MLi - 3H_2O]^+$, 559 $[MLi - 4H_2O]^+$ (see Fig. 2); ^1H NMR (200 MHz, $CDCl_3$) and ^{13}C NMR (50 MHz, $CDCl_3$): Fig. 1.

Annogalene (**2**). Obtained in an amorphous form by prep HPLC: μ-Bondapak C_{18}; MeOH–H_2O 17:3; R_t 21.8 min.; $C_{37}H_{66}O_7$; $[\alpha]_D + 14°$ ($CHCl_3$; *c* 0.31); IR ν_{max} (film) cm^{-1}: 3350, 2910, 2845, 1750; EIMS, *m/z*: 397, 379, 361, 343, 327, 309 (100%), 297, 295, 291, 273, 241, 225, 223, 141, 111; FABMS (NBa + LiCl), *m/z*: 629 $[M + Li]^+$(100%), 623 $[MH]^+$, 611 $[MLi - H_2O]^+$, 593 $[MLi - 2H_2O]^+$, 575 $[MLi - 3H_2O]^+$, 559 $[MLi - 4H_2O]^+$, (see Fig. 2); ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Fig. 3.

Annonacin-A (**3**). Obtained in an amorphous form by prep. HPLC: μ-Bondapak C_{18}; MeOH–H_2O 17:3; R_t 14.5 min.; $C_{35}H_{64}O_7$; IR ν_{max} (film) cm^{-1}: 3400, 2910, 2845, 1745; FABMS (NBa + LiCl), *m/z*: 603 $[M + Li]^+$ (100%), 597 $[MH]^+$, 585 $[MLi - H_2O]^+$, 567 $[MLi - 2H_2O]^+$, 549 $[MLi - 3H_2O]^+$, 531 $[MLi - 4H_2O]^+$, 491, 433, 403, 391, 361; see ref. [16].

Annonacin (**4**). Obtained in an amorphous form by prep. HPLC: μ-Bondapak C_{18}; MeOH–H_2O 17:3; R_t 17.2 min; $C_{35}H_{64}O_7$; IR ν_{max} (film) cm^{-1}: 3400, 2910, 2845, 1745; FABMS (NBa + LiCl), *m/z*: 603 $[M + Li]^+$ (100%); see ref. [17].

Gigantetrocin (**5**). Obtained in an amorphous form by prep. HPLC: μ-Bondapak C_{18}; MeOH-H_2O 17:3; R_t 19.3 min; $C_{35}H_{64}O_7$; IR ν_{max} (film) cm^{-1}: 3400, 2910, 2845, 1745; FABMS (NBa + LiCl), *m/z*: 603 $[M + Li]^+$ (100%), 597 $[MH]^+$, 585 $[MLi - H_2O]^+$, 567 $[MLi - 2H_2O]^+$, 549 $[MLi - 3H_2O]^+$, 531 $[MLi - 4H_2O]^+$, 491, 461, 405, 375, 317, 287; see ref. [18].

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