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New Method for the Determination of the Absolute Stereochemistry in Antitumoral Annonaceous Acetogenins

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Abstract: The absolute configurations at the carbinol centers in several acetogenins were determined through *p*-bromophenylurethane derivatives and subsequent Mosher ester methodology. This method has been applied on α,α'-dihydroxylated adjacent bis-THF acetogenins with a *threo/cis/threo/cis/erythro* relative configuration {membrarollin (**1**), a new acetogenin isolated from *Rollinia membranacea* seeds, rollimembrin (**2**), membranacin (**3**) and rolliniastatin-1 (**4**)}, and a *threo/trans/threo/trans/erythro* relative configuration {motrilin (**5**), squamocin (**6**), and desacetyluvaricin (**7**)}. **1** was found to be the most potent inhibitor of the mammalian mitochondrial complex I.

Annonaceous acetogenins are a group of secondary metabolites that have attracted a great interest because of their original structures and their cytotoxic, antitumor, parasitic and insecticide activities.[1-3] These biological effects have been related with the ability of acetogenins to inhibit the NADH:ubiquinone oxidoreductase (complex I) of the mitochondrial electron transport chain.[4,5] Classical acetogenins can be included into three subgroups: mono-tetrahydrofuranic (THF), adjacent bis-THF, and non adjacent bis-THF. One of the most frequently found,[6] the α,α'-dihydroxylated adjacent bis-THF acetogenins, have six stereogenic centers across the THF system.

Because of their waxy nature, they do not produce crystals for an adequate X-ray crystallographic analysis. Thus, the determination of the absolute stereochemistry represents the major problem in the elucidation of these structures; in addition, the stereochemistries, in many cases, influence the relative potencies and biological specificities.[2,3,7] Several approaches have been made based on the preparation of different derivatives and then the application of Mosher ester methodology, or by simply preparing per-Mosher ester derivatives.[8,9] A method based on the formation of formaldehyde acetal derivatives and the subsequent application of the Mosher ester has been described for several acetogenins having 1,2-, 1,4-, and 1,5-diols along their aliphatic chains.[10] Frequently, very small differences between the proton chemical shifts of (*S*)- and (*R*)- MTPA esters (α-methoxy-α-(trifluoromethyl) phenylacetates) on both sides of the chiral carbinol centers are observed (< 0.001 and sometimes described as approximately 0). Moreover, systematic irregular $\Delta\delta_{S-R}$ values of the per-Mosher ester derivatives in α,α'-dihydroxylated adjacent bis-THF acetogenins were obtained by several authors.[9,11,12] This effect should be explained as being due to the steric interactions between the bulky bis-THF rings and the MTPA esters. Thus, only an absolute configuration prediction could be envisaged by this method.

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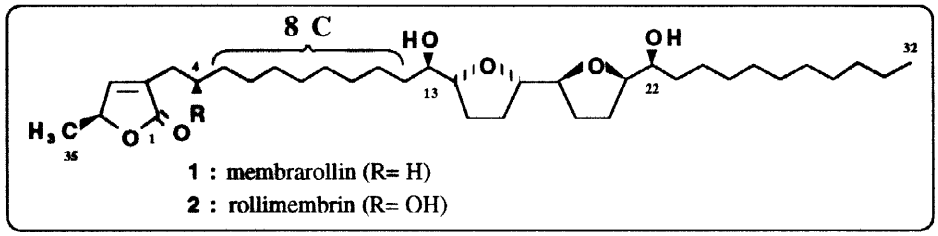
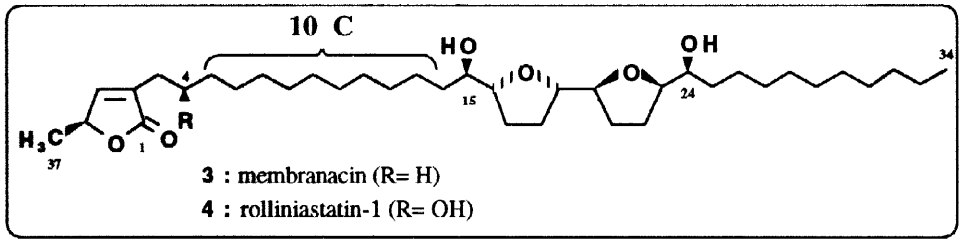
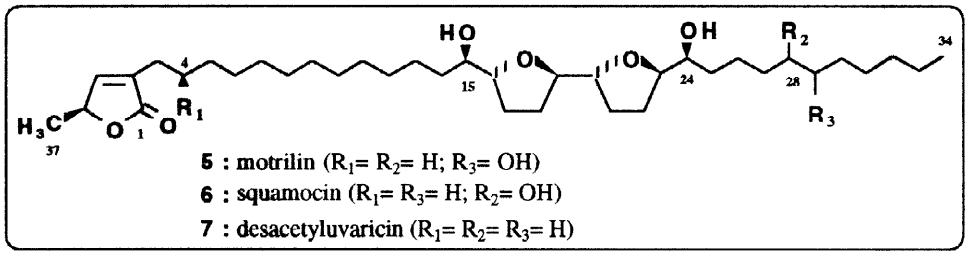
* **Adjacent bis-THF Acetogenins:** ***threo/cis/threo/cis/erythro***





* **Adjacent bis-THF Acetogenins:** ***threo/trans/threo/trans/erythro***



In order to establish unambiguously the absolute stereochemistry of a carbinol center in polyhydroxylated adjacent bis-THF acetogenins, we describe here a new method to prepare the monoalcohol derivatives by treatment with *p*-bromophenylisocyanate, formation of the corresponding mono- or di-urethane derivatives, and subsequent (*S*) and (*R*) mono-Mosher esters. The method was applied to several adjacent bis-THF acetogenins with a *threo/cis/threo/cis/erythro* relative configuration {membrarollin (**1**), rollimembrin (**2**), membranacin (**3**) and rolliniastatin-1(**4**)}, and *threo/trans/threo/trans/erythro* relative configuration {motrilin (**5**), squamocin (**6**) and desacetyluvaricin (**7**)}. The validity of this method is discussed.

On the other hand, one of them, membrarollin (**1**), is a new compound whose structural elucidation and inhibitory effect on the NADH oxidase of the mitochondrial electron transport chain, are described.

Results and Discussion

Membrarollin (**1**) was isolated as a white waxy compound from the MeOH extract of *Rollinia membranacea* seeds[13] by the usual chromatographic methods followed by semipreparative HPLC. The molecular weight of **1** was determined by peaks at *m/z* 601 $[M+Na]^+$ and *m/z* 579 $[MH]^+$ in the LSIMS. The presence of two OH groups was suggested by an IR band at 3430 cm^{-1}, by succesive losses of two H_2O molecules from $[MH]^+$ in LSIMS, and the preparation of the diurethane derivative with *p*-bromophenyl-isocyanate. A prominent IR carbonyl absorption in **1** at 1750 cm^{-1} and a positive reaction with Kedde reagent, suggested the presence of an α,β-unsaturated γ-lactone ring.[2,3] This 4-pentanolide moiety in **1**, as well as the absence of an hydroxyl group at the C-4 position, were deduced by 1D and 2D NMR spectroscopy (Table 1).

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

H	δ (*J*, Hz)	Coupling in COSY 45	Coupling in HMQC (multiplicity DEPT ^{13}C)
1			174.00 (C)
2			134.12 (C)
3	2.26 *tdd* (7.7; 1.5; 1.5)	H-4, H-33, H-34	25.15 (CH_2)
4	1.55 *m*	H-3	27.37 (CH)
5-10	1.24 *m*		29.59 (CH_2)
11	1.24 *m*		26.00 (CH_2)
12	1.46 *m*	H-13	34.26 (CH_2)
13	3.40 *dt* (7.3; 5.0)	H-14, H-12	74.00 (CH)
14	3.81 *m*	H-13	83.02 (CH)
15,16	1.77; 1.93 *m*	H-14, H-17	28.76, 27.88 (CH_2)
17	3.87 *m*	H-16	81.10 (CH)
18	3.87 *m*	H-19	80.99 (CH)
19,20	1.77; 1.93 *m*	H-18, H-21	28.42, 23.70 (CH_2)
21	3.90 *m*	H-20, H-22	83.02 (CH)
22	3.85 *m*	H-23, H-21	71.91 (CH)
23	1.36 *m*	H-22	32.76 (CH_2)
24	1.30 *m*		25.78 (CH_2)
25-29	1.24 *m*		29.59 (CH_2)
30	1.24 *m*		31.87 (CH_2)
31	1.24 *m*	H-32	22.66 (CH_2)
32	0.87 *t* (7.0)	H-31	14.10 (CH_3)
33	6.98 *dt* (1.5; 1.5)	H-3, H-34	148.82 (C)
34	4.98 *qq* (1.5; 6.8)	H-33, H-35	77.41 (CH)
35	1.40 *d* (6.8)	H-34	19.20 (CH_3)

An α,α'-dihydroxylated adjacent bis-THF system with a *threo/cis/threo/cis/erythro* relative stereochemistry was deduced for **1** by comparing their ^1H- and ^{13}C-NMR methine bearing oxygenated values with those of the only other three acetogenins with an identical system: rollimembrin (**2**),[12] membranacin (**3**)[13] and rolliniastatin-1 (**4**).[14] The placement of the flanked carbinol centers (at C-13 and C-22) and the adjacent bis-THF moieties (between C-14 and C-21) along the aliphatic chain, were determined by the classic diagnostic of the EIMS fragment ions of **1** (Figure 1).

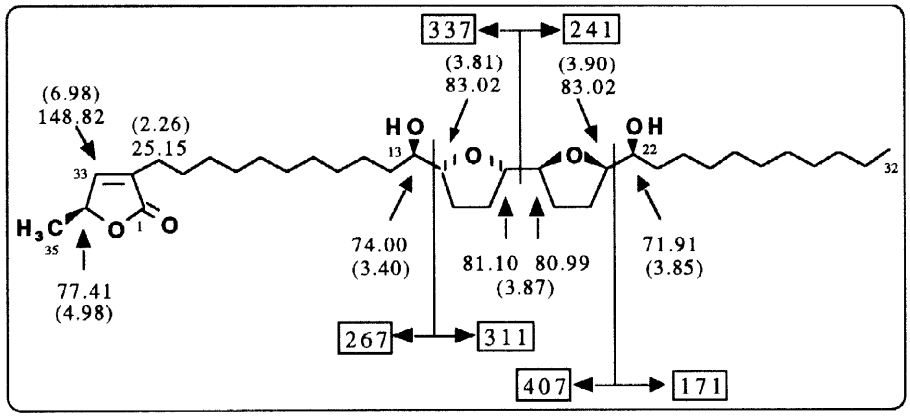


Figure 1. Diagnostic values of ^{13}C-NMR, ^1H-NMR (in parentheses) and EIMS of membrarollin (**1**)

Therefore, membrarollin (**1**) is a new C_{35} acetogenin and the fourth member of the unusual bis-THF acetogenins with a *threo/cis/threo/cis/erythro* relative configuration.

As mentioned above, we assessed the absolute configurations of several adjacent bis-THF acetogenins through formation of monourethane or diurethane ester derivatives by treatment with *p*-bromophenylisocyanate and subsequent application of Mosher ester methodology. This procedure permits us to obtain monoalcohols of the parent acetogenins, to decrease the number of free hydroxyls, and does not change the configuration at the carbinol centers. Consequently, the assignments of the proton chemical shifts affected by their mono-Mosher ester derivatives become more feasible. Thus, the obtention of these monoalcohols in good yields was the first aim of this procedure and several assay conditions were needed.

Pettit[15] referred to the obtention of a 15-*O*-*p*-bromophenylurethane derivative from rolliniastatin-1 (a trihydroxylated bis-THF acetogenin) by the addition of *p*-bromophenylisocyanate, but no reaction conditions were reported. When we applied this method for rolliniastatin-1 (**4**), adding millimolar equivalent of *p*-bromophenylisocyanate for 4, 6, 10 or 12 hours at room temperature, no reaction product was obtained and the starting acetogenin was almost completely recovered. By increasing the amount of reagent to 2 millimolar equivalents and refluxing, a mixture of two compounds **4a** (yield: 9.4 %) and **4b** (yield: 27.5 %) were obtained (Table 2).

Table 2.- Urethane derivatives obtained after treatment of **1-7** by *p*-bromophenylisocyanate* refluxed 24h

Compound (mg)	Reagent (eq.)*	Reaction Compounds	Yield (%)
1 (33.5)	1.5	**1a**: 13-*O*-*p*-Br-phenylurethane-membrarollin	43.9
		1b: 13,22-*O*-*p*-Br-phenylurethane-membrarollin	7.0
2 (29.5)	3.0	**2a**: 4,13-*O*-*p*-Br-phenylurethane-rollimembrin	51.0
		2b: 4,13,22-*O*-*p*-Br-phenylurethane-rollimembrin	36.7
3 (29.9)	1.5	**3a**: 15-*O*-*p*-Br-phenylurethane-membranacin	41.7
		3b:15,24-*O*-*p*-Br-phenylurethane-membranacin	6.2
4 (200)	2.0	**4a**: 4,15-*O*-*p*-Br-phenylurethane-rolliniastatin-1	9.4
		4b: 15-*O*-*p*-Br-phenylurethane-rolliniastatin-1	27.5
4 (200)	3.0	**4a**: 4,15-*O*-*p*-Br-phenylurethane-rolliniastatin-1	47.5
		4c: 4,15,24-*O*-*p*-Br-phenylurethane-rolliniastatin-1	18.7
5 (30.1)	3.0	**5a**: 15,29-*O*-*p*-Br-phenylurethane-motrilin	20.3
		5b: 15,24,29-*O*-*p*-Br-phenylurethane-motrilin	6.7
6 (150)	3.0	**6a**: 15,28-*O*-*p*-Br-phenylurethane-squamocin	10.0
		6b: 24,28-*O*-*p*-Br-phenylurethane-squamocin	6.9
		6c: 15,24,28-*O*-*p*-Br-phenylurethane-squamocin	22.1
		6d: 28-*O*-*p*-Br-phenylurethane-squamocin	15.4
7 (35.2)	1.5	**7a**: 15-*O*-*p*-Br-phenylurethane-desacetyluvaricin	24.1
		7b: 15,24-*O*-*p*-Br-phenylurethane-desacetyluvaricin	8.4

The structure of **4b** was determined by LSIMS (*m/z* 843 $[M+Na]^+$ and *m/z* 821 $[MH]^+$) and by ^1H and COSY 45 NMR spectral data. The absence of the hydroxymethine proton signal resonance at δ 3.40 corresponding to H-15 in **4** and the appearance of a downfield multiplet at δ 4.82 lead us to identify **4b** as the 15-*O*-*p*-bromophenylurethane of rolliniastatin-1. The structure of **4a** was also confirmed by LSIMS (*m/z* 1041 $[M+Na]^+$ and *m/z* 1019 $[MH]^+$) and by NMR spectral data. In the ^1H-NMR spectrum of **4a**, in addition to that described above for **4b**, the characteristic ABX system corresponding to the nonequivalent protons H-3a, H-3b and H-4 observed in **4** and **4b** is changed by a *tdd* at δ 2.56 (H-3) correlated with a proton signal resonance at δ 5.03 (H-4) in the ^1H-^1H COSY spectrum. Thus, compound **4a** was identified as the 4,15-*O*-*p*-bromophenylurethane of **4** and confirmed the position of the free hydroxyl group at C-24 in this monoalcohol.

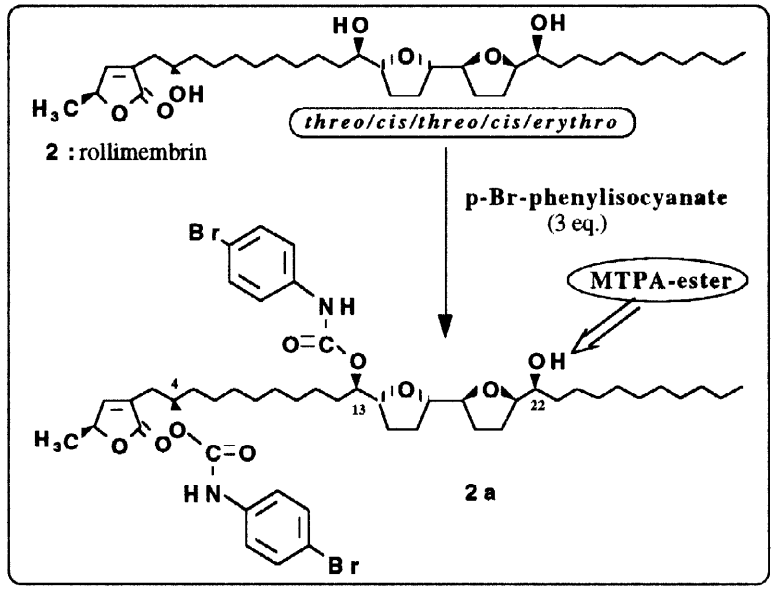


Figure 2.
Formation of diurethane monoalcohol bis-THF acetogenin derivative (**2a**) from rollimembrin (**2**)

Because of the low yield in the monoalcohol **4a**, a third set of experiments were carried out by refluxing for 24 h with 3 millimolar equivalents of reagent. In this condition, **4c** (triurethane, 18.7%) and a most important yield of **4a** (47.5%) were obtained. Addition of 3 eq. of *p*-bromophenylisocyanate was considered as the adequate for further assays with other trihydroxylated acetogenins: rollimembrin (**2**), motrilin (**5**) and squamocin (**6**) (Table 2 and Figure 2). Several conditions were also assayed to obtain a monoalcohol from the dihydroxylated adjacent bis-THF acetogenin membrarollin (**1**). Addition of 1.5 millimolar eq. of *p*-bromophenylisocyanate was selected in order to prepare the monourethane monoalcohol derivatives from membrarollin (**1**), membranacin (**3**) and desacetyluvaricin (**7**) (Table 2). The structural elucidation of these monoalcohols was also achieved by LSIMS, and ^1H- and COSY 45 NMR spectral data (Table 3).

The monoalcohols (**1a-7a** and **6b**) were converted into their corresponding (*S*)-MTPA and (*R*)-MTPA esters. Detailed ^1H-^1H COSY analysis allowed the assignment of the proton chemical shifts affected by these mono-Mosher ester derivatives, and the absolute configurations at C-15, C-22 or C-24 was unambiguously established (Table 4). Because the relative configurations around the bis-THF system were already well known,[2,3,14] the absolute configurations of all the carbinol centers was concluded to be: 13*R*, 14*R*, 17*S*, 18*S*, 21*R*, 22*S* for **1** and **2**, 15*R*, 16*R*, 19*S*, 20*S*, 23*R*, 24*S* for **3** and **4**, 15*R*, 16*R*, 19*R*, 20*R*, 23*R*, 24*S* for **5**, **6** and **7**.

Table 3. Comparison of affected ^{1}H-NMR δ values of monoalcohols (**1a-7a** and **6b**) with those of the parent acetogenins with a *threo/cis/threo/cis/erythro* (**1-4**) and a *threo/trans/threo/trans/erythro* relative configuration (**5-7**)

	Compounds							
Proton	**1**	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**
H-3a	2.26	2.26	2.39	2.56	2.25	2.25	2.36	2.56
H-3b	2.26	2.26	2.52	2.56	2.25	2.25	2.52	2.56
H-4	1.55	1.55	3.81	5.05	1.53	1.53	3.81	5.03
H-13	3.40	4.83	3.40	4.85				
H-15					3.40	4.85	3.40	4.82
OH free		*C-22*		*C-22*		*C-24*		*C-24*

Proton	**5**	**5a**	**6**	**6a**	**6b**	**7**	**7a**
H-15	3.38	4.80	3.39	4.82	3.38	3.37	4.81
H-16	3.85	4.10	3.85	4.07	3.79	3.80	4.08
H-23	3.85	3.86	3.85	3.86	4.06	3.80	3.84
H-24	3.88	3.74	3.85	3.74	4.86	3.85	3.78
H-28			3.52	4.82	4.86		
H-29	3.55	4.80					
OH free		*C-24*		*C-24*	*C-15*		*C-24*

Table 4.- Diagnostic ^{1}H-NMR values (400 MHz, $CDCl_3$) of (*S*)- and (*R*)-MTPA ester of monoalcohols **1a-7a** and **6b**

Monoalcohol	(*S*)-MTPA ester		(*R*)-MTPA ester		$\Delta\delta_{(S-R)}$	Config
1a	H-21	3.925	H-21	3.870	+ 0.055	22*S*
	H-23	1.601	H-23	1.670	- 0.069	
2a	H-21	3.900	H-21	3.830	+ 0.070	22*S*
	H-23	1.573	H-23	1.615	- 0.042	
3a	H-23	3.890	H-23	3.860	+ 0.030	24*S*
	H-25	1.550	H-25	1.655	- 0.105	
4a	H-23	3.875	H-23	3.840	+ 0.035	24*S*
	H-25	1.540	H-25	1.625	- 0.085	
5a	H-23	4.024	H-23	3.967	+ 0.057	24*S*
	H-25	1.573	H-25	1.608	- 0.035	
6a	H-23	4.024	H-23	3.971	+ 0.053	24*S*
	H-25	1.549	H-25	1.603	- 0.054	
6b	H-14	1.597	H-14	1.470	+ 0.127	15*R*
	H-16	4.017	H-16	4.061	- 0.044	
7a	H-23	4.043	H-23	3.997	+ 0.046	24*S*
	H-25	1.530	H-25	1.580	- 0.050	

In conclusion, with this new method, we have succeeded in the determination of the absolute stereochemistries across the THF system for compounds **1-7**. In the first step, monoalcohols at the α' position (C-22 or C-24) were readily obtained in good yields (40-51%) for acetogenins with a *threo/cis/threo/cis/erythro* relative configuration (**1-4**) after treatment by *p*-bromophenylisocyanate (Table 2). The yields were considerably lower (10-24 %) in compounds with a *threo/trans/threo/trans/erythro* relative configuration (**5-7**) whose two α and α' monoalcohols were obtained. The results also furnish experimental support for this new approach in applying the Mosher ester method. So, the application of Mosher methodology to monoalcohol acetogenin derivatives allowed us to know easily the absolute configurations of a group of adjacent bis-THF acetogenins. Moreover regular $\Delta\delta_{(S-R)}$ values (0.03-0.12) were observed and the usual ambiguous results obtained in Mosher ester acetogenins were eluded.

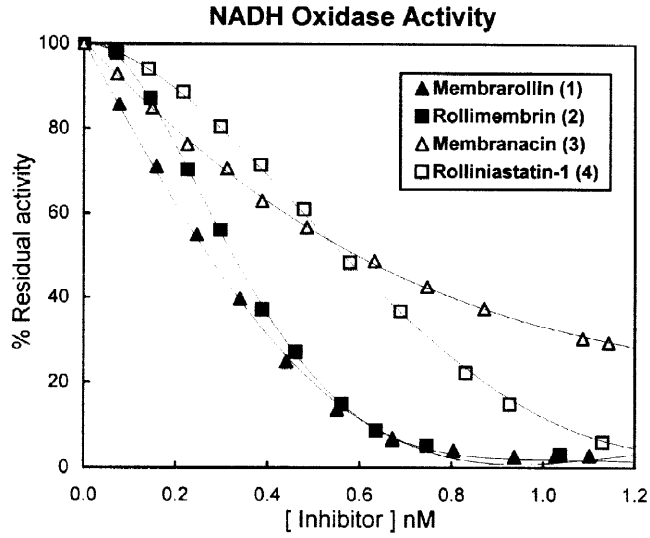


Figure 3.- Titration curves of acetogenins **1-4** against NADH oxidase activity

In an attempt to advance our study of the structure activity relationship (SAR) of the unique acetogenins with a *threo/cis/threo/cis/erythro* relative configuration we assayed the new membrarollin (**1**) as inhibitor of the mitochondrial electron transport chain. We compared the potency found for **1** with the one previously published for compunds **2-4**.[5,12] Figure 3 shows the titration curves for the four acetogenins, membrarollin (**1**), rollimembrin (**2**), membranacin (**3**) and rolliniastatin-1 (**4**) against the NADH oxidase activity, that represents an integrated activity including the respiratory complexes I, III and IV. Because of acetogenins only inhibit the mitochondrial complex I,[4,5] NADH oxidase activity is a good method to establish a SAR of these compounds.

As expected, membrarollin (**1**) was found to be a very potent inhibitor of the NADH oxidase activity. Moreover, from IC_{50} values (Figure 4) we confirm that a shorter alkyl chain between the γ-lactone ring and the THF moiety plays an important role in the inhibitory potency, as membrarollin (**1**) and rollimembrin (**2**) resulted more potent inhibitors of mitochondrial complex I than membranacin (**3**) and rolliniastatin-1 (**4**), respectively.

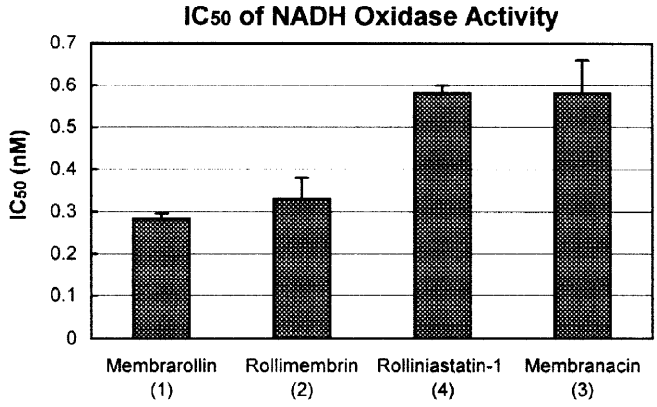


Figure 4.- IC_{50} values of acetogenins **1-4** for the NADH oxidase activity

The hyperbolic shape of the titration curve found for membrarollin (**1**) was similar to that of membranacin (**3**), probably due to the absence of a hydroxyl group at the C-4 position (Figure 3). The same tendency, but with a sigmoidal shape, was previously reported[12] for rollimembrin (**2**) and rolliniastatin-1 (**4**). Therefore the hydroxyl at C-4 position could affect the interaction of the acetogenins with the enzyme.

Experimental Section

General Experimental Procedures. Optical rotation was determined with a Perkin-Elmer 241 polarimeter. IR spectra (film) were run on a Perkin-Elmer 843 spectrometer. UV spectrum was taken on a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer in MeOH solution. EIMS and LSIMS were determined on a VG Auto Spec Fisons spectrometer. NMR spectra were recorded on a Bruker AC-250 or a Varian Unity-300 and 400 spectrometer at 250, 300 or 400 MHz for ^{1}H, and 100 MHz for ^{13}C, using the solvent signal as reference ($CDCl_3$ at δ 7.26 and δ 77.0). Multiplicities of ^{13}C-NMR resonances were assigned by DEPT experiments. COSY 45 and HMQC correlations were run using a Varian Unity-400 MHz instrument. Semipreparative HPLC was carried out on a LiChroCart^R 100 RP-18 column (25 x 1 cm i.d., 10 μm particle size) using MeOH-H_2O 85:15 (flow rate: 2 ml/min, detector: UV 210 nm). Rt: 14.49 min for **1**. Compounds **2-7** were isolated by our group from Annonaceous species.[1,12]

Bioassays. The bioactivity of membrarollin (**1**) was assayed using inverted submitochondrial particles (SMP) from beef heart. They 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. They were ultracentrifugated, resuspended in 250 mM sucrose, 10 mM Tris-HCl buffer, pH 7.4, and stored frozen at -80 °C. For the inhibitor tritrations, **1** was diluted in absolute ethanol at 2 mM. The stock solution was kept in the dark at -20 °C. Appropiate dilutions between 2 and 10 μM were make before the tritrations. Beef heart SMP were diluted to 0.5 mg.ml^{-1} in sucrose-Tris buffer and treated with 300 μM NADH to activate complex I. Increasing concentrations of the ethanolic solution of inhibitor were added to this preparation, with about 5 min incubation on ice between each addition. Maximal ethanol concentration never exceeded 2% of volumen and control activity was not affected by this concentration. After each addition of inhibitor, NADH oxidase activity was measured. This integrated enzymatic activity was assayed at 22 °C in 50 mM potassium phosphate buffer, pH 7.4, 1 mM EDTA, in a double beam spectrophotometer. SMP were diluted to 6-7 μg.ml^{-1} in the cuvette. Aerobic NADH oxidation was measured in presence of 75 μM NADH and following the decrease in absorbance at 340 nm (ε = 6.22 mM^{-1}.cm^{-1}). Data from four titrations were pulled and fitted for graphics, and to assess the means and standard deviations.

Membrarollin, 1. $C_{35}H_{62}O_6$; $[\alpha]_D$+ 10° (*c*= 0.8, MeOH); IR (film) ν_{max} 3370, 2940, 2880, 1750, 1456, 1380, 1039 cm^{-1}; UV (EtOH) λ_{max} (log ε) 210 (3.95) nm; EIMS (%), 578 (6) [M]$^+$, 560 (3), 542 (5), 436 (4), 407 (24), 390 (12), 337 (44), 319 (72), 311 (12), 267 (100), 241 (53), 223 (12), 171 (4), 153 (6), 111 (15), 97 (43) see Fig. 1; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1 and Fig. 1.

General procedure to obtain monoalcohol bis-THF acetogenins:

A. Preparation of p-bromophenylurethane ester derivatives from the dihydroxylated membrarollin (**1**). A solution of **1** (33.5 mg, 0.057 mmol) in CH_2Cl_2 (2 mL) was treated with 1.5 millimolar eq. of *p*-bromophenylisocyanate (17 mg in CH_2Cl_2, 1 mL). After refluxing for 24 h the reaction mixture was washed by water, evaporated, and the residue (50.2 mg) purified on silicagel 60H with hexane/EtAcO 74:26, to afford 13-*O*-*p*-bromo-phenylurethane monoester derivative **1a** (19.6 mg, 0.025 mmol, 43.9%) and 13,22-*O*-*p*-bromophenylurethane diester derivative **1b** (4 mg, 0.004 mmol, 7.0 %). For other dihydroxylated acetogenins assayed, see Table 2.

13-*O*-*p*-bromophenylurethane of membrarollin, 1a. $C_{42}H_{66}{}^{80}BrNO_7$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^{1}H (250 MHz) and COSY 45 (400 MHz) NMR δ: 8.29 (1H, NH), 7.39-7.28 (4H, Ar), 6.97 (1H, H-33), 4.97 (1H, H-34), 4.83 (1H, H-13), 3.88 (5H, H-14, H-17, H-18, H-21, H-22), 2.26 (2H, H-3); LSIMS: *m/z* 799 [M+Na]$^+$ and *m/z* 777 [MH]$^+$.

13,22-Di-*O*-*p*-bromophenylurethane of membrarollin, 1b. $C_{49}H_{70}{}^{80}Br_2N_2O_8$; IR (film): 1745 (C=O, lactone), 1720 (C=O, urethane); ^{1}H (250 MHz) and COSY 45 (400 MHz) NMR δ: 8.29 (2H, NH), 7.40-7.27 (8H, Ar), 6.98 (1H, H-33), 4.99 (2H, H-22, H-34), 4.89 (1H, H-13), 3.89 (4H, H-14, H-17, H-18, H-21), 2.26 (2H, H-3); LSIMS: *m/z* 997 [M+Na]$^+$ and *m/z* 975 [MH]$^+$.

15-*O*-*p*-bromophenylurethane of membranacin, 3a. $C_{44}H_{70}{}^{80}BrNO_7$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^{1}H- and COSY-45 NMR (400 MHz) δ: 8.27 (1H, NH), 7.39-7.28 (4H, Ar), 6.98 (1H, H-35), 4.97 (1H, H-36), 4.87 (1H, H-15), 3.92 (5H, H-16, H-19, H-20, H-23, H-24), 2.25 (2H, H-3); LSIMS: *m/z* 827 [M+Na]$^+$ and *m/z* 805 [MH]$^+$.

15,24-Di-*O*-*p*-bromophenylurethane of membranacin, 3b. $C_{51}H_{74}{}^{80}Br_2N_2O_8$; LSIMS: *m/z* 1025 [M+Na]$^+$ and *m/z* 1003 [MH]$^+$.

15-*O*-*p*-bromophenylurethane of desacetyluvaricin, 7a. $C_{44}H_{70}{}^{80}BrNO_7$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (250 MHz) and COSY 45 (400 MHz) NMR δ: 7.49-7.28 (4H, Ar), 6.98 (1H, H-35), 6.78 (1H, NH), 5.00 (1H, H-36), 4.81 (1H, H-15), 4.08 (1H, H-16), 3.89 (2H, H-19, H-20), 3.84 (1H, H-23), 3.78 (1H, H-24), 2.25 (2H, H-3); LSIMS: *m/z* 827 $[M+Na]^+$ and *m/z* 805 $[MH]^+$.

15,24-Di-*O*-*p*-bromophenylurethane of desacetyluvaricin, 7b. $C_{51}H_{74}{}^{80}Br_2N_2O_8$; LSIMS: *m/z* 1025 $[M+Na]^+$ and *m/z* 1003 $[MH]^+$.

B. Preparation of p-bromophenylurethane ester derivatives from the trihydroxylated rolliniastatin-1 (**4**). A solution of **4** (200 mg, 0.32 mmol) in CH_2Cl_2 (3 mL) was treated with 3 millimolar eq. of *p*-bromophenylisocyanate (190.5 mg in CH_2Cl_2, 3 mL). After refluxing for 24 h the reaction mixture was washed by water, evaporated, and the residue (390 mg) purified on silicagel 60H with CH_2Cl_2/EtAcO 93:7, to afford 4,15-*O*-*p*-bromophenylurethane diester derivative **4a** (155.5 mg, 0.152 mmol, 47.5%) and 4,15,24-*O*-*p* bromophenylurethane triester derivative **4c** (73.6 mg, 0.06 mmol, 18.7%). For other trihydroxylated acetogenins assayed, see Table 2.

4,15-Di-*O*-*p*-bromophenylurethane of rolliniastatin-1, 4a. $C_{51}H_{74}{}^{80}Br_2N_2O_9$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (250 MHz) and COSY 45 (400 MHz) NMR δ: 8.29 (1H, NH), 7.35-7.29 (8H, Ar), 7.18 (1H, H-35), 7.10 (1H, NH), 5.03 (1H, H-4), 4.94 (1H, H-36), 4.82 (1H, H-15), 3.86 (5H, H-16, H-19, H-20, H-23, H-24), 2.56 (2H, H-3); LSIMS: *m/z* 1041 $[M+Na]^+$ and *m/z* 1019 $[MH]^+$.

15-*O*-*p*-bromophenylurethane of rolliniastatin-1, 4b. $C_{44}H_{70}{}^{80}BrNO_8$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H and COSY 45 NMR (400 MHz) δ: 8.29 (1H, NH), 7.36-7.24 (4H, Ar), 7.15 (1H, H-35), 5.01 (1H, H-36), 4.82 (1H, H-15), 3.86 (6H, H-4, H-16, H-19, H-20, H-23, H-24), 2.50 (1H, H-3a), 2.36 (1H, H-3b); LSIMS: *m/z* 843 $[M+Na]^+$ and *m/z* 821$[MH]^+$.

4,15,24-Tri-*O*-*p*-bromophenylurethane of rolliniastatin-1, 4c. $C_{58}H_{78}{}^{80}Br_3N_3O_{10}$; IR (film): 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (250 MHz) and COSY 45 (400 MHz) NMR δ: 8.29 (2H, NH), 7.45-7.29 (12 H, Ar), 7.12 (1H, H-35), 6.76 (1H, NH), 5.08 (1H, H-4), 4.96 (2H, H-24, H-36), 4.79 (1H, H-15), 3.85 (4H, H-16, H-19, H-20, H-23), 2.56 (2H, H-3); LSIMS: *m/z* 1239 $[M+Na]^+$ and *m/z* 1217 $[MH]^+$.

4,13-Di-*O*-*p*-bromophenylurethane of rollimembrin, 2a. $C_{49}H_{70}{}^{80}Br_2N_2O_9$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (250 MHz) and COSY 45 (400 MHz) NMR δ: 8.29 (1H, NH), 7.41-7.28 (8H, Ar), 7.12 (1H, H-33), 6.69 (1H, NH), 5.04 (1H, H-4), 4.94 (1H, H-34), 4.85 (1H, H-13), 3.91 (5H, H-14, H-17, H-18, H-21, H-22), 2.56 (2H, H-3); LSIMS: *m/z* 1013 $[M+Na]^+$ and *m/z* 990 $[M]^+$.

4,13,22-Tri-*O*-*p*-bromophenylurethane of rollimembrin, 2b. $C_{56}H_{74}{}^{80}Br_3N_3O_{10}$; IR (film): 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (250 MHz) and COSY 45 (400 MHz) NMR δ: 8.29 (2H, NH), 7.46-7.29 (12H, Ar), 7.11 (1H, H-33), 6.79 (1H, NH), 5.06 (1H, H-4), 4.95 (2H, H-22, H-34), 4.78 (1H, H-13), 3.85 (4H, H-14, H-17, H-18, H-21), 2.56 (2H, H-3); LSIMS: *m/z* 1211 $[M+Na]^+$ and *m/z* 1188 $[M]^+$.

15,29-Di-*O*-*p*-bromophenylurethane of motrilin, 5a. $C_{51}H_{74}{}^{80}Br_2N_2O_9$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (250 MHz) and COSY 45 (400 MHz) NMR δ: 8.29 (1H, NH), 7.40-7.27 (8H, Ar), 6.95 (1H, H-35), 6.75 (1H, NH), 4.99 (1H, H-36), 4.80 (2H, H-15, H-29), 4.10 (1H, H-16), 3.86 (3H, H-19, H-20, H-23), 3.75 (1H, H-24), 2.25 (2H, H-3); LSIMS: *m/z* 1041 $[M+Na]^+$ and *m/z* 1019 $[MH]^+$.

15,24,29-Tri-*O*-*p*-bromophenylurethane of motrilin, 5b. $C_{58}H_{78}{}^{80}Br_3N_3O_{10}$; LSIMS: *m/z* 1239 $[M+Na]^+$ and *m/z* 1216 $[M]^+$

15,28-Di-*O*-*p*-bromophenylurethane of squamocin, 6a. $C_{51}H_{74}{}^{80}Br_2N_2O_9$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (300 MHz) and COSY 45 (400 MHz) NMR δ: 7.40-7.28 (8H, Ar), 6.98 (1H, H-35), 6.85 (1H, NH), 6.77 (1H, NH), 4.99 (1H, H-36), 4.82 (2H, H-15, H-28), 4.07 (1H, H-16), 3.87 (2H, H-19, H-20), 3.86 (1H, H-23), 3.74 (1H, H-24), 2.25 (2H, H-3); LSIMS: *m/z* 1041 $[M+Na]^+$ and *m/z* 1018 $[M]^+$.

24,28-Di-*O*-*p*-bromophenylurethane of squamocin, 6b. $C_{51}H_{74}{}^{80}Br_2N_2O_9$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (300 MHz) and COSY 45 (400 MHz) NMR δ: 7.38-7.28 (8H, Ar), 7.15 (1H, NH), 6.98 (1H, H-35), 6.75 (1H, NH), 4.98 (1H, H-36), 4.86 (2H, H-24, H-28), 4.06 (1H, H-23), 3.87 (2H, H-19, H-20), 3.79 (1H, H-16), 3.38 (1H, H-15), 2.25 (2H, H-3); LSIMS: *m/z* 1041 $[M+Na]^+$ and *m/z* 1018 $[M]^+$.

15,24,28-Tri-*O*-*p*-bromophenylurethane of squamocin, 6c. $C_{58}H_{78}{}^{80}Br_3N_3O_{10}$; LSIMS: *m/z* 1239 $[M+Na]^+$ and *m/z* 1216 $[M]^+$

28-*O*-*p*-bromophenylurethane of squamocin, 6d. $C_{44}H_{68}{}^{80}BrNO_8$; IR (film): 3330 (OH), 1745 (C=O, lactone), 1720 (C=O, urethane); ^1H (250 MHz) and COSY 45 (400 MHz) NMR δ: 7.39-7.29 (4H, Ar), 7.00 (1H, NH), 6.98 (1H, H-35), 4.98 (1H, H-36), 4.81 (1H, H-28), 3.86 (5H, H-16, H-19, H-20, H-23, H-24), 3.37 (1H, H-15), 2.25 (2H, H-3); **LSIMS**: *m/z* 833 $[M+Na]^+$ and *m/z* 810 $[M]^+$.

General procedure to prepare MTPA esters of urethane monoalcohol acetogenins:

To a solution of monoalcohol (**1a-7a and 6b**) in CH_2Cl_2 were sequentially added pyridine, 4-(dimethylamino)-pyridine and (*R*)-(-)- or (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride as previously described.[9,12] To the mixture after stirring at room temperature for 5 h, CH_2Cl_2 was added and washed with H_2O. The CH_2Cl_2 solution was washed using a saturated $NaHCO_3$ solution, H_2O and dried in vacuo to give the (*S*)- and (*R*)-Mosher mono-esters in quantitative yield.

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