Semisynthesis of Antitumoral Acetogenins: SAR of Functionalized Alkyl-Chain Bis-Tetrahydrofuranic Acetogenins, Specific Inhibitors of Mitochondrial Complex I

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Received May 29, 2000

The acetogenins of Annonaceae are known by their potent cytotoxic activity. In fact, they are promising candidates as a new future generation of antitumoral drugs to fight against the current chemiotherapic resistant tumors. The main target enzyme of these compounds is complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial respiratory chain, a key enzymatic complex of energy metabolism. In an attempt to characterize the relevant structural factor of the acetogenins that determines the inhibitory potency against this enzyme, we have prepared a series of bis-tetrahydrofuranic acetogenins with different functional groups along the alkyl chain. They comprise several oxo, hydroxylimino, mesylated, triazido, and acetylated derivatives from the head series compounds rolliniastatin-1, guanacone, and squamocin. Our results suggest a double binding point of acetogenins to the enzyme involving the α,α′-dihydroxylated tetrahydrofuranic system as well as the alkyl chain that links the terminal α,*β*-unsaturated-*γ*-methyl-*γ*-lactone. The former mimics and competes with the ubiquinone substrate. The latter modulates the inhibitory potency following a complex outline in which multiple structural factors probably contribute to an appropriate conformation of the compound to penetrate inside complex I.

Introduction

Annonaceous acetogenins (ACG) are very interesting compounds due to their well-known cytotoxic, antitumor, parasitic, and insecticidic activities.[1−3] ACG mainly acts by inhibiting the enzyme NADH:ubiquinone oxidoreductase, or complex I, of the respiratory chain,[4−6] which plays an important role in the maintenance of the bioenergetic function of the cells by driving approximately one-third of the ATP synthesis. Complex I is the most complicated and the least-well-known of the mitochondrial respiratory complexes. The interest in this enzymatic complex is growing due to its possible implication in the pathogenesis of a wide spectrum of human neurodegenerative diseases,[7,8] as well as to its identification as the enzyme affected by several therapeutic drugs and new commercial pesticides.[9] Both facts have propelled the search for new natural or synthetic inhibitors of this bioenergetic enzymatic complex, as well as its selection as a target for a new future generation of antitumoral drugs to control the tumors that are still resistant to current chemiotherapics.[3,10] As a consequence, an enormous number of inhibitory compounds have been identified in only a few years.[11] Nevertheless, these inhibitors belong to very different chemical groups, which hindered the establishment of a general structure−activity relationship (SAR).[9,12] Indeed, the identification of the chemical determinants required to obtain a potent complex I inhibition, as well as the contribution of the different functional groups present in the chemical structures of the inhibitors, has not been established without ambiguity. Therefore, the study of a series of derivatives, in which the chemical structures have been systematically modified, is very useful for understanding how complex I inhibitors interact with the enzyme.

The most powerful inhibitors of the mammalian complex I belong to the group of ACG.[13] In fact, some of them are considered the closest near-stoichiometric natural inhibitors of complex I.[14] However, the possibility to carry out semisynthetic modifications of these molecules has offered a valuable opportunity to advance the knowledge of how to modulate their potent cytotoxic action through the identification and characterization of the functional groups responsible for inhibitory potency. Several previous studies have shown that the relevant factors that affect the cytotoxicity and inhibitory potency of ACG are located mainly in the alkyl chain that links the α,α′-dihydroxylated tetrahydrofuranic (THF) system of the molecule with the terminal α,*β*-unsaturated-methyl-*γ*-lactone.[15−19] However, it seems that the stereochemistry of the THF system can also affect the inhibitory action,[6,13,14,20] whereas the possible contribution of other chemical groups, such as those that surround the THF system, has not been explored extensively.

With the purpose of advancing the knowledge of the inhibitory mode of action of the potent ACG, we have carried out some semisynthetic modifications of a series

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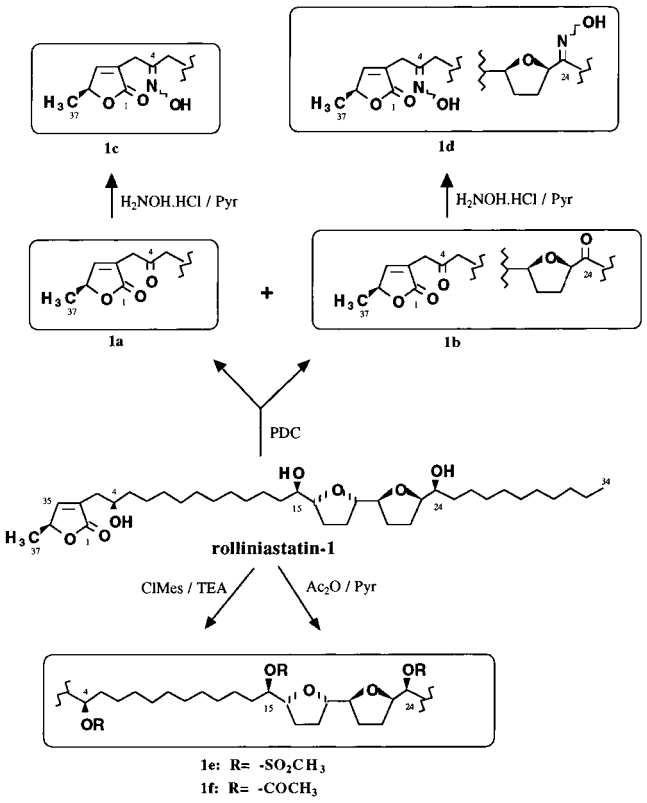
10.1021/jm000911j CCC: $19.00
Published on Web 11/16/2000

Scheme 1. Rolliniastatin-1 (**1**) Derivatives



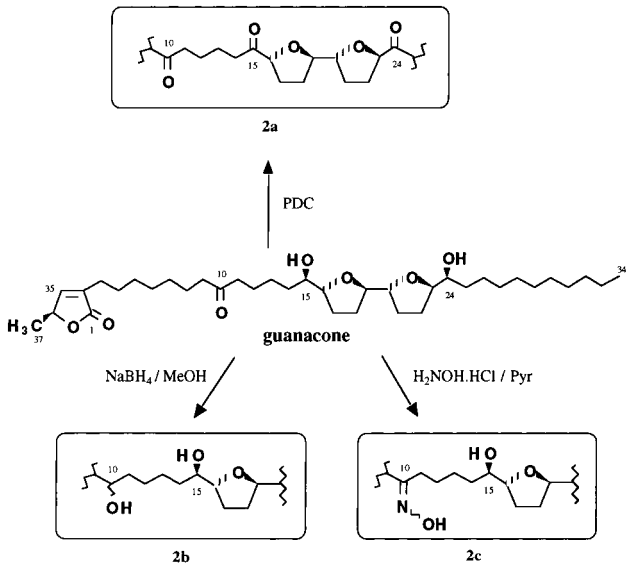
of bis-THF ACG, taking as starting material three representative compounds: rolliniastatin-1 (**1**), guanacone (**2**), and squamocin (**3**). These compounds differ by the stereochemistry of the THF system (*threo/cis/threo/cis/erythro* in **1** and *threo/trans/threo/trans/erythro* in **2** and **3**), and the number and position of the hydroxyl groups placed along the alkyl chain, susceptible to being conveniently functionalized. Semisynthetic modifications have been directed to oxidize hydroxyl groups to keto groups and to introduce nitrogenated groups, which produce an increase of the inhibitory potency in certain ACG series,[13,16,19] and also to block the hydroxyl groups as mesylated and acetylated derivatives. The wide array of derivatives obtained has allowed us to analyze the contribution of the functional groups placed in three parts of the molecule: the hydroxyl groups linked at the THF system, the alkyl chain placed between the terminal lactone moiety and the THF ring, and the terminal alkyl chain.

Results and Discussion

Semisynthesis. A group of semisynthetic derivatives was prepared as shown in Schemes 1−3, from three natural bis-THF acetogenins. Rolliniastatin-1 (**1**), isolated from *Rollinia membranacea*,[20] shows a *threo/cis/threo/cis/erythro* relative configuration; guanacone (**2**), isolated from *Annona* aff. *spraguei*,[16] and squamocin (**3**), isolated from *Annona cherimolia*,[21] show a *threo/trans/threo/trans/erythro* relative configuration.

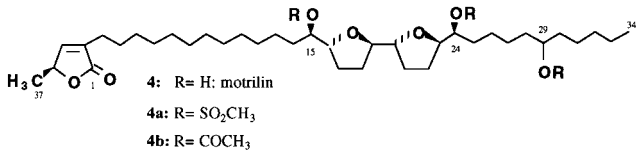
The preparation of the ketonic derivatives from those di- or trihydroxylated ACG (**1**−**3**) was accomplished by controlled oxidation using 2.5 equiv of pyridinium dichromate (PDC),[22] followed by usual purification. While rolliniastatin-1 (**1**) furnished both 4-dehydroxy-4-oxo- (**1a**) and 4,24-didehydroxy-4,24-dioxorolliniastatin-1 (**1b**), only one compound was obtained, 15,24-didehydroxy-15,24-dioxoguanacone (**2a**) or 28-dehydroxy-28-oxosquamocin (**3a**), when this procedure was applied to guanacone (**2**) or squamocin (**3**). These results demonstrate that oxidation is influenced by the relative configuration and it occurs only at the less sterically hindered hydroxyl groups, and in moderate yields. It is worth noting that cleavage of compounds occurs when more than 3 equiv of PDC was used.

Scheme 2. Guanacone (**2**) Derivatives



These ketonic derivatives were employed as starting material for preparing monohydroxylimine (**1c** and **3b**) or dihydroxylimine (**1d**) derivatives by treatment with $H_2NOH{\cdot}HCl$ in pyridine.[16] Compounds were obtained in good yields but, as expected and confirmed by NMR spectral data, as *cis* and *trans* mixtures.

The trimesylated (**1e**, **3c**, and **4a**) and triacetylated (**1f**, **3e**, and **4b**) derivatives, were prepared in nearly quantitative yield by treatment of the parent compounds with mesyl chloride and triethylamine[23] or acetic anhydride in pyridine, respectively.



In an attempt to obtain new nitrogen-containing substituents, **3c** was treated with sodium azide in dimethylformamide yielding the 15,24,28-tridehydroxy-15,24,28-triazidosquamocin (**3d**), in which absolute configuration at C-15, C-24, and C-28 was inverted because of the S_N2 type character of the mesylate displacement reaction by sodium azide.[24] As **3d** was less effective in the bioactivity assays (see below), no other azido derivatives were prepared.

Bioactivity. To gain knowledge of the mechanism of cytotoxicity of ACG, we have studied the inhibitory potency of the functionalized alkyl-chain derivatives described above against the mitochondrial complex I prepared as ultrasonic-disrupted submitochondrial particles from beef heart, the best model for studying the mammalian enzyme. We have evaluated their inhibitory

Scheme 3. Squamocine (**3**) Derivatives

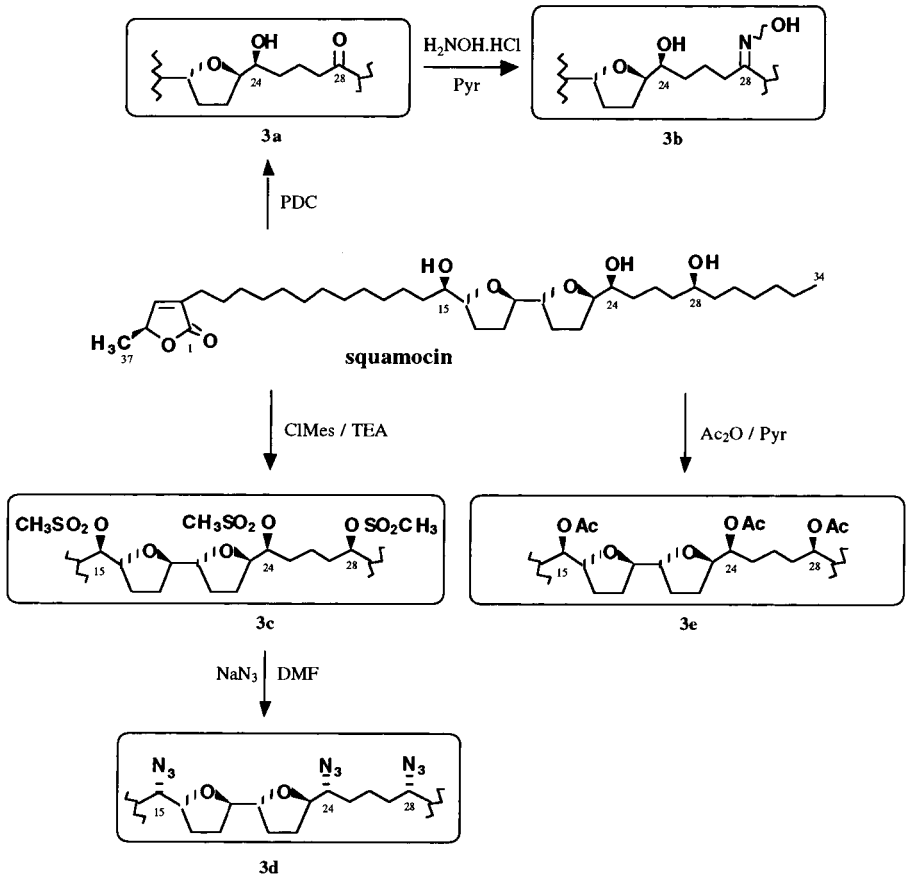


Table 1. Inhibitory Potency of Rolliniastatin-1 (**1**) Derivatives and Related Compounds

	IC_{50} (nM)			
compd	NADH oxidase	NADH:DB oxidoreductase	IC_{50} DB/Ox ratio	rel potency[a]
1	0.60 ± 0.04	0.74 ± 0.04	1.2	2.4
1a	0.42 ± 0.08	0.45 ± 0.07	1.1	1.7
1b	0.34 ± 0.02	0.60 ± 0.08	1.8	1.4
1c	0.25 ± 0.03	0.29 ± 0.03	1.1	1.0
1d	0.34 ± 0.02	0.39 ± 0.04	1.1	1.4
1e	1.68 ± 0.06	438 ± 272	260	6.7
1f	1.46 ± 0.09	1890 ± 160	1295	5.8
5	0.33 ± 0.07	0.83 ± 0.14	2.5	1.3

[a] Relative potency compared with the NADH oxidase IC_{50} of compound **1c**.

action by measuring NADH oxidase activity, a whole process of the respiratory chain that starts with oxidation of NADH in complex I by endogenous ubiquinone of the inner mitochondrial membrane, as well as NADH: ubiquinone oxidoreductase activity, a specific complex I activity that requires addition of decylubiquinone (DB) as ubiquinone-like substrate, thus named NADH:DB oxidoreductase activity. The former gives an evaluation of the inhibitory potency in a more unaltered enzyme environment, whereas the latter gives an approximate indication of the tightness of the binding, due to the existence of a partial competition between inhibitors and DB.[11,14,19]

The results are given in the tables grouping the derivatives obtained from each one of the three head series: rolliniastatin-1 (**1**) (Table 1), guanacone (**2**) (Table 2), and squamocin (**3**) (Table 3), also including some related compounds for comparison. The most

Table 2. Inhibitory Potency of Guanacone (**2**) Derivatives and Related Compounds

	IC_{50} (nM)			
compd	NADH oxidase	NADH:DB oxidoreductase	IC_{50} DB/Ox ratio	rel potency[a]
2	1.52 ± 0.12	2.1 ± 0.5	1.4	6.1
2a	1.65 ± 0.07	2.50 ± 0.02	1.5	6.6
2b[16]	0.95 ± 0.08	1.9 ± 0.3	2.0	3.8
2c[16]	0.34 ± 0.05	0.67 ± 0.16	2.0	1.4
6	0.51 ± 0.05	0.61 ± 0.04	1.2	2.0
7	0.32 ± 0.06	0.44 ± 0.08	1.4	1.3

[a] Relative potency compared with the NADH oxidase IC_{50} of compound **1c** (Table 1).

Table 3. Inhibitory Potency of Squamocin (**3**) Derivatives and Related Compounds

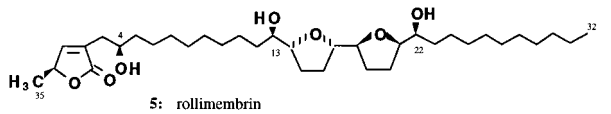
	IC_{50} (nM)			
compd	NADH oxidase	NADH:DB oxidoreductase	IC_{50} DB/Ox ratio	rel potency[a]
3	0.59 ± 0.07	0.77 ± 0.15	1.3	2.4
3a	0.65 ± 0.11	1.3 ± 0.3	1.9	2.6
3b	0.74 ± 0.19	0.9 ± 0.2	1.2	3.0
3c	14.1 ± 1.6	2037 ± 28	144	56
3d	18 ± 2	315 ± 19	17	72
3e	5.0 ± 1.3	2936 ± 564	587	20
4	0.41 ± 0.04	0.81 ± 0.13	2.0	1.6
4a	2.90 ± 0.11	64 ± 4	22	12
4b	2.1 ± 0.3	248 ± 17	118	8.4

[a] Relative potency compared with the NADH oxidase IC_{50} of compound **1c** (Table 1).

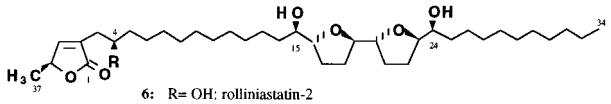
potent ACG found in these series was the semisynthetic derivative **1c**, whose IC_{50} (0.25 nM) is the lowest value compared with that of the most potent natural acetogenins known to date, [14−16,20] the semisynthetic deriva-

tives previously obtained,[16] and the new ones in this study. Indeed, **1c** inhibits complex I in a very near 1:1 stoichiometric ratio, taking into account that the complex I concentration in the assay media was 0.28 nM. Therefore, relative potency data given in the tables refer to this extremely potent compound taking the NADH oxidase IC_{50} as a major potency criterion. IC_{50} values from the NADH:DB oxidoreductase assay were often higher than those of the former assay, as is frequently observed.[14] However, most of the compounds studied gave IC_{50} DB/oxidase ratios between 1 and 2, which is indicative of tight binding to the enzyme, even with the presence of a high concentration of the quinone analogue used for the assay.[14,19] Nevertheless, some derivatives failed to maintain this ratio, as indicated below.

One of the most potent head series found in this study was rolliniastatin-1 (**1**), a natural adjacent bis-THF ACG with a *threo/cis/threo/cis/erythro* relative configuration. As shown in Table 1, potency increased with oxidation of the 4-OH group to a keto group (**1a**) and even more with oxidation of both 4- and 24-OH groups (**1b**). Indeed, the dioxo derivative has a potency similar to that of rollimembrin (**5**), a previously isolated ACG with the same relative configuration but a shorter alkyl chain.[20] As indicated above, the highest potency was obtained for compound **1c**, whereas **1d** was slightly less potent. On the other hand, the substitution of the three hydroxyl groups of **1** by mesyl (**1e**) and acetyl (**1f**) groups yielded a loss of potency. Moreover, these trimesylated and triacetylated derivatives showed a great loss of potency in the NADH:DB oxidoreductase assay, being highly displaced by the added ubiquinone analogue.



5: rollimembrin



6: R= OH: rolliniastatin-2

7: R= H: desacetyluvaricin

Guanacone (**2**) and its dioxo derivative (**2a**) were less potent than other related ACG with the same *threo/trans/threo/trans/erythro* relative configuration in the bis-adjacent-THF system, such as rolliniastatin-2 (**6**) and desacetyluvaricin (**7**), as shown in Table 2, the latter approaching the potency of the most potent compounds of the first series. Similar high potency was obtained with the previously reported 10-dihydroguanacone (**2b**) and 10-deoxo-10-hydroxyliminoguanacone (**2c**).[16]

The last series of this study (Table 3) includes squamocin (**3**) and motrilin (**4**), two adjacent bis-THF ACG with the same relative configuration as guanacone (**2**) but with the possibility of modifying the functional group located at either the 28- or 29-position. Potency was slightly increased with oxidation of the 28-OH group of squamocin (**3**) to a keto group (**3a**) as well as its substitution by a hydroxylimino group (**3b**). The most potent compound of this series was the natural unmodified **4**. As in the first series, the trimesylated (**3c** and **4a**) and also triacetylated (**3e** and **4b**) derivatives gave slightly less potency in the NADH oxidase assay, but they showed a great loss of potency in the NADH:DB oxidoreductase assay. Similar behavior was also observed with the triazido derivative **3d**.

The semisynthetic modifications obtained and assayed in this study have intended to evaluate the contribution of the functional groups placed in different parts of ACG: the THF system, the alkyl chain that links this THF system with the terminal lactone moiety, and the terminal alkyl chain. This wide array of derivatives can help to clarify what parts of the molecular structure are involved in binding complex I and therefore in exerting the inhibitory and cytotoxic actions. A recent study by Shimada et al.[18] on the membrane conformations of ACG and their relation to cytotoxicity has concluded that the dihydroxylated THF system acts merely as an anchor to the polar heads of the membrane phospholipids. Thus, it is thought that the terminal lactone moiety, and maybe part of the near alkyl chain, penetrates into complex I to exert the inhibitory action. This observation seems compatible with the identification of a unique inhibitor-binding domain in complex I[25] placed in the PSST subunit of the transmembrane part of the mammalian enzyme.[26] If so, the main structural factor for potent inhibitory activity should be located in this part of the molecule, i.e., between the γ-lactone and the THF system, whereas the central dihydroxylated THF system should play a minor role.

Nevertheless, we have found that this central part of the molecule is needed for potent inhibition. Indeed, compounds that exhibited the largest decrease in potency, especially in the NADH:DB oxidoreductase assay, were the mesylated, acetylated, and azido derivatives of the hydroxyl groups surrounding the THF rings. It strongly suggests a relevant role of these groups for binding the enzyme and especially for competing with the ubiquinone substrate. Moreover, oxidation of these hydroxyl groups to keto groups did not produce differences in potency (**2** vs **2a**), indicating a requirement for H-bonding acceptors close to the THF rings. Taken together, these observations further suggest that this central part of the molecular structure of ACG mimics the quinone/quinol head of the ubiquinone and thus antagonizes the ubiquinone substrate by competing for the same binding site. This proposal was advanced in previous reports, based on structural analogies of ACG with ubiquinone, but without direct evidence.[5,11]

Nevertheless, we have found that the inhibitory potency of ACG depends on other structural factors, mainly the functional groups located in the alkyl chain that links the THF system with the terminal lactone moiety. Table 4 shows the relative potencies of the most potent compounds studied here given as a percentage of the NADH oxidase assay IC_{50} for 4-dehydroxy-4-hydroxyliminorolliniastatin-1 (**1c**). This extremely potent compound combines a scarce relative THF configuration with the presence of a nitrogenated group near the γ-lactone. However, it is followed by the natural desacetyluvaricin (**7**) that presents a different stereochemistry and the shorter rollimembrin (**5**). The combination of a keto group near the lactone with another keto group near the THF system yields another potent compound (**1b**) with the same potency as the compound possessing a hydroxylimine group in the middle of this alkyl chain (**2c**).[16] Compounds with different stereo-

Table 4. Relative Potency of the Acetogenins

compd	name	rel potency (%)
1c	4-dehydroxy-4-hydroxyliminorolliniastatin-1	**100**
7	desacetyluvaricin	78.1
5	rollimembrin	75.8
1b	4,24-didehydroxy-4,24-dioxorolliniastatin-1	73.5
2c	10-deoxo-10-hydroxyliminoguanacone	73.5
1d	4,24-didehydroxy-4,24-dihydroxylimino-rolliniastatin-1	73.5
4	motrilin	61.0
1a	4-dehydroxy-4-oxorolliniastatin-1	59.5
6	rolliniastatin-2	49.0
3	squamocin	42.3
1	rolliniastatin-1	41.7
3a	28-dehydroxy-28-oxosquamocin	38.5
3b	28-dehydroxy-28-hydroxyliminosquamocin	33.8
2b	10-dihydroguanacone	26.3

chemistry and functional groups occupy the following positions of this ranking. It seems that functional groups located near the terminal lactone moiety or at least in the alkyl chain that links the lactone with the THF system are relevant for modulating the inhibitory potency of these compounds. Although these functional groups are not strictly required for strong potency (e.g., the high potency of desacetyluvaricin (**7**), motrilin (**4**), and squamocin (**3**)), several derivatives with different functional groups at the 4- and 10-positions show higher potency than the parent compounds (**1a**, **1b**, **1c**, and **1d** vs **1**; **2b** and **2c** vs **2**).

In partial accordance with Shimada et al.,[18] it is likely to be a direct interaction of this part of the molecule with complex I, in addition to the α,α′-dihydoxylated THF system. However, the structural requirements for this additional binding are less clear; therefore, it is thought that they could involve several factors affecting the relative position of the lactone with respect to the THF system. ACG are unusual structures compared with most groups of complex I inhibitors due to their high degree of flexibility. The presence of functional groups in this part of the alkyl chain (4- and 10-positions in the compounds of this study) could alter the orientation of the lactone by steric constraints or by binding the enzyme at secondary points, as has been previously suggested.[13,16,19,20] Moreover, a shorter length of this alkyl chain increases the potency, like in rollimembrin (**5**)[20] and other ACG series.[17] In addition, the number, position, and configuration of the THF rings, overlooked factors due to the limited compounds used in other studies,[3,10,17] also contribute to the inhibitory action of ACG.[6,13,14,19,20]

Taken together, these observations further suggest a second binding point to the enzyme. Indeed, there is evidence for at least two inhibitor-binding sites in complex I[5,6,11,14,27] and also two very close ubiquinone-binding sites,[26,28] likely to be located in the same domain of the enzyme.[25,26] Therefore, we propose a double binding of ACG with the enzyme. The main point is clearly a ubiquinone-binding site antagonized by the central part of the molecule in which the α,α′-dihydoxylated THF system competes with the quinone head of the substrate. The second binding point involves the terminal γ-lactone moiety and probably a part of the adjacent alkyl chain, with the inhibitory action of ACG having been modulated by an appropriate orientation of these groups with respect to the THF system. This second binding point might be correlated with a very near second ubiquinone-binding site. The proposed double binding could explain the noncompetitive inhibition kinetics showed by most ACG and the uncompetitive inhibition previously found for some compounds.[5,6,14] Furthermore, it opens an interesting perspective to investigate the exact contribution of the γ-lactone moiety to the inhibitory potency of ACG and its contribution to the inhibitory mechanism with new semisynthetic derivatives affecting this part of the chemical structure.

Experimental Section

General Instrumentation. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra (film) were taken on a Perkin-Elmer 843 infrared spectrometer. Mass spectra (LSIMS, HRLSIMS, EIMS or HREIMS) were determined on a VG Auto Spec Fisons spectrometer. ^{1}H NMR (300 or 400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Varian Unity-300 and Varian Unity-400 instruments, 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. Assignments of ^{1}H and ^{13}C NMR were made by 2D experiments. Column chromatography was performed with silica gel 60H (7736 Merck) and flash chromatography with silica gel 60 (9385 Merck). The empirical formula of the compounds was determined by HRLSIMS or HREIMS.

Acetogenins Used for Further Transformations. Rolliniastatin-1 (**1**) was isolated from *Rollinia membranacea* seeds.[20] Guanacone (**2**) was isolated from *Annona* aff. *spraguei* seeds.[16] Squamocin (**3**) and motrilin (**4**) were isolated from *Annona cherimolia* seeds.[21]

General Procedure for Preparing Oxidized Acetogenins. 1. Semisynthesis of 4-Dehydroxy-4-oxorolliniastatin-1 (1a) and 4,24-Didehydroxy-4,24-dioxorolliniastatin-1 (1b). To a CH_2Cl_2 solution (4 mL) of rolliniastatin-1 (**1**; 145 mg, 0.23 mmol) was added pyridinium dichromate (PDC; 2.5 equiv). After the mixture was stirred and refluxed for 2 h, water was added and the reaction mixture was extracted with 4 × 10 mL of CH_2Cl_2. The organic solutions were washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel 60 H column chromatography, eluting with hexane/CH_2Cl_2/EtOAc/MeOH 2:7:2:0.1, affording **1a** (42 mg, 29%) and **1b** (25 mg, 17%) as amorphous compounds.

Compound 1a: $C_{37}H_{64}O_7$; $[\alpha]_D$ +30° (*c* 0.66, EtOH); IR (film) ν_{max} cm^{-1} 3421, 2925, 2853, 1755, 1721, 1647, 1464, 1319, 1197, 1075, 953; HRLSIMS *m*/*z* 621.4725 (calcd 621.4730 for $C_{37}H_{65}O_7$); LSIMS *m*/*z* 643 $[M + Na]^+$, 621 $[MH]^+$; HREIMS *m*/*z* 509.4236 (calcd 509.4206 for $C_{31}H_{57}O_5$), 491.4072 (calcd 491.4100 for $C_{31}H_{55}O_4$), 449.2933 (calcd 449.2903 for $C_{26}H_{40}O_6$), 361.2385 (calcd 361.2378 for $C_{22}H_{33}O_4$), 309.2069 (calcd 309.2065 for $C_{18}H_{29}O_4$), 241.2159 (calcd 241.2167 for $C_{15}H_{29}O_2$); EIMS *m*/*z* (%) 509 (3), 491 (2), 473 (2), 449 (6), 431 (4), 413 (4), 379 (9), 361 (18), 341 (4), 326 (6), 311 (20), 309 (100), 293 (6), 291 (3), 281 (6), 241 (21), 141 (15), 139 (8), 111 (14); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37 (d, J = 1.6 Hz, H-35), 5.07 (dq, J = 1.6 and 7.2 Hz, H-36), 3.91 (m, H-23), 3.86 (m, H-19, 20), 3.85 (m, H-24), 3.81 (m, H-16), 3.41 (s, H-3), 3.40 (m, H-15), 2.50 (t, J = 6.8 Hz, H-5), 1.91, 1.77 (m, H-17, 18, 21, 22), 1.56 (m, H-6), 1.49 (m, H-14, 25), 1.44 (d, J = 7.2 Hz, H-37), 1.24 (m, H-7−13 and H-26−33), 0.87 (t, J = 6.8 Hz, H-34); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.6 (C-4), 173.4 (C-1), 153.0 (C-35), 126.4 (C-2), 83.0 (C-16), 82.9 (C-23), 81.1 (C-19), 80.9 (C-20), 78.1 (C-36), 74.0 (C-15), 71.8 (C-24), 43.2 (C-5), 37.8 (C-3), 34.2 (C-14), 32.7 (C-25), 31.8 (C-32), 29.6−29.0 (C-7−12 and 27−31), 28.7 (C-17), 28.4 (C-21), 27.8 (C-18), 25.9 (C-13), 25.7 (C-26), 23.7 (C-22), 23.6 (C-6), 22.6 (C-33), 18.9 (C-37), 14.1 (C-34).

Compound 1b: $C_{37}H_{62}O_7$; HRLSIMS *m*/*z* 619.4569 (calcd 619.4573 for $C_{37}H_{63}O_7$); LSIMS *m*/*z* 641 $[M + Na]^+$, 619 $[MH]^+$;

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37 (d, J = 1.5 Hz, H-35), 5.06 (dq, J = 1.5 and 6.8 Hz, H-36), 4.36 (dd, J = 6.0 and 8.4 Hz, H-23), 3.91 (m, H-19, 20), 3.82 (m, H-16), 3.40 (s, H-3), 3.37 (m, H-15), 2.53 (t, J = 7.0 Hz, H-25), 2.50 (t, J = 7.0 Hz, H-5), 2.18,1.97 (m, H-22), 1.93,1.76 (m, H-17, 18, 21), 1.56 (m, H-6 and H-26), 1.47 (m, H-14), 1.45 (d, J = 6.8 Hz, H-37), 1.25 (m, H-7−13 and H-27−33), 0.86 (t, J = 6.9 Hz, H-34); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.4 (C-24), 205.6 (C-4), 173.4 (C-1), 153.1 (C-35), 126.4 (C-2), 83.8 (C-23), 82.8 (C-16), 82.7 (C-19), 81.4 (C-20), 78.1 (C-36), 74.2 (C-15), 43.2 (C-5), 38.6 (C-25), 37.8 (C-3), 33.9 (C-14), 31.8 (C-32), 29.6−29.0 (C-7−12 and 27−31), 28.8 (C-17), 28.2 (C-21), 27.8 (C-18), 27.7 (C-22), 25.7 (C-13), 23.6 (C-6), 23.1 (C-26), 22.6 (C-33), 18.9 (C-37), 14.1 (C-34).

2. Semisynthesis of 15,24-Didehydroxy-15,24-dioxoguanacone (2a). A similar treatment of guanacone (**2**; 286 mg, 0.46 mmol) with PDC (2.5 equiv) and purification by silica gel 60 H column chromatography, eluting with hexane/CH_2Cl_2/EtOAc/MeOH 1:7:2:0.1, afforded **2a** (40 mg, 14%) as an amorphous compound.

Compound 2a: $C_{37}H_{60}O_7$; $[\alpha]_D$ +8° (*c* 0.60, EtOH); IR (film) ν_{max} cm^{-1} 2925, 2851, 1737, 1715, 1710, 1410, 1377, 1323, 1198, 1082; HREIMS *m*/*z* 309.2467 (calcd 309.2429 for $C_{19}H_{33}O_3$), 223.1338 (calcd 223.1334 for $C_{13}H_{19}O_3$), 195.1352 (calcd 195.1385 for $C_{12}H_{19}O_2$), 181.1226 (calcd 181.1228 for $C_{11}H_{17}O_2$), 169.1502 (calcd 169.1592 for $C_{11}H_{21}O$); EIMS *m*/*z* (%) $[M]^+$ 616 (12), 598 (3), 448 (21), 430 (100), 421 (7), 402 (8), 349 (7), 309 (42), 281 (5), 237 (5), 223 (26), 195 (8), 181 (21), 169 (8); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (d, J = 1.6 Hz, H-35), 4.99 (dq, J = 1.6 and 6.8 Hz, H-36), 4.40 (m, H-16, 23), 4.07 (m, H-19, 20), 2.42 (t, J = 6.8 Hz, H-14, 25), 2.37 (t, J = 7.0 Hz, H-9, 11), 2.24 (m, H-3), 1.95, 1.82 (m, H-18, 21), 1.85, 1.82 (m, H-17, 22), 1.55 (m, H-4, 8, 12 and 26), 1.40 (d, J = 6.8 Hz, H-37), 1.24 (m, H-5−7 and 27−33), 0.88 (t, J = 6.8 Hz, H-34); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.0 (C-10), 210.5 (C-15, 24), 173.9 (C-1), 148.9 (C-35), 134.1 (C-2), 83.9 (C-16, 23), 82.4 (C-19), 82.3 (C-20), 77.1 (C-36), 42.7 (C-9, 11), 42.5, 42.2 (C-14, 25), 31.8 (C-32), 29.5−29.0 (C-5−7 and 27−31), 27.2 (C-4), 25.1 (C-3), 23.7, 23.6, 23.2, 23.1 (C-8, 12, 13, 26), 22.6 (C-33), 19.9 (C-37), 14.0 (C-34).

3. Semisynthesis of 28-Dehydroxy-28-oxosquamocin (3a). A similar treatment of **3** (45 mg, 0.072 mmol) with PDC (2.5 equiv) and purification by silica gel 60 H column chromatography, eluting with hexane/CH_2Cl_2/EtOAc/MeOH 1:5:1:0.1, afforded **3a** (13 mg, 29%) as an amorphous compound.

Compound 3a: $C_{37}H_{64}O_7$; LSIMS *m*/*z* 643 $[M + Na]^+$, 621 $[MH]^+$; HREIMS *m*/*z* 602.4563 (calcd 602.4546 for $C_{37}H_{62}O_6$), 347.2574 (calcd 347.2586 for $C_{22}H_{35}O_3$), 295.2271 (calcd 295.2273 for $C_{18}H_{31}O_3$); EIMS *m*/*z* (%) 602 (11), 584 (4), 435 (2), 365(4), 347 (42), 307 (13), 295 (100), 265 (4), 255 (4), 237 (11), 185 (9), 167 (7), 113 (22), 85 (9); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (d, J = 1.2 Hz, H-35), 5.00 (dq, J = 1.2 and 6.4 Hz, H-36), 3.92 (m, H-20, 23), 3.82 (m, H-16, 19, 24), 3.39 (m, H-15), 2.42 and 2.37 (2t, J = 7.2 Hz, H-27 and H-29), 2.25 (t, J = 7.6 Hz, H-3), 1.54 (m, H-4, 26, 30), 1.42 (m, H-14, 25), 1.39 (d, J = 6.4 Hz, H-37), 1.24 (m, H-5−13 and 31−33), 0.87 (t, J = 7.0 Hz, H-34); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.4 (C-28), 173.8 (C-1), 148.8 (C-35), 134.1 (C-2), 83.2 (C-16), 82.6 (C-23), 82.5 (C-19), 82.2 (C-20), 77.3 (C-36), 74.1 (C-15), 71.1 (C-24), 42.8 (C-27), 42.4 (C-29), 33.2 (C-14), 31.8 (C-32), 31.5 (C-25), 29.7−29.2 (C-5−12, 31), 29.1 (C-21), 28.9 (C-18), 28.3 (C-17), 27.3 (C-4), 25.6 (C-13), 25.1 (C-3), 24.6 (C-22), 23.8 (C-30), 22.4 (C-33), 20.2 (C-26), 19.2 (C-37), 14.0 (C-34).

General Procedure for Preparing Hydroxylimino Derivatives. 1. Semisynthesis of 4-Dehydroxy-4-hydroxyliminorolliniastatin-1 (1c). To a EtOH solution (2 mL) of 4-oxorolliniastatin-1 (**1a**; 41 mg, 0.066 mmol) were added pyridine (50 μL) and H_2NOH·HCl (1 equiv). After the mixture was stirred and refluxed for 1 h, water was added and the reaction mixture was extracted with 4 × 10 mL of CH_2Cl_2. The organic solutions were washed with water, dried over anhydrous Na_2SO_4, evaporated in vacuo and purified by silica gel 60 H column chromatography, eluting with cyclohexane/CH_2Cl_2/EtOAc 4:5:1, affording **1c** (*cis* and *trans* mixture, 36 mg, 86%) as an amorphous compound.

Compound 1c: $C_{37}H_{65}O_7N$; $[\alpha]_D$ +40° (*c* 0.20, EtOH); HRLSIMS *m*/*z* 636.4818 (calcd 636.4839 for $C_{37}H_{66}O_7N$); LSIMS *m*/*z* 658 $[M + Na]^+$, 636 $[MH]^+$; HREIMS *m*/*z* 619.4813 (calcd 619.4811 for $C_{37}H_{65}O_6N$), 378.2643 (calcd 378.2644 for $C_{22}H_{36}O_4N$), 241.2175 (calcd 241.2167 for $C_{15}H_{29}O_2$); EIMS *m*/*z* (%) 635 (3), 619 (7), 601 (4), 465 (8), 449 (12), 394 (3), 378 (17), 360 (17), 324 (37), 311 (20), 309 (34), 294 (10), 281 (5), 241 (90), 154 (5); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.19 (d, J = 1.2 Hz, H-35 *cis* or *trans*), 7.12 (d, J = 1.6 Hz, H-35 *cis* or *trans*), 5.03 (dq, J = 1.2 and 6.8 Hz, H-36), 3.91 (m, H-23), 3.86 (m, H-19, 20), 3.81 (m, H-16, 24), 3.42 (m, H-15), 3.33 (d, J = 14.2 Hz, H-3a *cis*), 3.27 (d, J = 14.2 Hz, H-3b *cis*), 3.18 (s, H-3 *trans*), 2.36 (brt, J = 8 Hz, H-5 *cis*), 2.23 (td, J = 7.4 and 4.0 Hz, H-5 *trans*), 1.93, 1.77 (m, H-17, 18, 21, 22), 1.48 (m, H-6), 1.42 (d, J = 6.8 Hz, H-37 *cis* or *trans*), 1.41 (d, J = 6.8 Hz, H-37 *cis* or *trans*), 1.39 (m, H-14, 25), 1.26 (m, H-7−13 and 26−33), 0.87 (t, J = 6.8 Hz, H-34); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4 (C-1), 157.9 (C-4), 151.5 (C-35), 128.6 (C-2), 83.3 (C-16), 83.1 (C-23), 81.1 (C-19), 81.0 (C-20), 77.8 (C-36), 73.9 and 73.7 (C-15 *cis* and C-15 *trans*), 71.7 (C-24), 34.2 (C-14), 34.1 (C-3), 33.9 (C-5), 32.7 (C-25), 31.8 (C-32), 29.6−29.0 (C-7−12 and 27−31), 28.7 (C-17), 28.3 (C-21), 27.8 (C-18), 26.0 (C-6), 25.8 (C-13), 25.3 (C-26), 23.7 (C-22), 22.6 (C-33), 18.9 (C-37), 14.1 (C-34).

2. Semisynthesis of 4,24-Didehydroxy-4,24-dihydroxyliminorolliniastatin-1 (1d). To a EtOH solution (2 mL) of 4,-24-dioxorolliniastatin-1 (**1b**; 20 mg, 0.032 mmol) were added pyridine (50 μL) and H_2NOH·HCl (3 equiv). After the mixture was stirred and refluxed for 5 h, water was added and the reaction mixture was extracted with 4 × 10 mL of CH_2Cl_2. After usual workup the organic solutions were purified by silica gel 60 H column chromatography, eluting with cyclohexane/CH_2Cl_2/EtOAc 4:5:1, affording **1d** (*cis* and *trans* mixtures, 15 mg, 72%) as an amorphous compound.

Compound 1d: $C_{37}H_{64}O_7N_2$; HRLSIMS *m*/*z* 649.4783 (calcd 649.4791 for $C_{37}H_{65}O_7N$); LSIMS *m*/*z* 671 $[M + Na]^+$, 649 $[MH]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 (d, J = 1.2 Hz, H-35 *cis* or *trans*), 7.13 (d, J = 1.2 Hz, H-35 *cis* or *trans*), 5.01 (dq, J = 1.2 and 6.9 Hz, H-36), 4.01 (m, H-23), 3.88 (m, H-16, 19, 20), 3.40 (m, H-15), 3.30 and 3,20 (2m, H-3a and H-3b *cis*), 3.18 (s, H-3 *trans*), 2.32 (m, H-5, 25), 2.19, 1.93 and 1.78 (m, H-17, 18, 21, 22), 1.52 (m, H-6, 26), 1.41 (d, J = 6.9 Hz, H-37 *cis* or *trans*), 1.40 (d, J = 6.9 Hz, H-37 *cis* or *trans*), 1.39 (m, H-14), 1.25 (m, H-7−13 and 27−33), 0.87 (t, J = 6.8 Hz, H-34); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4 (C-1), 159.0 (C-24), 157.9 (C-4), 151.7 (C-35), 128.5 (C-2), 82.4 (C-16), 81.6 (C-19), 81.3 (C-20), 79.9 and 77.8 (C-23 *cis* and C-23 *trans*), 77.7 (C-36), 75.0 and 74.3 (C-15 *cis* and C-15 *trans*), 34.1 (C-14), 34.0 (C-3), 33.5 (C-5), 31.8 (C-32), 30.0 (C-25), 29.6−29.0 (C-7−12 and 27−31), 28.7 (C-17), 28.3 (C-21), 27.9 (C-18), 25.6 and 25.4 (C-6, 13, 26), 23.6 (C-22), 22.6 (C-33), 18.9 (C-37), 14.1 (C-34).

3. Semisynthesis of 28-Dehydroxy-28-hydroxyliminosquamocin (3b). After treatment of the EtOH solution (1.5 mL) of **3a** (10 mg, 0.016 mmol) with pyridine (25 μL) and H_2NOH·HCl (1 equiv) and usual workup, the organic solutions were purified by silica gel 60 H column chromatography, eluting with cyclohexane/CH_2Cl_2/EtOAc 4:5:1, affording **3b** (*cis* and *trans* mixture, 9 mg, 87%) as an amorphous compound.

Compound 3b: $C_{37}H_{65}O_7N$; IR (film) ν_{max} cm^{-1} 3421, 2925, 2854, 1754, 1651, 1461, 1070; HRLSIMS *m*/*z* 636.4825 (calcd 636.4839 for $C_{37}H_{66}O_7N$); LSIMS *m*/*z* 658 $[M + Na]^+$, 636 $[MH]^+$; EIMS *m*/*z* (%) 603 (3), 584 (6), 435 (2), 365(3), 347 (55), 295 (100), 277 (2), 265 (3), 199 (12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8 (C-1), 161.5 (C-28), 148.8 (C-35), 134.3 (C-2), 83.2 (C-16), 82.9 (C-23), 82.5 (C-19), 82.2 (C-20), 77.3 (C-36), 74.1 (C-15), 70.8 (C-24), 33.2 (C-14), 31.9 (C-27, 29), 31.8 (C-32), 31.5 (C-25), 29.6−29.2 (C-5−12, 31), 29.1 (C-21), 28.9 (C-18), 28.3 (C-17), 27.3 (C-4), 25.6 (C-13), 25.1 (C-3), 24.7 (C-22), 22.6 (C-26, 30), 22.4 (C-33), 19.2 (C-37), 14.0 (C-34).

General Procedure for Preparing Mesylated Derivatives. 1. Semisynthesis of 4,15,24-Trimesylrolliniastatin-1 (1e). To a dry CH_2Cl_2 solution (3 mL) of rolliniastatin-1

(**1**; 40 mg, 0.064 mmol) were added mesyl chloride (6 equiv) and triethylamine (10 equiv). After the mixture was stirred at room temperature for 2 h, water (20 mL) was added and the reaction mixture was extracted with 4 × 10 mL of CH_2Cl_2. After the combined organic solutions were washed with 0.1 M HCl, followed by 5% $NaHCO_3$ aq and water, and finally dried over anhydrous Na_2SO_4, concentration in vacuo afforded **1e** (52.6 mg, 96%) as an amorphous compound.

Compound 1e: $C_{40}H_{72}O_{13}S_3$; $[\alpha]_D$ +19° (*c* 0.8, EtOH); IR (film) ν_{max} cm^{-1}:2925, 2854, 1755, 1466, 1349, 1173, 1076, 1028, 912, 785, 722; HREIMS *m/z* 879.4056 (calcd 879.4032 for $C_{40}H_{72}O_{13}S_3Na$); HREIMS *m/z* 664.4379 (calcd 664.4372 for $C_{38}H_{64}O_7S$), 568.4480 (calcd 568.4491 for $C_{37}H_{60}O_4$), 415.2790 (calcd 415.2848 for $C_{26}H_{39}O_4$), 345.2414 (calcd 345.2429 for $C_{22}H_{33}O_3$); 223.2052 (calcd 223.2061 for $C_{15}H_{27}O$); LSIMS *m/z* 879 $[M + Na]^+$, 857 $[MH]^+$, 761, 665; EIMS *m/z* (%) 664 (48), 646 (6), 568 (62), 550 (40), 467 (7), 441 (22), 415 (37), 389 (6), 371 (40), 345 (65), 327 (71), 293 (16), 275 (9), 249 (55), 223 (33), 219 (7), 153 (22), 123 (40); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27 (d, J = 1.2 Hz, H-35), 5.04 (dq, J = 1.2 and 6.8 Hz, H-36), 4.90 (m, H-4), 4.72 (m, H-24), 4.50 (m, H-15), 3.96 (m, H-16, 23), 3.80 (m, H-19, 20), 2.69 (dd, J = 15 and 3.0 Hz, H-3b), 2.66 (dd, J = 15 and 7.8 Hz, H-3a), 1.92, 1.66 (m, H-17, 18, 21, 22), 1.69 (m, H-5, 25), 1.57 (m, H-14), 1.44 (d, J = 6.8 Hz, H-37), 1.24 (m, H-6−13 and H-26−33), 0.87 (t, J = 6.8 Hz, H-34), 2.99, 3.05 and 3.16 (3s, 3 CH_3SO_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4 (C-1), 152.8 (C-35), 128.5 (C-2), 86.5 (C-15), 83.2 (C-24), 82.2 (C-19), 81.9 (C-20), 80.9 (C-4), 80.4 (C-16), 79.8 (C-23), 77.8 (C-36), 34.4 (C-5), 31.8 (C-32), 31.7 (C-14), 31.5 (C-25), 30.4 (C-3), 29.4−29.1 (C-7−13 and 27−31), 25.4 (C-26), 22.5 (C-33), 18.6 (C-37), 14.0 (C-34), 38.8, 38.6, 38.5 (3 CH_3SO_2).

2. Semisynthesis of 15,24,28-Trimesylsquamocin (3c). A similar treatment of **3** (46 mg, 0.074 mmol) with mesyl chloride (6 equiv) and triethylamine (10 equiv) afforded **3c** (60 mg, 94%) as an amorphous compound.

Compound 3c: $C_{40}H_{70}O_{13}S_3$; LSIMS *m/z* 879 $[M + Na]^+$, 857 $[MH]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (d, J = 1.5 Hz, H-35), 4.98 (dq, J = 1.5 and 7.0 Hz, H-36), 4.75 (m, H-24), 4.67 (m, H-28), 4.50 (m, H-15), 4.02 (m, H-16, 23), 3.86 (m, H-19, 20), 2.23 (brt, J = 6.5 Hz, H-3), 1.92, 1.63 (m, H-17, 18, 21, 22), 1.54 (m, H-14, 25, 27, 29), 1.39 (d, J = 7.0 Hz, H-37), 1.24 (m, H-5−13), 0.85 (t, J = 7.0 Hz, H-34), 3.00, 3.04 and 3.14 (3s, 3 CH_3SO_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8 (C-1), 148.9 (C-35), 134.1 (C-2), 86.2 (C-15), 83.4 (C-28), 83.5 (C-24), 81.7 (C-19), 81.2 (C-20), 80.2 (C-16), 79.9 (C-23), 77.3 (C-36), 34.6 and 33.9 (C-27, 29), 31.5 (C-32), 31.1 and 31.0 (C-14, 25), 29.4−29.1 (C-5−12 and C-31), 29.0 (C-21), 28.8 (C-18), 28.4 (C-17), 27.2 (C-4), 26.0 (C-22), 25.05 (C-3), 24.9 and 24.8 (C-13, 30), 22.4 (C-33), 20.9 (C-26), 19.1 (C-37), 13.9 (C-34), 38.7, 38.5, 38.3 (3 CH_3SO_2).

3. Semisynthesis of 15,24,29-Trimesylmotrilin (4a). A similar treatment of **4** (67 mg, 0.09 mmol) with mesyl chloride (6 equiv) and triethylamine afforded **4a** (72 mg, 90%) as an amorphous compound.

Compound 4a: $C_{40}H_{72}O_{13}S_3$; IR (film) ν_{max} cm^{-1} 2926, 2854, 1754, 1652, 1463, 1349, 1173, 1048, 911, 784; LSIMS *m/z* 879 $[M + Na]^+$, 857 $[MH]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.97 (d, J = 1.5 Hz, H-35), 4.97 (dq, J = 1.5 and 7.0 Hz, H-36), 4.73 (m, H-29), 4.65 (m, H-24), 4.48 (m, H-15), 4.01 (m, H-16, 23), 3.83 (m, H-19, 20), 2.22 (brt, J = 6.5 Hz, H-3), 1.91, 1.62 (m, H-17, 18, 21, 22), 1.51 (m, H-14, 25, 28, 30), 1.39 (d, J = 7.0 Hz, H-37), 1.22 (m, H-5−13), 0.85 (t, J = 6.4 Hz, H-34), 2.97, 3.01, 3.11 (3s, 3 CH_3SO_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8 (C-1), 148.9 (C-35), 134.1 (C-2), 86.2 (C-15), 83.7 (C-24), 83.6 (C-29), 81.7 (C-19), 81.2 (C-20), 80.2 (C-16), 79.9 (C-23), 77.2 (C-36), 34.3 and 34.0 (C-28, 30), 31.7 and 31.1 (C-14, 25), 31.4 (C-32), 29.4−29.2 (C-5−12 and C-26, 27), 29.0 (C-21), 28.7 (C-18), 28.4 (C-17), 27.2 (C-4), 26.0 (C-22), 25.0 (C-3), 24.9 and 24.8 (C-13, 31), 22.3 (C-33), 19.1 (C-37), 13.8 (C-34), 38.8, 38.6, 38.3 (3 CH_3SO_2).

General Procedure for Preparing Acetylated Derivatives. In separate experiments, 10 mg of **1**, **3** and **4** were treated with pyridine (1 mL) and acetic anhydride (1.5 mL) overnight at room temperature. After usual workup **1f**, **3e** and **4b** were obtained as amorphous compounds in quantitative yields.

Compound 1f (4,15,24-triacetylrolliniastatin-1): $C_{43}H_{72}O_{10}$; ^{1}H NMR ($CDCl_3$, 300 MHz) identical to that previously described in ref 29; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.5 (C-1), 170.8, 170.7 and 170.6 (3 *C*OCH$_3$), 150.9 (C-35), 130.2 (C-2), 81.9 and 81.6 (C-16, 23), 80.3 and 79.8 (C-19, 20), 77.5 (C-36), 75.5, 75.1 (C-15, 24), 71.9 (C-4), 34.1 (C-3), 31.8 (C-32), 31.7, 31.5 and 30.4 (C-5, 14 and 25), 29.4−25.2 (C-6−13 and 26−31), 22.6 (C-33), 21.2 and 21.1 (3 CO*C*H$_3$), 18.9 (C-37), 14.1 (C-34).

Compound 3e (15,24,28-triacetylsquamocin): $C_{43}H_{72}O_{10}$; ^{1}H and ^{13}C NMR ($CDCl_3$) identical to that previously described in refs 29 and 30.

Compound 4b (15,24,29-triacetylmotrilin): $C_{43}H_{72}O_{10}$; ^{1}H NMR ($CDCl_3$, 300 MHz) identical to that previously described in ref 21; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.8 (C-1), 170.9 and 170.8 (3 *C*OCH$_3$), 148.8 (C-35), 134.1 (C-2), 81.5 and 81.1 (C-16, 23), 80.4 and 80.0 (C-19, 20), 77.4 (C-36), 75.2 (C-15, 24), 74.2 (C-29), 34.0 (C-28, 30), 31.6 (C-32), 31.0 and 30.8 (C-14, 25), 29.4−25.2 (C-3−13 and 26−31), 22.5 (C-33), 21.1 (3 CO*C*H$_3$), 19.1 (C-37), 13.9 (C-34).

Semisynthesis of 15,24,28-Tridehydroxy-15,24,28-triazidosquamocin (3d). To a DMF solution (3 mL) of 15,24,-28-trimesylsquamocin (**3c**; 127 mg, 0.159 mmol) was added NaN_3 (621 mg, 60 equiv). After stirring overnight at 50 °C, water (20 mL) was added and the reaction mixture was extracted with 5 × 10 mL of CH_2Cl_2. The combined organic solutions were then washed with water, dried over anhydrous Na_2SO_4 and purified by silica gel 60 H column chromatography, eluting with CH_2Cl_2/EtOAc 99:1, affording **3d** (62 mg, 56%), as an amorphous compound.

Compound 3d: $C_{37}H_{63}O_4N_9$; $[\alpha]_D$ +22.5° (*c* 0.4, EtOH); IR (film) ν_{max} cm^{-1} 2925, 2854, 2100, 1757, 1462, 1319, 1260, 1196, 1173, 1074, 801; HREIMS *m/z* 669.4935 (calcd 669.4941 for $C_{37}H_{63}N_7O_4$), 641.4794 (calcd 641.4880 for $C_{37}H_{63}N_5O_4$), 627.4855 (calcd 627.4849 for $C_{37}H_{63}N_4O_4$), 599.4747 (calcd 599.4787 for $C_{37}H_{63}N_2O_4$), 585.4676 (calcd 585.4757 for $C_{37}H_{63}NO_4$), 432.3154 (calcd 432.3113 for $C_{26}H_{42}NO_4$), 362.2720 (calcd 362.2695 for $C_{22}H_{36}NO_3$), 292.2287 (calcd 292.2276 for $C_{18}H_{30}NO_2$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (d, J = 1.5 Hz, H-35), 4.98 (dq, J = 1.5 and 6.6 Hz, H-36), 4.03 (m, H-16, 23), 3.96 (m, H-19, 20), 3.57 (m, H-24), 3.23 (m, H-28), 3.11 (m, H-15), 2.26 (brt, J = 7.5 Hz, H-3), 1.99, 1.83 (H-17, 18, 21, 22), 1.54 (m, H-4, 14, 25, 27, 29), 1.41 (d, J = 7.0 Hz, H-37), 1.27 (m, H-5−13 and H-30−33), 0.88 (t, J = 6.0 Hz, H-34); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9 (C-1), 148.8 (C-35), 132.8 (C-2), 82.1 (C-16), 81.8 (C-23), 81.3 (C-19), 81.2 (C-20), 77.3 (C-36), 65.5, 65.2 and 62.8 (C-15, 24, 28), 34.3 and 34.0 (C-27, 29), 31.6 (C-32), 31.0 (C-14), 30.5 (C-25), 29.0 (C-21), 28.6 (C-18), 28.3 (C-17), 27.3 (C-4), 26.03 (C-22), 25.1 (C-3), 22.9 (C-26), 22.5 (C-33), 19.2 (C-37), 14.0 (C-34).

Biochemical Methods. The inhibitory potency of the compounds was assayed using submitochondrial particles (SMP) from beef heart. SMP were obtained by extensive ultrasonic disruption of frozen-thawed mitochondria to produce open membrane fragments where permeability barriers to substrates were lost. Active complex I content in SMP preparations was estimated as previously described,[31] giving a concentration of 45.8 ± 0.3 pmol·mg^{-1}. SMP were diluted to 0.5 mg·mL^{-1} in 250 mM sucrose, 10 mM Tris-HCl buffer, pH 7.4, and treated with 300 μM NADH to activate complex I before starting experiments.

The enzymatic activities were assayed at 22 °C in 50 mM potassium phosphate buffer, pH 7.4, 1 mM EDTA with the SMP diluted to 6 mg·mL^{-1} (0.28 ± 0.01 nM complex I) in the cuvette. NADH:ubiquinone oxidoreductase was measured with 75 μM NADH and 30 μM DB as a soluble short-chain analogue of ubiquinone in the presence of 2 μM antimycin and 2 mM potassium cyanide to block any reaction downstream from complex I.[32] NADH oxidase activity was measured as the aerobic oxidation of 75 μM NADH in the absence of external quinone substrates and other inhibitors of the respiratory

chain. Reaction rates were calculated from the linear decrease of NADH concentration ($\lambda = 340$ nm, $\epsilon = 6.22$ $mM^{-1}\cdot cm^{-1}$) in an end-window photomultiplier spectrophotometer (ATI-Unicam UV4-500).

Stock solutions (2 mM in absolute ethanol) of the acetogenins used in this study were prepared and kept in the dark at −20 °C. Appropriate dilutions between 5 and 50 μM were made before the titrations. Increasing concentrations of these ethanolic solutions were then added to the diluted SMP preparations with 5 min incubation on ice between each addition. Maximal ethanol concentration never exceeded 2% of volume, and control activity was not affected by this concentration.[5] After each addition of inhibitor the enzymatic activities involving complex I were measured as described. The inhibitory concentration 50 (IC_{50}) was taken as the final compound concentration in the assay medium that yielded 50% inhibition of either NADH oxidase or NADH:DB oxidoreductase activities. Given values are means ± SD of four assays for each compound.

Acknowledgment. This research was supported by the Spanish Dirección General de Investigación Científica y Técnica (DGICYT) under Grant SAF 97-0013.

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JM000911J