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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 629–632

# Synthesis and mitochondrial complex I inhibition of dihydroxy-cohibin A, non-THF annonaceous acetogenin analogue

Hiroyuki Konno,[a,*] Naoki Hiura,[a] Hidefumi Makabe,[b] Masato Abe[c] and Hideto Miyoshi[c,*]

[a]*Department of Biological Science and Technology, Faculty of Engineering, University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan*
[b]*Integrated Department of Sciences of Functional Foods, Graduate School of Agriculture, Shinshu University, 8304 Minamiminowa, Kamiina, Nagano 399-4598, Japan*
[c]*Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*

Received 3 October 2003; accepted 18 November 2003

**Abstract**—To elucidate the inhibitory action of acetogenins, we synthesized an acetogenin derivative which possesses tetraol in place of the tetrahydrofuran ring and examined its inhibitory activity against bovine heart mitochondrial complex I. Our results indicate that these hydroxy groups are an essential structural factor though it is not effective as bis-THF hydroxy groups combination.


## 1. Introduction

Among the rapidly growing family of the Annonaceous acetogenin,[1,2] these are endemic to certain plants of the Annonaceae and have been shown to possess a broad spectrum of biological activities involving cytotoxic, antitumor, pesticidal, antifeedant, and immunosuppressive properties. Although action of their compounds is thought the inhibition of the complex I (NADH-ubiquinone oxidoreductase) in mammalian and insect mitochondrial electron transport systems[3,4] and of the NADH oxidase found in the plasma membranes of cancer cells,[5,6] there is still no hard experimental evidence to verify whether the inhibitors bind to the ubiquinone reduction site. Although bullatacin (**2**),[4] bis-THF acetogenin isolated from *Annona bullata,* is actually one of the most potent inhibitors of this enzyme identified to date, there are few structural similarities between the acetogenin and ordinary complex I inhibitors such as piericidin A and rotenone. Thus, studying their essential structural sites as well as inhibitory property of acetogenin, structural–activity relationship is important to elucidate the terminal electron transfer system of complex I.

In previous structure–activity relationships using natural and synthetic acetogenin, these are shown following: (i) acetogenin acts as a potent inhibitor only when the γ-lactone and THF ring moieties are directly linked by an alkyl spacer (13 carbon atoms is better); (ii) number of THF rings, the presence of polar functional groups such as hydroxy group in the spacer, and stereochemistry around the THF are not essential structural factors for the potent activity.[4] On the other hand, Shimada et al. proposed a model of active conformation of acetogenins, wherein THF ring with flanking hydroxy groups work as hydrophilic anchor in mitochondrial membrane (Fig. 1).[7] There are few studies of structural simplification of THF ring based on natural acetogenin without catechol and ether linked acetogenins.[6] Taking into consideration the fact that hydrophilicity of the place of THF site is essential, this finding is very interesting to elucidate the mode of action of acetongenin.

In the present study, we synthesized an acetogenin analogue which possesses tetraol in place of the THF ring, named dihydroxy-cohibin A (**1**), non-THF acetogenin (Fig. 2). There are minor group of non-THF ring compounds which can be assumed to be degradation products and intermediary precursors in the biosynthesis onto acetogenins. Cohibin A has been isolated by

*Keywords:* Annonaceous acetogenin; Biological activity; Complex I inhibitor; Stereoselective synthesis; Aetogenin analogue.
* Corresponding author. Tel.: +81-88-656-9213; fax: +81-88-656-9213; e-mail: konno@bio.tokushima-u.ac.jp

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doi:10.1016/j.bmcl.2003.11.057

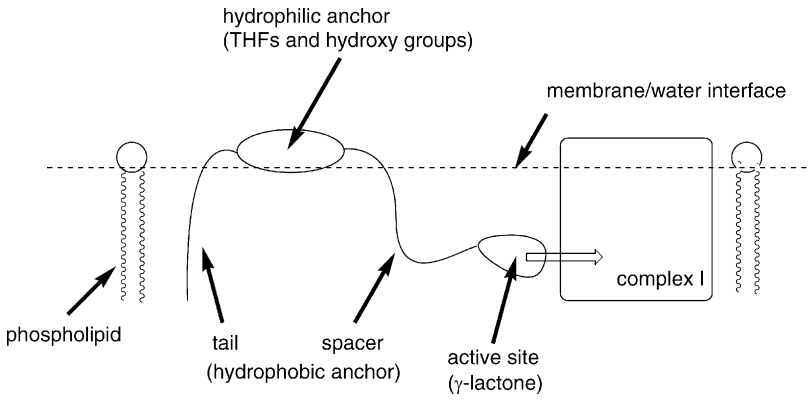



**Figure 1.** Model of the active conformation of acetogenins interacting with complex I in the mitochondrial membrane proposed by Shimada et al.[7]

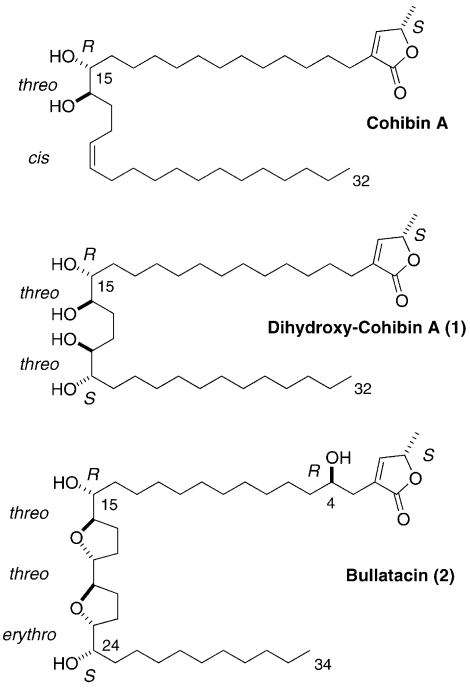



**Figure 2.** Structure of cohibin A, synthetic dihydroxy-cohibin A (**1**) and bullatacin (**2**).

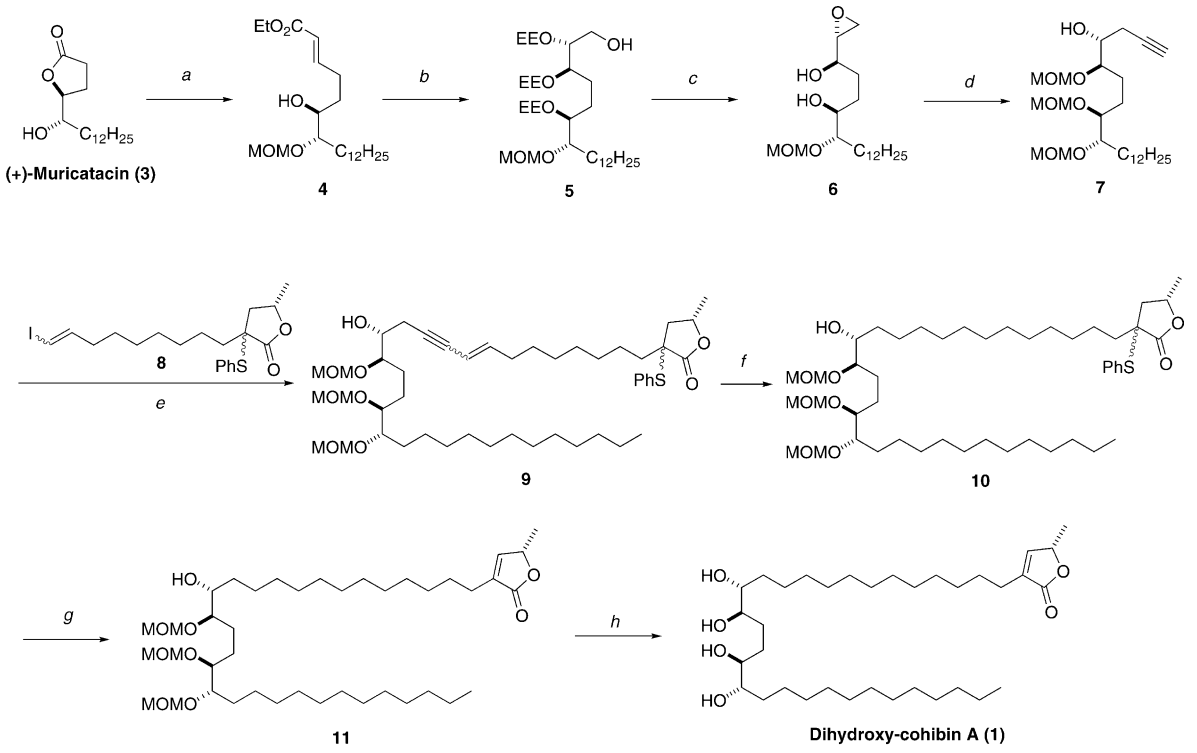
Cavé group from the roots of the tropical fruit, *Annona muricata*.[8] Dihydroxy-cohibin A (**1**), acetogenin analogue that possessed dihydroxy function in the place of olefin based on cohibin A, was designed to be increase the hydrophilicity. Stereochemistry of dihydroxy-cohibin A (**1**) was led to cohibin A and previous publications.[9,10] The inhibitory action of dihydroxy-cohibin A (**1**) and **11** was examined with bovine heart mitochondrial complex I.

## 2. Chemistry

The synthetic procedures are outlined in Scheme 1. The key intermeditate **6** of enantiomer form was synthesized earlier by us[9] and all stereoisomers of muricatacin **3** could be easily obtained in an enantiomerically pure form.[11] We commenced synthesis using (+)-muricatacin **3**. After protecting the hydroxy group of **3** as a MOM ether, the partial reduction of the resulting lactone with DIBAL afforded acetal, which was then submitted to a careful Horner–Emmons reaction at −78 °C to give the chain-extented α,β-*E*-unsaturated ester **4**. Protection of the hydroxy group of **4** as an EE ether and subsequent Sharpless asymmetric dihydroxylation using AD-mix β furnished dihydroxy ester, which, after protecting the hydroxy group with ethyl vinyl ether, was reduced with $LiAlH_4$ to yield **5**. A three step sequence of reactions involving treatment with *p*-TsCl, hydrolysis of the EE group and oxirane ring formation with KOH provided dihydroxy epoxide **6**, which was proved to have a 92% diastereomeric excess by $^1H$ NMR analysis. Fortunately, the undesired diastereomer could be removed from **6** by column chromatography. Protection of the hydroxy group of **6** as a MOM ether, a coupling reaction with lithum TMS-acetylide in the presence of $BF_3{\cdot}Et_2O$ and deprotection of TMS group with KF afforded **7**. The γ-lactone moiety **8** was prepared by application of the method that had been reported earlier by our group.[12] A Pd-mediated cross-coupling reaction between **7** and **8** with $Pd(PPh_3)_4$, CuI, and pyrrolidine without any solvent yielded enyne **9**.[13] Catalytic hydrogenation of **9** with Wilkinson's catalyst gave **10**. Sequential thermal elimination of the sulfide moiety were performed according to the reported method to afford **11**. Finally deprotection of MOM group of **11** with $BF_3{\cdot}Et_2O$ in $Me_2S$ gave dihydroxy-cohibin A (**1**).

## 3. Bioactivity

The inhibition of complex I activity was determined by NADH oxidase assay using bovine heart submitochondrial particles.[14] The potency of bullatacin (**2**), one of the most potent natural acetogenins, in terms of $IC_{50}$ value was 0.8 nM in the present study. Under the same experimental conditions, $IC_{50}$ of **1** and **11** were 20 and 4100 nM, respectively. Thus, free hydroxy groups between the spacer and the tail are essentially important for the activity though it is not effective as bis-THF hydroxy groups combination. This was supported by the marked loss of inhibitory activity of **11**, which has lonely free hydroxy group with tri-MOM protected hydroxy groups located in the middle of the inhibitor.




**Scheme 1.** (a) (i) MOMCl, $i$Pr$_2$NEt, $CH_2Cl_2$, 65%; (ii) DIBAL-H, $CH_2Cl_2$, 95%; (iii) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 75%; (b) (i) $EtOCH{=}CH_2$, PPTS, $CH_2Cl_2$, 87%; (ii) AD-mix β, $MeSO_2NH_2$, $t$BuOH–$H_2O$, 92%; (iii) $EtOCH{=}CH_2$, PPTS, $CH_2Cl_2$, 81%; (iv) $LiAlH_4$, THF, 99%; (c) (i) $p$TsCl, pyridine; (ii) PPTS, MeOH; (iii) KOH, 50%; (d) (i) MOMCl, $i$Pr$_2$NEt, $CH_2Cl_2$; (ii) trimethylsilyl acetylene, $n$BuLi, $BF_3{\cdot}Et_2O$, THF; (iii) KF, MeOH, 15%; (e) $Pd(PPh_3)_4$, CuI, pyrrolidine, 58%; (f) $H_2$, $Rh(PPh_3)_3Cl$, benzene, 97%; (g) (i) $m$CPBA, $NaHCO_3$, $CH_2Cl_2$; (ii) toluene reflux, 73%; (h) $BF_3{\cdot}Et_2O$, $Me_2S$, 56%.

## 4. Discussion

The present study indicated that free hydroxy groups in the place of THF ring are preferable for the activity but is not effective as bullatacin type structure. The present results do not support Shimada's model from hydrophilic property of tetraol unit and previous publications.[4] If the hydrogen bond-donating ability of the hydroxy groups as hydrophilic anchor at the liposomal membrane surface is important for Complex I inhibition, potent activity of tetraol acetogenin would have shown to the intermolecular hydrogen bonds between the hydroxy groups and oxygen in phospholipid. Molecular structure of hydrophilic site, gylcerol backbone of the phospholipid, can be regarded high degree of flexibility in the liposomal membrane. Structure of hydrophilic anchor at the liposomal membrane surface also can permit to be flexible structure. However bullatacin type structure contained rigid bis THF unit remains to be the most potent inhibitor. We propose that both γ-lactone ring and THF ring act in cooperative manner on the enzyme with the support of some specific conformation of the spacer.[15] Therefore, we guess that bioactivity of dihydroxy-cohibin A (**1**) are diminish (20 times-fold) by the high degree of flexibility of the tetraol unit and more rigid structure contained with free hydroxy groups need to increase in activity. Thus, in order to examine the potential of these compounds as novel agents for pharmaceutical and agrochemical use, it seems worthwhile to design and study more simple analogue incorporating key elements of Annonaceous acetogenin. Taking into account our hypothesis the synthesis and active conformation of complex I, but simplified analogues design is underway.

## Acknowledgements

We thank Prof. Hitoshi Hori (University of Tokushima), and Prof. Hiromasa Kiyota (Tohoku University) for useful discussion. This work was supported by the Agricultural Chemical Research Foundation and Mitsubishi Chemical Corporation Fund.

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