C08102. Tucupentol, a Novel Mono-tetrahydrofuranic Acetogenin from *Annona montana*, as a Potent Inhibitor of Mitochondrial Complex I

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Ten acetogenins, one of them new, were isolated from leaves and twigs of a Solivian collection of *Annona montana.* The new compound that we named tucupentol (1), is a mono-tetrahydrofuran-pentahydroxy-acetogenin. The inhibitory potency of tucupentol (1) on the mitochondrial complex I was evaluated, and this activity was compared with that of the known acetogenins, annonacin-A, *cis*-annonacin-10-one, aromin, and gigantetronenin, also isolated from this plant material. The mentioned acetogenins acted as selective inhibitors of mitochondrial complex I in the 0.8-5.4-nM range.

Introduction. - Plants of the family Annonaceae, found mainly in the tropical áreas of America, África, and Southeast Asia, are important sources of edible fruits, fragrance oils, and drugs employed in folk medicine for various purposes. Several species have been intensely investigated over the last 20 years, mainly because of the discovery of annonaceous acetogenins (ACGs), a group of C_32/C_{34} fatty-acid-derived natural producís that show a wide variety of biological activities. The edible fruit tree *Annona montana* (Annonaceae), known as 'simn/', grows widely in Santa Cruz de la Sierra, Bolivia, where the population employs its leaves with medicinal purposes [1]. Previous chemical investigations of seeds from Tonate, French Guyane [2], leaves from Hainan Province, China [1][3], and seeds from Chia-Yi City, Taiwan [4] [5] have resulted in the isolation of more than 20 new bioactive ACGs [6]. It has been repeatedly reported that acetogenins play an important role in the protection of plañís against pathogens and herbivorous insects [7] [8], and inhibit the NADH :ubiquinone oxidoreductase (complex I) in the mitochondrial electron-transport chain [9] [10], among other bioactivities. Their structural features, as the substitution pattern in the lactone ring, the number and location of the OH groups and THF rings, and the presence and location of C=O groups, determine the potency of these compounds. Particularly, it has been pointed out that acetogenins, with a C(10)=O group are potent inhibitors of complex I [11]. A sight on the type of acetogenins isolated from *A. montana* [4] [5] reveáis that many bioactive mono-THF keto acetogenins had been obtained from this species. Since there were no reports on previous studies of Bolivian collections of *A. montana,* we collected leaves and twigs from trees growing in Santa Cruz de la Sierra, Bolivia.

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CHEMISTRY & BIODIVERSITY
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Druck: K. Triltsch, Print und digitale Medien GmbH,
D-97199 Ochsenfurt-Hohestadt

Number of reprints? **Ex.** D with D covers without

Results and Discussion. - *Compounds.* Chromatographic fractionation of the MeOH extract furnished a new acetogenin that we named tucupentol (1) together with nine known acetogenins, aromin (2) [12], annonacin-A (3) [13], cw-annonacin-10-one (4) [14], gigantetronenin (5) [15], densicomacin-1 (6) [16], murihexocin-B (7) [17], murihexocin-A (8) [17], muricatocin-A (9) [18], and annonacin (10) [19]. The acetogenins 2, 3, and 6-9 had not been previously isolated from *A. montana.* The molecular weight of the new compound 1 was established by the $[A/ + H]^+$ ion observed at *miz* 613.46396 (cale. 613.46794 for $C_{35}H_{65}O_8$) in the HR-LSI-MS. The succesive EI-MS peaks observed at *miz* 576 ($[M-2\ H_2O]^+$), 558, and 540 suggested the presence of five OH groups. Absorptions at 223 nm and 1730 cm~' in the UV and IR spectra, respectively, are in good agreement with the presence of an a,/8-unsaturated y-lactone ring in 1 [11]. :H-NMR resonances at *ó* 7.19, 5.04, 1.43, and 2.40-2.50, as well as the ^{13}C-NMR resonances at *ó* 174.7, 152.2, 130.9, 78.1, 69.4, and 19.0 *(Table 1),* provided characteristic spectroscopic evidences for the presence of an a,/3-unsaturated y-lactone with an 4-OH group (full 'H- and ^{13}C-NMR spectra and HMBC correlations in *Table 1).* The (S)-configuration at C(34), as depicted, has been assigned by comparison of the 'H-NMR signáis of 1 with previously reported NMR data of identical type of acetogenins [6]. In the mass spectrum, the ions at *miz* 183 and 213 (cleavage between C(7)/C(8) and C(8)/C(9), resp.) as well as the fragments at *miz* 393 (cleavage between C(7)/C(8) -2 H_2O) and 375 (C(7)/C(8) -3 H_2O) suggested that a free OH group should be located at C(8) *(Fig.).* The presence of an a-hydroxylated mono-THF system with a *transIthreo* relative configuration was deduced by ^{1}H,^{13}C NMR heteronuclear correlations between the signáis at *ó* 3.42 and 74.4 (a CH-OH group) , as well as the signal at *ó* 3.85 (H-C(12), H— C(15)) correlated with resonances at *ó* 82.0 and 79.4 assigned to two tetrahydrofuran methines. The MS peaks at *miz* 269 and 325 allowed the placement of the a-hydroxylated THF system between C(12) and C(16) *(Fig).* The two additional OH groups were located at vicinal C-atoms. The fragment ions at *miz* 395, 377, and 359 suggested that the OH groups should be located at C(19) and C(20) (Fig.). The 'H-NMR resonance at *ó* 3.35-3.45 (2 H) assigned to the H-atoms at C(19) and C(20), as well as the ^{13}C-NMR resonance at *ó* 74.6 (C(19) and C(20)) indicated that the vicinal diol was in a í/zreoconfiguration [6]. Therefore, tucupentol (1) is an a,/?-unsaturated y-methyl y-lactone mono-THF acetogenin with a *transí threo-threo* relative configuration. Structures of the known compounds 2-10 were deduced by comparison of their spectroscopic data with those of the authentic compounds reported in the literature.

Inhlbition of Mitochondríal Complex I. The complex-I inhibitory potency of tucupentol (1) was evaluated against the integrated NADH oxidase activity of beef heart open submitochondrial particles [9] [10] . For comparison, aromin (2) , annonacin-A (3), cis-annonacin-10-one (4), and gigantetronenin (5) were selected. Other isolated compounds were not tested, because it is well-known that an excessive polarity led to a loss of potency [10]. As shown in *Table 2,* cw-annonacin-10-one (4; /C_{50} 0.8 ±0.1 nM) was the most powerful inhíbitor of NADH oxidase among the tested compounds. Apparently, besides the íra«5-THF moiety with an adjacent OH, the OH group at C(4), and the C(10)=O group play an importan! role in the potency. Replacement of the C(10)=O with C(10)— OH group, as in annonacin-A (3), produces a less active compound (7C_{5n} 5.4 ±1.3 nM). Tucupentol (1), with an /C_{50} valué of 5. 3 ±0.3 nM, has a

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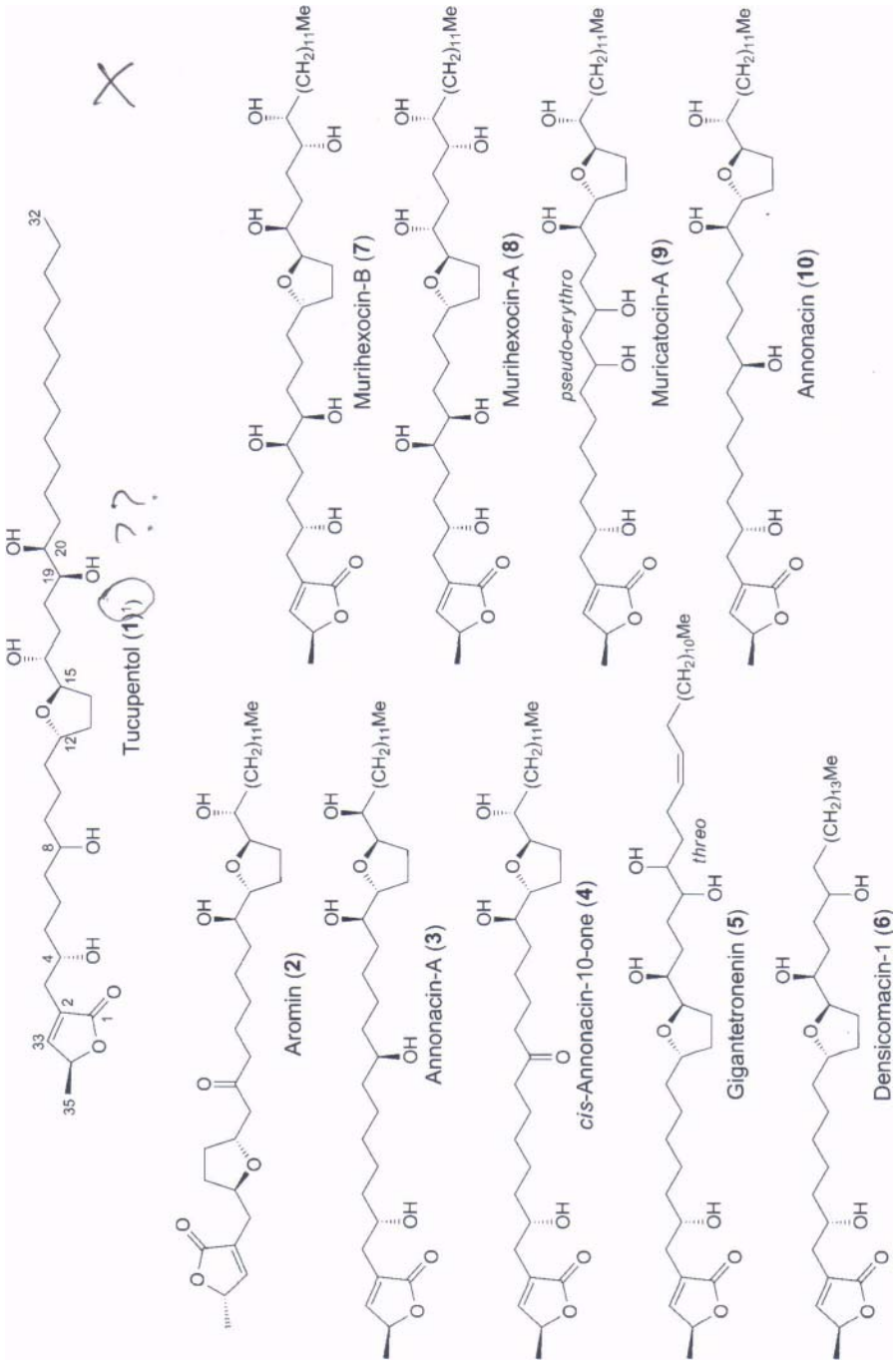

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Table 1. *'H- and "C-NMR Spectral Data, and HMBC Correlations of Tucupentol* (1) *in CDC1₃* (at 400 and 100 MHz, resp.; <5 in ppm, 7 in Hz)

	Ó(H¡0 Mot Wd^	ári/) ó(C)	HMBC(H-*C)
C(l)	_	174.7	
C(2)	-	130.9	
CH2(3)	2.40-2.50*	33.3	1, 2, 4, 5, 33
y H-C(4)	3.80-3.90* / t- e ^í	=• 69.4	
X CH2(5)	• •) 'I-25" ^Zr-	^ 37.2	
y? CH2(6)	••) / 2 S- 1 56'	* 2L6	
> CH2(7) jx- H-C(8)	••H.2S- <.SS 3 61-B(m) 3 55"	3,7o S	
y CH2(9)	••)¡ lr v/?/	5 •*. 36.8	
> CH2(10)	•') 4 . 2. 6 - ^ • 5	S 1 22 3	
CH2(11)	• •) {,~2,<£~ \ C.	^tt 33.3	
H-C(12)	3.80-3.90*	79.4	
CH2(13)	1.60-2.00*	28.4	
CH2(14)	1.60-2.00*	32.3	
H-C(15)	3.80-3.90*	82.0	
H-C(16)	3.35-3.45*	i¿ 74.4	
X CH2(17)	••) 1.2S- Le	JC,^ 35.3	
^ CH2(18)	•') (2 s-'A -£-	á ^ * 33.6	
H-C(19)	3.35-3.45*	74.6	
H-C(20)	3.35-3.45*	74.6	
CH2(21)	1.25-1.55*	30.3	
CH2(22-29)	1.25-1.55*	25.8-29.7	
CH2(30)	1.25-1.55*	31.9	
CH2(31)	1.25-1.55*	22.6	
Me(32)	0.87 (*t, J* =7)	14.1	30, 31
H-C(33)	7.19 (br. *s)*	152.2	1, 2, 34
H-C(34)	5.04 *(dq,J* = 6.8, 1.5)	78.1	2, 35
Me(35)	1.43 (d, 7 = 6.8)	19.0	33, 34

*: Overlapping signáis.

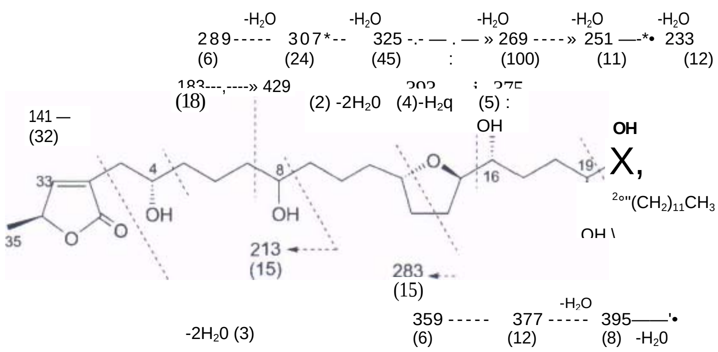

Figure. *Diagnostic Fragment ¡ons of Tucupentol* (1). Numbers in parentheses indícate relative abundances in the MS.

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potency similar to annonacin-A (3) and rotenone, a very well-known inhibitor of mitochondrial complex I.

Table 2. *NADH Oxidase Inhibitory Activity of Acetogenins*

Compound	/C_{50} [nM][a])
Aromin (2)	3.9 ±0.5''
Annonacin-A (3)	5.4±1.3[a]
cw-Annonacin-10-one (4)	0.8±0.1[C]
Gigantetronenin (5)	3.7±0.1[b]
Tucupentol (1)	5.3 ± 0.3[a]
Rotenone	5.1±0.1[a]

'') MeaniSEM. Means within the row, followed by the same superscript, are not significantly different (/>>0.05, Tukey múltiple range test).

This research was supported by grants from *Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT),* and *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).*

Experimental Parí

General. Chromatographic separations were performed by column chromatography (CC) on silica gel (230-400 mesh; *Merck).* Semiprep. HPLC: *LiChroCart'' 100 RP-18* column (25 x 1 cm i.d., 10-um particle size), flow rate 2.0ml/min, with MeCN/H_2O. Anal. TLC: *Merck* precoated silica-gel *60 F_{254}* plates. Optical rotations: *Horiba High Sensitive Polarimeter*. IR Spectra: *Shimadzu IR-408* spectrometer. 'H- and ^{13}C- NMR, DEPT, HMBC, HMQC, 'H,'H-COSY: *Varían Unity* spectrometer at 400 MHz, using the solvent signal as reference ($CDC1_3$ at ó 7.26 and 77.0 ppm). MS: *VG Auto Spec Fisons* spectrometer.

Compounds. The leaves and twigs of *Annona montana (sinin?)* were collected at Santa Cruz de la Sierra, Solivia, in February 1999. A voucher sample (Lil. 607345) is deposited with the Herbarium of Fundación Miguel Lulo, Tucumán, Argentina. The dried and powdered leaves and twigs of *'sininf* (550 g) were percolated with MeOH (2x31). Evaporation of the solvent yield a crude MeOH extract (25 g) which was further partitioned between CH_2C1_2 (3 x 100 mi) and H_2O (100 mi). The CH_2C1_2 layer was washed with H_2O, dried (anh. Na_2SO_4), filtered, and evaporated *in vacuo* to give 10 g of a solid extract with an acetogenin mixture that was further cromatographed on a silica-gel column (300 g), with gradients of hexane/AcOEt (90:10; 70:30; 50:50; 30:70; 10:90) to give five fractions (/to *V*, 300 mi each). *Fr. H* (320 mg) was submitted to semiprep. HPLC with CH,CN/H_2O 90 : 10 to afford *aromin* (2; 26.4 mg; $í_R$ 27.5 min, [a]g = +10.3 (CHC1,, c = 0.25)). *Fr. ¡II* (660 mg), after semiprep. HPLC with CH_3CN/H_2O 90:10, afforded *annonacin-A (3;* 130 mg; $Í_R$ 16.0 min, [<x]g=+23.8 (CH_2C1_2, c = 0.4)), *_annonacin* (10; 136 mg; fu 22.5 min), *CK-annonacin-1 0-one* (4; 17.5 mg; /$_R$ 24.5 min, [a]g = +6.2 ($CHC1_3$, ~*c* = O.Ó7^p|énsicomacin-í}(6; 57.8 mg; $í_K$ 26.5 min, [ct]g = + 26 (MeOH, c = 0.05)), and *gigantetronenin* (5; 68.6 mgT^ Só.O'miñT [a]g = + 10 (CHC1,, c = 0.2). *Fr. IV* (50 mg), after semiprep. HPLC with 80:20, afforded *murícatocin-A* (9; 5 mg; ($_R$ 19.0 min, [a]g= +21.8 (EtOH, c = 0.001)).

dYactionFA5(250 mg) was submitted to semiprep. HPLC with MeCN/H_2O 70 : 30 to afford *murihexocin-A* (8 ; 28.4 mg *; t_R* 7.0 min) , *murí hexocin-B* (7; 53 .8 mg ; $í_R$ 8.5 min) , and *tucupentol* (1; 69 mg ; /$_R$ 1 8.0 min) . *Tucupentol* (1). Waxy compound. [a]g= -7.1 (CHC1,, c = 0.17). UV (EtOH): A_{max} 223 nm (loge 3.56) . IR (film): i>$_{mM}$ 3400, 1730, 1650, 1465, 1052. 'H- and "C-NMR: see *Table 1.* HR-LSI-MS; 613.46396 ([M + H] $^+$, CjsHjsOji''; cale. 613.46794). Copies of the original spectra may be obtained upon request from the corresponding author.

Inhibitory Potency of Acetogenins. The ¡nhibitory potency of acetogenins as complex-I inhibitors was determined using submitochondrial particles from beef heart as reported in [9] [10] [20]. The 50%

inhibitory concentration (IC_{50}) was taken as the final compound concentration in the assay médium that yielded 50% inhibition of NADH oxidase activity. NADH Oxidase activity was measured as the aerobic oxidation of 75 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 statistical means and the standard deviations.

Statistical Analysis. The results are reported as mean ± SEM. The differences in the mean valúes were evaluated by analysis of variance (ANOVA). The *Tukey* test was used for all pairwise múltiple comparisons of groups. In all statistical analysis, $P > 0.05$ was considered not significan!.

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Received March 11, 2008

Keywords für Beitrag »C08102«

Annona montana

Tucupentol

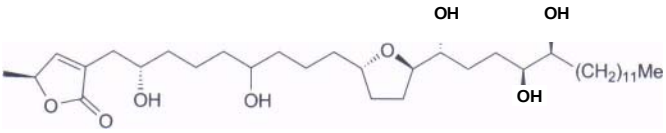
Acetogenins

Inhibitors

Mitochondrial complex I

NADH Oxidase inhibitory activity

C + B 08102



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