Semisynthesis and Antiplasmodial Activity of the Quinoline Alkaloid Aurachin E

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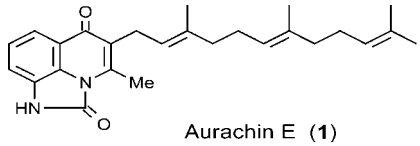
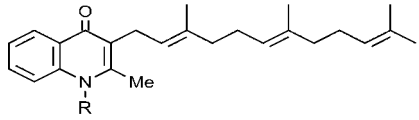
A one-step synthesis of the rare aurachin E (**1**) from the easily accessible aurachin C (**2**) and cyanogen bromide is described. 3-Bromocarbamoylquinoline (**5**) is formed in a side reaction with concomitant loss of the 3-farnesyl residue. In an alternative approach, aurachin D (**3**) was reacted with phosgene and sodium azide to form the imidazolone ring of **1** via *N*-acylation. Unexpectedly, the initial reaction occurred at the carbonyl group of **3** to give 1*H*-pyrrolo[3,2-*c*]quinoline **4**. The reaction sequence represents a novel route to this type of compound. Aurachin E, contrary to other aurachins, combines a high *in vitro* antiplasmodial activity with low cytotoxicity and absence of mitochondrial respiratory inhibition.

The aurachins form a large family of isoprenoid quinoline alkaloids isolated from the myxobacteria *Stigmatella aurantiaca*[1] and *Stigmatella erecta*.[2] Their biosynthesis has been investigated recently by a feeding study[1] and by cloning part of the biosynthesis gene cluster.[3] Whereas anthranilic acid and acetate were directly identified as precursors of the quinoline nucleus and the side chain of the predominant aurachins A−D, the formation of the minor aurachins followed directly from biosynthetic considerations.[1] Only aurachin E (**1**), with its extra imidazolone ring, did not fit in this scheme.



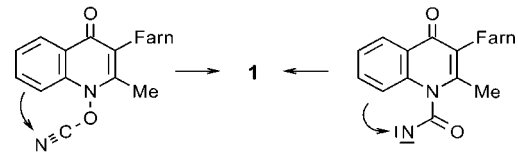
Aurachin E (**1**)



Aurachin C (**2**) R = OH
D (**3**) R = H

On the basis of the fact that feeding of anthranilic acid increased the production of aurachin C (**2**) up to 20-fold,[1b] 2,3-diaminobenzoic acid, the presumed precursor of **1**, was fed to *S. aurantiaca*. As this had no influence on the production of **1**, it was hypothesized that it might be derived from aurachin C (**2**) or D (**3**) by an unknown biosynthetic mechanism. Alternatively, it could not be excluded that **1** was an artifact formed during fermentation or isolation of the aurachins, e.g., via reactive intermediates such as **I** or **II** (Scheme 1). Particularly nitrene **II** was suspected as an intermediate, being formed from aurachin D (**3**), phosgene, and sodium azide. Both compounds may have been present during downstream processing of the fermenter: phosgene in the chloroform used for extraction and sodium azide used for equipment sterilization. The fact that 4-quinolones are attacked at the nitrogen by methyl and benzyl chloroformate[4] suggested this was a possibility. Indeed, **3** reacted smoothly with phosgene followed by sodium azide to give an 85% yield of a new compound, **4**, showing the bright blue fluorescence of **1**. However, its chromatographic behavior was

Scheme 1. Proposed Reactive Intermediates **I** and **II** in the Formation of Aurachin E (**1**) from Aurachin C (**2**) and Aurachin D (**3**), Respectively (Farn = farnesyl)



I **II**

different, and HRMS indicated the unexpected elemental composition $C_{25}H_{32}N_2$. Thus, in a formal sense, the oxygen of **3** ($C_{25}H_{33}NO$) had been replaced by nitrogen, and another double-bond equivalent introduced. As the NMR spectra showed only modifications at C-1′ to C-3′ of the farnesyl side chain, the azidocarbonyl group must have been introduced in the C-4 position, followed by insertion of the intermediate nitrene in the adjacent double bond to give an intermediate *N*-acylaziridine.[5] Rearrangement and extrusion of CO_2 eventually leads to pyrroloquinoline **4** (Scheme 2). Characteristic signals in the ^{1}H NMR spectrum of **4** are a sharp singlet at δ 6.47 for H-1′, a sextet at δ 3.04 for H-3′, and a doublet at δ 1.40 for H_3-13′. Moreover, all ^{1}H and ^{13}C NMR signals of the heterocyclic moiety are nearly identical with those reported for 2-butyl-4-methyl-1*H*-pyrrolo[3,2-*c*]quinoline.[6] Preliminary work indicated that this novel synthesis of pyrroloquinolines has broad applicability.[7] At this point, we became aware of a recent report on another case of exclusive *O*-acylation of 2-alkylquinolones with ethyl chloroformate.[8] Thus, depending on structural restraints and reaction conditions either *N*-[4] or *O*-acylation[8] as in our case is observed with 4-quinolones. The presence of pyridine or DMAP had no influence on the outcome of the reaction, and no trace of aurachin E (**1**) was detected.

Following an alternative approach via intermediate **I**, aurachin C (**2**) was treated with cyanogen bromide in CH_2Cl_2. A rapid reaction occurred, producing a complex mixture that on TLC showed the conspicuous fluorescent spot for **1**. After removal of some insoluble material by filtration, chromatographic separation on silica gel gave **1** in 14% yield, and in a later fraction 5% of **3**. The insoluble material (**5**) showed an IR band at 1812 cm^{-1} and liberated HBr when dissolved in methanol. After partition between ethyl acetate and buffer a neutral compound was obtained, which from its IR band at 1767 cm^{-1}, elemental composition $C_{11}H_8N_2O_2$, and NMR spectra was identified as the cyclic carbamate **7** ($\delta_{C=O}$ 153.1). From these results the original material must have been carbamoyl bromide, which on dissolution in DMSO or protic solvents rapidly cyclized to the hydrobromide of carbamate **7**. A

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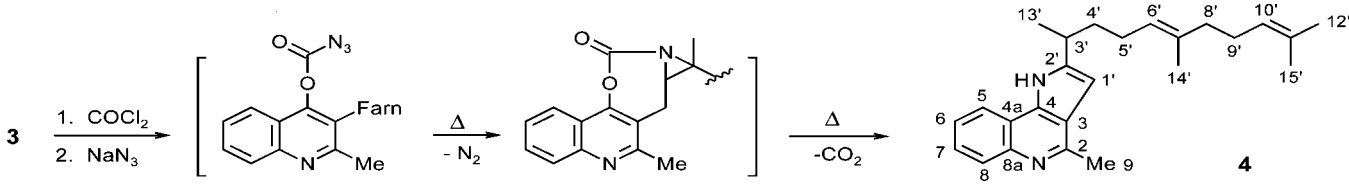
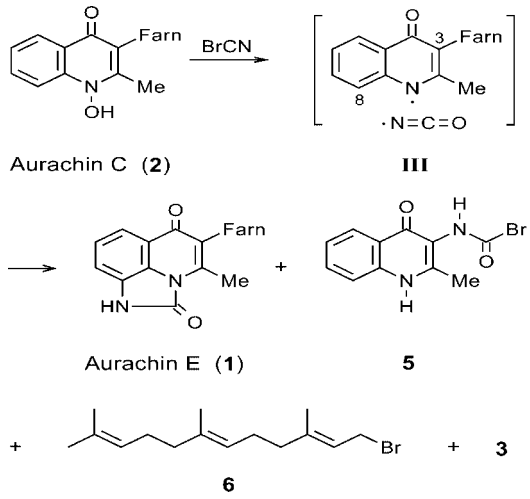
Scheme 2. Reaction of Aurachin D (**3**) with Phosgene and Sodium Azide (Farn = farnesyl)

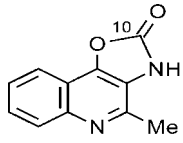


fourth compound observed in significant amounts in the proton NMR spectrum of the reaction mixture was identified as farnesyl bromide (**6**). The characteristic doublet at δ 3.95 and triplet at δ 5.50 for 1-H_2 and 2-H were in good agreement with the expected values,[15] and in the HPLC/MS a peak appearing at the end of the gradient showed negative molecular ions of **6** at *m/z* 281/283.

Scheme 3. Reaction of Aurachin C (**2**) with Cyanogen Bromide



Mechanistically these four compounds are derived from an intermediate radical pair **III**, which recombines through either C-8 or C-3 (Scheme 3). Whereas the 8-isocyanato intermediate cyclizes to give aurachin E (**1**), the corresponding C-3 isomer loses a farnesyl cation followed by addition of hydrogen bromide to give carbamoyl bromide **5**.[8] The latter on aqueous workup affords the cyclic carbamate **7**. The farnesyl cation reacts with bromide to give **6**, and in a side reaction, the quinolone radical in **III** abstracts a hydrogen atom from the solvent to give aurachin D (**3**).



Even though, the yield of **1** is low, gram amounts can now be produced in a single step from the easily accessible aurachin C (**2**)[1a] and introduced in biological tests. From early on antimalaria activity has been suspected for the aurachins.[11] A first *in vitro* screening against *Plasmodium falciparum* provided by the WHO (Geneva) showed good activity for aurachins C and E comparable to that of chloroquine and artemisinine (Table 1). Remarkably, the cytotoxicity of aurachin E (**1**) was also as low, and, in contrast to aurachins A−D, no inhibition of mitochondrial respiration nor antibacterial activity was observed. While the antiplasmodial activity of aurachins B−D may be ascribed to the inhibition of respiration of the malaria parasites, another mechanism must be operative with **1**. For unknown reasons, no *in vivo* activity was observed in a murine malaria model with *Plasmodium berghei* at 100 mg/kg, whereas chloroquine showed an ED_{90} of 2.8 mg/kg.[14] Nevertheless, investigations into the mechanism of action and SAR studies are needed to ascertain whether **1** is a useful lead for the development of an antimalarial drug.

Table 1. Inhibition of Mitochondrial Respiration, Cytotoxicity, and *in Vitro* Antiplasmodial Activity of Aurachins A−E, Chloroquine, and Artemisinine

aurachin	inhibition of respiration[a] IC_{50} (μg/mL)	cytotoxicity[b] IC_{50} (μg/mL)	antiplasmodial activity[c] IC_{50} (ng/mL)	
			W-2 clone	D-6 clone
A	28[d]	1.4	>12 000	>12 000
B	14[d]	3.2	36	78
C (**2**)	16[d]	1.8	26	0.90
D (**3**)	18[d]	1.3	370	250
E (**1**)	1100[e]	25	13	0.4
chloroquine	>8100[e]	14	35	1.2
artemisinine	>8100[e]	>40	0.43	1.1

[a] NADH oxidation was tested in submitochondrial particles of bovine heart as described previously.[1,15] [b] Serial dilutions of the compounds were incubated with L929 mouse fibroblasts in 96-well plates. Metabolic activities were measured after 5 days using a MTT assay.[16] [c] The activity was tested against *Plasmodium falciparum* Indochina W-2 and Sierra Leone D-6 clones according to a procedure described previously.[14] [d] Values were taken from ref 1. [e] Protein concentration was 46 μg/mL, and the rate of NADH oxidation in the control was 1.6 μmol·mg^{-1}·min^{-1}.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-2102 PC scanning spectrometer. IR spectra were measured on a Nicolet 20DXB FT-IR spectrometer. NMR spectra were recorded on Bruker ARX-400 and DMX-600 NMR spectrometers. EI and DCI mass spectra (reactant gas NH_3) were obtained on a Finnigan MAT 95 spectrometer, with high-resolution data acquired using peak matching (M/DM = 10000). HPLC/DAD/ESIMS: PE Sciex Api-2000. LC/MS; Nucleodur C18 column, 5 μm, 2 × 125 mm (Machery-Nagel) (0.3 mL/min CH_3CN/5 mM NH_4OAc buffer pH 5.5, isocratic 5:95 for 5 min, then gradient to 95:5 in 30 min). Analytical TLC: aluminum sheets silica gel Si 60 F_{254}; Merck, detection UV absorption at 254 nm. Cyanogen bromide was supplied by EGA Chemie, Steinheim, Germany.

4-Methyl-2-[(*E*)-1,5,9-trimethyldeca-4,8-dienyl]-1*H*-pyrrolo[3,2-*c*]quinoline (4). Aurachin D (**3**) (110 mg, 0.3 mmol) was dissolved in a solution of phosgene in toluene (1 mL, 20%, 2 mmol) and kept at room temperature for 24 h. Excess phosgene and solvent were evaporated in a stream of N_2, the residue was dissolved in acetone (2 mL), NaN_3 (130 mg, 2 mmol) was added, and the suspension was stirred at 80 °C for 24 h. The acetone was evaporated and the residue extracted with CH_2Cl_2. TLC of the extract indicated the presence of some educt **3** and a single more polar product. The reaction mixture was separated by flash chromatography on silica gel (3.3 g) with a CH_2Cl_2/MeOH gradient of 98:2 to 50:50. In addition to **3** (20 mg, 18%), **4** was obtained as a tan-colored oil (77 mg, 71%, based on recovered starting material 85%; TLC R_f = 0.33, CH_2Cl_2/MeOH, 9:1).

Compound 4: colorless solid; TLC, red-violet spot on staining with vanillin/H_2SO_4 followed by heating to 120 °C; UV (MeOH) λ_{max} (log ε) 210 (4.50), 250 (4.60), 256 (4.64), 284 (3.83), 328 nm (3.41); IR (KBr) ν_{max} 3427, 2963, 2924, 2853, 1594, 1569, 1515 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.90 (1H, br s, NH), 8.10 (1H, J = 7.5 Hz, d, J = 7.5 Hz, H-8), 8.02 (1H, d, J = 6.5 Hz, H-5), 7.49 (1H, t, J = 7.5 Hz, H-7), 7.40 (1H, t, J = 7.5 Hz, H-6), 6.47 (H, s, H-1′), 5.09 (H, m, H-6′, H-10′), 3.04 (1H, tq, J = 7.5 Hz, H-3′), 2.88 (3H, s, H_3-9), 1.90−2.10 (6H, m, H_2-5′, H_2-8′, H_2-9′), 1.60−1.90 (2H, m, H_2-4′), 1.67

(3H, br s, H_3-12′), 1.60 (3H, br s, H_3-15′), 1.52 (3H, br s, H_3-14′), 1.40 (3H, d, J = 7.5 Hz, H_3-13′); ^{13}C NMR/HMBC correlation ($CDCl_3$, 75 MHz) δ 153.8 (C-2/H-9, H-1′), 144.2 (C-2′/H-1′, H-3′, H-4′, H_3-13′), 143.0 (C-8a/H-7, H-8), 135.8 (C-7′/H_2-5′, H_2-8′, H_3-14′), 134.4 (C-4/H-5, H-1′), 131.5 (C-11′/H_2-9′, H_3-12′, H_3-15′), 128.5 (C-8/H-6), 126.3 (C-7/H-5), 124.9 (C-6/H-8), 124.3 (C-10′/H_2-8′, H_2-9′, H_3-12′, H_3-15′), 123.9 (C-6′/H_2-4′, H_2-5′, H_2-8′, H_3-14′), 120.9 (C-3/H-1′, H_3-9), 119.7 (C-5/H-7), 116.9 (C-4a/H-5, H-6, H-8), 99.0 (C-1′/H-3′), 39.7 (C-8′/H-6′, H-9′, H-10′, H_3-14′), 37.4 (C-4′/H-3′, H_2-5′, H_3-13′), 32.8 (C-3′/H-1′, H-4′, H-5′, H_3-13′), 26.6 (C-5′/H-3′, H-4′, H-6′), 25.7 (C-9′, C-12′/H_2-8′, H-10′, H_3-12′, H_3-15′), 22.3 (C-9), 20.9 (C-13′/H-3′, H_2-4′), 17.8 (C-15′/H_3-12′), 16.1 (C-14′/H-6′, H_2-8′); HRESI/MS $[M + H]^+$ m/z 361.2604 (calcd for $C_{25}H_{33}N_2$, 361.2644).

Reaction of 2 with Cyanogen Bromide. A solution of BrCN (0.6 g, 5.7 mmol) was added quickly to a solution of **2** (2.0 g, 5.3 mmol) in 30 mL of CH_2Cl_2 with rapid stirring. The color turned red-brown and faded within a few minutes, and a yellow precipitate formed slowly. After 30 min the suspension was filtered and the crystals were washed with CH_2Cl_2 to give 390 mg (37%) of carbamoylbromide **5**. The combined solutions were separated by flash chromatography on silica gel. Elution with a gradient of CH_2Cl_2/MeOH from 100:0 to 95:5 gave **1** (310 mg, 14%; TLC R_f 0.53, CH_2Cl_2/MeOH, 95:5) and in a later fraction yielded **3** (93 mg, 5%). Both compounds were identified by comparison with authentic samples.[1a]

Aurachin E (1): TLC, bright blue fluorescence on irradiation at 366 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.90 (1H, br s, NH), 7.86 (1H, s, J = 7.5 Hz, H-5), 7.36 (1H, t, J = 7.5 Hz, H-6), 7.23 (1H, d, J = 7.5 Hz, H-7), 5.00−5.10 (3H, m, H-2′, H-6′, H-10′), 3.41 (2H, d, J = 6.5 Hz, H_2-1′), 1.85−2.11 (8H, m, H_2-4′, H_2-5′, H_2-8′, H_2-9′, 2.82 (3H, s, H_3-10), 1.82 (3H, br s, H_3-13′), 1.65 (3H, br s, H_3-15′), 1.57 (3H, br s, H_3-14′), 1.56 (3H, br s, H_3-12′).

3-Bromocarbonylamino-2-methyl-1*H*-quinoline-4-one (5): pale yellow powder, mp 245 °C; IR (KBr) ν_{max} 3460 (m), 3400 (m), 3100 ← 2700 (s), 1812 (vs), 1667 (m), 1621 (m), 579 cm^{-1} (s).

4-Methyl-3*H*-oxazolo[4,5-*c*]quinoline-2-one (7). A fresh solution of **5** (20 mg) in 1 mL of methanol was evaporated at 30 °C to dryness. The residue was partitioned between pH 7 buffer and ethyl acetate. The organic phase was dried with $MgSO_4$ to give 11 mg of **7** as a colorless solid (TLC R_f 0.32, CH_2Cl_2/MeOH, 95:5; decomposition on attempted crystallization from organic solvents); UV (MeOH) λ_{max} (log ε) 228 (4.38), 316 (3.36), 338 (3.40); IR (KBr) ν_{max} 3200−2850, 1767, 1650, 1610 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.06, 8.02 (2H, d, J = 7.5 Hz, H-5, H-8), 7.72, 7.65 (2H, t, J = 7.5 Hz, H-6, H-7), 2.77 (3H, s, H_3-9); HPLC/ESIMS, $[M + H]^+$ m/z 201; EIMS m/z 200 M^+ (100%), 145 (18), 117 (16); HREIMS m/z 200.0584 (calcd for $C_{11}H_8N_2O_2$, 200.0586).

Hydrobromide of 7: ^{1}H NMR (DMSO-d_6 solution of **5**, 400 MHz) δ 8.25; 8.22 (2H, d, J = 7 Hz, H-5, H-8), 7.99; 7.87 (2H, t, J = 7 Hz, H-6, H-7), 2.48 (3H, s, H_3-9); ^{13}C NMR (DMSO-d_6 solution of **5**, 100 MHz) δ 153.1 (C-10), 148.4 (C-4), 142.4 (C-8a), 137.4 (C-2), 132.1 (C-8), 129.0 (C-7), 124.6 (C-3), 121.5 (C-6), 120.7 (C-5), 112.8 (C-4a), 17.4 (C-9).

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References and Notes

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