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Rollimembrin, a Novel Acetogenin Inhibitor of Mammalian Mitochondrial Complex I

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Abstract: Rollimembrin (**3**), is a new adjacent bis-tetrahydrofuranic acetogenin with a scarce relative configuration, *threo/cis/threo/cis/erythro*, isolated from *Rollinia membranacea* seeds. The mechanism of cytotoxic activity, determined by NADH-oxidase experiments, establish that rollimembrin (**3**) is the most potent inhibitor of mammalian mitochondrial complex I. © 1997 Elsevier Science Ltd.

Annonaceous acetogenins are known by their potent biological activity as inhibitors of tumoral cell growth and also as insecticides, acaricides, fungicides, antiparasitics and herbicides. These compounds, derived from polyketides as biogenetic precursors, have been isolated exclusively from several species of Annonaceae.[1-3] Their mode of action mainly targets on the mitochondrial NADH:ubiquinone oxidoreductase,[4,5] also known as complex I of the mitochondrial respiratory chain. Inhibition of complex I, which is the main gate for the energy production in the cell, opens an interesting perspective for the development of a new generation of antitumor drugs. Recently, it has been described that some acetogenins also inhibit a new NADH oxidase located in the cytoplasmic membrane of hepatocytes, which is involved in cellular growth and signal recognition.[6] This second target of Annonaceous acetogenins could increase the sensitivity of tumoral cells to these compounds.

We have studied the mode of action of a number of acetogenins on the complex I of inverted submitochondrial particles from beef heart.[5] From this study, the adjacent bis-tetrahydrofuranic (bis-THF) acetogenin rolliniastatin-1, (**1**) was considered the most potent inhibitor of complex I, closely followed in potency by its diastereoisomer rolliniastatin-2 [19,20-diepimer of rolliniastatin-1(**1**)]. Moreover, the mode of action of both rolliniastatins-1 and -2, is not identical: **1** acts by blocking two different sites in complex I,[5] one of them probably related to the ubiquinone reducing site of other enzymes,[7] including the plasmatic-membrane NADH oxidase.[6]

During the re-isolation of rolliniastatin-1 (**1**) from *Rollinia membranacea* seeds, three other adjacent bis-THF acetogenins were isolated, the known rolliniastatin-2[1] and membranacin (**2**),[1,8] and the new rollimembrin (**3**), which is the first report of a C_{35} acetogenin with a very scarce relative configuration: *threo/cis/threo/cis/erythro*. The structure of rollimembrin (**3**) was determined by ^{1}H-, ^{13}C-NMR, DEPT, COSY, HMQC and FABMS experiments, and chemical derivations. The inhibitory potency on the mammalian complex I of **3** was compared with that of rolliniastatin-1 (**1**) and membranacin (**2**), the only other acetogenins with an identical relative configuration.[1,9]

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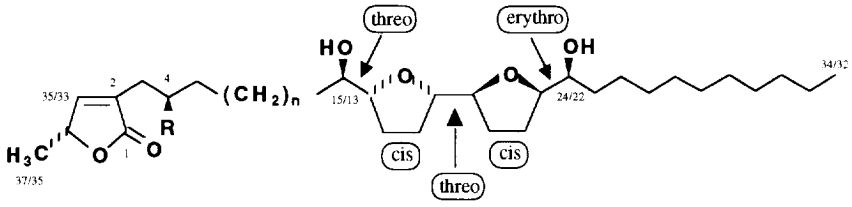

1: R= OH; n= 9; **rolliniastatin-1** [$C_{37}H_{66}O_7$]
2: R= H; n= 9; **membranacin** [$C_{37}H_{66}O_6$]
3: R= OH; n= 7; **rollimembrin** [$C_{35}H_{62}O_7$]

Rollimembrin (**3**) is a white amorphous compound, isolated and purified by semi-preparative HPLC.[12] The molecular weight of **3** was indicated in FABMS by peaks at *m/z* 617 $[M+Na]^+$ and *m/z* 595 $[MH]^+$. HREIMS of **3** gave a peak at *m/z* 594.4470 $[M^+]$ corresponding to the molecular formula $C_{35}H_{62}O_7$.[13] The presence of three OH moieties in the structure of **3** was suggested by an IR absorption at 3430 cm^{-1}, by succesive losses of three H_2O molecules from $[MH]^+$ in FABMS, and by the formation of a triacetyl derivative (**3a**).[14] A prominent IR carbonyl absorption at 1750 cm^{-1} and a positive reaction with Kedde reagent, suggested the presence in **3** of an α,β-unsaturated γ-lactone ring. The ^1H NMR resonances at δ 7.17 (d, H-33), δ 5.04 (dq, H-34), δ 3.81 (m, H-4), δ 2.39 (dd, H-3a), δ 2.52 (dd, H-3b) and δ 1.42 (d, H-35) were correlated respectively with the ^{13}C NMR signals at δ 151.71 (C-33), δ 77.90 (C-34), δ 69.93 (C-4), δ 33.28 (C-3) and δ 19.08 (C-35) by HMQC (Figure 1 and Table 1). These results confirmed the presence of a 4-pentanolide moiety (α,β-unsaturated γ-methyl γ-lactone) with an hydroxyl group in the alkyl chain at the 4 position, characteristic of the acetogenins.[1-3]

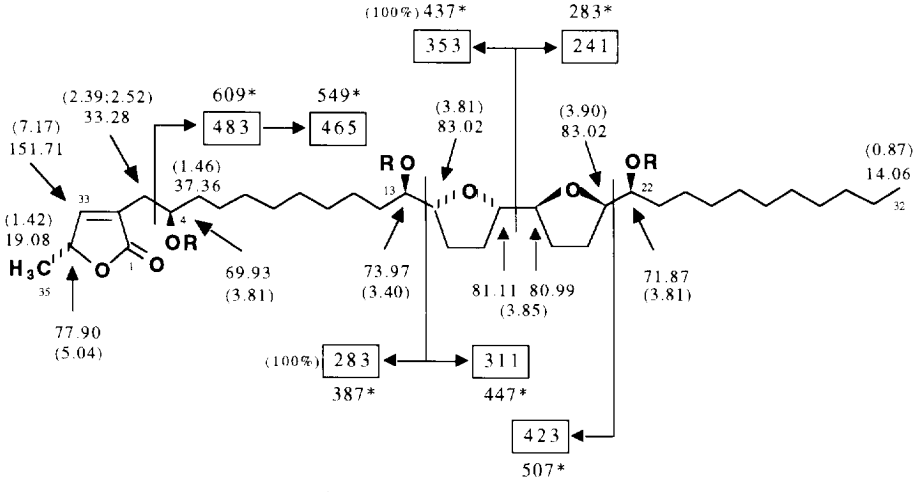

Figure 1. Diagnostic values of ^{13}C-NMR and ^1H-NMR (in parentheses) of rollimembrin (**3**); and EIMS of **3** (R= H) and triacetyl-rollimembrin (**3a**)* (R= Ac)

The remaining ^{1}H- and ^{13}C-NMR signals of the oxygenated methines of **3**, assigned by 2D experiments (COSY 45 and HMQC), at δ 3.40/73.97, δ 3.81/83.02, δ 3.85/81.11-80.99, δ 3.90/83.02, and δ 3.81/71.87, correspond to the bis-THF α,α'-dihydroxylated system with the scarce relative configuration *threo/cis/threo/cis/erythro*.[1,9,15] This configuration is identical to that of rolliniastatin-1 (**1**),[16] which was established by NMR comparison with synthetic models[17] (see Table 1 and Figure 1). Analysis of mass fragments showed that rollimembrin (**3**) has a structure with two methylene groups less than **1** in the alkyl chain, between the γ-lactone and the bis-THF α,α'-dihydroxylated system (Figure 1). Therefore the hydroxyl groups of this system are placed at C-13 and C-22.

Table 1. 1D and 2D NMR experiments (400 MHz, $CDCl_3$) of rollimembrin (**3**)

H	δ (*J*, Hz)	Coupling in COSY 45	Coupling in HMQC (multiplicity DEPT ^{13}C)
1			174.56 (C)
2			131.17 (C)
3a	2.39 *dd* (15, 8)	H-3b (2.52), H-4 (3.81)	33.28 (CH_2)
3b	2.52 *dd* (15, 3)	H-3a (2.39)	
4	3.81 *m*	H-3a (2.39), H-5 (1.46)	69.93 (CH)
5	1.46 *m*	H-4 (3.81)	37.36 (CH_2)
6	1.30 *m*		25.98 (CH_2)
7-10	1.25 *m*		29.56 (CH_2)
11	1.30 *m*		25.69 (CH_2)
12	1.46 *m*	H-13 (3.40)	34.19 (CH_2)
13	3.40 *m*	H-12 (1.46), H-14 (3.81)	73.97 (CH)
14	3.81 *m*	H-13 (3.40), H-15 (1.76,1.92)	83.02 (CH)
15,16	1.76,1.92 *m*	H-14 (3.81), H-17 (3.85)	28.70,27.86 (CH_2)
17	3.85	H-16 (1.76,1.92)	81.11 (CH)
18	3.85	H-19 (1.76,1.92)	80.99 (CH)
19,20	1.76,1.92 *m*	H-18 (3.85), H-21 (3.90)	28.40,23.72 (CH_2)
21	3.90 *m*	H-20 (1.76,1.92), H-22 (3.81)	83.02 (CH)
22	3.81 *m*	H-23 (1.36), H-21 (3.90)	71.87 (CH)
23	1.36 *m*	H-22 (3.81)	32.77 (CH_2)
24	1.30 *m*		25.51 (CH_2)
25-29	1.25 *m*		29.56 (CH_2)
30	1.25 *m*		31.87 (CH_2)
31	1.25 *m*	H-32 (0.87)	22.64 (CH_2)
32	0.87 *t* (6.8)	H-31 (1.25)	14.06 (CH_3)
33	7.17 *d* (1.25)		151.71 (CH)
34	5.04 *dq* (7, 1.25)	H-35 (1.42)	77.90 (CH)
35	1.42 *d* (7)	H-34 (5.04)	19.08 (CH_3)

The absolute stereochemistry of the carbinol centers was determined by preparing the (*R*)- and (*S*)-methoxy-trifluoromethyl-phenyl acetates from **3** by the Mosher's method.[18] Thus, rollimembrin (**3**) was converted to the (*S*)-MTPA (**3b**) and (*R*)-MTPA (**3c**) esters.[19] The negative $\Delta\delta_{S-R}$ values of H-3 and γ-lactone protons, as well as the negative values for the H-14 to 17 THF ring protons (the irregular positive value for H-14 was also found in other acetogenins[21]), and positive values for the H-21 to 18 THF ring protons, indicated *R*, *R* , and *S* configurations for C-4, C-13, and C-22, respectively (see Table 2).

Table 2. ^{1}H-NMR (400 MHz, $CDCl_3$) data of Mosher esters of **3** (**3b** and **3c**)

	H-35	H-34	H-33	H-3	H-5	H-12	H-14	H-15/16	H-21	H-23
S-MTPA -**3b**	1.27	4.85	6.71	2.57	≈1.61	≈1.61	3.98	1.90-1.75	4.04	≈1.65
R-MTPA-**3c**	1.31	4.91	6.97	2.63	≈1.50	≈1.50	3.90	2.00-1.80	3.96	≈1.69
Δδs-r	- 0.04	- 0.06	- 0.26	- 0.06	+ 0.11	+ 0.11	+ 0.08	-0.10 / -0.05	+0.08	-0.04
configurations			**C-4 *R***			**C-13 *R***			**C-22 *S***	

Figure 2 shows the IC_{50} values of rolliniastatin-1 (**1**), membranacin (**2**) and rollimembrin (**3**), the only three acetogenins with a *threo/cis/threo/cis/erythro* configuration, against the NADH oxidase activity in submitochondrial particles from beef heart.[22,23] The three acetogenins were found to be potent inhibitors of the mitochondrial NADH:ubiquinone oxidoreductase (complex I). Rollimembrin (**3**) was found to be the most potent inhibitor of the integrated respiratory chain with an IC_{50} and full-inhibition concentration lower than rolliniastatin-1 (**1**).

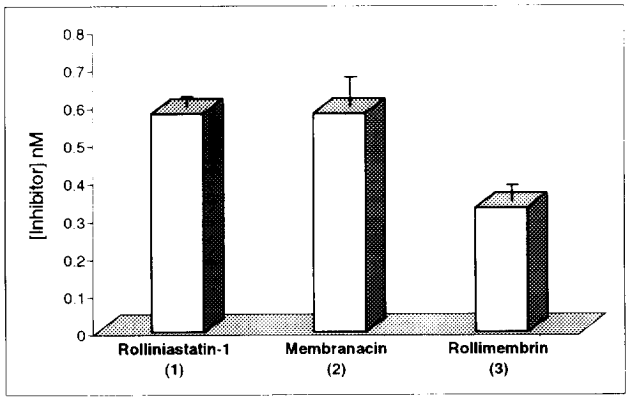

Figure 2. Inhibitory concentration 50 (IC_{50}) of acetogenins (**1**, **2** and **3**) for the NADH oxidase activity in bovine heart submitochondrial particles. Experimental conditions described below.[24] Control activity 0.95 μmol min^{-1} mg^{-1} approximately. Data are means from four determinations for each product.

Figure 3 shows the titration curves of the three acetogenins.[24] Membranacin (**2**), which showed the same IC_{50} as rolliniastatin-1 (**1**), did not inhibit completely the aerobic NADH oxidation at the relatively low concentrations used in this study. Moreover, the shape of the titration curve was different. While rolliniastatin-1 (**1**) and rollimembrin (**3**) gave sigmoidal curves, membranacin (**2**) produced consistently an hyperbolic curve. We think that, although the three acetogenins are potent inhibitors of mitochondrial complex I, the mechanism by which inhibition is achieved may be non identical between membranacin (**2**) and the two others (**1** and **3**).

The sigmoidal titration curve given by rollimembrin (**3**) associates it to the mode of action of **1** (and also of piericidin A) by blocking the two cooperative sites of complex I.[5,27,28] The second site, which is not inhibited by membranacin (**2**), could be related to the ubiquinone reducing site of other bacterial enzymes[7] and probably with the site of the mammalian plasmatic-membrane NADH oxidase.[6] Therefore, the highest inhibitory potency of rollimembrin (**3**) makes it an interesting compound for antitumoral trials.

From the close chemical structure of the three acetogenins, we can conclude that: a) acetogenins with a *threo/cis/threo/cis/erythro* configuration are the most potent inhibitors of mammalian complex I; b) the proximity of the γ-lactone ring to the THF ring plays an important role in the inhibitory potency, rollimembrin (**3**), with a shorter alkyl chain, being the most potent; and c) the hydroxyl group at the C-4 position could affect the mode of action of the acetogenin, because membranacin (**2**), without this OH group, behaves in a different manner.

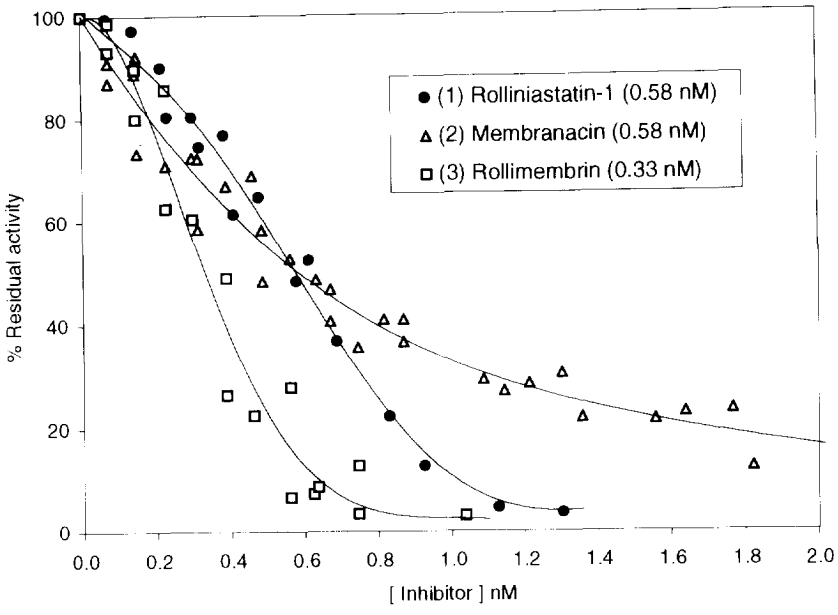

Figure 3. Titration of acetogenins (**1**, **2** and **3**) against NADH oxidase activity. Experimental conditions described below [24] and in Figure 2. IC_{50} indicated in parentheses.

Acknowledgments

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12. Bis-THF acetogenins from *R. membranacea* seeds were purified by semipreparative HPLC, carried out on a LiChroCart^{R} 100 RP-18 column (25 x 1 cm i.d., 10 µm particle size) using CH_3CN-H_2O 70:30 (flow rate: 2 ml/min, detector: UV 210 nm).
13. Rollimembrin (**3**), $[\alpha]_D$ +17.5 (c 0.4, MeOH); IR (dry film) ν max: 3430, 2924, 2849, 1750, 1647, 1462, 1316, 1200, 1070 cm^{-1}; UV (EtOH) λ max (log ε): 210 (3.95); HREIMS *m/z* 594.4470 $[M]^+$ (calc. 594.4495 for $C_{35}H_{62}O_7$), 483.4032 (calc. 483.4045 for $C_{29}H_{55}O_5$), 423.2755 (calc. 423.2746 for $C_{24}H_{39}O_6$), 353.2348 (calc. 353.2328 for $C_{20}H_{33}O_5$), 283.1922 (calc. 283.1908 for $C_{16}H_{27}O_4$); FABMS *m/z* 617 $[M+Na]^+$, 595 $[MH]^+$; EIMS *m/z* (%): 483 (3), 465 (3), 423 (35), 353 (8), 335 (32), 317 (13), 311 (4), 293 (4), 283 (100), 265 (16), 247 (4), 241 (10), 223 (11), 153 (2) 141 (8), 123 (5), 111(4) (Figure 1); ^{1}H-NMR ($CDCl_3$, 400 MHz), ^{13}C-NMR ($CDCl_3$, 100 MHz), COSY-45 and HMQC NMR data, Table 1 and Figure 1.

14. Prepared from **3** (10 mg) by Ac_2O and pyridine at r.t. for 8h, to yield 10.7 mg of **3a**. Compound **3a** (4,13,22-triactyl-rollimembrin), $C_{41}H_{68}O_{10}$; IR (film) ν max: 2923, 2851, 1750, 1736, 1462, 1316, 1239, 1073 cm^{-1}; FABMS *m/z* 743 $[M+Na]^+$, 720 $[M]^+$; EIMS, see Figure 1; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.87 (3H, *t*, *J*= 7 Hz, CH_3-32), 1.39 (3H, *d*, *J*= 7 Hz, CH_3-35), 2.02 (3H, *s*, $OCOCH_3$-4), 2.04 (3H, *s*, $OCOCH_3$-22), 2.07 (3H, *s*, $OCOCH_3$-13), 2.50 (2H, *m*, H-3), 3.78-3.98 (4H, 2*m*, H-14,17,18,21), 4.90 (2H, *m*, H-13,22), 5.00 (1H, *dq*, *J*= 7 Hz, *J'*= 1.5 Hz, H-34), 5.09 (1H, *m*, H-4), 7.07 (1H, *d*, *J*= 1.5 Hz, H-33); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.00 (C-1), 170.64 ($O\underline{C}OCH_3$), 150.86 (C-33), 130.19 (C-2), 81.91 and 81.69 (C-14,21), 80.30 and 79.85 (C-17,18), 77.53 (C-34), 75.48 (C-22), 75.12 (C-13), 71.96 (C-4), 34.11 (C-5), 31.90 (C-30), 30.86 (C-12,23), 29.86 (C-3), 27.62 and 27.07 (C-15,16,19,20), 25.36 and 25.24 (C-6,11,24), 22.67 (C-31), 21.20 ($OCO\underline{C}H_3$), 18.92 (C-35), 14.11 (C-32).
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19. Preparation of the C(4,13,22)-(*S*)- and (*R*) MTPA esters of rollimembrin (**3**). To a stirred solution of **3** (2.5 mg) in CH_2Cl_2 at r.t., was added pyridine, 4-(dimethylamino) pyridine and (*R*)-MTPA-Cl or (*S*)-MTPA-Cl.[20] The mixture was allowed to sit for 2h at r.t., saturated after with $NaHCO_3$ and extracted with CH_2Cl_2. By usual treatment the (S) and (R)-MTPA esters of rollimembrin were obtained: 2 mg of **3b** and 2.5 mg of **3c**.
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22. Inverted submitochondrial particles (SMP) from beef heart were obtained by extensive ultrasonic disruption of frozen-thawed mitochondria in such way to produce open membrane fragments where permeability barriers to substrates were lost. After ultracentrifugation they were finally resuspended in 250 mM sucrose, 10 mM Tris-HCl buffer, pH 7.4, and stored frozen at -80 °C.[23]
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