# Jahonin and asimicin acetogenins from *Annona jahnii (Annonaceae)*

## Jahonin y Asimicin Acetogenins de *Annona jahnii (Annonaceae)*

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**Resumen**

Una nueva acetogernina citotoxica, Jahonin (**1**) y la llamada asimicin (**2**) fueron aisladas a partir de las ramas de *Annona jahnii* Saff. (Annonaceae) usando fraccionamiento dirigido por ensayos de letalidad sobre camaroncitos de salina. **1** representa una acetogenina del tipo C-35, con ausencia de anillos de tetrahidrofurano (THF) y de epóxido, con un grupo ceto en C-10 y con un doble enlace, separado por dos metilenos de un diol vecinal. La estructura y la configuración relativa del diol vecinal en **1**, fueron establecidas mediante $^{1}$H- RMN, $^{13}$C-RMN, COSY, HMBC, HMQC y mediante un derivado químico. El acetónido derivado (**3**) fue preparado a partir del compuesto **1**, por reacción de **1** con una mezcla de HCl-acetona. Jahonin (**1**) mostró citotoxicidad frente a tres líneas de células tumorales humanas mediante un ensayo MTT de 24 h (Fadu, Hep-2, SVKO3 y HeLa).

**Palabras clave:** *Annona jahnii;* jahonin; acetogenin; citotoxicidad.

**Abstract**

A new cytotoxic Annonaceous acetogenin, jahonin (**1**) and asimicin (**2**), were isolated from the twigs of *Annona jahnii* Saff. (Annonaceae) by bioactivity-directed fractionation using lethality to brine shrimp. The known acetogenin, asimicin (**2**) is the first time has been reported from this species. **1** represents an unusual type of C-35 Annonaceous acetogenin, lacking either tetrahydrofuran (THF) or epoxide rings, bearing a keto group at C-10, and possessing a double bond located two methylenes away from a vicinal diol. The structure and relative configuration of vicinal diol in **1** were elucidated by $^{1}$H and $^{13}$C-NMR, COSY, HMBC, HMQC and from chemical derivatives. The acetonide derivative (**3**) was prepared from **1** by reactions with HCl-acetone mixture. Jahonin (**1**) showed cytotoxicities, among three human solid tumor cell lines in in our 24 h MTT human solid tumor panel (Fadu, SVKO3, and Hep-2 and HeLa).

**Key words:** *Annona jahnii;* jahonin; acetogenin; cytotoxicity.

## Introduction

Annonanceous acetogenins are bioactive plant secondary metabolites found only in several genera of the Annonaceous family. They are a unique class of long-chain fatty acids derivatives with potent *in vivo* and *in vitro* anticancer, apoptotic, pesticidal antimicrobial, antimalarial, antileishmanial activities (Bermejo, 2005; Zeng, 1996; Raynaud-LeGrandic, 2004; Chiu, 2003; Motoyama, 2002). Most of the 350 acetogenins, until now reported, contain a α-β-unsaturated γ-lactone ring and a mono or bis tetrahydrofuran (THF) core. Only few of them lack the THF ring (Zeng, 1996; Bermejo, 2005).

Annonaceous acetogenins are powerful inhibitors of glutamate-dependent mitochondrial respiration, in both mammalian and insect systems, where they inhibit mitochondrial NADH: CoQ oxidoreductase activity (Zeng, 1996; Motoyama, 2002; Bermejo, 2005; Barrachina, 2007; Cavé, 1997); they also are powerful inhibitors of the plasma membrane NADH oxidase of tumor cells (Murai, 2006; Morrè, 1995). They show selectivity for tumorous vs. normal cell

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(Oberlies, 1995); and they are equally effective against multidrug resistant tumor cells (Oberlies, 1997, (Oberlies, 1997).

The twigs of *Annona jahnii* Saff. (Annonaceae), collected in the state of Monagas (Venezuela), using brine shrimp lethality (BST)-directed fractionation (Meyer, 1982) of the ethanol extract, have yielded a new linear acetogenin jahonin (**1**) and the known acetogenin, asimicin; this is the first time that asimicin has been reported from this species. We already have reported the isolation of annojahnin (Colman-Saizarbitoria, 1998), annodienin and janhnonacin (Colman-Saizarbitoria, 1999) from the same species. Others linear acetogenins have been recently isolated (Liaw, 2005) Jahonin (**1**) is a new keto-acetogenin with a double bond and with neither a tetrahydrofuran (THF) nor an epoxide rings are present.

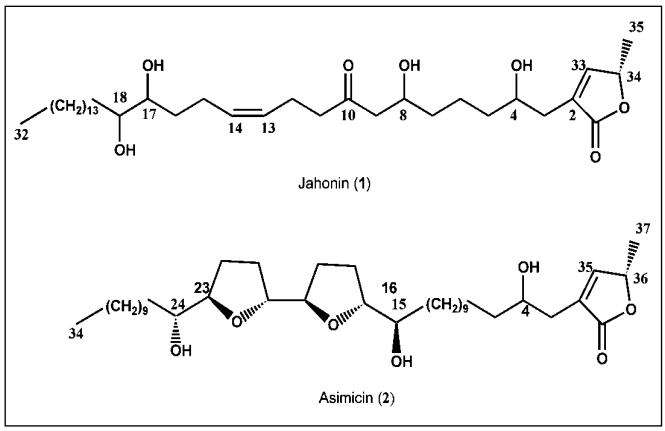


**Figure 1. Structures of Jahonin (1) and Asimicin (2)**

## Materials and Methods

### PLANT MATERIAL

Twigs of *Annona jahnii* Saff. Were collected in state Monagas of Venezuela, and authenticated by Professor Stephen Tillet at the Herbario Ovalles, Universidad Central de Venezuela, where a voucher specimen of twigs is deposited.

### CELL LINES

*The Human larynx carcinoma (Hep-2), Human pharynx carcinoma (Fadu), human ovary carcinoma (SVKO3), Human fibroblast and Human uterus carcinoma (HeLa) lines cells were grown in monolayer culture in Minimum Essential Medium (MEM) complemented with the addition of 10% of bovine fetal serum and 1% of a mixture (1:1) of penicillin/streptomycin. Medium was changed every 2 days and the cells cultivated at 37 °C, in a humidified atmosphere containing 5% of $CO_2$.*

### GENERAL PROCEDURES

Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra (film) were recorded on a Perkin-Elmer 1420 spectrometer. UV spectra were taken on a Beckman DU-7. $^1$H-NMR and $^{13}$C-NMR spectra were recorded in $CDCl_3$ solutions in a Bruker AV-400 MHz. The mass spectra were taken at 70 eV (probe) in a Shimadzu QP-2000, and in a VARIAN Saturn 2000. Silica Gel (200-300 mesh) was used for Column chromatography (CC) and silica HF-254 for TLC. Spots were detected on TLC by heating after spraying with 5% phosphomolybdic acid in EtOH.

### PRODUCTS, EXTRACTION AND ISOLATION.

The pulverized twigs (4.0 kg) were extracted with EtOH in a soxhlet for 24 h, and partitioned, as described above, to obtain FOO5. The bioactive (BST $LC_{50}$ 194 ppm) (Meyer, 1982) residue of FOO5 (60 g) was subjected to column chromatography over silica gel (2 kg) eluted with a gradient of hexane-EtOAc-MeOH. Fractions ($F_1$-1 to $F_1$-92) were combined based on TLC analysis. The active pool P6 ($F_1$-8 - $F_1$-15) (4 g, BST $LC_{50}$ 23 ppm) (Meyer, 1982) was further resolved on another silica gel (160 g) column, eluted with 33% of acetone in hexane and re-chromatographed on reverse phase column eluted with MeOH: $H_2O$ (gradient) to afford compounds **1** and asimicin (**2**) (Rupprecht, 1989).

The vicinal diol structure and relative configuration in **1**, where established by $^1$H- RMN, $^{13}$C-RMN, COSY, HMBC, HMQC and a chemical derivative. The acetonido derived **3** was a result of the reaction of compound **1** with HCl-acetona mixture.

***Jahonin* (1):** White waxy solid (17 mg); mp: 70-72 $^o$C; UV (1 max, MeOH, nm): 220, log e 3.31; IR (film) cm$^{-1}$: 3360, 2918, 2897, 1737, 1703, 1648, 1467, 1282, 1199, 669. CIMS m/z $(MH)^+$ 595 (50%), EIMS m/z $M^+$ 594 ( 15%) and fragmentation (figure 2); HREIMS m/z 594. 450008 $(MH)^+$ (calcd 594.449555 for $C_{35}H_{62}O_7$). $^1$H-NMR ($CDCl_3$, 400 $MH_2$ δ ppm). 2.35 (ddd, 1H, $H_{3a}$ J: 15; 3.3; 1.1 Hz), 2.21 (ddd, 1H, $H_{3b}$ J: 15; 8.6; 1.1 Hz), 3.83 (m, $H_4$), 1.49 (m, $H_5$), 1.22-1.54 (m $H_6$, $H_7$), 3.55 (m , $H_8$), 2.4(m, $H_9$), 2.38 (m, 1H, $H_{11}$), 2.39 (m, 1H, $H_{12}$), 5.35 (ddd, 1H, $H_{13}$ J:11.0; 7.0; 6.4 Hz), 5.34 (ddd, 1H, $H_{14}$ J: 11.0; 7.0; 6.5 Hz ), 2.25(m, 1H, $H_{15}$ ), 1.5 (m, 1H, $H_{16}$ ), 3.45 (m,1H, $H_{17}$), 3.45 (m, 1H, $H_{18}$), 1.22-174 (m, 1H, $H_{19}$), 1.22-1.74 (m, 2H, $H_{20}$ and $H_{30}$), 0.90 (t, 1H, $H_{32}$ J: 7.0), 7.12 (q,1 H, $H_{33}$), 4.96 (qd 1H, $H_{34}$) H-34, 1.39 (d, 1H, $H_{35}$, J: 7.0 Hz).

$^{13}$C-NMR ($CDCl_3$; 125 MHz, δ ppm): 175(C-1), 130.9 (C-2), 31.9 (C-3), 69.9 (C-4), 37.4 (C-5), 25.7-

32.0 (C-6-C-7), 75.5(C-8), 209(C-10), 128.8(C-13), 130.6(C-14), 74.3 (C-17), 74.1 (C-18), 33.4-33.5 (C-19), 25.5-32.0 (C-20-C-30), 22.7 (C-31), 14.2 (C-32), 151.1 (C-33), 78.0 (C-34), 19.2 (C-35).

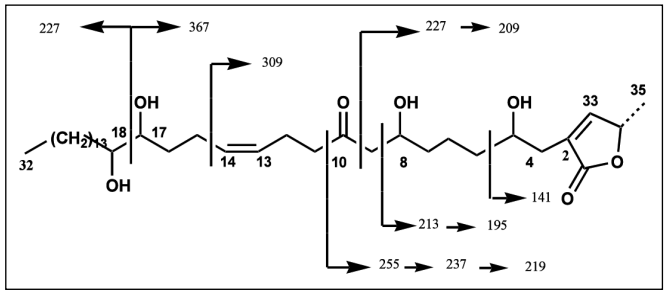


**Figure 2. Diagnostic eims fragmentation ions of Jahonin (1).**

**Asimicin (2)**: White waxy solid (8 mg); CIMS m/z $(MH)^+$ 622 (50%), $C_{37}H_{66}O_7$). IR (film) $cm^{-1}$: 3425, 2920, 2837, 1737, 2643, 1455, 1314, 1073, 667. $^1$H-NMR ($CDCl_3$, 400 MHz, δ ppm): 2.53 (m, 1H, $H_{3a}$), 2.40 (m, 1H, $H_{3b}$), 3.86 (m, 1H, $H_4$), 1.49 (m, 1H, $H_5$), 1.22-1.74 (m, 2H, $H_6$ and $H_{13}$), 1.41(m,1H, $H_{14}$), 3.39 (m,1H, $H_{15}$), 3.86 (m,1H,$H_{16}$), 1.65 and 1.98 (m,2H, $H_{17}$ and $H_{18}$), 3.86(m,1H, $H_{19}$), 3.86 (m,1H, $H_{20}$), 1.65 and 1.98 (m,2H, $H_{21}$ and $H_{22}$), 3.85 (m,1H, $H_{23}$), 3.39 (m,1H, $H_{24}$), 1.22-1.74 (m,2H, $H_{25}$ and H-$_{33}$), 0.88 (t,1H, $H_{34}$), 7.19 (q, 1H, $H_{35}$), 5.06 (qd,1H, $H_{36}$), 1.44 (d, 1H, $H_{37}$); $^{13}$C-NMR ($CDCl_3$; 125 MHz, δppm ): 174.6 (C-1), 131.0 (C-2), 33.4 (C-3), 69.9 (C-4), 37.4 (C-5), 25.7 (C-6), 29.7-29.3 (C-7-C-13); 33.4 (C-14), 74.0 (C-15), 83.1 (C-16), 28.4 ( C-17), 29.0 (C-18), 81.8 (C-19), 81.8 (C-20), 28.8 (C-21, C-22), 83.1 (C-23), 74.0 (C-24), 33.4 (C-25), 25.6 (C-26), 29.3-29.7 (C-27-C-33), 14.1 (C-34), 151.7 (C-35), 78.0 (C-36), 19.1 (C-37).

**Acetonide derivative 3**. To **1** (1.5 mg) was added 0.5 ml of HCl-acetone (0.7 mg HCl in 1 ml acetone), and the solution was left overnight; the mixture was then dried at rt in vacuum (Colman-Saizarbitoria, 1999) to yield compound **3**. $^1$H-NMR ($CDCl_3$, 500 MHz, δ ppm): 2.54 (dddd, 1H, $H_{3a}$ , J:15; 3.3; 1.1 Hz), 2.40 (dddd , 1H, $H_{3b}$ , J:15; 8.6; 1.1 Hz), 3.5 (m, $H_4$), 1.49 (m, 1H, $H_5$), 1.22-1.54 (m, 2H, $H_6$ , $H_7$ ), 3.55 (m, 1H, $H_8$ ), 2. 4(m, 2H, $H_9$ and $H_{11}$), 2.15 (m, 1H, $H_{12}$), 5.34 (ddd, 1H, H-$_{13}$ J: 11.0; 7.0; 6.5 ), 2.25(m, 1H, $H_{15}$), 3.62 (m,1H, $H_{17}$), 3.58 (m,1H, $H_{18}$), 0.90 (t, 1H, $H_{32}$ , J:7.0), 7.12 (q,1H, $H_{33}$), 4.96 (qd,1H, $H_{34}$), 1.39 (d, 1H, J:7.0) and acetonyl methyl protons 1.375 and 1.382 ppm.

## Bioassays

The cytotoxicity was determined by dye reduction assay, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT). Cells were seeded at 5 x $10^3$ cells per well in MEM, complemented with 10% of bovine fetal serum, 1% of a penicillin/streptomycin mixture. Following a 48 h incubation period, at 37 °C and 5% $CO_2$, for all the cells, the culture medium was changed and the cells were treated with extract at 10, 100 and 1000mg/ml. After 24 hours incubation, medium was replaced by the one containing 100µl MTT (2mg/ml) in phosphate buffer (PBS) and pH 7.2. The cells were incubated for another 4 h and then, the medium was removed and the formazan crystals (Colored fiber mesh is characteristic of the presence of living cells) were dissolved in DMSO. After 30 minutes absorbance at 570 nm was measured using Micro-plate Reader. Results were expressed as a percentage of viable cells compared with control (unexposed cells) (Denizot, 1986; Mosmann, 1983).

The cytotoxicities of the Jahonin (**1**) (BST $LC_{50}$ 23) (Meyer, 1982) are summarized in Table I. Jahonin (**1**) showed cytotoxicities against the four human tumor cells lines in 24 h MTT human solid tumor panel.

## Results and Discussion

The dried twigs were extracted with ethanol. The ethanol residue (FOO1) was partitioned between water (FOO2) and chloroform (FOO3), and the residue of FOO3 was partitioned between hexane (FOO6) and 10% water in methanol (FOO5). The most bioactive extract, as tested by the BST (Meyer, 1982), was the residue of FOO5 ($LC_{50}$=194 ppm). FOO5 was submitted to successive fractionations by CC normal and reverse phase and preparative TLC, directed by the BST assay (Meyer, 1982), to yield two compounds, jahonin (**1**) (Figure 1) and asimicin (**2**); the latter was identified by IR, MS, $^1$H and $^{13}$C data analysis and by comparison with those reported (Rupprecht., 1989). Jahonin (**1**) was isolated as a waxy solid with MP 70-72ºC. The CIMS of **1** gave an $MH^+$ at 595, and EIMS an $M^+$ 594. The molecular formula was established to be $C_{35}H_{62}O_7$ on the basis of HREIMS and CIMS and RMN spectroscopy.

The existence of four OH groups in compound **1** was indicated by an IR OH absorption at 3360 $cm^{-1}$ and four successive losses of $H_2O$ (m/z) from the $MH^+$ in the CIMS and EIMS. As with most other acetogenins, the presence of a methyl substituted α, β-unsaturated γ-lactone was suggested by the IR (V max 1737 $cm^{-1}$), UV ( max 218 nm), and the corresponding resonances (δ) in the $^1$H and $^{13}$C -NMR spectra: $^1$H-RMN at δ 7.12 ppm, q (H-33), 4.96 ppm, dq (H-34), 1.39 ppm (H-35), 2.35 ppm, dd and 2.00

Table I
**Bioactivity of Extract FOO5 of the seeds of *Annona jahnii***

| Fraction | BST [a] $DE_{50}$ g/ml | Hep-2[b] $LC_{50}$ ppm | SVKO3[b] $LC_{50}$ ppm | Fadu[b] $LC_{50}$ ppm | HELA $LC_{50}$ ppm | Fibroblastos[b] $LC_{50}$ ppm |
|---|---|---|---|---|---|---|
| **(1)** | 11.45 | < 40 | <20 | <20 | <40 | 66.80 |

[a]Brine shrimp lethality (Meyer, 1982). [b]Cytotoxicities (Denizot, 1986; Mosmann, 1983) in human larynx carcinoma (Hep-2), Human pharynx carcinoma (Fadu), human ovary carcinoma (SVKO3), Human uterus carcinoma (HeLa) lines cells and human fibroblast.

ppm, dd (H-3a) and 3b) and 3.83 ppm, m (H-4), and carbons resonances at d 175 ppm (C-1), 151.1 ppm (C-33), 130.9 ppm (C-2), 78.0 ppm (C-34), 19.2 ppm (C-35), y 69.9 ppm (C-4). Two olefinic protons, coupled to each other, were discerned in the $^1$H-NMR spectrum at δ 5.35 ppm (ddd, J=11.0; 7.0; 6.4 Hz) and 5.34 ppm (ddd, J=11.0; 7.0; 6.4 Hz), suggesting the presence of an isolated *cis*-double bond; this group was further confirmed by two carbon resonances at δ 130.6 ppm and 128.8 ppm. The position of the double bond was determined by the EIMS fragmentation (Figure 2) and from the COSY and HMBC spectra, to be at C-13 and C-14. The COSY spectrum of **1** showed coupling correlation of H-13, H-14, H-12, and H-11. HMBC correlation cross peaks between H-17 (δ 3.45)/ C-15 (δ 27.3), H-16 (δ 1.5); C-16 (δ 33.5)/ C-18 (74.1), H-15 (δ 2.3 C-13 (δ 128.8) and C-17( 74.3), also cross peaks between C-10 (δ 209)/ H-11 (δ 2.40); C-11(δ 42.7 ppm)/H-13 (δ 5.34 ppm); H-11 (δ 1.50 ppm)/ C13 (δ 128.8 ppm) were clearly shown in the spectrum. Examination of the $^1$H-$^1$H COSY spectrum revealed that the double bond and vicinal diol moieties are separated by two methylene units, and double bond and keto group are separated by two methylene units.

Analyses of the EIMS fragmentation of compound **1** demonstrated that the four OH groups were located at C-4, C-8, C-17 and C-18 as shown in Figure 2. The presence of the vicinal diol moiety (C-17/18) was also evidenced in the $^1$H and $^{13}$C-NMR spectra by a proton signal at δ 3.49 ppm for two carbinol methine protons and signals for two oxygenated carbons at δ 74.3 ppm and 74.1 ppm, similar to others linear acetogenins with vicinal diol (Liaw, 2005). To determine the relative configuration at C-17/18 of the vicinal diol, the acetonide derivative (**2**) was prepared (Figure 1). The $^1$H-NMR signals for H-l7 and H-18 at δ 3.49 ppm and the signals for the acetonyl methyl protons, showing two separate singlet peaks at δ 1.375 ppm and 1.382 ppm, suggested the *trans* assignment for the dioxolane ring; the methyl protons of *cis* dioxolane rings show two singlet peaks, at δ 1.43 ppm and 1.33 ppm, and the methine protons are at δ 3.62 ppm and d 3.58 ppm (Colman-Saizarbitoria, 1999); thus, the configuration of the diol was determined to be *threo*, since the *trans* configuration at C-17/18 in **2** could only be derived from a vicinal diol with a *threo* configuration (Colman-Saizarbitoria, 1999).

The presence of an additional carbonyl signal at 1703 $cm^{-1}$ in the IR spectrum suggested that compound **1** is a keto-acetogenin compound. The $^1$H-NMR data also suggested the location of the keto group at C-10 since two additional two-proton triplets (J=7.5 Hz) are shown in the spectrum of **1**, at δ 2.38 ppm and 2.39 ppm, consistent with two methylene groups at C-9 and C-11, flanking the keto group. The location of the keto group was then clearly confirmed on the basis of EIMS fragmentation (Figure 3).

The cytotoxicities of the fraction P6 (BST $LC_{50}$ 23) (Meyer, 1982) are summarized in Table I. P6 showed cytotoxicities against the four human tumor cells lines in 24 h MTT human solid tumor panel.

## Conclusion

A new cytotoxic against the four human tumor cells lines in 24 h Annonaceous acetogenin, jahonin (**1**) and asimicin (**2**), were isolated from the twigs of *Annona jahnii* (Annonaceae). The structure and relative configuration of vicinal diol in **1** were elucidated by spectroscopy methods and chemical derivative type acetonide (**3**).

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## References


Bermejo A, Figadere B, Zafra-Polo MC, Barrachina I, Estornell E, Cortes D. 2005. Acetogenins from annonaceous: recent progress in isolation, synthesis and mechanisms of action. Nat Prod Rep 22:269-303.

Colman-Saizarbitoria T, Johnson H, Alali FQ, Hopp DC, Roberts LL, McLaughlin J. 1998. Annojahnin from Annona Jahnii: a possible precursor of mono-Tetrahydrofuran Acetogenins. Phytochemistry 49:1609-1616.

Colman-Sazarbitoria T, Liu X, Hopp C, Johnson H, Alali FQ, Rogers LL, McLaughlin J. 1999. Annondienin and Jahnonacin new bioactive nontetrahydrofuran Annonaceous Acetogenins from the twigs of Annona Jahnii. Nat Prod Letters 14:65-75.

Denizot F, Lang R. 1986. Rapid colorimetric assay for cell growth and survival. J. Immunol. Methods 89:271-275.

Glye C, Raynaud S, Fourneaus C, Laurens A, Lprévote O, Serani L, Fournet A, Hocquemiller R. 2000. Cohibin C and D, two important metabolites In the biogenesis af Acetogenins From Annona Muricta and Annona Nutans. J Nat Prod 63:1192-1196.

Hui-Fen Chiu H-F, Tsai-Tung Chih, Yun-Mei Hsian, Chih-Hua Tseng, Ming-Jung Wu, Yang-Chang Wu. 2003. Bullatacin a potent antitumor Annonaceous acetogenin, induces apoptosis through a reduction of intracellular camp and cGMP levels in human hepatoma 2.2.15 Cells. Biochem Pharmacol. 65:319-327.

Ichimaru N, Abe M, Murai M, Senoh M, NishiokaT, Miyoshi H. 2006. Function of the side chains of D-lac-acetogenin in the inhibitory effect on mitochondrial complex I (NADH-Ubiquinone Oxidoreductase). Biorg Med Chem 16: 3555-3558.

Liaw C-C, Chang F-R, Chen S-L, Wu C-C, LeeK-H, Wu Y-C. 2006. Novel Cytotoxic Monotetrahydrofuranic Acetogenins from Annona Montana. Biorg Med Chem 13: 4767-4776.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp: convenient general bioassay for active constituens. Planta Medica 45: 31-34.

Morré DJ, De Cabo R, Farley C, Oberlies NH, McLaughlin JL. 1995. Mode of action of bullatacin, a potent antitumor acetogenin: Inhibition of NADH oxidase of HeLa and HL-60, but not liver, plasma membranes. Life Sci 56: 343-347.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. J Immunol Methods 65:55-59.

Motoyama T, Yabunaka H, Miyoshi H. 2002. Essential Structural Factors Of Acetogenin, Potent inhibitors of mitochondrial tumor cell growth inhibition by several Annonaceous Acetogenins in an *in vitro* disk diffusion assay complex I. Biorg Med Chem 12: 2089-2092.

Murai M, Ichimaru N, Abe M, Nishioka T, Miyoshi H. 2006. Mode of inhibitory action of -Lac-Acetogenin, a new class of inhibitors of bovine heart mitochondria complex I. Biochemistry 45:9778-9787.

Oberlies NH, Jones JL, Corbett TH, Fotopoulos SS, McLaughlin JL. 1995. Tumor cell growth inhibition by several Annonaceous acetogenins in an in vitro disk diffusion assay. Cancer Letters 96: 55-58.

Oberlies NH, Croy VL, Harrison M L, McLaughlin JL. 1997. The Annonaceous acetogenin bullatacin is cytotoxic against multidrug-resistant human mammary adenocarcinoma cells. Cancer Letters 115: 73-79.

Oberlies NH, Chang C-J, McLaughlin JL. 1997. Structure-activity relationships of diverse Annonaceous acetogenins against multidrug resistant human mammary Adenocarcinoma (MCF-7/Adr) cells. J Med Chem 40: 2102-2108.

Raynaud-LeGrandic S, Foureau C, Laurens A, Bories C, Hocquemiller R, Loiseau PM. 2004. *In vitro* antileishmanial activity of Acetpgenins from Annonaceae. Biomedicine & Pharmacotherapy 58: 388-392.

Royo B, Baldoni H, Chaboune N, Uvire F, Depedro N, Zafra-Polo C, Bermejo A, El Aoud N, Cabedo N, Saez J, Toeno J, Enriz RD, Cortes D. 2007. New antitumoral Acetogenin «Guanacone Type» derivatives: isolation and bioactivity. molecular dynamics simulation of diacety-guanacone. Biorg Med Chem 15: 4369-4381.

Rupprecht J, Chang C, Cassady J, McLaughlin J, Mickolajczak K, Weisleder D. 1989. Asimicin a new cytotoxic and pesticidal acetogenin from the pawpaw.Asimina triloba (Annonaceae). Tetrahedron 45: 6941-6946.

Zeng L, Ye Q, Oberlies NH, Shi G, Gu Z-M, He K, McLaughlin J. 1996. Recent advances in annonaceous Acetogenins. J Nat Prod Rep. 13: 275-280.


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