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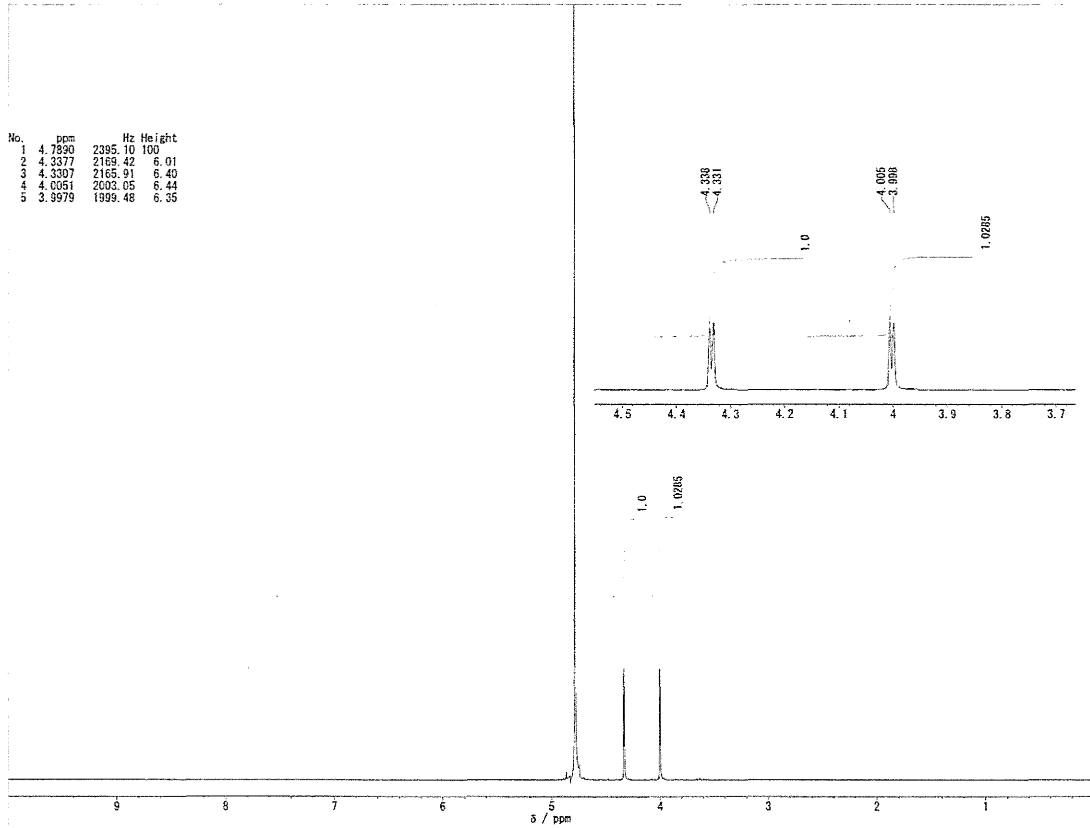

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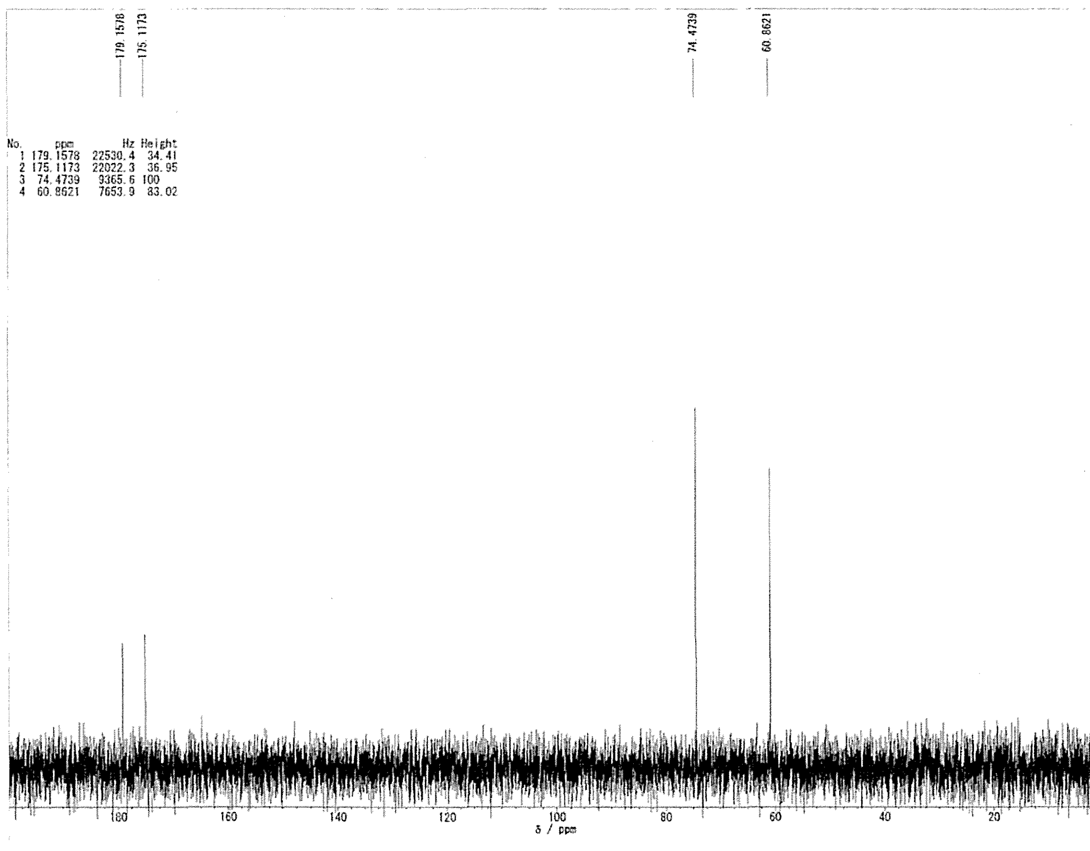

Figure S8 (a) ^{1}H NMR spectra recorded at 500 MHz and (b) ^{13}C NMR spectra recorded at 125 MHz of D-EHA obtained by enzymatic optical resolution. Note that the ^{1}H signal at 4.79 ppm is attributed to deuterium oxide (D_2O).

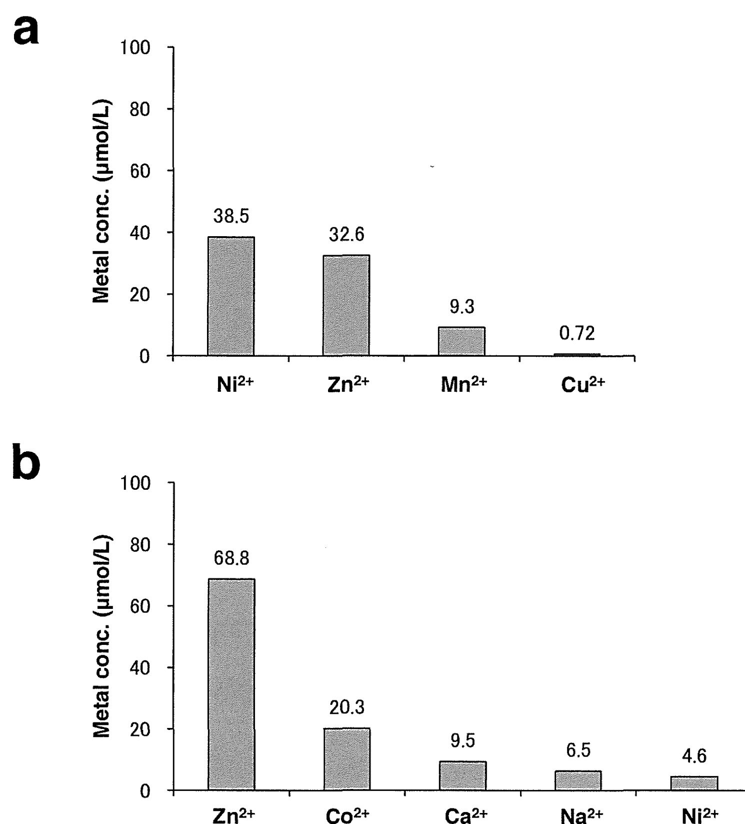

Figure S9 The metal concentration in the solution of recombinant D-THA DH purified by (a) HisTrap Ni^{2+}-affinity column (GE Healthcare, Little Chalfont, UK) and (b) HisTALON Co^{2+}-affinity column (Clontech Laboratories, Inc., Mountain View, CA). These were determined by inductively coupled plasma mass spectrometry (ICP-MS; ELAN DRC-e; Perkin Elmer, Waltham, MA). Enzyme concentrations were taken as 100 μmol/L. Shown are the five top-ranked metals for each analysis (Only four metals were detected in the sample of *a*). Large amount of Zn^{2+} was detected along with a relatively high content of Ni^{2+} and Co^{2+} after purification by Ni^{2+}- and Co^{2+}-affinity chromatography, respectively. There was a slight or no concentration of Mg^{2+}.

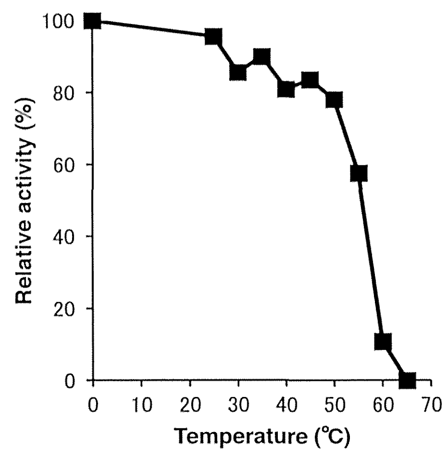

Figure S10 Thermal stability of D-THA DH. Purified D-THA DH solution was incubated for 15 min at different temperatures (0-65 °C). After incubation, it was placed on ice for 5 min and then the residual dehydratase activity was assayed by the protocol described in the Materials and methods. The activity of the enzyme incubated at 0 °C was taken as 100%. Over 80% of the residual activity was present in the range of temperature between 0 and 45 °C.

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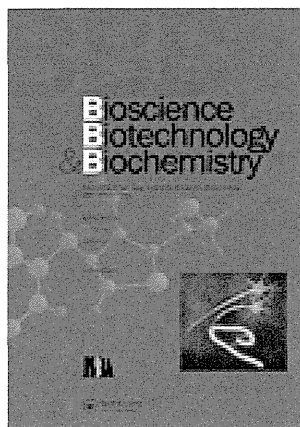

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Total synthesis of aurachins C, D, and L, and a structurally simplified analog of aurachin C

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Note

Total synthesis of aurachins C, D, and L, and a structurally simplified analog of aurachin C

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The quinoline antibiotics aurachins C, D, and L, and a structurally simplified analog of aurachin C were synthesized from 1-(2-nitrophenyl)butane-1,3-dione via reductive cyclizations of δ-nitro ketone intermediates, with zinc or iron as key steps. The results of antimicrobial tests indicate that the *N*-hydroxyquinolone nucleus mimics the electron carrier in the respiratory chain more strongly than the quinoline *N*-oxide nucleus.

Key words: aurachins; quinoline antibiotics; reductive cyclization

Bacterial respiratory chain inhibitors are promising antibiotics, since there are some differences between the mammalian respiratory chain and the bacterial one. In particular, the quinoline alkaloids have received considerable attention because of their significant activity in the quinol oxidation sites of the bacterial respiratory chain.[1,2] Quinoline alkaloids aurachins C (**1**), D (**2**), and L (**3**) were isolated from the culture broth of *Stigmatella aurantica* by Höfle et al.[3,4] Kitagawa et al. isolated the 9′-hydroxyaurachin C from a culture broth of *Rhodococcus erythropolis* and named it aurachin RE (**4**).[5–7] While **1**, **2**, and **4** have been reported to exhibit antimicrobial activity against many Gram-positive bacteria, no biological activity of **3** has been reported thus far. Considering the structural similarity among aurachins and 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) (**5**), which is an electron transport inhibitor of the respiratory chain,[8] the *N*-hydroxyquinolone nucleus is likely responsible for the activity.[9,10] On the other hand, the side chain would not be so important for antibiotic activity, because substrates in the bacterial respiratory chain like menaquinone (**6**) have different side chain lengths. The unique structures and the potential of aurachins as a new lead compound for practical antibiotics have prompted synthetic efforts toward aurachins and their analogs by organic chemists. Two research groups have succeeded in the total synthesis of **2** and examined the effect of the side chain lengths so far.[11,12] Both two groups reported that the geranyl chain analog retained the antibiotic activity but the prenyl chain analog was considerably less active than **2**. According to their studies on aurachin D, a structurally simplified analog of aurachin C with a shorter side chain would show as strong of an antimicrobial activity as aurachin C. We describe herein the total synthesis of aurachins C (**1**), D (**2**) and L (**3**), and the structurally simplified analog of aurachin C together with biological activities of **3** and the analog (Fig. 1).

As shown in Scheme 1, our synthesis of **1** began with the alkylation of known ketone **7**[13] with farnesyl bromide to give **8** as a 1:1 keto–enol tautomeric mixture. Then the alkylation product **8** was reductively cyclized with zinc dust in the presence of ammonium chloride to afford aurachin C (**1**) in 46% yield.[14] In contrast, the synthesis of aurachin D (**2**) was accomplished in 63% yield by a reductive cyclization with iron dust in the presence of hydrochloric acid. Although the acids used in these reactions were different, these results are likely due to the smaller first ionization energy of iron atoms (762.5 kJ/mol) than that of zinc atoms (906.4 kJ/mol).[15] Stronger reducing power of the iron atom compared to that of the zinc would make it possible to convert the nitro group into the amine. The ^{1}H- and ^{13}C NMR of **1** and **2** were identical to those of natural products.

Having completed the synthesis of aurachins C and D, we then turned our attention to the synthesis of aurachin L (**3**). The plausible biogenesis[4] proposed by Höfle et al. suggests that 2*H*-pyran moiety of **3** is constructed by 6π-electrocyclization from the corresponding dienone precursor. In light of the biogenesis of **3**, the moiety would be concisely constructed by a domino Knoevenagel/6π-electrocyclization. Thus we subjected **7** to farnesal under the Knoevenagel conditions

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Abbreviations: HMPA, hexamethylphosphoric triamide; RCM, ring-closing metathesis; MIC, minimum inhibitory concentration; SAR, structure-activity relationship.

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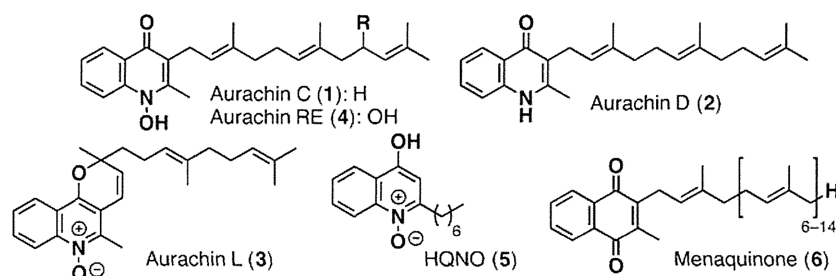

Fig. 1. Structures of aurachins C (**1**), D (**2**), L (**3**), RE (**4**), HQNO (**5**) & menaquinone (**6**).

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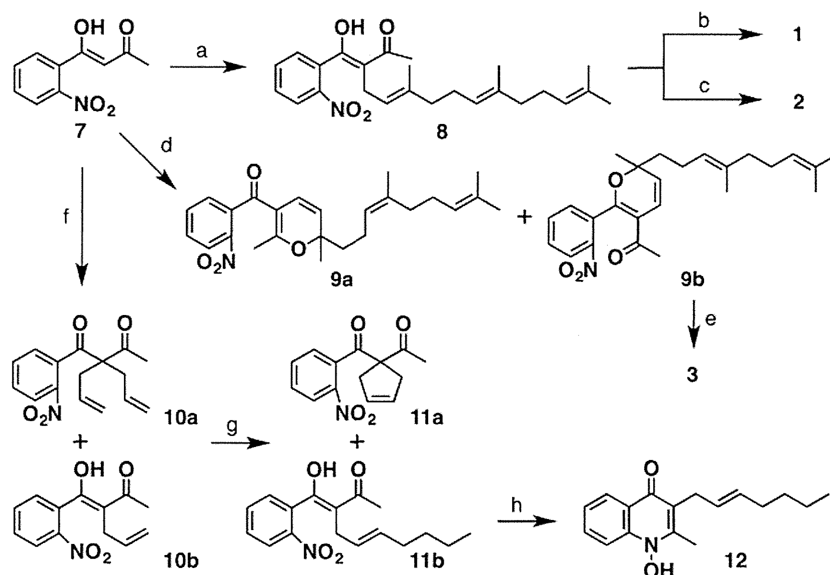

Scheme 1. Synthesis of aurachins C (**1**), D (**2**), L (**3**) & aurachin C analong **12**.

Notes: Reagents and conditions: (a) NaH, farnesyl bromide, HMPA, THF, 0–60 °C (52%, 69% based on recovered **7**); (b) Zn dust, NH_4Cl, $EtOH/H_2O$ (1:1), 80 °C (46%); (c) Fe dust, 6 M HCl, EtOH, 90 °C (63%); (d) farnesal, piperidine, toluene, rt to 60 °C, (39%); (e) Zn dust, NH_4Cl, $EtOH/H_2O$ (1:1), 80 °C (29%); (f) NaH, allyl bromide, HMPA, THF, 0–50 °C; (g) 1-hexene, Grubbs' 2nd generation catalyst, CH_2Cl_2, reflux (**11a**: 9% from **7**, **11b**: 12% from **7**, 28% based on recovered **7**); (h) Zn dust, NH_4Cl, $EtOH/H_2O$ (1:1), 80 °C (49%).

to afford an inseparable mixture of desired cycloadduct **9b** and presumably its isomer **9a** in 39% combined yield.[1] Finally, the mixture was exposed to the reductive cyclization conditions using zinc dust to afford aurachin L (**3**) in 29% yield together with a separable by-product derived from **9a**. The ^{1}H- and ^{13}C NMR of **3** exhibited good agreement with those of natural aurachin L.

Next, we synthesized a structurally simplified analog of **1**. According to the SAR study reported by Nay's and Speicher's group, the geranyl chain (i.e. 3,7-dimethyl-2,6-octadienyl chain) analog almost conserved the antibiotic activity.[11,12] Thus, we set **12** as a target molecule to consider whether the 7-carbon straight side chain analog is able to retain the activity or not. By following the same procedure conducted for the preparation of **8**, **7** was alkylated with allyl bromide to give an inseparable 2:3 mixture of **10a** and **10b**. For side chain elongation of **10b**, we employed cross-metathesis with 1-hexene and obtained a separable 4:5 mixture of desired product **11b** and RCM product **11a**, which was removed by SiO_2 chromatography at this stage. The reductive cyclization of **11b** with zinc dust proceeded smoothly to afford **12** in 49%. The ^{1}H- and ^{13}C NMR of **12** were analogous to those of **1** except for signals due to the side chain moiety.

The antimicrobial activities of **3** and **12** were tested by an agar diffusion assay. As shown in Table 1, **3** showed 10–17 mm zones of inhibition when 500 ng/disks of the compounds were applied on paper disks. On the other hand, the analog **12** was found to exhibit strong antimicrobial activity at only 50 ng/disks. These results indicated that the branching in the side chain was not important for the activity and the analog (**12**) with a shorter side chain than the geranyl group in aurachin C (**1**) conserved the activity. We are considering that the difference in biological activity between **3** and **12** would be ascribable to the structural difference in their nitrogen-containing heterocyclic rings (quinoline *N*-oxide nucleus in **3** and *N*-hydroxyquinolone nucleus in **12**). Since the *N*-hydroxyquinolone nucleus included in **12** seems to be more similar to the naphthoquinone moiety of the electron carrier like **6** than the quinoline *N*-oxide nucleus in **3**, **12** might better mimic the electron carrier than **3**.

In conclusion, we successfully achieved two-step syntheses of aurachins C and D from readily available starting material **7** in 24 and 33% overall yields, respectively. It is noteworthy that the two different types of the nucleus, *N*-hydroxyquinolone and quinolone, could be synthesized by changing the metal employed in the reductive cyclization. The first synthesis of aurachin L

Table 1. Antimicrobial spectra of **3** & **12**.

Test organism	Diameter of inhibition zone (mm)	
	3[a]	**12**[b]
Sinorhizobium meliloti JCM 20682 (IAM 12611)	0	8
Pseudomonas putida JCM 13063 (IAM 1236)	0	0
Escherichia coli K-12	0	0
Bacillus subtilis JCM 1465 (IAM 12118)	0	0
Sinomonas atrocyanea JCM 1329 (IAM 12339)	13	19
Corynebacterium glutamicum JCM 1318 (IAM 12435)	10	8
Streptomyces griseus JCM 4047 (IAM 12311)	17 (turbid)	12
Rhodococcus erythropolis JCM 3201 (IAM 12122)	0	0

[a]About 500 ng of the compounds were applied on paper disks (6 mm diameter).
[b]About 50 ng of the compounds were applied on paper disks (6 mm diameter).

was also accomplished in 11% overall yield by utilizing a domino Knoevenagel/6π-electrocyclization for the construction of 2*H*-pyran moiety. The results of antimicrobial tests for **3** and **12** indicated that *N*-hydroxyquinolone nucleus better mimics the electron carrier than the quinoline *N*-oxide nucleus.

Supectral data of aurachins C (1), D (2) & L (3)

Aurachin C (1): IR ν_{max}: 2300–3500 (s), 2919 (s), 1533 (s), 1430 (s), 1530 (s), 756 (m); ^{1}H NMR (270 MHz, CD_3OD) δ: 1.53 (3H, s), 1.56 (3H, s), 1.61 (3H, s), 1.81 (3H, s), 1.85–2.15 (8H, m), 2.57 (3H, s), 3.47 (3H, d, *J* = 6.8 Hz), 4.95–5.17 (2H, m), 7.40 (1H, ddd, *J* = 8.2, 7.2, 1.35 Hz), 7.71 (1H, ddd, *J* = 8.5, 7.2, 1.2 Hz), 7.98 (1H, d, *J* = 8.5 Hz), 8.28 (1H, dd, *J* = 8.2, 1.2 Hz); ^{13}C NMR (67.5 MHz, CD_3OD) δ: 15.8, 16.8, 17.1, 18.4, 26.4, 26.6, 28.2, 28.5, 41.48, 41.52, 116.8, 121.0, 124.5, 125.7, 125.9, 126.0, 126.1, 127.0, 132.7, 133.5, 136.7, 137.0, 141.6, 151.7, 175.3; HRMS (FAB) *m/z*: calcd. for $C_{25}H_{34}O_2N$, 380.2590; found, 380.2592 ($[M + H]^+$).

Aurachin D (2): IR ν_{max}: 2916 (s), 1552 (m), 1494 (s); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.55 (3H, s), 1.55 (3H, s), 1.65 (3H, s), 1.74 (3H, s), 1.77–2.10 (8H, m), 2.45 (3H, s), 3.41 (2H, d, *J* = 6.5 Hz), 5.00–5.18 (3H, m), 7.27 (1H, t, *J* = 8.3 Hz), 7.40 (1H, d, *J* = 8.3 Hz), 7.50 (1H, d, *J* = 6.8 Hz), 8.36 (1H, d, *J* = 7.3 Hz), 9.83 (1H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ: 16.1, 16.4, 17.8, 18.9, 24.2, 25.8, 26.8, 26.9, 39.8, 39.8, 117.3, 120.1, 122.5, 123.2, 124.3, 124.5, 126.3, 131.3, 131.4, 135.0, 135.3, 139.2, 146.3, 177.3; HRMS (FAB) *m/z*: calcd. for $C_{25}H_{34}ON$, 364.2638; found, 364.2640 ($[M + H]^+$).

Aurachin L (3): IR ν_{max}: 2924(s), 1325 (m), 1063 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.52 (3H, s), 1.53 (3H, s), 1.57 (3H, s), 1.66 (3H, s), 1.78–1.84 (1H, m), 1.85–1.90 (1H, m), 1.90–1.95 (2H, m), 2.00–2.04 (2H, m), 2.14–2.20 (2H, m), 2.75 (3H, s), 5.06 (1H, tt, *J* = 6.0, 1.0 Hz), 5.11 (1H, td, *J* = 5.5, 2.0 Hz), 5.76 (1H, d, *J* = 8.5 Hz), 6.59 (1H, d, *J* = 8.5 Hz), 7.55 (1H, td, *J* = 7.0, 2.0 Hz), 7.72 (1H, td, *J* = 7.0, 0.8 Hz), 8.15 (1H, dd, *J* = 7.0, 0.8 Hz), 8.72 (1H, d, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1, 16.0, 17.7, 22.4, 25.7, 26.5, 26.6, 39.6, 41.2, 80.5, 111.1, 118.4, 119.9, 120.6, 122.1, 123.2, 124.2, 127.1, 130.1, 130.7, 131.4, 135.9, 140.9, 143.6, 147.4; HRMS (FAB) *m/z*: calcd. for $C_{25}H_{32}O_2N$, 378.2428; found, 378.2437 ($[M + H]^+$).

Spectral Data for **8**, **11b**, and **12**, and experimental procedures for **1**, **2**, **3**, **8**, **11b**, and **12** are available on *Biosci. Biotechnol. Biochem.* Web site.

Supplemental material

The supplemental material for this paper is available at http://dx.doi.org/10.1080/09168451.2014.918494.

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We thank Ms Yamada (Tohoku University) and Mr Kurashina for measuring NMR, IR, and MS spectra.

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Note

1. Comparison of the peak integrations of the double bond proton signals in the resulting pyran rings of **9a** ([270 MHz, $CDCl_3$] δ: 6.83 [1H, d, *J* = 12.2 Hz], 6.39 [1H, d, *J* = 12.2 Hz]) and **9b** ([270 MHz, $CDCl_3$] δ: 5.86 [1H, d, *J* = 10.3 Hz], 5.06 [1H, d, *J* = 10.3 Hz]) indicated their ratio to be 1:1.2.

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Supplemental Information

IR spectra were recorded by a Jasco FT/IR-4100 spectrometer using an ATR (ZnSe) attachment, and reported in cm^{-1}. NMR spectra were recorded with TMS as an internal standard ($\delta = 0.00$) in $CDCl_3$ by a JEOL JNMA-270 spectrometer (270 MHz for ^1H and 67.5 MHz for ^{13}C) unless otherwise stated. The mass spectra were obtained with JEOL JMS-700 spectrometer operated in the FAB mode. Kanto Silica Gel 60N (spherical neutral) was used for column chromatography. Solvents for reactions were purchased and used as received. All air- or moisture-sensitive reactions were conducted under a nitrogen atmosphere.

*(3Z,5E,9E)-3-(hydroxy(2-nitrophenyl)methylene)-6,10,14-trimethylpentadeca-5,9,13-trien-2-one (**8**).* To a suspension of NaH (60% dispersion in mineral oil, 10.6 mg, 0.266 mmol) and HMPA (92 μl, 0.531 mmol) in THF (0.5 ml) was added **7** (50 mg, 0.241 mmol) in THF (0.5 ml) at 0 ˚C under Ar. After 15 min, farnesyl bromide (72 μl, 0.266 mmol) in THF (0.1 ml) was added to the mixture at 0˚ C, which was allowed to gradually warm to room temperature over 1 h and stirred at 60 ˚C for 2 h. The mixture was poured into saturated aq. NH_4Cl, then extracted with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (hexane/EtOAc = 40:1 to 50:1) to give 51.2 mg (52%, 69% based on recovered **7**) of a 1:1 keto-enol tautomeric mixture of **8** as a yellow oil along with the recovered starting material (12.2 mg, 24%): IR ν_{max}: 2977 (m), 1725 (m), 1770 (m), 1530 (s), 1346 (s); ^1H-NMR (270 MHz) δ: 1.52 (3H, s), 1.58 (6H, s), 1.68 (3H, s), 1.77-2.22 (8H, m), 2.21 (0.5×3H, s), 2.24 (0.5×3H, s), 2.70 (2H, d, $J = 6.5$ Hz), 4.05 (0.5×1H, dd, $J = 6.2, 8.4$ Hz), 4.85-4.95 (0.5×1H, m), 5.00-5.15 (0.5×1H + 2H, m), 7.36 (1H, d, $J = 7.3$ Hz), 7.54-7.75 (2H, m), 8.14 (0.5×1H, dd, $J = 1.6, 5.9$ Hz), 8.17 (0.5×1H, dd, $J = 1.4, 5.9$ Hz), 16.1 (0.5×1H, s); ^{13}C-NMR (67.5 MHz) δ: 15.7, 16.0, 16.0, 16.2, 17.7, 22.7, 25.7, 26.3, 26.4, 26.6, 26.71, 26.773, 28.1, 29.5, 39.5, 39.63,

39.67, 39.71, 67.00, 109.5, 119.3, 122.3, 123.6, 123.75, 123.82, 124.26, 124.29, 124.4, 124.5, 128.3, 128.7, 129.9, 130.8, 131.29, 131.33, 133.4, 133.7, 134.4, 135.2, 135.3, 136.1, 136.9, 139.0, 145.5, 145.9, 188.5, 190.6, 197.9, 204.0; HRMS (FAB) *m/z*: calcd. for $C_{25}H_{34}O_4N$, 412.2488; found, 412.2484 ($[M+H]^+$).

Aurachin C (1). To a solution of **8** (42.0 mg, 0.102 mmol) in EtOH/water (1:1, 3 ml) were added NH_4Cl (25. 7 mg, 0.480 mmol) and zinc dust (54.2 mg, 0.829 mmol) at room temperature, and the mixture was stirred for 10 min at 80 ˚C. Saturated aq. $NaHCO_3$ was then added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (hexane/EtOAc = 1:1) to give 17.8 mg (46%) of **1** as a yellow oil.

Aurachin D (2). To a solution of **8** (21.1 mg, 51.3 μmol) in EtOH (2.7 ml) were added 6 M aq. HCl (50 μl) and iron dust (22 mg, 0.394 mmol) at room temperature and the mixture was stirred at 90 ˚C. Additional iron dust (112 mg in total) and 6 M aq. HCl (350 μl in total) were added portionwise to the reaction mixture over a period of 6 h until **8** was completely consumed (TLC monitoring). The reaction mixture was diluted with EtOAc/water (ca. 1:1), then passed through a pad of Celite, and the filtrate was extracted with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (hexane/EtOAc = 2:1) to give 11.8 mg (63%) of **2** as a yellow oil.

Aurachin L (3). To a solution of **7** (26.3 mg, 0.127 mmol) in toluene (8.5 ml) were added farnesal (33.6 mg, 0.153 mmol) and piperidine (16 μl, 0.165 mmol) at room temperature under Ar. After being stirred for 1 h at the same temperature, the mixture was heated at 60 ˚C for 3.5 h. Then, additional farnesal (25.4 mg, 0.115 mmol) in toluene (0.5 ml) was added to the reaction mixture, and the resulting mixture was stirred

at 60 ˚C for additional 2.5 h. The mixture was passed through a pad of SiO_2 (ca. 1 g) eluted with EtOAc and the filtrate was concentrated *in vacuo*. The residue was chromatographed over SiO_2 (hexane/EtOAc = 30:1 to 10:1) to give 20.1 mg (39%) of an inseparable 1:1.2 mixture of **9a** and **9b** as a yellow oil. To a solution of 6.3 mg of the mixture obtained above {containing 2.9 mg (7.1 μmol) of **9a** and 3.4 mg (8.3 μmol) of **9b**} in EtOH (0.8 ml) /water (0.25 ml) were added NH_4Cl (3.9 mg, 70 μmol) and zinc dust (8.1 mg, 0.12 mmol) at room temperature, and the mixture was heated at 80 ˚C. After being stirred for 5 min, the mixture was passed through a pad of Celite, the filter cake was washed with EtOAc, and the combined filtrate was concentrated *in vacuo*. The residue was purified by TLC ($CHCl_3$/acetone/MeOH = 75:20:5) to give 1.7 mg (4.5 μmol, 29% from the mixture of **9a** and **9b**) of **3** as a yellow oil, along with 0.7 mg (1.8 μmol, 12% from the mixture of **9a** and **9b**) of by-product derived from **9a**.

*(3Z,5E)-3-(hydroxy(2-nitrophenyl)methylene)dec-5-en-2-one (**11b**).* To a suspension of NaH (29.0 mg, 0.724 mmol, 60% dispersion in mineral oil) in THF (1.0 ml) were added HMPA (796 μl, 6.58 mmol) and **7** (136 mg, 0.658 mmol) in THF (1.0 ml) at 0 ˚C under Ar. After 15 min, allyl bromide (57 μl, 0.658 mmol) in THF (0.5 ml) was added to the mixture at 0˚ C, which was allowed to gradually warm to room temperature over 20 min and stirred at 50 ˚C for 1.5 h. The mixture was poured into saturated aq. NH_4Cl, and the resulting mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (hexane/EtOAc = 20:1) to give 38.7 mg of an inseparable 2:3 mixture of **10a** and **10b** as a yellow oil, along with the recovered starting material (79.5 mg, 58%). To a solution of the mixture obtained above (38.7 mg) in CH_2Cl_2 (5.3 ml) were added 1-hexene (97 μl, 0.783 mmol) and the second-generation Grubbs catalyst (10.3 mg, 0.013 mmol) at room temperature under Ar. After being stirred at reflux for 1.5 h, the mixture was concentrated *in vacuo*. The residue was chromatographed over SiO_2 (hexane/EtOAc = 30:1 to 20:1) to give 16.1 mg

of **11a** (9% from **7**) and 23.1 mg (12% from **7**, 28% based on recovered **7**) of a 3:2 keto-enol tautomeric mixture of **11b** as a yellow oil: IR ν_{max} 2926 (m), 1528 (s), 1346 (s); ^{1}H-NMR (270 MHz) δ: 0.80–0.95 (3H, m), 1.17–1.35 (4H, m), 1.85–2.00 (2H, m), 2.22 (0.6×3H, s), 2.25 (0.4×3H, s), 2.63–2.76 (2H, m), 4.08 (0.4H, t, J = 7.4 Hz), 5.16–5.23 (0.6×2H, m), 5.24–5.40 (0.4H, m), 5.43–5.55 (0.4H, m), 7.35–7.40 (1H, m), 7.53–7.77 (2H, m), 8.13–8.20 (1H, m), 16.23 (0.6H, s); ^{13}C-NMR (67.5 MHz) δ: 13.87, 13.89, 22.13, 22.20, 22.8, 29.5, 30.7, 31.4, 31.5, 32.0, 32.1, 32.5, 67.1, 108.2, 124.4, 124.5, 124.9, 126.9, 127.1, 128.3, 128.6, 130.0, 130.9, 131.8, 133.2, 133.6, 134.4, 134.5, 136.8, 137.1, 188.4, 191.3, 197.7, 203.9; HRMS (FAB) *m/z*: calcd. for $C_{17}H_{22}O_4N$, 304.1550; found, 304.1549 ($[M+H]^+$).

*(E)-3-(hept-2-en-1-yl)-1-hydroxy-2-methylquinolin-4(1H)-one (**12**).* To a solution of **11b** (11.5 mg, 39.7 μmol) in EtOH/water (1:1, 0.7 ml) were added NH_4Cl (10.0 mg, 0.187 mmol) and zinc dust (20.0 mg, 0.307 mmol) at room temperature, and the mixture was stirred for 10 min at 80 °C. Saturated aq. $NaHCO_3$ was then added to the mixture, and the resulting mixture was extracted with EtOAc. The combined organic extracts were washed with water, brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (hexane/EtOAc = 1:1 to 0:1) to give 5.3 mg (49%) of **12** as a yellow oil: IR ν_{max} 2250–3200 (s), 2926 (s), 1590 (s), 1428 (s), 1349 (s), 756 (m); ^{1}H-NMR (270 MHz, CD_3OD) δ: 0.89 (3H, t, J = 7.0 Hz), 1.25–1.45 (4H, m), 1.95–2.05 (2H, m), 2.62 (3H, s), 3.46 (2H, d, J = 2.0 Hz), 5.45–5.55 (2H, m), 7.45 (1H, ddd, J = 0.8, 7.3, 8.1 Hz), 7.76 (1H, ddd, J = 1.4, 7.0, 8.6 Hz), 8.00 (1H, d, J = 8.4 Hz), 8.31 (1H, dd, J = 0.8, 8.1 Hz); ^{13}C-NMR (75 MHz, CD_3OD) δ: 21.5, 25.0, 30.6, 31.4, 35.6, 40.9, 46.8, 64.3, 64.4, 108.7, 121.6, 139.2, 214.7; HRMS (FAB) *m/z*: calcd. for $C_{17}H_{22}O_2N$, 272.1651; found, 272.1653 ($[M+H]^+$).

Agar diffusion assay. The antimicrobial activity of aurachin compounds was tested by an agar diffusion assay (paper disc assay). About 10^7 bacteria in a 5 ml of a

LB soft agar (0.5% agar) were poured and solidified on a LB agar media in Petri dish (9 cm in diameter). Filter paper discs (6 mm diameter) containing 50 or 500 ng of test substance were placed in the center. After 24 h of incubation, diameters of each growth inhibition zones were measured.