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NOTES

Chloroquinocin, a Novel Chlorinated Naphthoquinone Antibiotic from *Streptomyces* sp., *LL*-A9227

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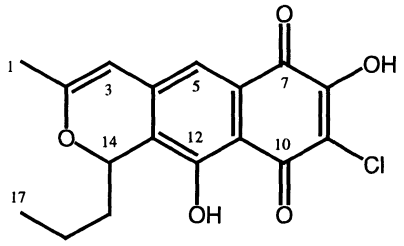
Synthesis investigations of naphthoquinone compounds have shown that chlorinations at certain positions can enhance *in vivo* antitumor activity[1] or increase therapeutic windows in treatment of fungal infection.[2] In natural products, the chlorinated naphthoquinone moieties were found in several members of naphthomycin group[3,4] as part of large ansa-macrolide structures, and in 3-chloro-plumbagin.[5] In our continuing search for lead compounds from natural sources for development of new antibiotics,[6,7] we examined culture *LL*-A9227, a strain of *Streptomyces*. A small aromatic antibiotic, chloroquinocin (**1**, Fig. 1), which contained a 2-chloro-3-hydroxy-naphtho-1,4-quinone moiety, was isolated from liquid cultures of the organism. In this paper, the production, isolation, structural elucidation, and antibiotic activity of **1** are reported.

Culture *LL*-A9227 was originally isolated in the 1950's from a soil sample collected near Litchfield Illinois, USA, and preserved through lyophilization. On Bennetts's agar, a plated culture of *LL*-A9227 changes in surface color from a cream to a reddish brown. Following initial growth, white to tan aerial mycelia and tan spores emerge over 14 days and the surface takes on a pitted appearance. The reverse on Bennett's exhibits a blackish brown color deepening over 14 days with an observable brown diffusable pigment.

A 7-day fermentation broth of *LL*-9227 was found to contain a new chlorinated quinone compound that showed activity against Gram-positive bacteria. Chromatography of the mycelial extract by reverse phase HPLC led to the isolation of chloroquinocin (**1**). The physico-chemical data of this compound are listed in Table 1, ^{1}H and ^{13}C NMR spectral data in Table 2, and circular dichroism (CD) spectrum is shown in Figure 2.

The molecular formula of chloroquinocin (**1**) was determined to be $C_{17}H_{15}ClO_5$ by high-resolution Fourier

Fig. 1. Structure of **1**.



1

Table 1. Physico-chemical data of **1**.

Appearance		red powder
Molecular formula		$C_{17}H_{15}ClO_5$
Molecular weight		334
ESIMS (neg, *m/z*)		333 (100%), 335 (33%)
HRFTICRMS (neg, *m/z*)	found	333.05303 $(M-H)^-$
	calcd	333.05352
UV λ_{max} (1:1 MeCN/H_2O, nm)		497, 433, 362, 295, 236
IR ν_{max} ($CHCl_3$, cm^{-1})		3415, 2956, 2932, 2872, 1647, 1604, 1559, 1314, 1284

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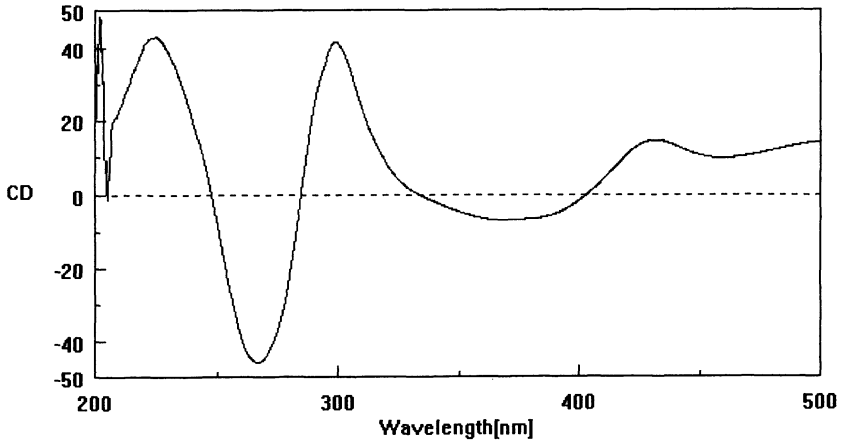
Table 2. ^{13}C and ^{1}H NMR spectral data of **1** in DMSO-d_6.

Atom	^{1}H (400 MHz, mult, J in Hz)	^{13}C (100 MHz)	HMBC (J = 8 Hz)
1	1.95 (s)	20.04 (CH_3)	C-2, C-3
2		157.93 (C)	
3	5.90 (s)	100.10 (CH)	C-1, C-2, C-4, C-5, C-13
4		138.97 (C)	
5	7.25 (s)	114.11 (CH)	C-3, C-4, C-7, C-11, C-13
6		132.27 (C)	
7		175.36 (C)	
8		155.66 (C)	
9		114.89 (C)	
10		184.58 (C)	
11		111.27 (C)	
12		155.92 (C)	
13		119.74 (C)	
14	5.55 (dd, 9.36, 2.96)	72.05 (CH)	C-2, C-13
15	1.44, 1.90 (m)	34.46 (CH_2)	
16	1.46 (m)	17.65 (CH_2)	C-15, C-17
17	0.92 (t, 7.05)	13.60 (CH_3)	C-15, C-16
OH	11.80 (s)[a]		C-11, C-12, C-13

[a]—Exchanged in D_2O.

Fig. 2. Circular dichroism spectrum of **1** in MeOH (0.15 mg/2 ml).



transform ion cyclotron resonance (FTICR) mass spectrometry. The ^{13}C NMR spectrum displayed signals of two carbonyls at δ 184.58 and 175.36, and ten olefinic (or aromatic) carbons between 100.10 and 157.93. The ^{13}C signals in the aliphatic region were assigned by a DEPT experiment to one oxygenated CH (δ 72.05), two CH_2's (20.04 and 34.46), and two CH_3's (17.65 and 13.60). In the ^{1}H NMR spectrum, a sharp signal at δ 11.80, exchanged in D_2O, was assigned to a phenolic OH.

Detailed analysis of 2-D NMR spectra, COSY, HMBC and HSQC, revealed a tricyclic naphthopyran system. The COSY spectrum delineated a homonuclear spin system from H-14 at δ 5.55 to H_2-15 at 1.90 and 1.44, to H_2-16 centered at 1.46, and to H_3-17 at 0.92. The 2- or 3-bond

Table 3. Antimicrobial activity of chloroquinocin (**1**).

Test organism	MIC (μg/ml)[a]
Bacillus subtilis	8
Staphylococcus aureus (Smith strain)	16
Staphylococcus aureus (methicillin-resistant)	16
Escherichia coli (imp)	64
Candida albicans	>128

[a] Microbroth dilution method in Mueller-Hinton II, incubated at 35 °C for 18 hours.

correlations in the HMBC spectrum (Table 2) between H_3-1 and C-2, and C-3, between H-3 and C-1, C-2, C-4, C-5, and C-13, between H-5 and C-3, C-4, C-11, and C-13, between 12-OH and C-11, C-12, and C-13, and between H-14 and C-2, and C-13 unambiguously established the benzopyran moiety. The allylic coupling between H_3-1 and H-3 in the COSY spectrum and NOEs between H_3-1 and H-3 and between H-3 and H-5 in a NOESY spectrum were observed as supporting evidence.

The remaining unaccounted elements were three oxygen, one chlorine, one hydrogen, and four carbon atoms. The latter were also indicated by quaternary ^{13}C NMR signals at δ 184.58, 175.36, 155.66, and 114.89 (broad). These data could best be assigned to a 2-chloro-3-hydroxy-naphtho-1,4-quinone moiety (note the change of numbering from **1**). In the HMBC spectrum, the strong 3-bond correlation between H-5 at δ 7.25 and the higher field keto carbon C-7 at 175.36 was observed, which placed the electron donating hydroxyl group at C-8, and therefore chlorine at C-9. The chemical shift data of the 12-OH at δ 11.80 and the keto carbon C-10 at 184.58 indicated that both these OH and C=O groups were associated with a hydrogen bond. The structural elucidation of **1** was thus completed.

Chloroquinocin (**1**) exhibited moderate *in vitro* activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*. Chloroquinocin showed weak activity for the Gram-negative bacterium *Escherichia coli* (imp) and no activity for the yeast *Candida albicans*. The minimum inhibition concentration (MIC) data obtained by the broth dilution method are listed in Table 3.

In summary, we have isolated and identified a new chlorinated naphthoquinone compound, chloroquinocin (**1**), from fermentation broth of *LL*-A9227. To our knowledge, the 2-chloro-3-hydroxy-naphtho-1,4-quinone moiety contained in this compound has not been reported previously in natural products. Its biological properties, other than antimicrobial activity, will be investigated.

Experimental

Fermentation of *LL*-A9227

Culture *LL*-A9227 was plated onto Bennett's medium from frozen stock and was incubated at 28°C until there was sufficient growth to inoculate into the first stage seed. A colony of the culture was transferred into six 25×150 mm tubes, containing 11 ml of seed broth each (10 g Difco glucose, 5 g Difco yeast extract, 20 g Difco soluble starch, 1 g $CaCO_3$, and 5 g NZ-Amine A, per liter distilled water). The tubes were shaken at 200 rpm, 28°C, 50% Rh, and a 50 mm throw for 4 days. The first stage seeds were then pooled and 10 ml portions were transferred to each of six 250-ml Erlenmeyer flasks containing 40 ml of culture broth (same as the first stage seeds). The second stage seeds were incubated for an additional 4 days. The combined second stage seeds were then used to inoculate (at 5.0%) six 2.8-liter Fernbach flasks, containing 1 liter of the production medium each (15 g Difco soluble starch, 50 g polyethylene glycol 8000, 1 g Difco bacto peptone, .01 g $MgSO_4 \cdot 7H_2O$, 0.5 g KH_2PO_4, MOPS at 50 mM, and 0.4 g Difco agar, per liter distilled water, pH 7.0). The fermentation was carried out under the same conditions as the first and second stage seeds for 7 days.

Isolation of **1**

The harvested fermentation broth (6 liters) was centrifuged at 3800 rpm for 30 minutes and the cell mass was extracted with methanol (2×2.5 liters). The combined solution was filtered through celite and evaporated under reduced pressure. A portion (51 g) of the total residue (170 g) was loaded onto a column containing LH-20 in methanol (350 ml bed volume). The column was then eluted with

methanol to obtain a pink band at 1.0~1.5 liters. The material (42.0 mg) from this fraction was separated by HPLC (YMC ODS-A column, 10×250 mm in size, 5 μm particle size), using a linear gradient of 60~100% acetonitrile/water containing 0.01% TFA in 20 minutes to obtain a broad peak centered at 11.0 minutes, which was further purified by HPLC, using a gradient of 85~100% methanol/water containing 0.01% TFA to afford chloroquinocin (**1**, 3.6 mg).

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