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Synthesis, determination of the absolute configuration of tonkinelin, and inhibitory action with bovine heart mitochondrial complex I

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Abstract—The first synthesis of two possible diastereomers of tonkinelin was achieved. By comparison of the optical rotation of two candidates of tonkinelin and the natural compound, it is suggested that the absolute configuration of natural tonkinelin is likely to be (17*S*,18*S*). The inhibitory activity of these compounds was examined with bovine heart mitochondrial NADH–ubiquinone oxidoreductase. These compounds showed remarkably weak inhibitory activity compared to ordinary acetogenins such as bullatacin.

1. Introduction

The annonaceous acetogenins, which are isolated from a number of plants of *Annonaceae*, have attracted much attention due to a wide variety of biological activities such as antitumoral, cytotoxic, antimalarial, immunosuppressive, pesticidal, and antifeedant activities. So far, more than 400 compounds have been isolated.[1–3] Their unique structure is characterized by one or more tetrahydrofuran rings, together with a terminal γ-lactone moiety on C-35 or C-37 carbon chain. The inhibitory effect of acetogenin on mitochondrial NADH–ubiquinone oxidoreductase (complex I) is of particular importance since their diverse biological activities are thought to be attributable to this effect. On the basis of studies of the structure–activity relationship (SAR) carried out by Miyoshi and co-worker using systematically selected natural and synthetic acetogenins, the important structural factor responsible for the potent inhibition of mitochondrial complex I is the length of the alkyl spacer linking the two taxophores (i.e., the hydroxylated THF and the γ-lactone rings). The optimal length of the spacer for inhibition is approximately 13 carbon atoms.[4] Consequently, significant effort has been devoted toward the synthesis of acetogenins.[5] Tonkinelin (**1**), which has a simple structure in the acetogenins, was isolated from *Uvaria tonkinesis* in 1996 by Chen and a co-worker.[6] This compound has two hydroxyl groups at C-17 and C-18 position, and possesses α,β-unsaturated γ-lactone which can be seen in ordinary annonaceous acetogenins. The absolute configuration of **1** has not been reported yet. However, because the *threo* relative stereochemistry of the dihydroxyl part of **1** has been determined by Chen and a co-worker,[6] and the well-known (*S*) configuration of the secondary methyl group of the γ-lactone moiety[7] was determined by the CD spectrum,[6] it follows that the absolute stereochemistry

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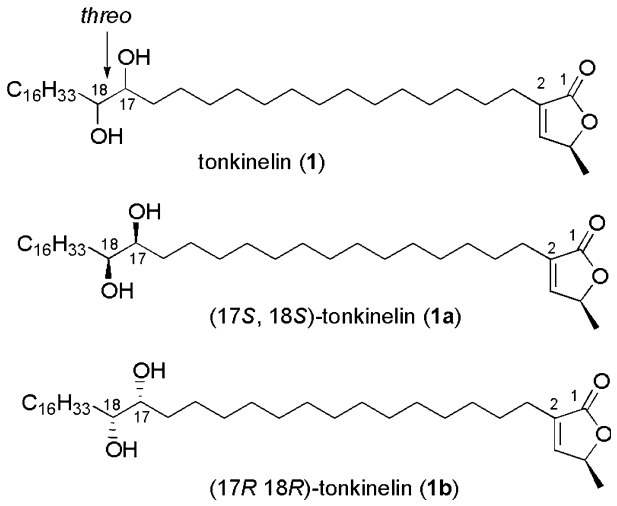
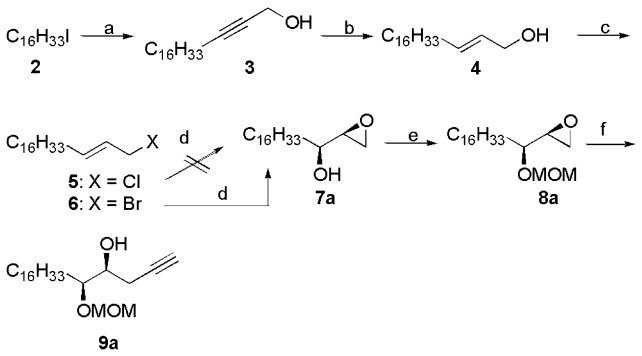


Figure 1. The possible structure of tonkinelin.



Scheme 1. Synthesis of dihydroxyl part of **1a**. Reagents and conditions: (a) propargyl alcohol, *n*-BuLi (55%); (b) $LiAlH_4$, THF, reflux, (85%); (c) NBS, PPh_3, CH_2Cl_2, (92%); (d) AD mix β, Me_2SONH_2, (74%); (e) MOMBr, *i*-Pr_2NEt, CH_2Cl_2, (91%); (f) lithium acetylide, an ethylenediamine complex (95%).

of **1** is (17*S*,18*S*) or (17*R*,18*R*). Two possible structures, **1a** and **1b**, would be difficult to differentiate by ^1H NMR or ^{13}C NMR spectroscopic data, since the two stereogenic regions, that is, the optically active dihydroxyl part and the γ-lactone moiety part, are separated by a long carbon chain. X-ray analysis is also very difficult due to the waxy nature of this compound. Even ^1H NMR data of the both of (*R*)- and (*S*)-bis-Mosher esters of **1a** and/or **1b** would not be differentiated because the (*R*)- and (*S*)-bis-MTPA esters of **1a** and **1b** should give the same spectra.[8] The optical rotation and/or melting point values would serve the clue to determine the absolute configuration. To establish the absolute configuration of tonkinelin (**1**) and evaluate its biological activity, we planned to synthesize the two candidates **1a** and **1b** (Fig. 1).

2. Results and discussion

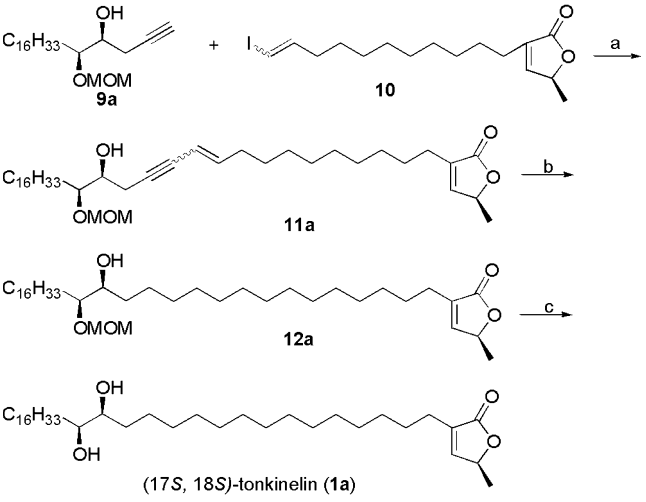
2.1. Synthesis

Our synthesis of dihydroxyl part of **1a** is shown in Scheme 1. Iodide **2** was converted to allylic bromide **6** by a routine three-step reaction sequence: (i) alkynylation, (ii) reduction (LAH) and (iii) bromination (NBS). Asymmetric dihydroxylation of **6** by the Sharpless procedure[9] using AD mix α and spontaneous epoxidation afforded epoxy alcohol **7a**, which showed 98% ee by a ^1H NMR analysis of the corresponding Mosher esters. When allylic chloride **5** was used in this reaction, spontaneous epoxidation did not proceed. The hydroxyl group of **7a** was protected as methoxymethyl ether (MOM ether) to give compound **8a**. Alkynylation of **8a** with lithium acetylide, an ethylenediamine complex, afforded **9a** (Scheme 1).

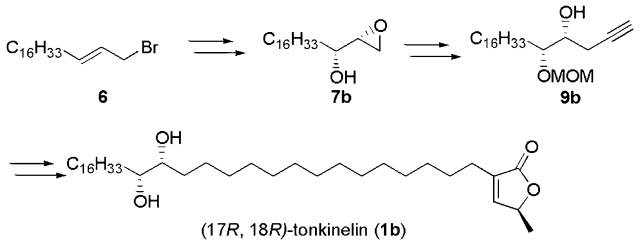
γ-Lactone part **10** was prepared as we have reported before.[10] Sonogashira cross-coupling reaction[11] of **9a** with **10** gave enyne **11a**. Diimide reduction of **11a** with *p*-$TsNHNH_2$ and sodium acetate in ethylene glycol diethyl ether afforded **12a**. Finally, deprotection of the MOM ether with $BF_3{\cdot}Et_2O$ afforded **1a** (Scheme 2).

Synthesis of (17*R*,18*R*)-tonkinelin (**1b**) was achieved from **7b** using AD mix β from **6** as described in Scheme 2 (Scheme 3).

The spectroscopic data (^1H NMR, ^{13}C NMR, IR, and MS spectra) of synthetic **1a** and **1b** were in good agreement with those of natural ones.[6] On the other hand, their optical rotations showed a clear difference. While the specific rotation of synthetic **1a** $[\alpha]_D^{18}$ +11.6 (*c* 0.14, $CHCl_3$) is similar to the reported value of naturally occurring tonkinelin $[\alpha]_D^{16}$ +14.49, (*c* 0.07, $CHCl_3$), that of **1b** $[\alpha]_D^{16}$ +0.80, (*c* 0.20, $CHCl_3$) showed a much lower value. On the basis of these results, we suggested that the absolute configuration of natural tonkinelin is likely to be **1a**.



Scheme 2. Synthesis of (17*S*,18*S*)-tonkinelin (**1a**). Reagents and conditions: (a) 5 mol % of $Cl_2Pd(PPh_3)_2$, 10 mol % of CuI, Et_3N (65%); (b) *p*-$TsNHNH_2$, NaOAc, diethoxyethane (64%); (c) $BF_3{\cdot}Et_2O$, Me_2S (56%).



Scheme 3. Synthesis of (17*R*,18*R*)-tonkinelin (**1b**).

2.2. Inhibitory action with bovine heart mitochondrial complex I

Inhibitory effects of compounds **1a** and **1b** on bovine heart mitochondrial complex I were examined according to the previous method (Fig. 2).[4] The IC_{50} values of **1a** and **1b** were 580 and 690 nM, respectively.

Compounds **1a** and **1b** exhibited almost same inhibitory potency, indicating that the stereochemistry around the dihydroxyl moiety does not affect the inhibitory action.[12] It is noteworthy that compared to potent natural ordinary acetogenins such as bullatacin (IC_{50} = 0.83 nM)[4] and *cis*-solamin (IC_{50} = 2.6 nM),[13] **1a** and **1b** are much weaker inhibitors of the enzyme. Further comparison with dihydroxy-cohibin-A (IC_{50} = 20 nM),[14] which was synthesized by us, the inhibitory activity is quite weak. The extra hydroxy groups that can be seen in dihydroxy-cohibin A may play an analogous role with the ether oxygen(s) of THF derivatives. Another reason for the weak activity may be due to the length of the spacer. The spacer of **1a** and/or **1b** is longer (15 carbon atoms) than the optimal length (13 carbon atoms). Miyoshi and co-workers revealed that the decrease in the strength of the inhibitory effect caused by elongating the spacer from 13 carbons was much more drastic than that caused by shortening (Table 1).[4]

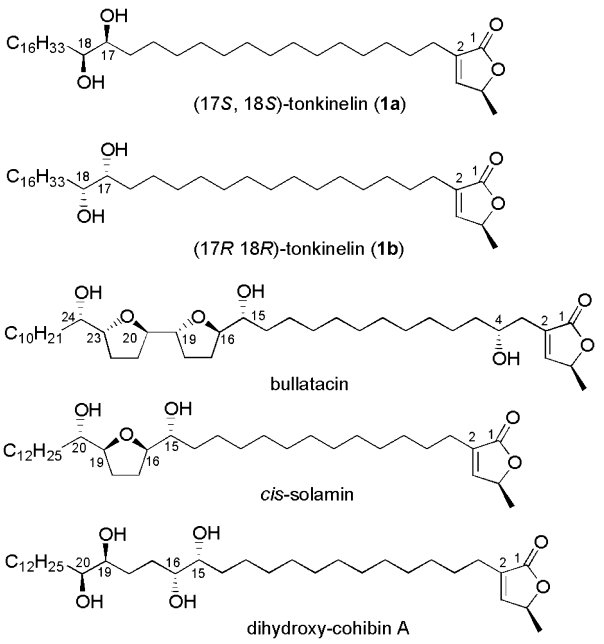


Figure 2. The structures of test compounds.

Table 1. Inhibitory activity of mitochondrial complex I

Sample	IC_{50} (nM)
1a	580
1b	690
Bullatacin	0.83
cis-Solamin	2.6
Dihydroxy-cohibin A	20

3. Conclusion

In conclusion, we have achieved a synthesis of (17*S*,18*S*)-tonkinelin (**1a**), (17*R*,18*R*)-tonkinelin (**1b**), and determined the absolute configuration of natural tonkinelin to be **1a**. We also examined their inhibitory actions with bovine heart mitochondrial complex I. These compounds elicited much weaker activity compared to ordinary annonaceous acetogenins.

4. Experimental

4.1. General

All melting points were uncorrected. ^1H and ^{13}C NMR spectra were measured with a Bruker DRX 500 FT NMR spectrometer in $CDCl_3$ at 500 and 125 MHz, respectively. Chemical shifts were relative to tetramethylsilane as an internal standard. The coupling constants were given in Hz. Mass spectra were obtained on JEOL JMS-HX211A and JMS-HX110A mass spectrometer. IR spectra were recorded with JASCO FT-IR 480 Plus infrared spectrometer. Optical rotations were determined with a JASCO DIP-1000 polarimeter.

4.1.1. 2-Nonadecyn-1-ol (3). To a solution of propargyl alcohol (0.80 mL, 13 mmol) in THF was added *n*-BuLi (10 mL, 26 mmol) at −20 °C. The mixture was stirred for 30 min, and then iodide (3.87 g, 11 mmol) in HMPA (3.8 mL, 22 mmol) was added. The resultant mixture was stirred for 6 h at 0 °C. The reaction was quenched with saturated aqueous NH_4Cl (20 mL) and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 5:1) to give **3** (1.70 g, 55%) as a colorless solid. Mp 56–57 °C; IR (KBr) ν_{max} cm^{-1}: 3184, 2916, 2848, 2240, 1470, 1019, 716; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.30 (24H, m), 1.31–1.38 (2H, m), 1.47–1.53 (2H, m), 1.61 (1H, s), 2.21 (2H, tt, J = 2.2, 7.1 Hz), 4.25 (2H, dt, J = 2.2, 5.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.11, 18.72, 22.68, 28.59, 28.87, 29.14, 29.35, 29.51, 29.62, 29.65, 29.67, 29.68, 31.91, 51.44, 78.20, 86.70; HREIMS (M^+): calcd for $C_{19}H_{36}O$, 280.2766; found, 280.2760.

4.1.2. (*E*)-2-Nonadecen-1-ol (4). To a suspension of $LiAlH_4$ (470 mg, 12 mmol) in THF was added acetylenic alcohol **3** (1.70 g, 6.2 mmol) at 0 °C. The mixture was

stirred for 2 h under reflux. The reaction was quenched with water and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 5:1) to give **4** (1.50 g, 85%) as a colorless solid. Mp 44–46 °C; IR (KBr) ν_{max} cm^{-1}: 3253, 3020, 2917, 2848, 1464, 1082, 961, 719; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.30 (26H, m), 1.31–1.39 (2H, m), 1.57 (1H, d, J = 7.1 Hz), 2.02–2.06 (2 H, m), 4.08 (2H, d, J = 5.1 Hz), 5.61–5.71 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.09, 22.68, 29.14, 29.35, 29.43, 29.50, 29.60, 29.65, 29.66, 29.69, 31.92, 32.21, 63.87, 128.82, 133.62; HREIMS (M^+): calcd for $C_{19}H_{38}O$, 282.2922; found, 282.2927.

4.1.3. (*E*)-1-Bromo-2-nonadecene (6). To a solution of alcohol **4** (150 mg, 0.53 mmol) were added NBS (95 mg, 0.53 mmol) and PPh_3 (140 mg, 2.8 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 10 min at rt. The reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL) and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50:1) to give crude **6** (160 mg, 92%) as a colorless oil. This compound was immediately used for the next step without further purification. IR (film) ν_{max} cm^{-1}: 2923, 2852, 1465, 963; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.30 (32H, m), 1.34–1.39 (2H, m), 2.03–2.07 (2H, m), 3.95 (2H, d, J = 7.4 Hz), 5.66–5.79 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.09, 22.68, 29.11, 29.35, 29.44, 29.57, 29.64, 29.66, 29.66, 29.69, 31.93, 32.06, 33.59, 126.26, 136.79.

4.1.4. (2*S*,3*S*)-1,2-Epoxynonadecan-3-ol (7a). To a suspension of AD mix α (690 mg) in *t*-BuOH/H_2O (1:1) (10 mL) were added allylic bromide **6** (160 mg, 0.49 mmol) and $MeSO_2NH_2$ (47 mg, 0.49 mmol). The mixture was stirred for 16 h at 0 °C. The reaction was quenched with aqueous Na_2SO_3 (5 mL) and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 5:1) to give **7a** (100 mg, 74%) as a colorless solid. Mp 61–63 °C; $[\alpha]_D^{18}$ −0.17 (*c* 0.50, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3344, 2954, 2916, 2848, 1466, 1125, 962, 868, 755, 720; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.30 (26H, m), 1.37–1.41 (2H, m), 1.58–1.63 (2H, m), 1.73 (1H, d, J = 6.0 Hz), 2.72 (1H, dd, J = 2.8, 4.9 Hz), 2.82 (1H, dd, J = 4.1, 4.9 Hz), 2.98 (1H, ddd, J = 2.8, 4.1, 4.9 Hz), 3.44 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.09, 22.68, 25.35, 29.35, 29.53, 29.57, 29.60, 29.66, 29.69, 31.92, 34.49, 55.31, 71.64; HRFABMS [(M+H)$^+$]: calcd for $C_{19}H_{39}O_2$, 299.2950; found 299.2941.

4.1.5. (2*S*,3*S*)-1,2-Epoxy-3-methoxymethoxynonadecane (8a). To a solution of alcohol **7a** (50 mg, 0.18 mmol) and *i*-Pr_2NEt (0.045 mL, 0.27 mmol) in CH_2Cl_2 (1.0 ml) was added MOMBr (0.02 mL, 0.23 mmol) at 0 °C. The mixture was stirred for 12 h at rt. The reaction was quenched with saturated aqueous NH_4Cl (2.0 mL) and the mixuture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 10:1) to give **8a** (55 mg, 91%) as a colorless oil. $[\alpha]_D^{20}$ −24.2 (*c* 1.70, $CHCl_3$); IR (film) ν_{max} cm^{-1}: 3046, 2925, 2853, 1467, 1401, 1377, 1257, 1216, 1152, 1102, 1036, 920, 848, 811, 721; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.30 (24H, m), 1.36–1.43 (2H, m), 1.46–1.52 (2H, m), 1.55–1.64 (2H, m), 2.53 (1H, dd, J = 2.7, 4.9 Hz), 2.77 (1H, dd, J = 4.3, 4.7 Hz), 2.96–2.99 (1H, m), 3.26 (1H, dd, J = 7.2, 12.8 Hz), 3.40 (3H, s), 4.67 (1H, d, J = 6.7 Hz), 4.87 (1H, d, J = 6.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.09, 22.68, 25.44, 29.35, 29.52, 29.58, 29.65, 29.69, 31.92, 32.32, 43.84, 54.70, 55.57, 78.01, 95.54; HRFABMS [(M+H)$^+$]: calcd for $C_{21}H_{43}O_3$, 343.3212; found, 343.3207.

4.1.6. (3*S*,4*S*)-5-Methoxymethoxy-1-docosyn-4-ol (9a). To a suspension of lithium acetylide, an ethylenediamine complex (34 mg, 0.17 mmol) in DMSO (1.0 mL) was added epoxide **8a** (22 mg, 0.066 mmol) in DMSO (0.20 mL) at 0 °C. The mixture was stirred for 12 h at rt. The reaction was quenched with saturated aqueous NH_4Cl (1.0 mL). The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 10:1) to give **9a** (22 mg, 95%) as a colorless oil. $[\alpha]_D^{20}$ 18.2, (*c* 0.74, $CHCl_3$); IR (film) ν_{max} cm^{-1}: 3445, 3312, 2920, 2851, 1467, 1377, 1257, 1213, 1150, 1100, 1037, 920, 721, 636; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.30 (24H, m), 1.33–1.48 (4H, m), 1.48–1.54 (1H, m), 1.58–1.64 (1H, m), 2.02 (1H, t, J = 2.7 Hz), 2.42 (1H, ddd, J = 2.7, 6.3, 16.8 Hz), 2.49 (1H, ddd, J = 2.7, 5.8, 16.8 Hz), 2.90 (1H, d, J = 5.6 Hz), 3.40 (3H, s), 3.58–3.62 (1H, m), 3.69–3.73 (1H, m), 4.70 (1H, d, J = 6.8 Hz), 4.72 (1H, d, J = 6.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.07, 22.66, 23.65, 25.26, 29.54, 29.57, 29.64, 29.65, 29.67, 30.92, 31.91, 55.87, 70.26, 71.11, 80.73, 81.15, 97.01; HREIMS (M^+): calcd for $C_{23}H_{44}O_3$, 368.3290; found, 368.3307.

4.1.7. (*EZ*,3*RS*,5*S*,15′*S*,16′*S*)-3-(15′-Hydroxy-16′-methoxymethoxy-10′-ditriaconten-12′-ynyl)-5-methyl-2,5-dihydrofuran-2-one (11a). To a solution of lactone **10** (12 mg, 0.031 mmol) in Et_3N (1.0 mL) was added $Cl_2Pd(PPh_3)_2$ (1.3 mg, 0.0031 mmol). After being stirred for 60 min, a solution of **9a** (11 mg, 0.031 mmol) in Et_3N (2.0 mL) and CuI (0.6 mg, 0.0031 mmol) were added to the solution. After being stirred for 12 h, the reaction was quenched with saturated NH_4Cl (1 mL) and the mixture was extracted with diethyl ether. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 5:1) to give **11a** (13 mg, 65%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 3466, 2925, 28531, 1759, 1466, 1373, 1318, 1202, 1149, 1101, 1032, 954, 920, 722; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.7 Hz), 1.20–1.40 (40H,

m), 1.41 (3H, d, J = 6.8 Hz), 1.45–1.67 (4H, m), 2.07 (1H, t, J = 7.0 Hz), 2.25–2.30 (3H, m), 2.50–2.67 (2H, m), 2.86–2.88 (1H, m), 3.42 (3H, s), 3.55–3.64 (1H, m), 3.70–3.74 (1H, m), 4.70 (1H, d, J = 6.8 Hz), 4.72 (1H, d, J = 6.8 Hz), 5.00 (1H, dq, J = 1.4, 6.8 Hz), 5.42–5.46 (1H, m), 5.84 (0.6H, dt, J = 10.7, 7.0 Hz), 6.07 (0.4H, dt, J = 15.8, 7.3 Hz), 6.99 (1H, d, J = 1.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.10, 19.19, 22.66, 24.43, 24.55, 25.15, 25.23, 25.34, 27.34, 27.37, 28.72, 28.87, 29.04, 29.13, 29.25, 29.29, 29.34, 29.39, 29.44, 29.46, 29.56, 29.60, 29.63, 29.68, 29.72, 30.19, 30.86, 30.93, 31.89, 32.94, 55.84, 71.06, 71.31, 71.35, 79.63, 81.13, 81.15, 81.19, 81.26, 84.24, 89.93, 96.96, 108.96, 109.44, 134.27, 143.27, 144.16, 148.84, 173.89; HRFABMS [(M+Na)$^+$]: calcd for $C_{39}H_{68}O_5Na$, 639.4964; found, 639.4980.

4.1.8. (3*RS*,5*S*,15′*S*,16′*S*)-3-(15′-Hydroxy-16′-methoxymethoxy-ditriacontyl)-5-methyl-2,5-dihydrofuran-2-one (12a). To a refluxing solution of **11a** (13 mg, 0.020 mmol) and *p*-toluenesulfonylhydrazide (270 mg, 1.4 mmol) in diethoxyethane (1.0 mL) was added a solution of sodium acetate (140 mg, 1.7 mmol) in H_2O (3.0 mL) over a period of 4 h. After being cooled to room temperature, the mixture was extracted with diethyl ether. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 5:1) to give **12a** (8.0 mg, 64%) as a colorless solid. Mp 38–40 °C; $[\alpha]_D^{18}$ +17 (c 0.16, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3488, 2924, 2853, 1759, 1466, 1373, 1318, 1200, 1147, 1099, 1035, 951, 919, 721; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.40 (48H, m), 1.41 (3H, d, J = 6.8 Hz), 1.42–1.63 (8H, m), 2.26 (2H, tt, J = 1.6, 7.7 Hz), 2.76 (1H, d, J = 4.1 Hz), 3.34 (1H, m), 3.42 (3H, s), 3.50 (1H, m), 4.70 (1H, d, J = 6.8 Hz), 4.72 (1H, d, J = 6.8 Hz), 5.00 (1H, qd, J = 6.8, 1.5 Hz), 6.99 (1H, d, J = 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.12, 19.20, 22.68, 25.16, 25.62, 27.37, 29.17, 29.31, 29.35, 29.52, 29.57, 29.59, 29.62, 29.64, 29.69, 29.75, 29.79, 31.91, 33.29, 55.83, 72.74, 77.41, 83.45, 97.08, 134.31, 148.84, 173.92; HRFABMS [(M+Na)$^+$]: calcd for $C_{39}H_{74}O_5Na$, 645.5434; found, 645.5426.

4.1.9. (17*S*,18*S*)-Tonkinelin (1a). To a solution of **12a** (8.0 mg, 0.012 mmol) in dimethyl sulfide (1.0 mL) was added $BF_3{\cdot}Et_2O$ (0.10 mL, 1.0 mmol) at 0 °C. After being stirred for 1 h at this temperature, the reaction was quenched with saturated $NaHCO_3$ (1.0 mL) and the mixture was extracted with AcOEt. The organic phase was washed brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to give **1a** (4.0 mg, 6.9 μmol, 56%) as a colorless solid. Mp 73–76 °C; $[\alpha]_D^{18}$ +11.6 (c 0.14, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3234, 2915, 2847, 1741, 1465, 1322, 1066, 1022, 720; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.40 (52H, m), 1.41 (3H, d, J = 6.8 Hz), 1.43–1.58 (4H, m), 2.01 (2H, br), 2.27 (2H, t, J = 7.8 Hz), 3.41 (2H, m), 5.00 (1H, qd, J = 6.8, 1.5 Hz), 6.99 (1H, d, J = 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.13, 19.21, 22.69, 25.16, 25.65, 27.38, 29.17, 29.29, 29.36, 29.50, 29.59, 29.60, 29.62, 29.65, 29.67, 29.69, 31.92, 33.59, 74.53, 77.43, 134.31, 148.87, 173.96; HRFABMS [(M+H)$^+$]: calcd for $C_{37}H_{71}O_4$, 579.5352; found, 579.5359.

4.1.10. (17*R*,18*R*)-Tonkinelin (1b). Mp 77–79 °C; $[\alpha]_D^{18}$ +0.80 (c 0.20, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3234, 2915, 2847, 1741, 1465, 1322, 1066, 1022, 720; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.88 (3H, t, J = 6.9 Hz), 1.20–1.40 (52H, m), 1.41 (3H, d, J = 6.8 Hz), 1.43–1.58 (4H, m), 2.01 (2H, br), 2.27 (2H, t, J = 7.8 Hz), 3.41 (2H, m), 5.00 (1H, qd, J = 6.8, 1.6 Hz), 6.99 (1H, d, J = 1.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.09, 19.22, 22.68, 25.19, 25.66, 27.43, 29.18, 29.30, 29.35, 29.51, 29.59, 29.61, 29.63, 29.66, 29.69, 31.93, 33.68, 74.55, 77.36, 134.41, 148.78, 173.85; HRFABMS [(M+H)$^+$]: calcd for $C_{37}H_{71}O_4$, 579.5352; found, 579.5344.

4.2. Biochemical methods

Bovine heart submitochondrial particles were prepared by the method of Matsuno-Yagi and Hatefi,[15] 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, ε = 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 the equilibration of the particles with inhibitor for 5 min. The IC_{50} values were averaged from three independent experiments.

References and notes

1. Bermejo, A.; Figadére, B.; Zafra-Polo, M.-C.; Barrachina, I.; Estornell, E.; Cortes, B. L. *Nat. Prod. Rep.* **2005**, *22*, 269–303.
2. Alali, F. Q.; Liu, X.-X.; McLaughlin, J. L. *J. Nat. Prod.* **1999**, *62*, 504–540.
3. Zafra-Polo, M. C.; Figadère, B.; Gallardo, T.; Tormo, J. R.; Cortes, D. *Phytochemistry* **1998**, *48*, 1087–1117.
4. Abe, M.; Murai, M.; Ichimaru, N.; Kenmochi, A.; Yoshida, T.; Kubo, A.; Kimura, Y.; Moroda, A.; Makabe, H.; Nishioka, T.; Miyoshi, H. *Biochemistry* **2005**, *44*, 14898–14906.
5. For recent syntheses of annonaceous acetogenins: (a) Curran, D. P.; Zhang, Q.; Richard, C.; Lu, H.; Gudipati, V.; Wilcox, C. S. *J. Am. Chem. Soc.* **2006**, *128*, 9561–9573; (b) Göksel, H.; Stark, B. W. *Org. Lett.* **2006**, *8*, 3433–3436; (c) Hattori, Y.; Kimura, Y.; Moroda, A.; Konno, H.; Abe, M.; Miyoshi, H.; Goto, T.; Makabe, H. *Chem. Asian. J.* **2006**, *1*, 894–904; (d) Narayan, R. S.; Borhan, B. *J. Org. Chem.* **2006**, *71*, 1416–1429; (e) Tominaga, H.; Maezaki, N.; Yanai, M.; Kojima, N.; Urabe, D.; Ueki, R.; Tanaka, T. *Eur. J. Org. Chem.* **2006**, 1422–1429; (f) Marshall, J. A.; Sabatini, J. J. *Org. Lett.* **2006**, *8*, 3557–3560; (g) Zhao, H.; Gorman, J. S. T.; Pagenkopf, B. *Org. Lett.* **2006**, *8*, 4379–4382; (h) Strand, D.; Norrby, P.-O.; Rein, T. *J. Org. Chem.* **2006**, *71*, 1879–1891; (i) Crimmins, M. T.; Zhang, Y.; Diaz, F. A. *Org. Lett.* **2006**, *8*, 2369–2372; (j) Hoye, T. R.; Eklov, B. M.; Jeon, J.; Khoroosi, M. *Org. Lett.* **2006**, *8*, 3383–3386; (k) Bandur, N. G.; Brückner, D.; Hoffmann,

R. W.; Koert, U. *Org. Lett.* **2006**, *8*, 3829–3831; (l) Takahashi, T.; Hongo, Y.; Ogawa, N.; Koshino, H.; Nakata, T. *J. Org. Chem.* **2006**, *71*, 6305–6308; (m) Donohoe, T. J.; Harris, R. M.; Burrows, J.; Parker, J. *J. Am. Chem. Soc.* **2006**, *128*, 13704–13705; (n) Makabe, H.; Kimura, Y.; Higuchi, M.; Konno, H.; Murai, M.; Miyoshi, H. *Bioorg. Med. Chem.* **2006**, *14*, 3119–3130.
6. Chen, Y.; Yu, D. Q. *Planta Med.* **1996**, *62*, 512–514.
7. Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Ramirez, E. A.; Zhuang, Z. P. *Tetrahedron Lett.* **1994**, *35*, 8529–8532.
8. Curran, D. P.; Zhang, Q.; Lu, H.; Gudipati, V. *J. Am. Chem. Soc.* **2006**, *128*, 9943–9956.
9. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483–2547.
10. Makabe, H.; Miyawaki, A.; Takahashi, R.; Hattori, Y.; Konno, H.; Abe, M.; Miyoshi, H. *Tetrahedron Lett.* **2004**, *45*, 973–977.
11. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetarhedron Lett.* **1975**, *16*, 4467–4470.
12. Miyoshi, H.; Ohshima, M.; Shimada, H.; Akagi, T.; Iwamura, H.; McLaughlin, J. L. *Biochim. Biophys. Acta* **1998**, *1365*, 443–452.
13. Makabe, H.; Hattori, Y.; Kimura, Y.; Konno, H.; Abe, M.; Miyoshi, H.; Tanaka, A.; Oritani, T. *Tetrahedron* **2004**, *60*, 10651–10657.
14. Konno, H.; Hiura, N.; Makabe, H.; Abe, M.; Miyoshi, H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 629–632.
15. Matsuno-Yagi, A.; Hatefi, Y. *J. Biol. Chem.* **1985**, *260*, 14424–14427.