J. Pestic. Sci., **31**(2), 156–158 (2006)

Note

Synthesis of photolabile Δlac-acetogenin for photoaffinity labeling of mitochondrial complex I

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(Received December 7, 2005; Accepted February 6, 2006)

Δlac-Acetogenins are a novel type of inhibitor acting at the terminal electron transfer step of mitochondrial NADH-ubiquinone oxidoreductase (complex I). To identify the binding site of Δlac-acetogenins by photoaffinity labeling, we synthesized a photolabile Δlac-acetogenin that possesses a biotin probe to enable the detection and the isolation of the labeled peptide without the use of a radioisotope. The photolabile Δlac-acetogenin synthesized in this study elicited potent inhibition of bovine heart mitochondrial complex I at the nanomolar level.

Keywords: complex I, acetogenin, respiratory inhibitor.

Introduction

Mitochondrial NADH-ubiquinone oxidoreductase (complex I) is one of the important targets of modern synthetic insecticides and acaricides.[1)] Much however remains to be learnt with regard to the mechanism of complex I because of its complex composition and the lack of a high-resolution 3D structure.[2)] We recently synthesized new acetogenin mimics named Δlac-acetogenins, that consist of the hydroxylated adjacent bis-THF ring and two alkyl side chains *without* a α,β-unsaturated γ-lactone ring (Fig. 1).[3,4)] Some Δlac-acetogenins elicited very potent inhibition with bovine heart mitochondrial complex I at the nanomolar level despite the lack of a γ-lactone ring which is a structural feature common to a large number of natural acetogenins.[5,6)] Interestingly, several lines of evidence, *e.g.* the competition test using a fluorescent ligand and the effect on superoxide production from complex I, revealed that the binding site of Δlac-acetogenins is different from that of natural acetogenins as well as ordinary complex I inhibitors such as rotenone and piericidin A.[3,4)] Thus

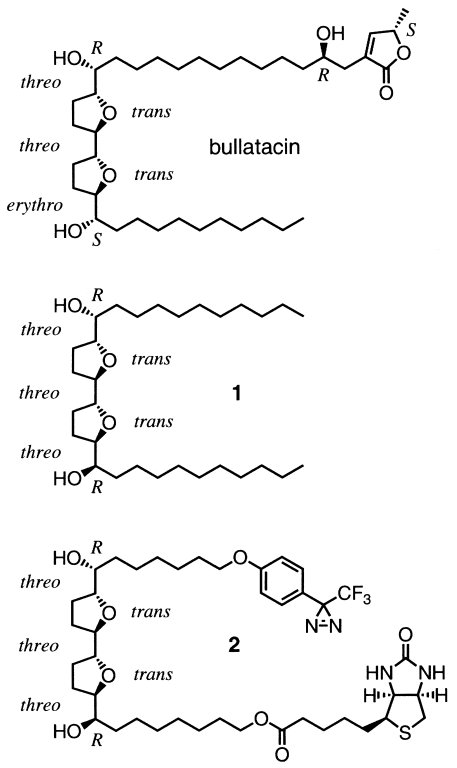

Fig. 1. Structures of bullatacin, a typical Δlac-acetogenin (**1**) and the photolabile derivative (**2**) synthesized in this study.

Δlac-acetogenins were shown to be a novel type of inhibitor acting at the terminal electron transfer step of complex I. Accordingly, a detailed analysis of their inhibitory actions would provide valuable insights into functional features of complex I, one of the largest known membrane protein complexes.

Identification of the binding site of Δlac-acetogenins at the amino acid level is essential for fully understanding the inhibitory action of the inhibitors. Toward this end, photoaffinity labeling followed by mass spectroscopic analysis of the labeled peptide is a powerful technique. In the present study, we synthesized a Δlac-acetogenin derivative possessing an aromatic diazirine as a photolabile functional group (Fig. 1). In addition, we attached a biotin probe to the inhibitor to enable the detection and the separation of the labeled peptide by Western blotting using the streptavidin-enzyme conjugate and streptavidin affinity chromatography, respectively.

Results and Discussion

Compound **2** was designed on the basis of the following concepts. First, the aromatic diazirine was selected as a photolabile group since this group is efficiently photolyzed at around 360 nm to generate a highly reactive carbene.[7)] Second, as the hydroxylated bis-THF ring is essential for the potent inhibitory effect, the aromatic diazirine and biotin were attached to the end of the side chain moiety, though the length of the chain was not optimized.

The synthesis of compounds **2** is outlined in Scheme 1(A). The key intermediate **3** was synthesized as described previously.[8,9)]

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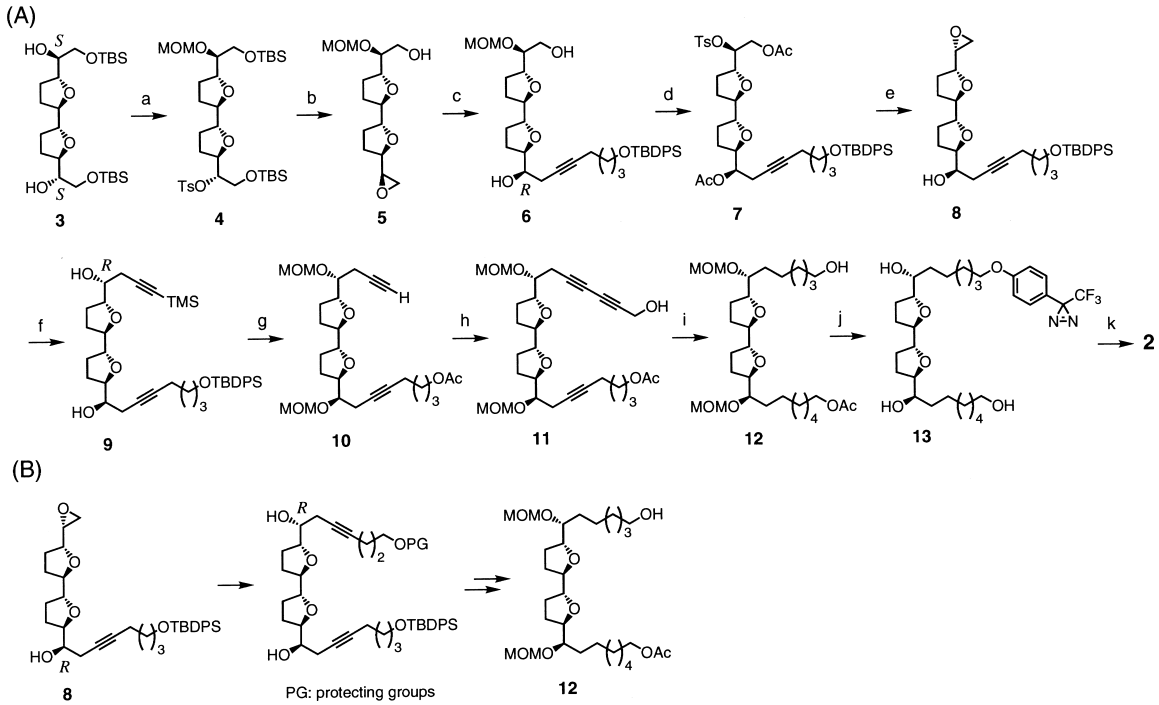

Scheme 1. Reagents and conditions: (a) i) TsCl (0.5 equiv.), 4-DMAP (4-dimethylaminopyridine), Et_3N, CH_2Cl_2, rt, overnight, 55% (repeated twice), ii) MOMCl (methoxymethyl chloride), $(i\text{-Pr})_2NEt$, CH_2Cl_2, rt, overnight, 98%; (b) TBAF (tetra-*n*-butylammonium fluoride), THF, 0 to 50°C, 2 hr, 88%; (c) $CH{\equiv}C(CH_2)_4OTBDPS$, *n*-BuLi (10 eq.), Et_2AlCl (10 eq.), toluene, 0°C, 1 hr, 72%; (d) i) AcCl, 4-DMAP, CH_2Cl_2, rt, 1 hr, 99%, ii) $BF_3 \cdot Et_2O$, Me_2S, −20°C, 1.5 hr, 96%, iii) TsCl, Et_3N, 4-DMAP, CH_2Cl_2, 30°C, overnight, 98%; (e) K_2CO_3, MeOH, 30°C, 1 hr, 80%; (f) TMS-acetylene, *n*-BuLi (10 eq.), Et_2AlCl (10 eq.), toluene, 0°C, 1 hr, 91%; (g) i) MOMCl, $(i\text{-Pr})_2NEt$, CH_2Cl_2, 0°C to rt, overnight, 92%, ii) TBAF, THF, rt, 3 hr, iii) AcCl, 4-DMAP, CH_2Cl_2, rt, 1 hr, 78% (2 steps); (h) 3-bromo-2-propyn-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, $(i\text{-Pr})_2NH$, THF, rt, 3 hr, 65%; (i) H_2, 10% Pd/C, EtOH, rt, overnight, 46%; (j) i) 3-(4-hydroxyphenyl)-3-trifluoromethyldiazirine, PPh_3, diisopropyl azodicarboxylate, THF, rt, 0.5 hr, 95%, ii) K_2CO_3, MeOH, rt, 1 hr, iii) 5% AcCl (in MeOH), CH_2Cl_2, rt, 2 hr, 93% (2 steps); (k) (+)-biotin, EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride), 4-DMAP, CH_2Cl_2, rt, 2 d, 46% (with 32% recovered **13**).

Treatment of **3** with 0.5 mol equivalent TsCl (repeated twice) and sequential MOM ether protection of the secondary hydroxy group afforded **4**. Desilylation of **4** with TBAF provided epoxide **5**. The opening of epoxide **5** with lithium acetylide, derived from 6-(*tert*-butyldiphenylsilyloxy)-1-hexyne, in the presence of Et_2AlCl[10] provided **6**. After acetyl protection of both hydroxy groups, deprotection of the MOM ether and sequential treatment with TsCl afforded **7**. Hydrolysis of the acetyl groups gave epoxide **8**. Initially, we examined the direct opening of epoxide **8** with $LiC{\equiv}C(CH_2)_3OR$ (R; benzyl or TBS) in the presence of Et_2AlCl to obtain **12**, as shown in Scheme 1(B), but the yield of the product was very poor. We attempted to improve this reaction step under various reaction conditions; for instance, $BF_3 \cdot Et_2O$[11] was used in place of Et_2AlCl at several molar ratios and temperatures. All cases, however, resulted in unsatisfactory results. Therefore we prepared **12** through the alternate approach described below.

The opening of epoxide **8** with lithium (trimethylsilyl) acetylide in the presence of Et_2AlCl provided **9**. After protection of the secondary hydroxy groups by MOM ether, desilylation and acetyl protection gave **10**. Pd(0)-catalyzed coupling of alkyne **10** with 3-bromo-2-propyn-1-ol gave **11** and subsequent hydrogenation with 10% Pd/C afforded **12**. After the reaction of **12** with 3-(4-hydroxyphenyl)-3-trifluoromethyldiazirine, which was synthesized by the method of Lu *et al.*,[12] under the Mitsunobu reaction conditions,[13] hydrolysis of the acetyl group and sequential deprotection of MOM ether afforded **13**. The reaction of **13** with (+)-biotin in the presence of EDC and 4-DMAP provided **2**: a colorless oil; $[\alpha]_D^{21}$ +42.1 (*c* 0.25, EtOH); ^1H NMR δ (400 MHz, $CDCl_3$): 7.12 (2H, d, J=8.5 Hz, ArH), 6.88 (2H, d, J=8.5 Hz, ArH), 5.39 (1H, br s, NH), 5.17 (1H, br s, NH), 4.51 (1H, m, NHC<u>H</u>), 4.32 (1H, m, NHC<u>H</u>), 4.06 (2H, t, J=6.6 Hz, OCH_2), 3.94 (2H, t, J=6.5 Hz, OCH_2), 3.92–3.83 (4H, m), 3.39 (2H, m), 3.17 (1H, m, SC<u>H</u>CH_2), 2.92 (1H, dd, J=12.7, 5.0 Hz, $SCH_a\underline{H}_b$), 2.75 (1H, d, J=12.7 Hz, $SC\underline{H}_aH_b$), 2.73 (2H, br s, OH), 2.33 (2H, t, J=7.3 Hz, $COCH_2$), 1.97 (4H, m), 1.77–1.26 (32H, m); ^{13}C NMR δ (100 MHz, $CDCl_3$): 173.69, 163.17, 160.17, 128.07 (2C), 121.51 (q, $^1J_{C\text{-}F}$=272.9 Hz), 120.14, 114.84 (2C), 83.23, 83.19, 81.86, 77.23, 73.99, 73.97, 68.07, 64.54, 61.90, 60.11, 55.30, 40.53, 33.99, 33.44, 33.29, 30.95, 29.56, 29.36, 29.28, 29.17, 29.03, 29.01, 28.59, 28.37, 28.35, 28.29, 25.92, 25.88, 25.58, 25.55, 24.87; HRMS (ESI-IT) m/z $(M+H)^+$: Calcd. for $C_{41}H_{62}O_8F_3N_4S$: 827.4240, Found: 827.4271. Details on the synthesis of **2** will be reported in due course.

The inhibition of complex I activity was determined by NADH oxidase assay using bovine heart submitochondrial particles.[4] Previous study indicated that the inhibitory potency of compound

1 is comparable to that of bullatacin,[3] one of the most potent natural acetogenins.[14,15] The potency of **1** in terms of the IC_{50}, *i.e.* the molar concentration needed to halve the control NADH oxidase activity, was 1.8 (±0.08) nM with the present submitochondrial particle preparations. The IC_{50} value of compound **2** was 3.9 (±0.3) nM and complete inhibition (>95%) was achieved at about 12 nM. The result indicates that compound **2** sufficiently retains a potent inhibitory effect on complex I irrespective of the presence of a hydrophilic biotin moiety. To find a clue as to whether natural acetogenins and compound **2** bind to different sites, we examined whether the effect of bullatacin and **2** is additive in double-inhibitor titration of steady state complex I activity.[3] As the result, no additivity of the inhibition was observed between the two inhibitors, indicating that their binding sites are different (data not shown). On the basis of these results, a photoaffinity labeling study using the bovine enzyme is currently underway in our laboratory.

Acknowledgments

We thank Nobuhiro Hirai and Naoki Mori (Kyoto Univ.) for HRMS analysis. This work was supported in part by a Grant-in-aid for Scientific Research from the Japan Society for the Promotion of Science (Grant 17380073 to H.M.).

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