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Essential structural features of acetogenins: role of hydroxy groups adjacent to the bis-THF rings

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Abstract—The presence of two hydroxy groups adjacent to the THF ring(s) is a common structural feature of natural acetogenins. To elucidate the role of each hydroxy group in the inhibitory action of acetogenins, we synthesized three acetogenin analogues which lack either or both of the hydroxy groups, and investigated their inhibitory activities with bovine heart mitochondrial complex I. Our results indicate that the presence of either of the two hydroxy groups sufficiently sustains a potent inhibitory effect.
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Acetogenins have diverse biological effects such as antitumor, antimalarial, pesticidal and antifeedant activities.[1,2] 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.[1,2] Actually some acetogenins, such as bullatacin (=rolliniastatin-2, Fig. 1) and rolliniastatin-1, are the most potent inhibitors of this enzyme identified to date.[3–6] Acetogenins are thought to act at the terminal electron transfer step of complex I,[5,6] but there is still no hard experimental evidence to verify whether the inhibitors bind to the ubiquinone reduction site. Considering the unusual structural characteristics as well as the very strong inhibitory effect of acetogenins, a detailed study on the inhibitory action of these inhibitors is needed to elucidate the structural and functional features of the terminal electron transfer step of complex I. To this end, identification of the crucial structural factors of acetogenins required for their potent inhibition would be very useful.

In previous structure–activity studies using a series of natural and synthetic acetogenins with mitochondrial complex I,[7–11] we showed that: (i) the presence of polar functional group(s) like an OH group in the spacer, number of THF rings and stereochemistry around the hydroxylated THF ring(s) are not essential structural factors for potent activity; (ii) natural γ-lactone ring itself is not crucial for the activity, and can be substitutable with an ubiquinone ring; and (iii) acetogenin acts as a strong inhibitor only when the γ-lactone and the THF ring moieties are directly linked by an alkyl spacer, the optimal length of which is about 13 carbon atoms. Thus, except for the important role of the alkyl spacer, essential structural factors including the active conformation of acetogenins remain to be elucidated.

The presence of two OH groups adjacent to the THF ring(s) is a common structural feature of a large number of natural acetogenins. Some reports suggested an important role for the OH group in the cytotoxicity of acetogenins,[12,13] whereas the interpretation of results of cytotoxic assays is somewhat complicated since one has to take into consideration factors such as membrane transport and metabolism. We previously showed that acetylation of both OH groups of a potent synthetic acetogenin (compound **1** in this study) resulted in a rather slight, just 6-fold, decrease in the inhibitory potency with mitochondrial complex I.[9] Although this result was quite unexpected, it suggests that high polarity (or hydrophilicity) around the THF ring moiety, rather than the hydrogen bond-donating ability of the OH groups, may be required for the inhibitor to adopt an active conformation.[14] To elucidate the role of each OH group, selective deoxygenation is necessary. In this study, we synthesized three acetogenin analogues which lack either or both of the two hydroxy groups located

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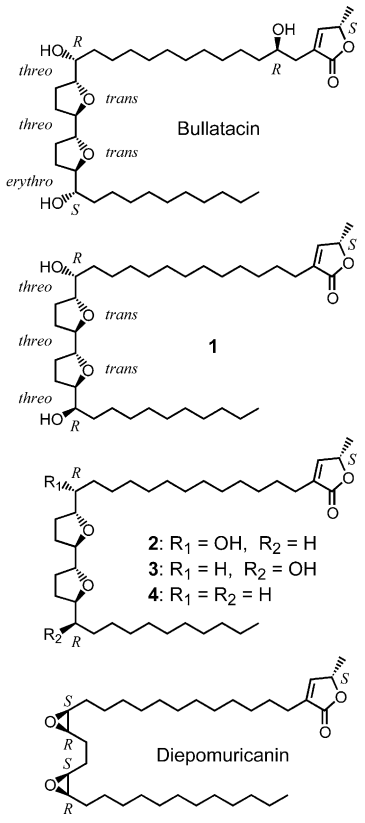


Figure 1. Structures of acetogenin analogues examined in this study.

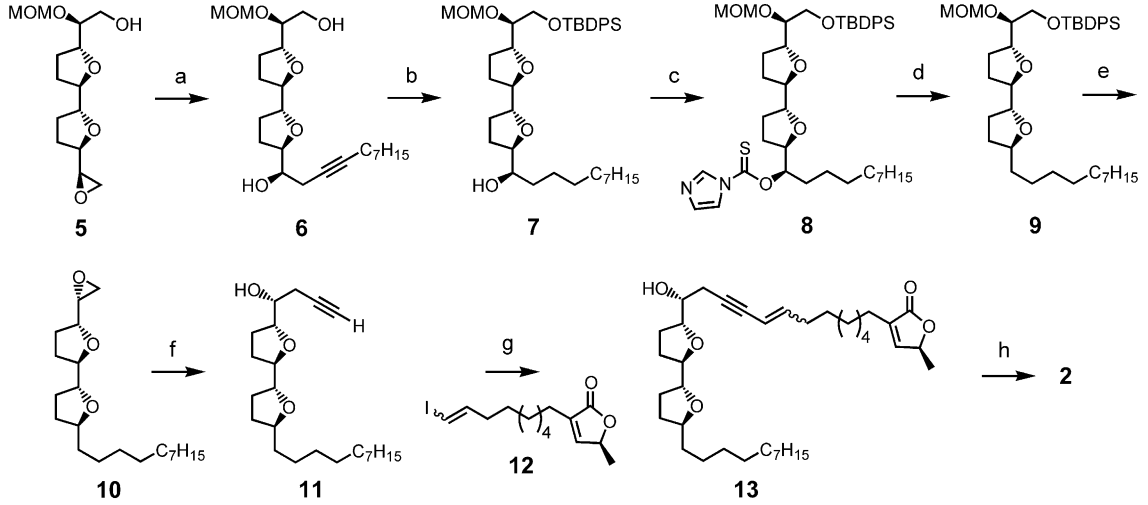
on the sides of bis-THF rings, and investigated their inhibitory activities with bovine heart mitochondrial complex I.

1. Synthesis

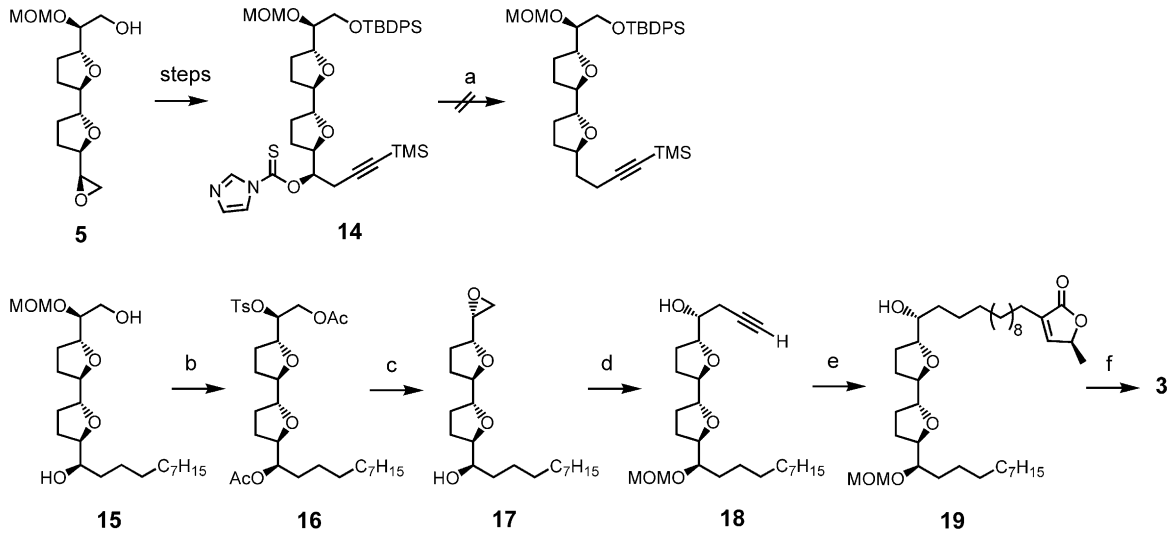
The synthesis of compound **2** is outlined in Scheme 1. The key intermediate **5** was synthesized as described previously.[10,11] The opening of epoxide **5** with 1-lithium-1-nonyne in the presence of BF_3 etherate[15] afforded **6**. Hydrogenation of **6** with Pd/C and sequential selective protection of the primary alcohol by TBDPSCl gave **7**. Deoxygenation of the free hydroxy group in **7** was carried out by the Barton two-step procedure,[16] that is conversion to the corresponding thiocarbonate **8** and then treatment with *n*-Bu_3SnH to produce **9**.

After deprotection of the MOM ether of **9**, tosylation of a free hydroxy group and sequential desilylation gave the epoxide **10**. Opening of the epoxide with lithium (trimethylsilyl)acetylide in the presence of BF_3 etherate and sequential desilylation afforded **11**. Pd(0)-catalyzed coupling[17] of alkyne **11** with vinyl iodide **12**, which was prepared as described,[18] gave the eneyne **13**. Since selective hydrogenation of **13** with Wilkinson's catalyst resulted in an appreciable reduction of the butenolide double bond as pointed out by Marshall and Chen,[19] hydrogenation was carried out with diimide, generated in situ from tosylhydrazine,[20] to obtain **2**.[21]

Initially, we tried to synthesize compound **3** by a similar sequence of reactions starting from **5**, as shown in Scheme 2. However, the radical deoxygenation of the thiocarbonate in **14** resulted in a very poor yield of the product. Several modifications of the reaction conditions were investigated, but attempts to improve the yield were unsuccessful. We therefore examined an alternate approach (Scheme 2). After acetylation of both hydroxy groups of **15**, which was obtained by catalytic hydrogenation of **6** with Pd/C, deprotection of MOM ether and sequential tosylation afforded **16**. Hydrolysis of the acetyl group gave epoxide **17**. The opening of epoxide **17** with lithium (trimethylsilyl)acetylide in the presence of BF_3 etherate and sequential desilylation provided **18**. Cross-coupling of **18** with the spacer moiety **12** and hydrogenation of the resultant eneyne by the above method afforded **19**. Deoxygenation of a free hydroxy group in **19** by the Barton method and sequential deprotection of the MOM ether afforded compound **3**.[21]



Scheme 1. (a) 1-Nonyne, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 0.5 h, 77%; (b) (i) H_2, Pd/C, EtOH, (ii) TBDPSCl (1.2 equiv), DMAP, CH_2Cl_2, rt, 92%; (c) thiocarbonyldiimidazole, DMAP, CH_2Cl_2, rt, 93%; (d) *n*-Bu_3SnH, AIBN, dry toluene, rt, 2 h, 65%; (e) (i) TMSBr, CH_2Cl_2, −30 °C, 1 h, (ii) TsCl, DMAP, Et_3N, CH_2Cl_2, rt, (iii) TBAF, THF, rt, 65%; (f) lithium (trimethylsilyl)acetylide, $BF_3{\cdot}Et_2O$, THF, −78 °C, 15 min, (ii) K_2CO_3, MeOH, rt, 5 h, 69%; (g) $(Ph_3P)_4Pd$, CuI, Et_3N, rt, 5 h, 72%; (h) $TsNHNH_2$, NaOAc, DME/H_2O (1:1), reflux, 85%.



Scheme 2. (a) *n*-Bu_3SnH, AIBN, dry toluene, rt; (b) (i) AcCl, DMAP, CH_2Cl_2, 0 °C to rt, (ii) $BF_3{\cdot}Et_2O$, Me_2S, −20 °C, 2 h; (iii) TsCl, DMAP, Et_3N, CH_2Cl_2, rt, 80%; (c) KOH, MeOH, rt, 2 h, 95%; (d) lithium (trimethylsilyl)acetylide, $BF_3{\cdot}Et_2O$, THF, −78 °C, 15 min; (ii) K_2CO_3, MeOH, rt, 5 h, 75%; (e) (i) compound **12**, $(Ph_3P)_4Pd$, CuI, Et_3N, rt, 5 h; (ii) $TsNHNH_2$, NaOAc, DME/H_2O (1:1), reflux, 62%; (f) (i) thiocarbonyldiimidazole, DMAP, CH_2Cl_2, rt; (ii) *n*-Bu_3SnH, AIBN, dry toluene, rt, 2 h; (iii) 4% AcCl in MeOH, CH_2Cl_2, rt, 1 h, 54%.

Compound **4** was synthesized by deoxygenation of a free hydroxy group in compound **2** by the Barton method in 53% yield (two steps).[21]

2. Bioactivity

The inhibition of complex I activity was determined by NADH oxidase assay using bovine heart submitochondrial particles (SMP).[7] Previous studies indicated that the inhibitory potency of compound **1**, a standard compound in this study, is comparable to that of bullatacin, one of the most potent natural acetogenins.[7,9] The inhibitory potency of compound **1** in terms of the IC_{50}, that is the molar concentration needed to halve the control NADH oxidase activity, was 0.75 (±0.08) with the present SMP preparations. The IC_{50} values of compounds **2** and **3** were 3.1 (±0.4) and 2.7 (±0.3) nM, respectively. The IC_{50} value of compound **4** was 85 (±9) nM, and maximum inhibition was saturated at about 85% even at 2 μM.[22] These results indicate that a lack of both OH groups resulted in a drastic decrease in inhibitory potency, but the presence of either is enough to retain fairly strong activity. This is consistent with the observation that a mono-acetyl derivative of compound **1**, wherein the OH group located on the side of the spacer is acetylated, elicits fairly potent activity, the IC_{50} being 2.1 nM.[9] Our results for the first time revealed that the contributions of the two OH groups toward the inhibitory action are equivalent.

Taking into consideration the result that acetylation of both OH groups of compound **1** reduced the inhibitory potency by just 6-fold,[9] and that an acetyl group is a fairly hydrophilic substituent,[14] the present study strongly suggests that high polarity (or hydrophilicity) around the THF ring moiety, rather than the hydrogen bond-donating ability of the OH group, is required for the inhibitor to adopt an active conformation. The importance of the high polarity around the THF ring moiety may explain why both the number of THF rings and the stereochemistry surrounding the hydroxylated THF ring moiety affect little the inhibitory potency. Furthermore, from a molecular viewpoint, the importance of high polarity may be associated with the fact that the putative target subunit of acetogenin in bovine complex I (PSST subunit[23]) is hydrophilic and contains no transmembrane helices according to prediction of secondary structure.[24–26]

We previously showed that the IC_{50} value of diepomuricanin (Fig. 1), which also has no free OH group, is 2.8 μM.[9] Compared to diepomuricanin, compound **4** appeared to be a much more potent inhibitor. This is probably due to the relatively fixed spatial position (i.e., lower flexibility) of the two ether oxygen atoms of compound **4** which favors an active conformation. The present study not only is helpful in elucidating the active conformation of acetogenins, but also provides useful guiding principles for wide structural modification of acetogenins with the aim of developing practical agents.

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21. Compound **2**: colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (m, 1H), 5.00 (dq, $J = 1.5$, 7.0 Hz, 1H), 3.95–3.80 (m, 4H), 3.38 (m, 1H), 2.49 (s, 1H), 2.26 (m, 2H), 2.08–1.96 (m, 4H), 1.65–1.23 (m, 46H), 1.41 (d, $J = 7.0$ Hz, 3H), 0.88 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 148.9, 134.4, 83.0, 82.1, 81.2, 80.0, 74.1, 35.8, 33.4, 32.1, 31.9, 29.8, 29.6, 29.5, 29.4, 29.2, 28.9, 28.8, 28.4, 27.4, 26.2, 25.7, 25.2, 22.7, 19.2, 14.1. $[\alpha]_D^{23} = +20.0$ (*c* 0.46, EtOH). ESI-MS (*m*/*z*) 613.5 $[M + Na]^+$. Compound **3**: colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (m, 1H), 5.00 (dq, $J = 1.5$, 7.0 Hz, 1H), 3.95–3.80 (m, 4H), 3.38 (m, 1H), 2.49 (s, 1H), 2.26 (m, 2H), 2.08–1.96 (m, 4H), 1.65–1.23 (m, 46H), 1.41 (d, $J = 7.0$ Hz, 3H), 0.88 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 148.9, 134.4, 83.0, 82.1, 81.2, 80.0, 74.1, 35.8, 33.4, 32.1, 31.9, 29.8, 29.6, 29.5, 29.4, 29.2, 28.9, 28.8, 28.4, 27.4, 26.2, 25.7, 25.2, 22.7, 19.2, 14.1. $[\alpha]_D^{23} = +26.7$ (*c* 0.15, EtOH). ESI-MS (*m*/*z*) 613.5 $[M + Na]^+$. Compound **4**: colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (m, 1H), 5.00 (dq, $J = 1.5$, 7.0 Hz, 1H), 3.94–3.86 (m, 4H), 2.26 (m, 2H), 2.02–1.91 (m, 4H), 1.85–1.23 (m, 48H), 1.41 (d, $J = 7.0$ Hz, 3H), 0.88 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 148.9, 134.4, 81.4, 79.8, 35.8, 32.1, 31.9, 29.8, 29.6, 29.5, 29.3, 29.2, 28.5, 27.4, 26.2, 25.2, 22.7, 19.2, 14.1. $[\alpha]_D^{23} = +9.5$ (*c* 0.11, EtOH). ESI-MS (*m*/*z*) 597.5 $[M + Na]^+$.
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