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Essential Structural Factors of Acetogenins, Potent Inhibitors of Mitochondrial Complex I

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Abstract—To elucidate the role of the hydrophobic alkyl tail of acetogenins in the inhibitory action, we synthesized an acetogenin derivative possessing the shortest tail (i.e., methyl group) and examined its inhibitory activity against bovine heart mitochondrial complex I. Our results indicated that the alkyl tail, which is one of the common structural features of natural acetogenins, is not an essential structural factor required for the potent inhibition.

Introduction

Acetogenins have very potent and diverse biological effects including cytotoxic, antitumor, antimalarial, pesticidal and antifeedant activities.[1] The inhibitory effects of acetogenins on mitochondrial NADH-ubiquinone oxidoreductase (complex I) are of particular note as the diverse biological activities are thought to be attributable to this effect.[1] In fact, some of these compounds, such as bullatacin (Fig. 1) and rolliniastatin-1, are the most potent inhibitors of this enzyme identified to date.[2,3] Although the acetogenins act at the terminal electron transfer step of complex I similarly to the ordinary complex I inhibitors,[2,3] there are few structural similarities between the acetogenins and ordinary complex I inhibitors such as piericidin A and rotenone. Thus, considering their unusual structural characteristics as well as the very strong inhibitory potency of acetogenins, detailed analysis of the inhibitory actions of these inhibitors is important to elucidate the structural and functional features of the terminal electron transfer step of complex I. As the first step toward this purpose, identification of the crucial structural factors of acetogenins required for their potent inhibitory effects would be very useful.[3,4]

In previous structure/activity studies using a series of natural and synthetic acetogenins,[5] we showed that: (i) number of THF rings, the presence of polar functional groups such as hydroxy group in the spacer, and stereochemistry around the THF and the γ-lactone ring moieties are not essential structural factors for the potent activity; (ii) acetogenin acts as a potent inhibitor only when the γ-lactone and the THF ring moieties are directly linked by an alkyl spacer; and (iii) the optimal length of the spacer is about 13 carbon atoms. On the basis of these results, we proposed that the γ-lactone ring is not the only reactive species interacting directly with the enzyme and that both ring moieties act in a cooperative manner on the enzyme with the support of some specific conformation of the spacer. On the other hand, there have been no studies concerning the role of the long alkyl tail, although the importance of hydrophobic interactions of the tail with the enzyme (or its environment) was suggested.[6] We synthesized an acetogenin derivative (**1**) possessing the shortest tail (i.e., methyl group) and compared its inhibitory activity for bovine complex I with that of its mother compound **2**.

Chemistry

The synthetic procedures are outlined in Scheme 1. The key intermediate **8** was synthesized according to the procedures reported by Hoye and Ye,[7] except that **3** was prepared by reacting *tert*-butyl acetate and *trans*-1,4-dibromo-2-butene.[8] Reduction of **3** with DIBAL-H and Wittig extension of the resultant dialdehyde gave **4**. This was reduced with DIBAL-H followed by double Sharpless asymmetric epoxidation to afford **5**. Silylation with TBDPSCl and sequential Sharpless asymmetric dihydroxylation gave **6**, which was immediately treated with

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trifluoroacetic acid to produce **7**. Treatment with TsCl afforded **8**, the ^{1}H NMR data and an optical rotation of which were completely identical to those reported.[7] After brief treatment of **8** with TBAF (0.5 molar equiv, 20 min), the reaction mixture was treated with K_2CO_3 to produce a mixture of two useful products, **9** and **10** (43 and 17%, respectively, based upon recovered starting material).[9] The ^{1}H and ^{13}C NMR data and an optical rotation of **10** also completely matched the reported data.[7]

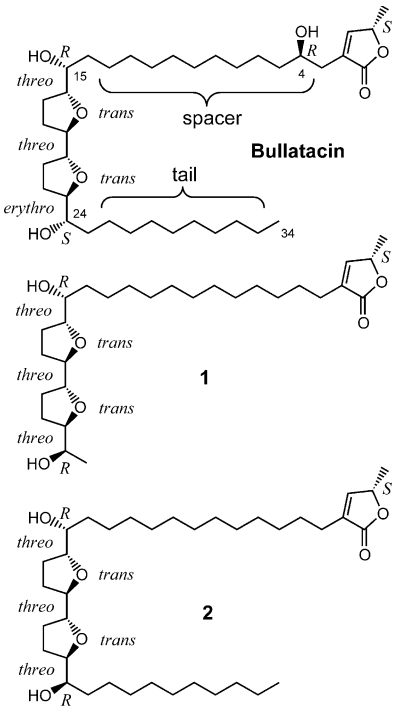


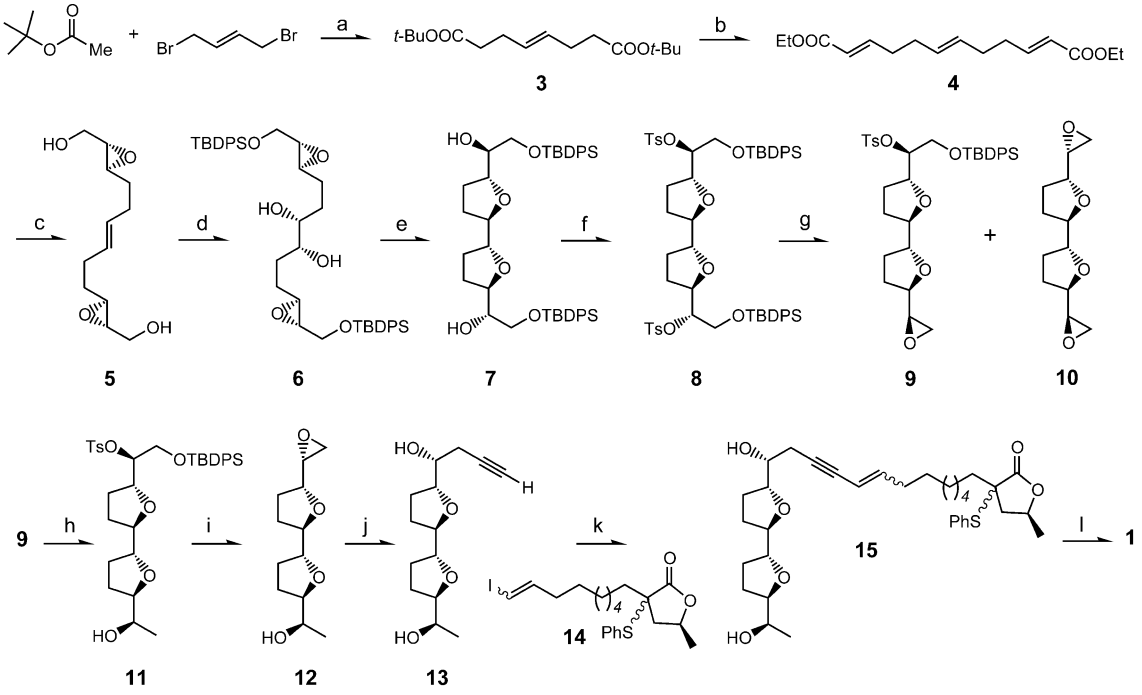
Figure 1. Structures of bullatacin and synthetic acetogenins examined in this study.

Opening of epoxide **9** by catalytic hydrogenation with Pd/C selectively yielded the secondary alcohol **11**.[10] Treatment of **11** with excess TBAF produced epoxide **12**. Opening of epoxide **12** with lithium (trimethylsilyl) acetylide in the presence of boron trifluoride etherate[11] and desilylation provided **13**. Pd(0)-catalyzed coupling[12] of alkyne **13** with vinyl iodide **14**, which was prepared by the reported method,[13] gave the eneyne **15**. Catalytic hydrogenation of **15** using Wilkinson's catalyst and sequential thermal elimination of the sulfide moiety were performed according to the reported method[7] to afford **1**.[14]

Compound **2** was the same sample as that synthesized previously.[5c]

Bioactivity

The inhibition of complex I activity was determined by NADH oxidase assay using bovine heart sub-mitochondrial particles.[15] Previous studies indicated that the inhibitory potency of synthetic acetogenin **2** is comparable to that of bullatacin, one of the most potent natural acetogenins.[5b,5c] The potency of **2** in terms of IC_{50} value, that is the molar concentration needed to halve the control NADH oxidase activity, was 0.9 nM in



Scheme 1. (a) LDA, THF, −40 °C, 1 h, 83%; (b) (i) DIBAL-H (2.0 molar equiv), CH_2Cl_2, −78 °C, 1 h; (ii) PH_3P=CHCOOEt, CH_2Cl_2, rt, 9 h, 65%; (c) (i) DIBAL-H, CH_2Cl_2, −78 °C, 2 h; (ii) *L*-(+)-DET, Ti(*i*-PrO)$_4$, *t*-BuOOH, CH_2Cl_2, −20 °C, 1 day, 58%; (d) (i) TBDPSCl, DMAP, Et_3N, CH_2Cl_2, rt, 16 h; (ii) AD-mix-β, $MeSO_2NH_2$, *t*-BuOH-H_2O, 0 °C, 16 h 68%; (e) TFA, CH_2Cl_2, rt, 1.5 h 82%; (f) TsCl, DMAP, Et_3N, CH_2Cl_2, 55 °C, 24 h, 91%; (g) (i) TBAF (0.5 molar equiv), THF, rt, 20 min; (ii) K_2CO_3, MeOH, rt, 16 h; (h) H_2, Pd/C, MeOH, 4 days, 95%; (i) TBAF, THF, rt, 5 h, 85%; (j) (i) lithium (trimethylsilyl)acetylide, BF_3·Et_2O, −78 °C, 15 min; (ii) K_2CO_3, MeOH, rt, 16 h, 62%; (k) $(Ph_3P)_2PdCl_2$, CuI, Et_3N, rt, 24 h, 72%; (l) (i) H_2, $(Ph_3P)_3RhCl$, benzene, rt, 1 day; (ii) *m*-CPBA, CH_2Cl_2, 0 °C, 30 min; (iii) toluene, 100 °C, 2 h, 32%.

the present study. Under the same experimental conditions, IC_{50} of **1** was 3.1 nM, and complete inhibition was attained at about 10 nM. Thus, irrespective of drastic shorting of the tail length, compound **1** retained sufficiently potent inhibitory activity, indicating that large hydrophobicity of the tail is not essentially important for the activity. This result is consistent with the observations that the presence of polar hydroxy group(s) in the tail of some natural acetogenins is not unfavorable for the activity.[2a,5a]

Discussion

In general, the activities of potent biologically active compounds are markedly diminished (by several orders of magnitude) by structural modification of essential structural unit(s). In this sense, essential structural factors of the THF and the γ-lactone ring moieties of acetogenins are not necessarily obvious, except for the important role of the alkyl spacer connecting both ring moieties, as described in the introductory section. In addition to the THF ring with flanking hydroxy groups and the γ-lactone ring, the presence of a long alkyl tail is also a common structural feature of a large number of natural acetogenins.[1a] Our results indicated that the hydrophobic tail is preferable for the activity, but is not an essential structural factor. Thus, regardless of the very potent activity, crucial structural features of acetogenins are quite ambiguous. This unusual characteristic may be related to the high degree of flexibility of the alkyl spacer that links the functionally important THF and γ-lactone ring moieties. The present study provided useful insight into design strategies of novel acetogenin mimics for further wide structural modifications, which would enable development of novel agents for pharmaceutical and agrochemical use.

Based on the results of ^{1}H NMR and differential scanning calorimetry studies of acetogenins in liposomal membrane, Shimada et al. proposed a model of active conformation of these inhibitors, wherein the alkyl tail and THF ring with flanking hydroxy groups work as hydrophobic and hydrophilic anchors, respectively, in the mitochondrial membrane, and only the γ-lactone interacts directly with the target site of complex I by lateral diffusion in the membrane.[6] As a prerequisite for the conformational model of Shimada et al., they noted a significant contribution of the intermolecular hydrogen bonds between the hydroxy groups in the adjacent THF rings and the oxygen atoms in the glycerol backbone of the phospholipid when the THF rings moiety acts as hydrophilic anchor at the liposomal membrane surface. If the hydrogen bond-donating ability of the hydroxy groups is important for complex I inhibition, acetylation of one or both hydroxy groups would result in a marked decrease in the inhibitory potency, but this was not the case.[5b] Furthermore, if the γ-lactone ring can be regarded as the only reactive species directly interacting to the enzyme, chemical modification of this moiety would result in drastic decrease in the inhibitory potency, but this was also not the case.[5b,5c] Thus, the present results along with the previous studies[5b,5c] do not support the model proposed by Shimada et al. The active conformation of acetogenins in complex I remains to be elucidated. However, we cannot necessarily exclude the validity of their model for *partitioning* of acetogenins into the liposomal membrane. In the liposomal membrane, the average location of THF and γ-lactone ring moieties could be primarily determined by their hydrophobicity.[16]

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9. Compound **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J = 8.2 Hz, 2H), 7.61 (brd, J = 6.0 Hz, 4H), 7.44–7.30 (m, 6H), 7.23 (d, J = 8.2 Hz, 2H), 4.65 (ddd, J = 4.6, 4.6 and 4.6 Hz, 1H), 4.28 (m, 1H), 3.92–3.80 (m, 3H), 3.69 (dd, J = 12.2 and 4.5 Hz, 1H), 3.59 (m, 1H), 2.94 (ddd, J = 4.2, 4.2 and 2.3 Hz, 1H), 2.72 (dd, J = 6.2 and 3.1 Hz, 1H), 2.64 (m, 1H), 2.39 (s, 3H), 2.12–1.60 (m, 8H), 1.02 (s, 9H). MS (ESI) m/z 659 $[M+Na]^+$. Compound **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97–3.89 (m, 4H), 2.96 (ddd, J = 4.2, 4.2 and 2.3 Hz, 2H), 2.76–2.70 (m, 4H), 2.18–2.05 (m, 2H), 2.03–1.92 (m, 2H), 1.88–1.75 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 82.1, 78.8, 54.2, 44.1, 28.8, 27.9. MS (ESI) m/z 249 $[M+Na]^+$. $[\alpha]_D^{23} = -15.9$ (c 3.34, ethyl acetate).
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14. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (m, 1H), 5.00 (dq, J = 1.5, 7.0 Hz, 1H), 3.83–3.90 (m, 3H), 3.78 (dt, J = 5.0, 7.5 Hz, 1H), 3.57 (dq, J = 6.3, 6.3 Hz, 1H), 3.37 (m, 1H), 2.79 (br, s, 2H), 2.28 (m, 2H), 1.96–2.04 (m, 4H), 1.43–1.80 (m, 8H), 1.21–1.43 (m, 18H), 1.41 (d, J = 7.0 Hz, 3H), 1.13 (d, J = 6.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 148.9, 134.4, 84.5, 83.2, 81.8, 81.7, 77.5, 77.0, 76.6, 74.1, 70.5, 33.5, 29.7, 29.6, 29.5, 29.3, 29.2, 29.0, 28.4, 27.4, 25.7, 25.2, 19.2, 18.8. $[\alpha]_D^{24} = +6.8$ (c 0.40, ethyl acetate). HRMS (FAB) for $C_{28}H_{49}O_6$ $(M+H)^+$ calcd 481.3529, found 481.3522.
15. Bovine heart submitochondrial particles were prepared by the method of Matsuno-Yagi and Hatefi (*J. Biol. Chem.* **1985**, 260, 14424) and stored in a buffer containing 0.25 M sucrose and 10 mM Tris–HCl (pH 7.4) at −82 °C. The NADH oxidase activity in the particles was followed spectrometrically with a Shimadzu UV-3000 (340 nm, $\varepsilon = 6.2\,mM^{-1}\,cm^{-1}$) at 30 °C. The reaction medium (2.5 mL) contained 0.25 M sucrose, 1 mM $MgCl_2$ and 50 mM phosphate buffer (pH 7.4). The final mitochondrial protein concentration was 30 μg of protein/mL. The reaction was started by adding 50 μM NADH after equilibration of the particles with inhibitor for 5 min. The IC_{50} values were averaged from two independent experiments.
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