Planta Medica

Natural Products and Medicinal Plant Research

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333 Seventh Avenue
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Reprint
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Tucumanin. a β-Hydroxy-γ-lactone Bistetrahydrofuranic Acetogenin from *Annona cherimolia,* is a Potent Inhibitor of Mitochondrial Complex I

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Abstract

A new {3-hydroxy-y-methyl-y-lactone bistetrahydrofuranic acetogenin, tucumanin, with the infrequent symmetrical *threo/trans/threo/trans/threo* relative configuration at the tetrahydrofuran rings was isolated from *Annona cherimolia* (Annonaceae) seeds. The inhibitory potency on the mitochondrial complex I of acetogenins with this relative configuration (tucumanin and asimicin) was compared with that shown by the corresponding pairs with an asymmetrical *threo/trans/threo/trans/erythro* relative configuration (laherradurin/rolliniastatin-2, and itrabin/molvizarin). All these compounds act as selective inhibitors of mitochondrial complex I in the 0.18 -1.55 nM range.

"Cherimoya" is an edible fruit from a tropical tree (Annona *cherimolia* Mill., Annonaceae) native to Peru that is cultivated worldwide. In previous chemical studies on the seeds of A. *cherimolia* cultivated in an small pseudo-tropical area in the south of Spain, on the coast of Granada, four monotetrahydrofuranic (mono-THF) and eleven bistetrahydrofuranic (bis-THF) acetogenins were isolated [1], [2]. Recently, three new cytotoxic mono-THF acetogenins, annocherin, annomolin, and annocherimolin, were isolated from seeds of "cherimoya" grown in plantations of Southern California [31,14].

The Annonaceous acetogenins are claimed as potent inhibitors of NADH:ubiquinone oxidoreductase (complex I) in the mitochondrial electron transport chain [5]. Among them, the {3-hydroxy-y-methyl-y-Iactone acetogenins, a type of acetogenin only isolated from the seeds of A. *cherimolia,* are more potent complex I inhibitors than any other natural acetogenin [6]. The high potency of this class of acetogenins led us to further investigate the composition of seeds collected in Tucuman (Argentina).

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Received: January 29, 2004 . Accepted: June 4. 2004

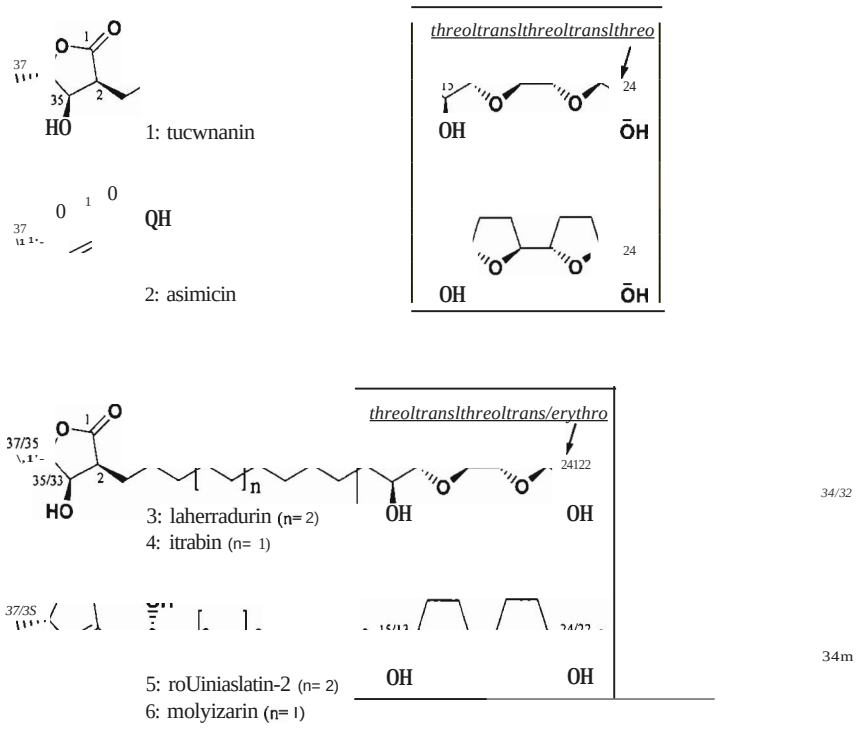
Bibliography: Planta Med 2004; 70: 866-868 . © Georg Thieme Verlag KG Stuttgart· New York· *DOI1O.1055/s-2004-827237*· ISSN 0032-0943

Fractionation of the methanolic extract furnished 11 known acetogenins (almunequin, asimicin, cherimolin-1, itrabin, laherradurin, molvizarin, motrilin, neoannonin, rolliniastatin-2, squamocin, squamocin-B) and a new acetogenin, tucumanin (1). The molecular weight of 1 was established by the $[M+Na]^+$ ion observed at *m/z* = 647.45183 (calcd. 647.44988 for $C_{37}H_{68}O_7Na$) in the HR-LSI-MS. Absorptions at 204 nm and at 1760 cm^{-1} in the UV and IR spectra, repectively, are in accordance with the presence of a saturated y-lactone ring in 1[1]. The ^{1}H-NMR spectrum and the 20 homonuclear correlation (lH-IH COSY 45) of 1. prove the existence of an ABX spin system, characteristic of the three lactone ring protons of a (3-hydroxy-y-methyl-y-lactone acetogenin [7], [8]: H-2 (α) at δ = 2.58, H-35 ({3) at δ = 4.19, and H-36 *(y)* at δ = 4.49. These protons are correlated in 20 heteronuclear NMR experiments (HMQC and HMBC) with three methine carbons at δ = 43.7, δ 73.7, and δ 82.4, respectively (Fig.1). The relative configuration of the lactone moiety deduced from the coupling constants, $J_{\alpha-\beta}$ = 5.5 Hz *(cis)* and $J_{\beta-\gamma}$ = 1.0 Hz *(trans),* was in agreement with that reported by Sibi et al. for the synthetic laherradurin [9].

The presence of an α, α′-dihydroxylated bis-THF system with the infrequent symmetrical *threo/trans/threo/trans/threo* relative configuration was deduced from IH-13C NMR heteronuclear correlations between the signals at δ = 3.38 and δ = 74.0 (two methine alcohol), and between the signals at δ = 3.81 and δ = 83.1 (two methine tetrahydrofuran) [lOJ. This system is placed between C-15 and C-24 based on the fragment ions observed in the EI-MS (Fig.1). Compound 1 is the first {3-hydroxy-y-methyl y-lactone adjacent bis-THF acetogenin with a *threo/trans/threo/trans/ threo* relative configuration.

In previous studies we have shown that the inhibitory potency of acetogenins is modulated by several structural factors. Therefore, it is relevant to compare the inhibitory potency of acetogenins with this type of configuration (tucumanin, 1 and asimicin, 2) with that of the corresponding pairs with an asymmetrical *threo/trans/threo/trans/erythro* relative configuration (laherradurin, 3/rolliniastatin-2, 5, and itrabin, 4/molvizarin, 6). As potency criterion we used the IC_{50} against the integrated NADH oxidase activity of beef heart open submitochondrial particles [5]. [6], [12]. which evaluates the complex I activity in a more physiological environment. Results are shown in Table 1.

The {3-hydroxyacetogenins with a *threo/trans/threo/trans/erythro* relative configuration, laherradurin (3) and itrabin (4) (lC_{50} = 0.18 and 0.21 nM, respectively) are more potent inhibitors of the mitochondrial complex I than the corresponding α,β-unsaturated y-Iactone acetogenins, rolliniastatin-2 (5) and molvizarin (6) (lC_{50} = 0.51 and 1.55 nM, respectively). However, tucumanin (1) (lC_{50} = 0.57 nM) shows an inverted tendency when compared to asimicin (2) (lc_{50} = 0.33 nM), both with a *threo/trans/threo/trans/threo* relative configuration. Therefore, our data show that, indeed, the terminal y-methyl-y-Iactone ring and the relative configuration at the THF system are both relevant structural factors for a potent inhibition of the mitochondrial complex I, because of a double binding to the active site of the enzyme to which both moieties probably contribute.



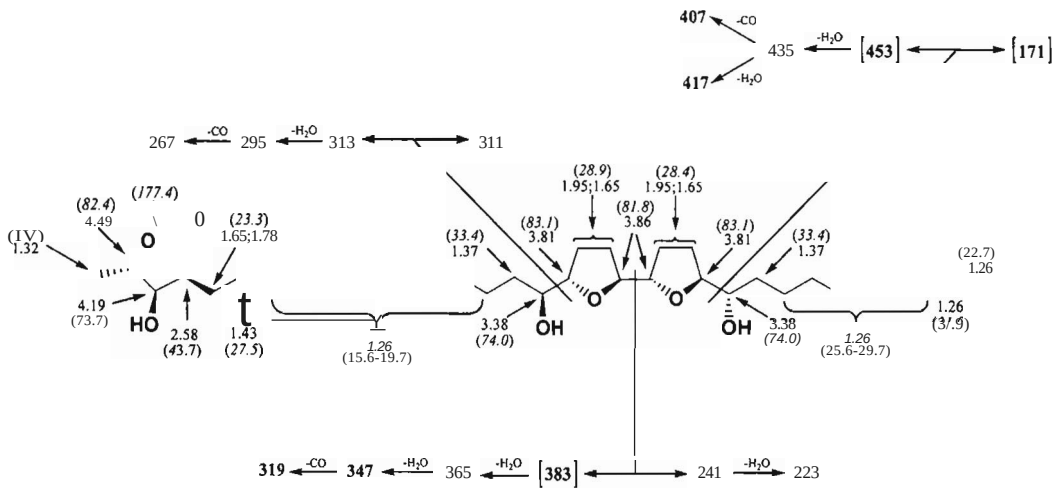


Fig. 1 1H_13C NMR heteronuclear correlations (13C in parentheses) spectral data, and significant EIMS fragment ions of tucumanin (1).

Table 1 Inhibitory potency of acetogenins 1- 6

	Compound						
	2	3	4	5	6	*rotenone* {6]
NADH oxidase 1(50 (nM)	0.57 ± 0.07*	0.33 ± 0.03'	0.18 ± 0.02*	0.21 ± 0.03'	0.5i ± 0.03'	1.551'0.17'	5.10 ± 0.09

Significant difference compared with rotenone; • P< 0.001.

Material and Methods

Optical rotation (Perkin-Elmer 241 polarimeter), lR spectra (Satellite FTIR-Mattson 980514 spectrometer), MS (VG Auto Spec Fisons spectrometer), lH_ and 13C-NMR, DEPT, HMBC, HMQC, tH-1H-COSY (Varian Unity 400 MHz spectrometer) using the solvent signal as reference (CDCl) at δ = 7.26 and 77.0). Chromatographic separations were carried out by column chromatography on silica gel 60 H (5 - 40 μm, 7336 Merck). Semi preparative HPLC was carried out on a LiChroCart^R 100 RP-18 column (25 x 1 em i. d., 10 μm particle size), flow rate 4.5 mLjmin. using MeOH-$H_2$0-THF. Analytical TLC was performed on Merck precoated silica gel 60 F_{254} plates.

The fruits of *Annona eherimo/ia* Mill. ("cherimoya") were collected at Yerba Buena, Tucuman, Argentina, in February 2000. A voucher sample (No. 515092) was deposited at the Herbarium of Instituto Li110 ofTucuman.

The dried and powdered seeds of cherimoya (350 g) were percolated with methanol (2 x 3 L). Evaporation of the solvent yielded a crude MeOH extract (15 g) which was further partitioned between CH_2Cl_2 (3 x 100 mL) and H_20 (100 mL). The CH_2Cl_2 layer was washed with water, dried over anhydrous Na2S04, filtered, and evaporated in vacuum to give 9 g of a solid extract with an acetogenin mixture, which was chromatographed on a silica gel 60 H (315 g) column, using gradients of hexane/EtOAc (90 : 10; 80: 20; 70: 30; 60: 40; 50: 50; 40: 60; 20; 80 and 10: 90) giving eight fractions (F-I to F-VIII, 250 mL each). Fraction F-IH (hexane/EtOAc 70: 30, 660 mg) was again chromatographed on silica gel 60 H (23 g) and eluted with CH_2Cl_2/MeOH (95: 5) to give five subfractions (50 mL each), one of them (F-III]) was applied to semipreparative HPLC with MeOH-H_20-THF (87: 13: 5) affording neoannonin {12 mg, R_t = 10.8 min, $[\alpha]_D^{25}$: +16.2° (CHCl], e 0.8)}; , F-IV (hexane/EtOAc, 60: 40, 2 g) was chromatographed on silica gel 60 H (70 g) and eluted with CH_2Cl_2/EtOAc/MeOH (50: 45: 5) to yield eight subfractions (100 mL each). F-IV_5 and F-IV_6 by semipreparative HPLC with MeOH-H_20-THF 80: 20: 5 afforded squamocin {90 mg, R[= 16.6 min, $[\alpha]_D^{25}$: +14.0° (CHCl], e 0.13), squamocin-B (16 mg, R_r = 8.7 min, $[\alpha]_D^{25}$: +27.6 (MeOH, e 0.2) and motrilin (5 mg, R_r = 14.8 min, $[\alpha]_D^{25}$: +10.8' (MeOH, c O.13)}, and asimicin {2, 5 mg, R_r = 29.4 min, $[\alpha]_D^{25}$: +11.0° (CHCl], c 0.22), molvizarin (6, 10 mg, R_r _ 13.9 min, $[\alpha]_D^{25}$: +10.0° (CHCl3, e 0.2) and rolliniastatin-2 (5, 5 mg, R_t = 11.3 min, $[\alpha]_D^{25}$: +12.8° (CHCl3, e 0.26)), respectively. F-VI (hexane/EtOAc, 40: 60, 500 mg) was chromatographed on silica gel (18 g) and eluted with CH_2Cl_2/EtOAc/MeOH (60: 13: 10) to give four subfractions (75 mL each). One of them (F-VI_2) was applied to semipreparative HPLC with MeOH-H_20-THF (80: 20: 5) affording cherimolin-1 {IS mg, R_t = 8.9 min, $[\alpha]_D^{25}$: +64.0° (MeOH, e 0.3) and almunequin (5 mg, R_r = 5.8 min, $[\alpha]_D^{25}$: +13.5° (CHCl3, c 0.14)}. F-VIII (hexane/HOAc, 10: 90, 250 mg) was chromatographed on silica gel (9 g) and eluted with CH_2Cl_2/HOAc/MeOH (10: 85: 5) to give three subfractions (50 mL each). F-$VIII_2$ was applied to semipreparative HPLC with MeOH-H_20-THF (80: 20: 5) affording itrabin {4. 40 mg, R_r = 2.1 min. $[\alpha]_D^{25}$: +21.0' (MeOH, c 0.2), laherradurin (3,60 mg, R_t = 3.5 min, $[\alpha]_D^{25}$: +21.0° (MeOH, e 0.09) and tucumanin (1,11 mg, R_r = 4.2 min)}.

Tucumanin (1): waxy compound, $[\alpha]_D^{25}$: +1.5° (EtOH, e 0.8); UV (EtOH): λ_{max} (log ε) = 204 nm (3.8). IR (film): ν_{max} = 3360, 2923,2853,1760,1465,1052 cm-'. HR-LSI-MS: *m/z* = 647.45183 [M + Na]+ (caled. for C37H6807Na: 647.44988). EI-MS, IH-NMR (400 MHz). and I3C-NMR (100 MHz), see Fig.1.

The inhibitory potency of acetogenins as complex r inhibitors was assayed using submitochondrial particles from beef heart as previously reported [5], [11], [12]. NADH oxidase activity was measured as the aerobic oxidation of75 ,uM NADH in the absence of external quinone substrates and other inhibitors of the respiratory chain. Rotenone (Sigma-Aldrich St. Louis, MO, USA) was used as the positive control. Data from four titrations were used to determine the statistic means and the standard deviations. Significance of differences was evaluated by the Student's *t* test, with P< 0.001 being regarded as significant.

Aclmowledgements

This research was supported by the Spanish Direccion General de Investigacion under grant SAF 2001 -3142. and by the Secretaria del Plan Valenciano de Investigacion Cientifica, DesarrolloTecnologico e Innovacion de la Generalitat Valenciana under grant GVOI-282.

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