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NOTE

Aurachin SS, a new antibiotic from *Streptomyces* sp. NA04227

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Aurachins comprise a small family of natural products featuring a characteristic quinoline chromophore substituted in position 3 or 4 by a farnesyl chain.[1–3] Owing to their structural similarity to analogs of the physiological ubiquinol and vitamin K, aurachins act as potent inhibitors of electron transport in the respiratory chain, more specifically as inhibitors of various cytochrome complexes.[4,5] With these structural properties, aurachin-type compounds are strong antimicrobial, antifungal and antiplasmodial agents. Aurachins were first isolated in 1987 from myxobacterium *Stigmatella aurantiaca* Sg a15.[6] Since then, additional members of aurachin-type compounds were isolated from the same strain and also from an actinobacterial strain, *Rhodococcus* sp.[7] During our continuous search for bioactive secondary metabolites from microorganisms living in special niche,[8–11] aurachin SS, a new aurachin-type compound together with two known compounds were isolated from *Streptomyces* sp. NA04227 (Figure 1).

Strain NA04227 was isolated from an earwig (*Forficula auricularia*) collected in Qixia Mountain, Nanjing, China. This strain grows and sporulates well on CPA agar medium, and was identified as a *Streptomyces* sp. on the basis of its 16 s ribosomal RNA gene sequence (accession number: KY465886). The preliminary antibacterial assay of the crude extract from NA04227 in YEME medium showed strong antibacterial activity. To isolate and identify the bioactive components, we did a large-scale fermentation, and subjected the extract to a combination of chromatographic methods including normal phase, C-18 silica gel and Sephadex LH-20, leading to isolation and characterizations of a new compound, named as aurachin SS (**1**), together with two known compounds, aurachin C (**2**)[12] and aurachin D (**3**)[12] (Figure 1). Herein, we report the isolation, structure elucidation, biological activity and biosynthesis of these aurachins.

Compound **1** was isolated as a brown oil, and the molecular formula was determined as $C_{21}H_{27}NO_2$ on the basis of HRESIMS analysis, indicating 9° of unsaturation. The ^1H, ^{13}C and HSQC NMR spectra indicated five methyl (with one of them oxygenated), three methylene, six methine groups and seven sp^2 hybridized quaternary carbons (Table 1). The ^1H-^1H COSY correlations of δ 8.67 (d, H-5)/7.77 (t, H-6)/7.70 (t, H-7)/8.12 (d, H-8) suggested the presence of an ortho-substituted aromatic ring (Figure 2). A methyl substituted quinoline moiety was disclosed by the HMBC correlations of H-5/C-4 and C-8a, H-8/C-4a, H-7/C-8a, and H-9/C-2 and C-3 (Figure 2). The ^1H-^1H COSY spectrum also revealed two other spin systems of H-1′/H-2′ and H-4′/H-5′/H-6′. The HMBC correlations of H-6′/C-8′ and C-10′; H-5′/C-7′ and C-3′, H-9′/C-2′ and C-4′, and H-1′/C-3′ suggested the presence of a geranyl group, which was anchored at C-3 according to the HMBC correlations of H-1′/C-2 and C-4, and H-2′/C-3. The methoxyl group was assigned to C-4 on the basis of HMBC correlation of H-10/C-4. Finally, the unusual downfield chemical shifts for H-8 (δ_H 8.12) and H-9 (δ_H 2.62) indicated that the presence of N-oxide group to consume one remaining oxygen atom.[12] Thus, the structure of **1** was established, and we named it as aurachin SS.

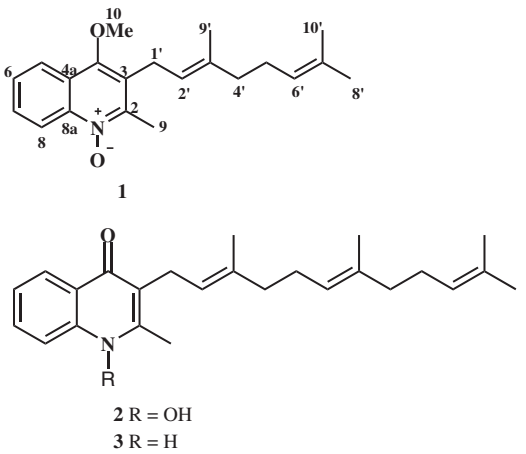

Figure 1 Structures of **1–3**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Inspired by the reported *aua* (in *S. aurantiaca* Sg a15) and *rau* (in *Rhodococcus erythropolis* JCM 6824) gene clusters, encoding the

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biosynthesis of aurachins[1] and aurachin RE,[7] respectively, we next aimed to identify *Streptomyces* aurachin biosynthetic genes (*sau* genes). The whole genome of *Streptomyces* sp. NA04227 was sequenced and then analyzed by antiSMASH online software,[13] revealing a mixed biosynthetic gene cluster that showed identical genetic organization, with 30–40% sequence identity, to the *aua* gene cluster[1] (Figure 3a; Table 2). Two genes *auaEII* and *auaF*, encoding an anthranilate-CoA ligase for activation of anthranilate[14] and a Rieske [2Fe–2 S] oxygenase for N-hydroxylation,[15] respectively, were missing from the *sau* cluster. The *sau* cluster has an additional set of genes, *sauL*, *-M* and *-N,* which were annotated as *trans*-isoprenyl diphosphate synthase, DXP synthase and HMBDP synthase, respectively, for supplying isopentenyl pyrophosphate and dimethylallyl pyrophosphate in MEP pathway.[16] At both end of *sau* gene cluster, there are two P450 genes, *sauPI* and *sauPII*, showing 45 and 38% protein sequence identity to *rauA* (Figure 3a).[7] Moreover, a thioesterase gene, *sauK*, was found in the middle of *sau* gene cluster, which is unique and missing in *aua* and *rau* gene clusters, and predicted to help the chain release from type II PKS and cyclization.

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of 1 in acetone-d_6

No.	δ(*^{1}H) (p.p.m.) J in Hz*	δ (*^{13}C)*	*p.p.m.*
2	—	147.7	C
3	—	127.8	C
4	—	151.0	C
4a	—	124.7	C
5	8.67, d (8.70)	120.8	CH
6	7.77, t (7.44)	128.5	CH
7	7.70, t (7.44)	130.2	CH
8	8.12, d (8.22)	123.4	CH
8a	—	141.8	C
9	2.62, s	15.4	CH_3
10	4.00, s	63.4	CH_3
1′	3.62, d (6.29)	26.3	CH_2
2′	5.17, t (6.21)	122.3	CH
3′	—	137.8	C
4′	2.06, m	40.2	CH_2
5′	2.09, m	27.1	CH_2
6′	5.07, t (6.74)	124.9	CH
7′	—	131.9	C
8′	1.62, s	25.8	CH_3
9′	1.86, s	16.5	CH_3
10′	1.56, s	17.7	CH_3

On the basis of the genetic organization and sequence identities, a biosynthetic pathway was proposed for biosynthesis of aurachins in NA04227 (Figure 3b). Anthranilic acid is activated by SauE,[14] followed by extension of **5** with two malonyl-CoA units catalyzed by minimal PKS to form **6**.[1] Prenylation with farnesyl diphosphate catalyzed by SauA would yield aurachin D (**3**),[17] and then hydroxylation presumably by SauPI or SauPII led to afford aurachin C (**2**). However, the substrate flexibility of SauA might also accept geranyl pyrophosphate instead of farnesyl pyrophosphate as substrate to produce **7**. Finally, one unidentified methyltransferase will recognize the geranyl pyrophosphate attached product (**8**) as substrate to generate auracin SS (**1**).

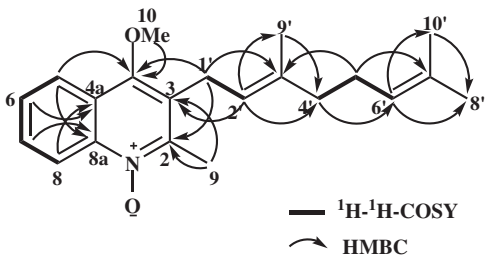

Figure 2 Key 2D correlations of compound **1**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

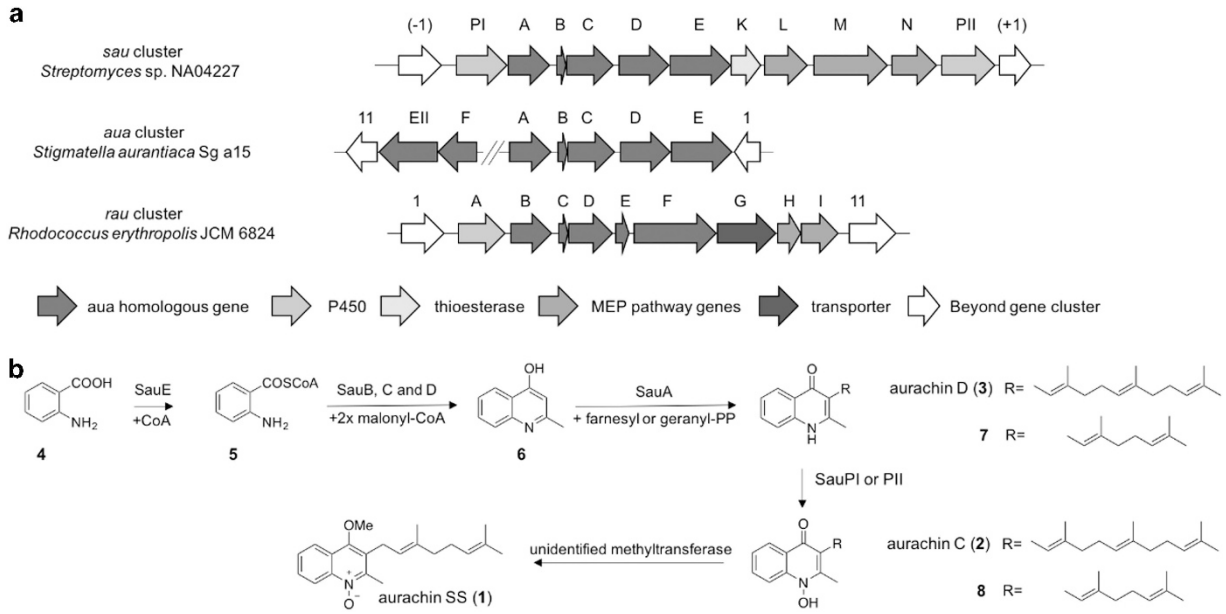

Figure 3 Putative biosynthetic gene cluster for aurachin production in *S.* sp. NA04227. (**a**) Genetic organization and comparison of aurachin gene cluster in *Stigmatella aurantiaca* Sg a15 (*aua* cluster) and *Rhodococcus erythropolis* JCM 6824 (*rau* cluster). (**b**) Proposed biosynthetic pathway for aurachins in *S.* sp. NA04227. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 2 Deduced functions of ORFs in the aurachin biosynthetic gene cluster

ORF	*Deduced function*	*Homolog/accession no.*	*Similarity/identity (%)*
sauPII	Cytochrome P450	rauA/BAN59735	38/55
sauN	HMBDP synthase (MEP pathway)	ptmM2/ACO31285	83/92
sauM	DXP synthase (MEP pathway)	ptmM3/ACO31286	58/66
sauL	Trans-isoprenyl diphosphate synthase	mcl2/AGH68887	54/66
sauK	Thioesterase	SEG54343	30/48
sauE	Anthranilate-CoA ligase	auaE/CCA65703	33/52
sauD	Beta-ketoacyl-CLF synthase II	auaD/CAL48956	31/45
sauC	Beta-ketoacyl-ACP synthase II	auaC/CAL48955	45/59
sauB	Acyl carrier protein	auaB/CAL48954	31/45
sauA	Prenyltransferase	auaA/CAL48953	34/50
sauPI	Cytochrome P450	rauA/BAN59735	45/61

Abbreviation: ORF, open reading frame.

Table 3 Antibacterial activities of 1–3 (MIC, μM)

Pathogens	***1***	***2***	***3***	*Rifampicin*	*Ampicillin*
S. aureus	64.0	8.0	8.0	1.0	4.0
MRSA	>128.0	128.0	128.0	4.0	64.0
S. pyogenes	32.0	8.0	4.0	0.50	2.0
B. subtilis	64.0	16.0	8.0	1.0	8.0
M. luteus	32.0	8.0	4.0	0.50	1.0

Abbreviations: *B. subtilis, Bacillus subtilis* CICC10283; *M. luteus, Micrococcus luteus*; MRSA, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300; *S. aureus*, *Staphylococcus aureus* CMCC(B)26003; *S. pyogenes*, *Streptococcus pyogenes* ATCC19615.

The isolated compounds **1–3** were evaluated for their antimicrobial activities. As shown in Table 3, compounds **2–3** showed potent bioactivities against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Micrococcus luteus* with MICs ranging from 4.0 to 16.0 μM. However, aurachin SS (**1**), which has a short side chain, only showed moderate activities against them with MICs from 32.0 to 64.0 μM.

In summary, one new (**1**) and two known (**2** and **3**) aurachins were isolated and identified from an earwig-associated *Streptomyces* sp. NA04227. The new compound, aurachin SS (**1**), features a unique geranyl side chain, differing from typical aurachin-type compounds discovered so far. A unified biosynthetic pathway was proposed based on the identified gene cluster. Antibacterial assay showed compounds **1–3** exhibited moderate to potent activity against four tested bacteria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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1 Sandmann, A. *et al.* A type II polyketide synthase from the Gram-negative bacterium *Stigmatella aurantiaca* is involved in aurachin alkaloid biosynthesis. *Angew. Chem. Int. Ed.* **46**, 2712–2716 (2007).
2 Kitagawa, W. & Tamura, T. A quinoline antibiotic from *Rhodococcus erythropolis* JCM 6824. *J. Antibiot.* **61**, 680–682 (2008).
3 Hofle, G. & Irschik, H. Isolation and biosynthesis of aurachin P and 5-nitroresorcinol from *Stigmatella erecta*. *J. Nat. Prod.* **71**, 1946–1948 (2008).
4 Junemann, S., Wrigglesworth, J. M. & Rich, P. R. Effects of decyl-aurachin D and reversed electron transfer in cytochrome bd. *Biochemistry* **36**, 9323–9331 (1997).
5 Debnath, J. *et al.* Discovery of selective menaquinone biosynthesis inhibitors against *Mycobacterium tuberculosis*. *J. Med. Chem.* **55**, 3739–3755 (2012).
6 Kunze, B., Hofle, G. & Reichenbach, H. Antibiotics from gliding bacteria.33. The aurachins, new quinoline antibiotics from Myxobacteria - production, physicochemical and biological properties. *J. Antibiot.* **40**, 258–265 (1987).
7 Kitagawa, W. *et al.* Cloning and heterologous expression of the aurachin RE biosynthesis gene cluster afford a new cytochrome P450 for quinoline N-hydroxylation. *Chembiochem* **14**, 1085–1093 (2013).
8 Zang, L. Y. *et al.* Sesquiterpenoids from the mangrove-derived endophytic fungus *Diaporthe* sp. *J. Nat. Prod.* **75**, 1744–1749 (2012).
9 Song, Y. N. *et al.* New ansamycin analogues from the mutant strain of *Streptomyces seoulensis*. *J. Antibiot.* **68**, 757–759 (2015).
10 Guo, Z. K. *et al.* Actinotetraoses A-H: tetrasaccharide derivatives from a Grasshopper-associated *Amycolatopsis* sp HCa1. *Planta Med.* **78**, 988–994 (2012).
11 Song, Y. N. *et al.* New ansamycin derivatives generated by simultaneous mutasynthesis. *Org. Lett.* **17**, 556–559 (2015).
12 Hoefle, G. & Kunze, B. Biosynthesis of aurachins A-L in *Stigmatella aurantiaca*: a feeding study. *J. Nat. Prod.* **71**, 1843–1849 (2008).
13 Weber, T. *et al.* antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res.* **43**, W237–W243 (2015).
14 Pistorius, D., Li, Y. Y., Mann, S. & Muller, R. Unprecedented anthranilate priming involving two enzymes of the acyl adenylating superfamily in aurachin biosynthesis. *J. Am. Chem. Soc.* **133**, 12362–12365 (2011).
15 Pistorius, D., Li, Y. Y., Sandmanny, A. & Muller, R. Completing the puzzle of aurachin biosynthesis in *Stigmatella aurantiaca* Sg a15. *Mol. Biosyst.* **7**, 3308–3315 (2011).
16 Smanski, M. J. *et al.* Dedicated ent-kaurene and ent-atiserene synthases for platensimycin and platencin biosynthesis. *Proc. Natl Acad. Sci. USA* **108**, 13498–13503 (2011).
17 Stec, E., Pistorius, D., Muller, R. & Li, S. M. AuaA, a membrane-bound farnesyltransferase from *Stigmatella aurantiaca*, catalyzes the prenylation of 2-methyl-4-hydroxyquinoline in the biosynthesis of Aurachins. *Chembiochem* **12**, 1724–1730 (2011).

Supplementary Information accompanies the paper on the The Journal of Antibiotics website (http://www.nature.com/ja)