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Note

Synthesis of ^{13}C-Labeled Ubiquinone-Acetogenin Hybrid Inhibitors of Mitochondrial Complex I

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Natural acetogenins are the most potent inhibitors of mitochondrial complex I. By synthesizing a ubiquinone-acetogenin hybrid inhibitor (named Q-acetogenin), we previously showed that a γ-lactone ring of acetogenins is completely substitutable with a ubiquinone ring. In this study, to open a new experimental approach to the study of acetogenin-complex I interaction, we report procedures for synthesizing ^{13}C-labeled Q-acetogenins, wherein the carbonyl carbon at the 1- or 4-position of the ubiquinone ring is specifically ^{13}C-labeled.

Keywords: mitochondrial complex I, acetogenin, ubiquinone.

INTRODUCTION

Mitochondrial NADH-ubiquinone oxidoreductase (complex I) is one of the important targets of modern synthetic insecticides and acaricides.[1] Among a wide variety of complex I inhibitors, some acetogenins, natural products isolated from the plant family *Uvaria accuminata* (Annonaceae), are the most potent.[2–5] Although acetogenins are thought to act on the terminal electron transfer step of complex I,[3,4] there is still no hard experimental evidence of whether the inhibitors bind to the ubiquinone reduction site. Additionally, there are few structural similarities between the acetogenins and ordinary complex I inhibitors such as piericidin A and rotenone. Thus, considering the unusual structural characteristics as well as the very strong inhibitory effect of acetogenins, a 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.

On the basis of structure-activity studies using a series of natural and synthetic acetogenins,[6–10] we showed that except for an important role of the long alkyl spacer linking the γ-lactone and the hydroxylated THF rings, crucial structural factors of acetogenins are rather ambiguous. Recent synthetic studies of ubiquinone-acetogenin hybrid inhibitors (Q-acetogenins), where the γ-lactone ring of acetogenins is replaced by a ubiquinone ring,[10,11] demonstrated that the ubiquinone ring is completely substitutable for the γ-lactone ring, though this finding does not necessarily mean that acetogenins occupy the ubiquinone reduction site of the enzyme, as discussed in Ref. 10. Nevertheless, the fact that Q-acetogenins are good mimics of potent acetogenins may open a new experimental approach to the study of ligand-complex I interaction, for example, the redox-reaction induced FTIR spectroscopic technique used for other electron-transfer enzymes in combination with [1- or 4-^{13}C]-ubiquinones.[12,13] In this study, we report procedures for producing ^{13}C-labeled Q-acetogenins (Fig. 1), wherein the carbonyl carbon at the 1- or 4-position of the ubiquinone ring is specifically ^{13}C-labeled.

RESULTS AND DISCUSSION

We have reported the procedures for producing Q-acetogenin[10] which is the same compound as that synthesized in this study, except for ^{13}C-labeling. However, as the previous procedures are not necessarily profitable for the synthesis of ^{13}C-labeled compounds, we examined an alternate approach. The absolute configuration around the hydroxylated THF ring moiety and length

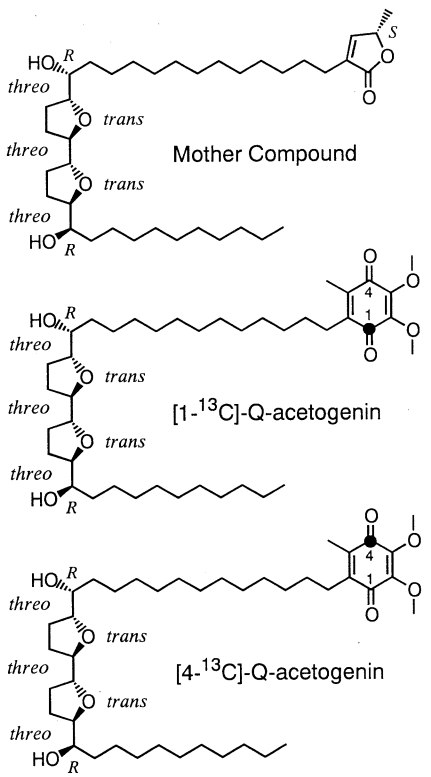

Fig. 1. The structures of ^{13}C-labeled ubiquinone-acetogenin hybrid inhibitors synthesized in this study.
The [1-^{13}C]- and [4-^{13}C]-Q-acetogenins are selectively ^{13}C-labeled at the 1- and 4-positions of the ubiquinone ring, respectively.

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of both the spacer and tail were set to be identical to those of the mother compound (Fig. 1). The inhibitory potency of this compound is comparable to that of bullatacin, one of the most potent natural acetogenins.[4-6)]

The synthetic procedure of [1-^{13}C]-Q-acetogenin is outlined in Scheme 1. Compound **2** was synthesized from **1** by five reaction steps as described previously.[9,10)] The opening of epoxide **2** with 11-(*tert*-butyldimethylsilyloxy)-1-undecyne in the presence of BF_3 etherate[14)] provided **3**. MOM ether protection and sequential hydrogenation afforded **4**. Desilylation of **4** with TBAF, mesylation and sequential iodination gave **5**. Introduction of the spacer moiety of **5** into the ^{13}C-labeled ubiquinone head **6** ([8-^{13}C]-1,4,4a,8a-tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methanonaphthalene-5,8-dione), which was prepared starting from [4-^{13}C]-methylsuccinic acid by the method of van Liemt *et al.*,[15)] was also carried out according to the method reported in Ref. 15 to obtain **7**. The best yield of this reaction step was obtained when potassium *tert*-butoxide was added to a mixture of **5** and **6**. Compound **7** readily underwent a retro-Diels-Alder reaction in refluxing toluene, and sequential deprotection of MOM ether afforded [1-^{13}C]-Q-acetogenin. ^{13}C NMR spectra showed that no scrambling of ^{13}C labels has taken place and the ^{13}C content is 98% or better.

[4-^{13}C]-Q-acetogenin was synthesized by the same procedures, except that [5-^{13}C]-1,4,4a,8a-tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methanonaphthalene-5,8-dione, which was synthesized starting from [1-^{13}C]-methylsuccinic acid,[15)] was used in place of **6**. The ^{13}C enrichment of the 4-position can be confirmed from the split 5-methyl proton signal ($^3J_{C\text{-}H}$=3.8 Hz). ^{13}C NMR spectra showed a ^{13}C content of 98% or better.

The FTIR spectroscopic technique in combination with a ^{13}C-labeled ligand is useful for studying ligand-enzyme interactions. Especially, the binding behavior of ubiquinone has been thoroughly examined with bacterial photosynthetic reaction centers and *Escherichia coli* cytochrome bo_3 complex using [1- or 4-^{13}C]-ubiquinones.[12,13,16,17)] A comparison of vibrational modes between labeled and unlabeled carbonyl groups facilitates an assignment of the functional group readily. The ^{13}C-labeled Q-acetogenins synthesized here should be useful for examining the binding behavior of the quinone head group with complex I.

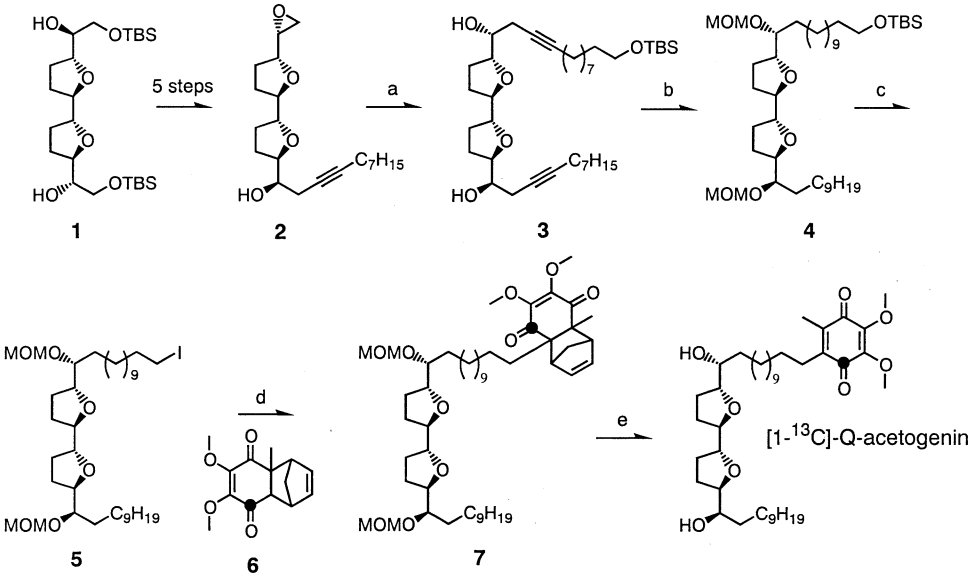
EXPERIMENTAL

1. *Compound 3*

To a solution of 11-(*tert*-butyldimethylsilyloxy)-1-undecyne (0.32 g, 1.14 mmol) in dry THF (20 ml) at −78°C was added a solution of *n*-BuLi (1.6 M in hexane, 0.72 ml, 1.15 mmol). After 30 min, BF_3•Et_2O (0.11 ml, 1.14 mmol) was added, and the mixture was stirred for 30 min. To the mixture was added a solution of epoxide **2** (0.40 g, 1.14 mmol) in THF (10 ml). The reaction mixture was stirred at −78°C for 30 min and worked up with saturated aqueous NH_4Cl. The crude product was purified by silica gel column chromatography (hexane–EtOAc, 7 : 3) to afford **3** (0.50 g, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.01 (m, 2H), 3.91 (m, 2H), 3.59 (t, J=6.6 Hz, 2H), 3.55 (m, 2H), 2.55 (br. s, 2H), 2.40 (m, 4H), 2.14 (m, 4H), 2.05–1.95 (m, 4H), 1.80–1.70 (m, 4H), 1.52–1.47 (m, 6H), 1.40–1.27 (m, 18H), 0.89 (s, 9H), 0.88 (t, J=6.6 Hz, 3H), 0.04 (s, 6H).

2. *Compound 4*

To a mixture of **3** (1.05 g, 1.66 mmol) and *i*-Pr_2NEt (2.57 g, 19.9 mmol) in anhydrous CH_2Cl_2 (8 ml) at 0°C was added MOMCl (1.01 g, 3.3 mmol). The reaction mixture was stirred at r.t. for 12 hr, quenched with a saturated aqueous NH_4Cl solution, washed with brine and then chromatographed on silica gel (hex-



Scheme 1. Synthetic procedure of [1-^{13}C]-Q-acetogenin.
Reaction conditions; a) 11-(*tert*-butyldimethylsilyloxy)-1-undecyne, *n*-BuLi, BF_3•Et_2O, THF, −78°C, 0.5 hr, 69%; b) i) MOMCl, (*i*-$Pr)_2NEt$, ii) H_2, Pd/C, EtOH, (97%); c) i) TBAF, THF, r.t., ii) MsCl, THF, $N(Et)_3$, 0°C, 20 min, iii) NaI, acetone, 35°C (83%); d) compound **6**, *tert*-BuO^-K^+, THF : DMF (1 : 3), −40 to −20°C, 1.5 hr, 95%; e) i) toluene, reflux, 1 hr, ii) 4% AcCl, MeOH, r.t., 4 hr (93%).

ane–EtOAc, 9 : 1) to give a MOM ether. Next, a mixture of the MOM ether (1.01 g, 1.4 mmol) and 10% Pd/C (0.11 g) in ethanol (20 ml) was stirred under a H_2 atmosphere at r.t. for 12 hr. The crude product was purified by silica gel column chromatography (hexane–EtOAc, 9 : 1) to afford **4** (1.21 g, 97% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.82 (d, J=6.9 Hz, 2H), 4.67 (d, J=6.9 Hz, 2H), 4.01 (m, 2H), 3.91 (m, 2H), 3.59 (t, J=6.6 Hz, 2H), 3.45 (m, 2H), 3.39 (s, 6H), 2.00–1.95 (m, 4H), 1.80–1.70 (m, 4H), 1.52–1.25 (m, 40H), 0.89 (s, 9H), 0.88 (t, J=6.6 Hz, 3H), 0.04 (s, 6H).

3. Compound 5

To a solution of **4** (0.99 g, 1.35 mmol) in dry THF (17 ml) was added TBAF (1.0 M in THF, 5.4 ml, 5.4 mmol) at 0°C, and the mixture was stirred at r.t. for 2 hr. The reaction mixture was worked up with saturated aqueous NH_4Cl and crude product was chromatographed on silica gel (hexane–EtOAc, 7 : 3) to afford the corresponding alcohol in a quantitative yield. Next, to a mixture of the alcohol (80 mg, 0.13 mmol) and Et_3N (0.26 g, 2.6 mmol) at 0°C was added MsCl (0.15 g, 1.3 mmol), and stirred for 40 min. The reaction mixture was worked up with water and purified by silica gel column chromatography (hexane–EtOAc, 4 : 1) to give the mesylate in a quantitative yield.

To a solution of the above mesylate in acetone (10 ml) was added NaI (0.19 g, 1.3 mmol) and stirred at 35°C for 12 hr. The reaction mixture was filtrated through Celite, concentrated *in vacuo* and purified by silica gel column chromatography (hexane–EtOAc, 7 : 3) to afford **5** (0.81 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 4.82 (d, J=6.9 Hz, 2H), 4.67 (d, J=6.9 Hz, 2H), 4.01 (m, 2H), 3.91 (m, 2H), 3.47 (m, 2H), 3.39 (s, 6H), 3.18 (t, J=7.2 Hz, 2H), 1.93–1.64 (m, 8H), 1.52–1.25 (m, 40H), 0.87 (t, J=6.6 Hz, 3H).

4. Compound 7

To a mixture of **5** (72 mg, 0.10 mmol) and **6** (30 mg, 0.12 mmol) in THF : DMF (1 : 3, 4 ml) was added *tert*-BuO^-K^+ (15 mg, 0.13 mmol) at −20°C and stirred for 1 hr. The reaction mixture was worked up with saturated aqueous NH_4Cl and purified by silica gel column chromatography (hexane–EtOAc, 1 : 1) to afford **7** (80 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 6.05 (m, 2H), 4.82 (d, J=6.9 Hz, 2H), 4.67 (d, J=6.9 Hz, 2H), 4.01 (m, 2H), 3.93 (s, 3H), 3.91 (m, 2H), 3.90 (s, 3H), 3.47 (m, 2H), 3.39 (s, 6H), 3.09 (m, 1H), 2.99 (m, 1H), 1.93–1.88 (m, 4H), 1.80–1.62 (m, 6H), 1.48–1.25 (m, 42H), 1.47 (s, 3H), 0.87 (t, J=6.6 Hz, 3H).

5. [1-^{13}C]-Q-acetogenin

A solution of **7** (51 mg, 0.065 mmol) in toluene (4 ml) was refluxed for 1 hr and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc, 4 : 1) to give the corresponding quinone in a quantitative yield. Next, to a solution of the quinone in CH_2Cl_2 (4 ml) was added a solution of AcCl (4% in MeOH) to produce [1-^{13}C]-Q-acetogenin (42 mg, 93% in two steps) as orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 3.98 (s, 6H), 3.88–3.82 (m, 4H), 3.42–3.34 (m, 2H), 2.45–2.41 (m, 4H), 2.00 (s, 3H), 2.05–1.96 (m, 4H), 1.74–1.59 (m, 4H), 1.56–1.47 (m, 2H), 1.46–1.25 (m, 38H), 0.87 (t, J=6.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) data were identical to those reported,[10] except for an intense signal at 184.1 ppm. ESI-MS (*m/z*) 714.5 $[M+Na]^+$.

6. [4-^{13}C]-Q-acetogenin

[4-^{13}C]-Q-acetogenin was synthesized by the same procedures using [5-^{13}C]-1,4,4a,8a-tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methanonaphthalene-5,8-dione in reaction step *d* in place of compound **6**. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 3.98 (s, 6H), 3.88–3.82 (m, 4H), 3.42–3.34 (m, 2H), 2.45–2.41 (m, 4H), 2.00 (d, $^3J_{C\text{-}H}$=3.8 Hz, 3H), 2.05–1.96 (m, 4H), 1.74–1.59 (m, 4H), 1.56–1.47 (m, 2H), 1.46–1.25 (m, 38H), 0.87 (t, J=6.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) data were identical to those reported,[10] except for an intense signal at 184.7 ppm. ESI-MS (*m/z*) 714.5 $[M+Na]^+$.

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