THE AURACHINS, NEW QUINOLINE ANTIBIOTICS FROM MYXOBACTERIA: PRODUCTION, PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES†

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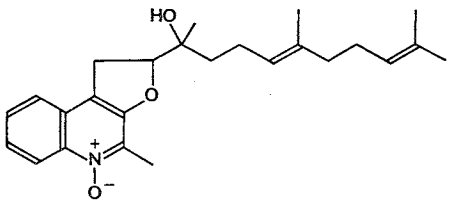
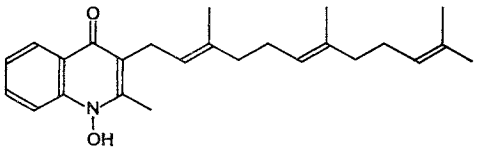
The aurachins, new quinoline alkaloids, were extracted with acetone from the biomass of the myxobacterium, *Stigmatella aurantiaca* strain Sg a15 and purified by column chromatography. The four described aurachins A, B, C and D, were inhibitory for Gram-positive bacteria and a few yeasts and molds. They blocked NADH oxidation in beef heart submitochondrial particles.

The myxobacterium, *Stigmatella aurantiaca* strain Sg a15, has been described to produce two structurally unrelated antibiotics, stigmatellin and a mixture of myxalamids[1~6]. ^{1}H NMR studies on cell mass extracts of the same strain led to the discovery of a third group of biologically active compounds (formerly Sg a15-A, -B, -C)[2]. They could be characterized as new quinoline alkaloids and were named aurachins. Thirteen variants, designated aurachins A~N, have been isolated so far. In all fermentations the aurachins A, B or C were the main components, while the aurachins D~N always represented minor components. In this article we report on the production, isolation and some physico-chemical and biological properties of the aurachins A, B, C and D. Fig. 1 shows the structures of the aurachins A~D, the elucidation of which will be published elsewhere[7].

Fig. 1. The chemical structures of the aurachins A, B, C and D[7].



Aurachin A



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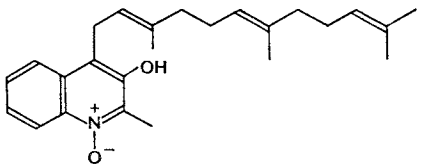
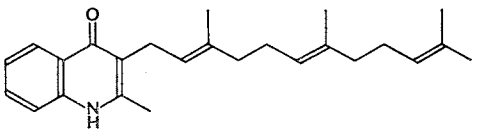


Aurachin B



Aurachin D

† Article No. 33 on antibiotics from gliding bacteria. Article No. 32: IRSCHIK, H.; R. JANSEN, K. GERTH, G. HÖFLE & H. REICHENBACH: J. Antibiotics 40: 7~13, 1987.

Microorganism

The producing organism was *Stigmatella aurantiaca* strain Sg a15 (=Stamm 1 Dawid). It was cultivated in peptone liquid medium as described before (peptone from casein, tryptically digested, Merck, Darmstadt 1%; $MgSO_4 \cdot 7H_2O$ 0.1%; pH 6.8)[1]. It also grew well on certain technical substrates[1]. In modified Zein liquid medium (Zein 1%; peptone from casein, tryptically digested, Merck, Darmstadt 0.1%; $MgSO_4 \cdot 7H_2O$ 0.1%; pH 7.3) the yield of certain aurachins was 50~100 times higher than in peptone liquid medium.

Production of Aurachins

To produce larger amounts of aurachins, strain Sg a15 was grown in volumes up to 320 liters in bioreactors equipped with a circulating pump stirrer system (Giovanola Frères, Monthey, Switzerland). For example 60 liters of modified Zein liquid medium in a type b 50 bioreactor were inoculated with 5 liters of shake cultures grown for 36~48 hours in the same medium. The fermentor was kept at 30°C and agitated at 500~650 rpm. The aeration rate was 0.03~0.048 vol/vol/minute. The pH drifted initially slightly into the acid range and rose then to 8.0. The initial pO_2 of about 90% saturation decreased to about 20% after 41 hours and was then maintained for the next 50 hours at this level by periodically increasing the aeration or stirring rate. Under these conditions aurachin C accumulated essentially during the growth phase (up to 4 mg/liter) and disappeared later, while aurachin A was produced essentially in the stationary phase (9 mg/liter). Aurachins B and D were only found in concentrations less than 1 mg/liter. Further studies showed that the proportion of aurachin A and aurachin B concentrations depended on the oxygen supply during fermentation. Under strong oxygen limitation we obtained higher yields of aurachin B and only very small amounts of aurachin A, while the concentrations of aurachins C and D remained more or less constant.

Table 1. Thin-layer chromatography of aurachins A, B, C and D*.

Chromatographic system	Rf values of aurachins			
	A	B	C	D
Hexane - 2-propanol (70 : 30)	0.57	0.68	0.77	0.79
Dichloromethane - acetone - methanol (70 : 20 : 5)	0.46	0.42	0.76	0.87
Ethyl acetate	0.16	0.11	0.53	0.73

* Silica gel 60 F_{254} (Merck, Darmstadt).

Table 2. Physico-chemical properties of aurachins A, B, C and D.

	A	B	C	D
MP (°C)	111~112	93~94	124~125	165~168
Optical rotation	−49.2° (*c* 0.4, MeOH)	—	—	—
MW	395	379	379	363
Molecular formula	$C_{25}H_{33}NO_3$	$C_{25}H_{33}NO_2$	$C_{25}H_{33}NO_2$	$C_{25}H_{33}NO$
UV λ_{max}^{MeOH} nm (ε)	369 (6,100), 354 (5,200), 317 (4,200), 304 (3,600), 249 (26,000), 239 (24,400), 219 (22,100),	352 (7,600), 342 (7,100), 315 (6,900), 302 (5,500), 288 (sh, 2,900), 242 (39,600), pH 2.5	346 (11,200), 336 (11,600), 299 (3,400), 287 (2,500), 251 (32,200), 245 (sh, 31,400), pH 2.5	334 (11,400), 321 (11,400), 308 (sh, 7,400), 291 (sh, 4,300), 280 (sh, 3,300), 245 (33,500), 240 (33,000),

Isolation and Physico-chemical Characterization

At the end of the fermentation the cells were separated from the supernatant by centrifugation. The cell mass containing about 90% of the antibiotics was extracted with acetone. ^{1}H NMR spectra of the crude extract showed multiplet signals at 8.6~8.8 ppm in addition to the signals of myxalamids, stigmatellin and lipids. The compounds were purified by chromatography on Florisil, silica gel, reversed-phase silica gel and Sephadex LH-20[2]. Aurachins A (Sg a15-A[2]), B (Sg a15-B[2]), C (Sg a15-C[2]) and D

Fig. 2. IR spectrum of aurachin A in KBr.

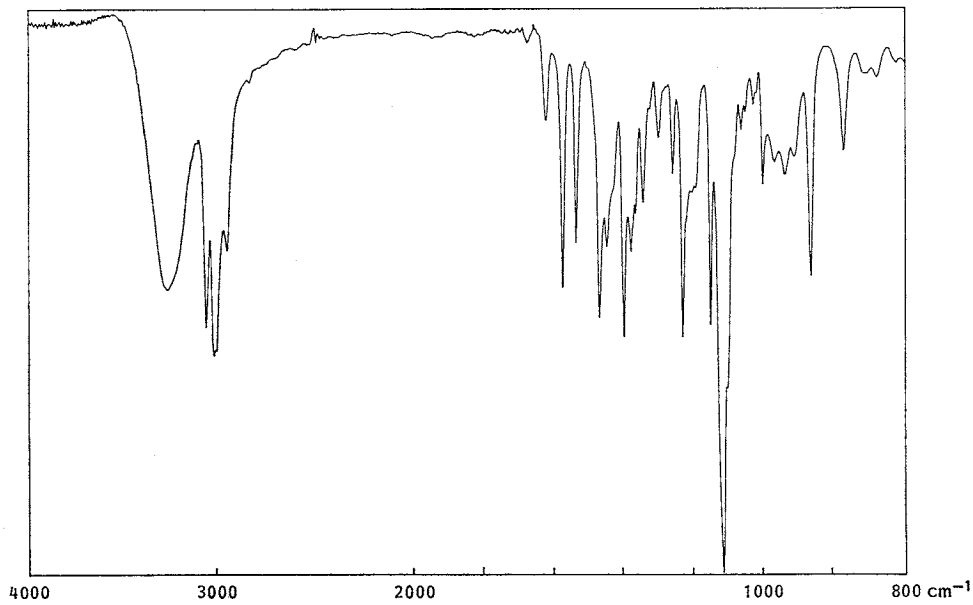
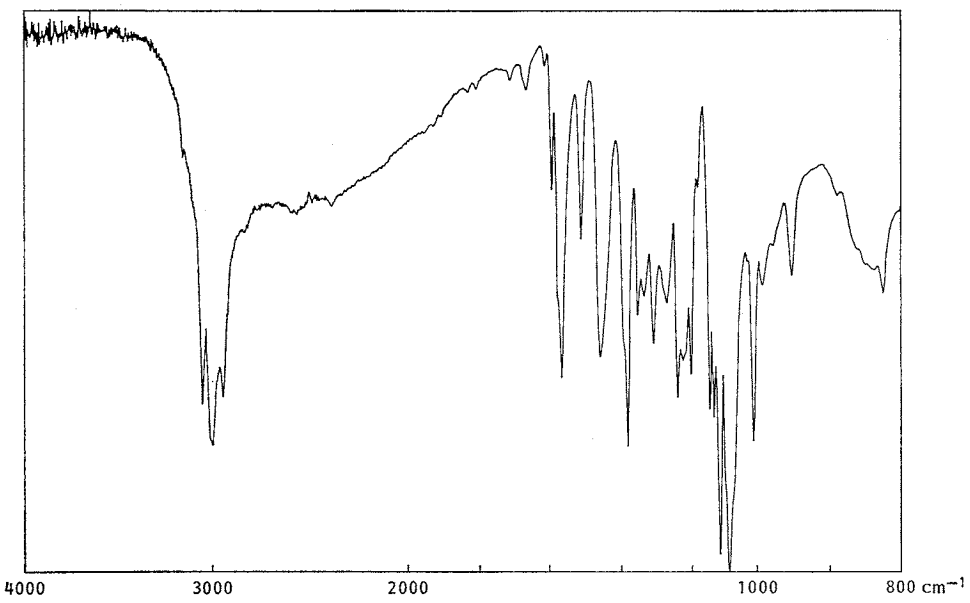


Fig. 3. IR spectrum of aurachin B in KBr.



crystallized from ether. They were soluble in methanol, acetone, ethyl acetate and chloroform, sparingly soluble in ether and water, and almost insoluble in hexane. They showed red spots on TLC after spraying with vanillin - sulfuric acid reagent and heating to 120°C. The Rf values of the aurachins A~D are given in Table 1. Further physico-chemical properties of the aurachins A~D are summarized in Table 2. The IR spectra of the aurachins A~D, measured in KBr with a Perkin-Elmer 297 IR spectrophotometer, are shown in Figs. 2~5.

Fig. 4. IR spectrum of aurachin C in KBr.

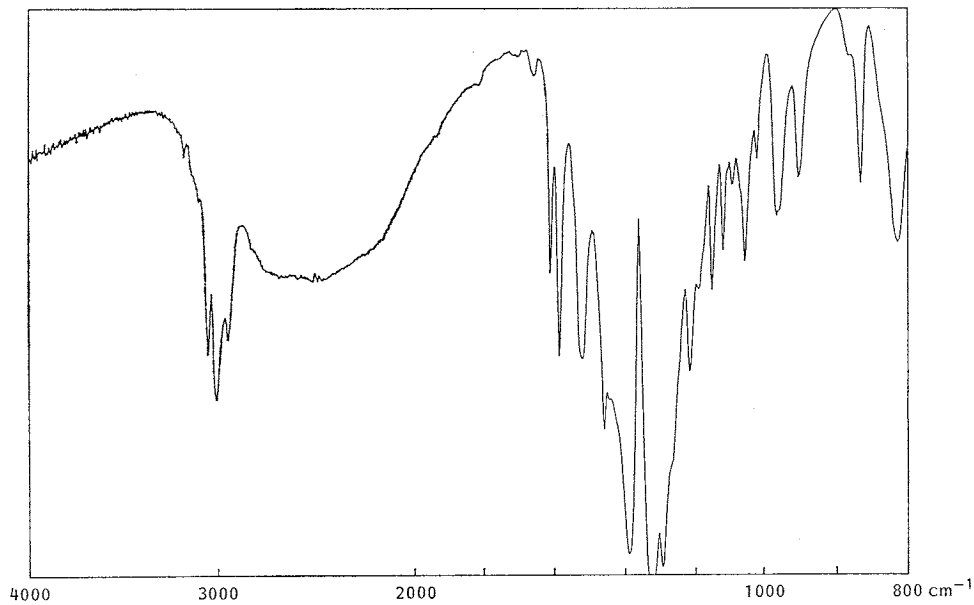
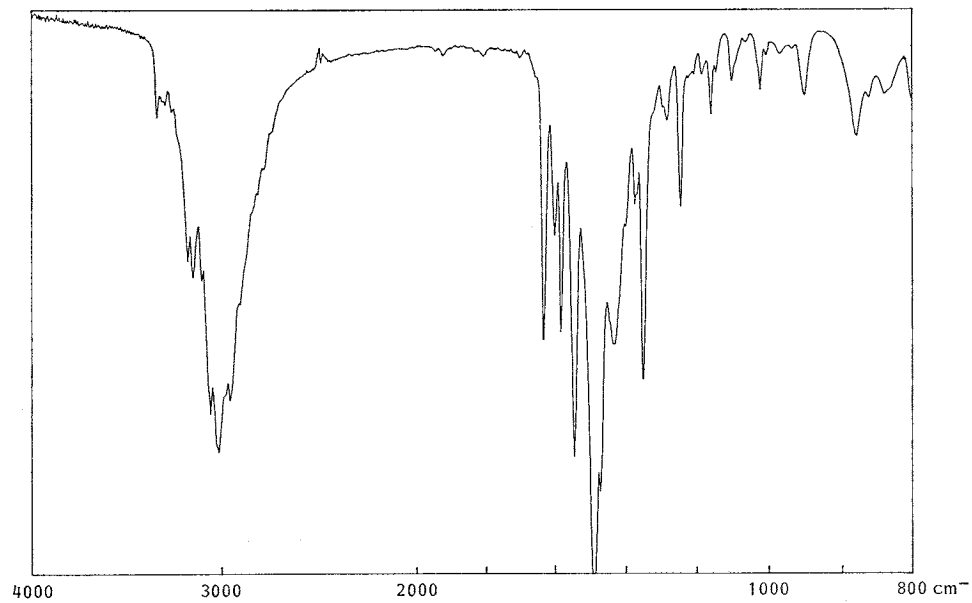


Fig. 5. IR spectrum of aurachin D in KBr.



Quantitation of Aurachins

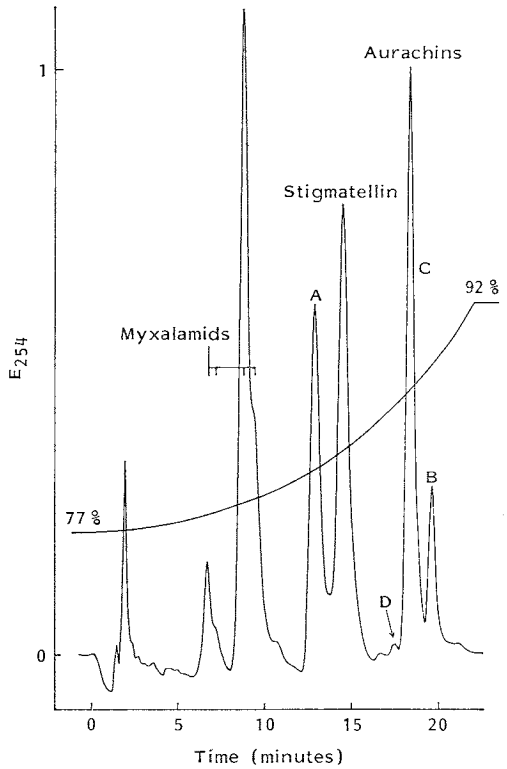
High performance liquid chromatography was used for the quantitative determination of the individual aurachins in cultures. Culture aliquots were first separated into cell mass and supernatant by centrifugation. The cell mass was then extracted with acetone, the broth with ethyl acetate, both under addition of a defined quantity of *n*-decyl benzoate as internal standard. Chromatography was performed on reversed-phase silica gel columns (Nucleosil RP-18, 5 μm, 25 cm×4 mm) with 0.5% acetic acid and a methanol - water gradient from 77% to 92% methanol as the mobile phase. The concentrations (C) of the antibiotics were calculated from the integrated peak areas (I) and the concentration (c) of the standard according to the equation:

$$C\ (\text{antibiotic}) = F \cdot \frac{I\ (\text{antibiotic})}{I\ (\text{standard})} \cdot c\ (\text{standard})$$

with the factor F=0.047 for aurachin A, 0.090 for aurachin B, 0.040 for aurachin C, and 0.110 for aurachin D. In addition to the aurachins the concentration of stigmatellin (F=0.030) and of myxalamids (F=0.205) also could be determined in the same HPLC run. Fig. 6 shows a HPLC profile of a cell mass extract of *S. aurantiaca* during fermentation.

Fig. 6. HPLC profile of a cell extract of *Stigmatella aurantiaca* strain Sg a15.

Chromatographic conditions: Column, 25 cm× 4 mm, with Nucleosil RP-18, 5 μm; solvent: MeOH - H_2O gradient from 77% to 92% MeOH with 0.5% acetic acid; flow rate: 2 ml/minute; detector: UV_{254}.



Biological Properties

As can be seen in Tables 3 and 4, the aurachins A~D were active against numerous Gram-positive bacteria and at higher concentrations also weakly against some fungi. In general, the aurachins C and D were more active than the aurachin A or B. Only some coryne-form bacteria were similarly sensitive to aurachin A and aurachin C. The inactivity of aurachin B in the agar-diffusion test may be due to the bad diffusion of this compound. Because of the structural analogy between aurachin C and 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO), which is an electron transport inhibitor of the respiratory chain[8], we tested the effect of aurachins on NADH oxidation in beef heart sub-mitochondrial particles. The preparation of the sub-mitochondrial particles and the performance of the experiments have been described previously[9]. For comparison NADH oxidation was also titrated with HQNO and stigmatellin. The results showed that the aurachins A, B, C and D block indeed NADH oxidation (Table 5).

Table 3. Antimicrobial spectrum of aurachins A, B, C and D.

Test organism[a]	Diameter of inhibition zone (mm)[b]			
	Aurachin A	Aurachin B	Aurachin C	Aurachin D
Bacillus brevis DSM 30	tr	0	13	10
B. cereus DSM 626	tr	0	12	11
B. megaterium DSM 32	0	tr	10	9
B. mycoides DSM 2048	0	tr	12	10
B. polymyxa DSM 36	0	tr	10	7
B. subtilis DSM 10	tr	tr	10	7
B. thuringiensis	0	8	14	10
Micrococcus luteus	9	tr	14	10
Staphylococcus aureus	9	8	8	9
Streptococcus faecalis	0	0	0	0
Arthrobacter aurescens DSM 20116	10	tr	12	10
A. simplex DSM 20130	10	tr	12	10
Brevibacterium linens DSM 20425	22	tr	15	14
B. ammoniagenes DSM 20306	23	tr	17	14
Corynebacterium fascians DSM 20131	13	tr	11	tr
C. glutamicum DSM 20300	(17)	0	14	14
Mycobacterium phlei	8	0	0	0
Nocardia corallina	11	0	tr	0
Streptomyces griseus DSM 40695	9	0	tr	14
S. fulvissimus DSM 40593	11	0	tr	tr
Enterobacter aerogenes	0	0	0	0
Escherichia coli K-12 DSM 498	0	0	0	0
E. coli K-12 W 945	0	0	0	0
Proteus mirabilis VI	0	0	0	0
Salmonella typhimurium	0	0	0	0
Candida albicans	0	0	0	0
Debaryomyces hansenii DSM 70238	0	0	0	0
Nadsonia fulvescens	0	0	tr	tr
Rhodotorula glutinis DSM 70398	0	(9)	tr	0
Saccharomyces cerevisiae GBF 36	8	0	0	0
Mucor hiemalis Tü 189	0	(10)	0	0
Polyporus sp. GBF 224	(8)	0	tr	tr
Rhizoctonia solani CBS 177.4	tr	tr	(8)	(8)

[a] The organisms were tested in standard complex media.
[b] The antibiotics (40 μg) were applied on paper discs of 6 mm diameter.
tr: Trace.

Table 4. Minimal inhibitory concentrations of aurachins A, B, C and D.

Test organism[a]	MIC (μg/ml)[b]			
	Aurachin A	Aurachin B	Aurachin C	Aurachin D
Bacillus subtilis	5	2.5	0.15	0.15
Staphylococcus aureus	2.5	1.25	0.39	0.39
Arthrobacter aurescens	0.19	0.78	0.19	0.19
Brevibacterium ammoniagenes	0.39	1.25	0.05	0.05
Corynebacterium fascians	1.56	1.56	0.78	0.78
Escherichia coli	>50	>50	>50	>50
Debaryomyces hansenii	50[c]	10[c]	10[c]	10[c]
Saccharomyces cerevisiae	50[c]	>50	0.19[c]	10[c]

[a] The organisms were grown in standard complex media.
[b] The minimal inhibitory concentrations were determined by the serial dilution assay.
[c] Inhibition was only incomplete. Remaining turbidity was up to 25%.

Discussion

The aurachins are a third group of new antibiotics produced by the myxobacterium, *Stigmatella aurantiaca* strain Sg a15. The previously described stigmatellin has a chromone structure[2] and the myxalamids are polyene-amide compounds[6]. The structure elucidation of the aurachins[7], which were discovered by ^{1}H NMR spectroscopy of cell extracts[2], revealed them to be new quinoline alkaloids. They are structurally related to 3- and 4-oxyquinolines. While aurachin C is related to 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) from *Pseudomonas aeruginosa*[10], no natural compounds are described, which are related to aurachin A or B. Only the mold product, viridicatin[11] contains a 3-hydroxyquinoline system, which originates *in vitro* and probably also *in vivo* from cyclopenin[11]. The time sequence of the appearance of the individual aurachins and preliminary experiments with ^{13}C-labeled aurachins suggest that aurachin D is the direct precursor of aurachin C, which is then transformed during fermentation into aurachins B and A. As HQNO, the aurachins A~D appear also to be potent inhibitors of the eukaryotic respiratory chain. The exact mechanism as well as the inhibitory effect on bacteria has, however, still to be determined. Although the three antibiotics of *S. aurantiaca* are structurally and biosynthetically completely different, they curiously seem to interfere all with eukaryotic respiration.

Table 5. The inhibition of NADH oxidation in beef heart sub-mitochondrial particles by aurachins*.

Antibiotic	Antibiotic concentration required for a 50%-inhibition (nM)
Aurachin A	71
Aurachin B	38
Aurachin C	42
Aurachin D	50
HQNO	494
Stigmatellin	15

* The antibiotics were dissolved in MeOH (maximum 2%). The test suspension (70 μg protein/ml) was preincubated with the antibiotic for 4 minutes before the reaction was started by the addition of NADH. The rate of NADH oxidation in the control without antibiotics was 1.6 nmol/minute/mg protein. Each value gives the average of 2~3 experiments.

Acknowledgments

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