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3,4a-Dichloro-10a-(3-chloro-6-hydroxy-2,2,6-trimethylcyclohexylmethyl)-6,8-dihydroxy-2,2,7-trimethyl-3,4,4a,10a-tetrahydro-2*H*-benzo[*g*]chromene-5,10-dione

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Key indicators

Single-crystal X-ray study
T = 218 K
Mean σ(C–C) = 0.003 Å
R factor = 0.038
wR factor = 0.095
Data-to-parameter ratio = 18.1

For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

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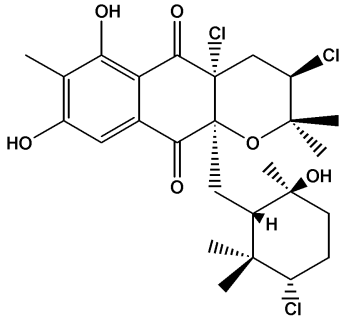
Antibiotic A80915C.

The title microbial antibiotic, $C_{26}H_{33}Cl_3O_6$, is an unusual trichlorinated dihydroquinone with cyclized prenyl and geranyl terpene substitution. The crystal structure provides an unambiguous assignment for this compound including its absolute stereochemistry, and shows the favored configuration of the chlorocyclohexane substituent.

Comment

In 1990, the Eli Lilly microbial natural products group (Fukuda, Mynderse & Raymond, 1990; Fukuda, Mynderse, Baker *et al.*, 1990) reported the isolation and antibiotic properties of a complex of chlorinated antibiotics, the A80915 complex, from a Palau Islands, soil-derived actinomycete, *Streptomyces aculeolatus*. The complex included various metabolites that were structurally related to a larger group of terpenoid dihydroquinones known as the napyradiomycins, isolated earlier by Gomi *et al.* (1987), Shomura *et al.* (1987) and Umezawa *et al.* (1995) from what appears to be the same species. These compounds were also isolated from other species (Hori *et al.* (1993; Shiomi, Iinuma *et al.*, 1986, Shiomi, Nakamura *et al.*, 1986, Shiomi *et al.*, 1987) isolated these compounds from other species. Whilst the three-dimensional structure of antibiotic A80915C has been reported (Fakuda, Mynderse, Baker *et al.*, 1990), and some NMR data were reported in a patent (Fukuda, Mynderse & Raymond, 1990), details of how the structure was assigned, complete NMR data, and the absolute stereochemistry of the antibiotic were never published.



(I)

As part of our interest in the isolation of new antitumor agents from marine-derived actinomycetes, we encountered a new obligate marine actinomycete strain (our strain CNQ-525) from bottom sediments (−500 m) collected offshore near San Diego, California. Cultivation of this bacterium under saline conditions resulted in a filtrate extract that was significantly cytotoxic toward HCT-116 human colon carcinoma (IC_{50} = 3.69 µg ml^{-1}). The potency of this extract was such that

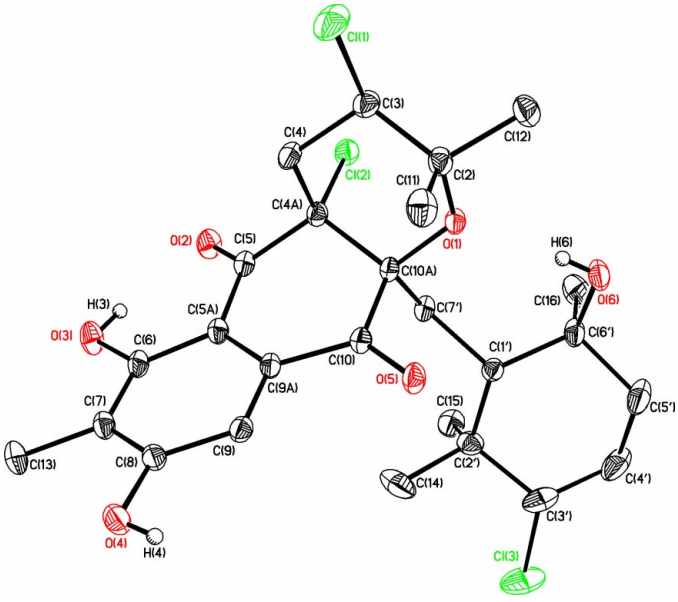


Figure 1
A view of (I), with ring-bound H atoms omitted for clarity. Displacement ellipsoids are drawn at the 30% probability level.

fractionation was undertaken and the isolation and purification of the major cytotoxic metabolite (I) was achieved. The purified dihydroquinone (I) showed significant cytotoxicity against HCT-116 colon carcinoma cells (IC_{50} = 4.22 μg ml^{-1}). Consistent with earlier reports (Fukuda, Mynderse & Raymond, 1990; Fukuda, Mynderse, Baker *et al.*, 1990), (I) showed antimicrobial activity against methicillin-resistant *Staphylococcus aureus* with a minimum inhibitory concentration of 1.9 μg ml^{-1} and vancomycin-resistant *Enterococcus faecium* (MIC = 3.9 μg ml^{-1}) in our assays.

The structure of (I) was unambiguously assigned by X-ray diffraction analysis. For X-ray studies, orthorhombic crystals of compound (I) were obtained from CH_3OH–CH_2Cl_2 (95:5) and recrystallized twice. The melting point of the compound is 456 K. The X-ray experiment fully defined the structure of (I), including its absolute stereochemistry (Fig. 1) The final structure (Fig. 1) clearly shows that the dihydronaphthoquinone is *cis* fused to the chlorotetrahydropyran ring. Furthermore, both the chlorotetrahydropyrone and chlorocyclohexane rings adopt chair conformations. The chlorine substituent at C4A is in the axial configuration, while the chlorine substituents at C3′ and C3 are equatorial.

Experimental

Marine actinomycete strain CNQ-525 was isolated using solid agar methods on a nutrient medium consisting of the following: 10 g starch, 4 g yeast extract, 2 g bacto-peptone, 18 g agar and 1 l sea water. The strain was cultured in the same medium (liquid) at 303 K for 9 d, and then extracted using Amberlite XAD7 resin (20–30 g l^{-1}) for 2 h. The resin was eluted with acetone (× 5) and the solvent was removed to generate the crude extract. The extract was then fractionated by C-18 column chromatography using H_2O, CH_3OH–H_2O (1:2), CH_3OH–H_2O (1:1), CH_3OH–H_2O (2:1), CH_3OH, EtOAc and CH_2Cl_2, to generate seven fractions. The fraction eluted with CH_3OH was triturated with CH_2Cl_2 and the CH_2Cl_2-soluble components were purified by HPLC on a C-18 column, eluting with H_2O–CH_3CN (1:9). Recrystallization from CH_3OH–CH_2Cl_2 (95:5) yielded pure antibiotic (I) as colorless orthorhombic crystals. The exact molecular weight of (I) was measured as 546.1342 atomic mass units by MALDI HRMS analysis. The optical rotation is $[\alpha]_D$ = −190° (*c*, 0.304 g 100 ml^{-1} $CHCl_3$) and $[\alpha]_D$ = − 86.4° (*c*, 0.318 g 100 ml^{-1} CH_3OH). The UV absorption is at 266 (ε = 21,500), 326 (ε = 8,800) and 357 nm (ε = 7799).

Crystal data

$C_{26}H_{33}Cl_3O_6$
M_r = 547.87
Orthorhombic, $P2_12_12_1$
a = 12.1542 (10) Å
b = 13.8359 (10) Å
c = 15.3928 (12) Å
V = 2588.5 (3) Å^3
Z = 4
D_x = 1.406 Mg m^{-3}
Mo *Kα* radiation
Cell parameters from 5719 reflections
θ = 2.6–28.2°
μ = 0.39 mm^{-1}
T = 218 (2) K
Block, colorless
0.40 × 0.25 × 0.15 mm

Data collection

Bruker SMART CCD area-detector diffractometer
φ and ω scans
Absorption correction: multi-scan (*SADABS*; Sheldrick, 1996)
T_{min} = 0.858, T_{max} = 0.943
19067 measured reflections
6052 independent reflections
5607 reflections with $I > 2\sigma(I)$
R_{int} = 0.033
θ_{max} = 28.0°
h = −15 → 15
k = −18 → 18
l = −19 → 18

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.038
$wR(F^2)$ = 0.095
S = 1.04
6052 reflections
334 parameters
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.051P)^2 + 0.3879P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.42 e Å^{-3}
$\Delta\rho_{min}$ = −0.25 e Å^{-3}
Absolute structure: Flack (1983), 2552 Friedel pairs
Flack parameter = −0.05 (4)

Table 1
Hydrogen-bonding geometry (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O3—H3···O2	0.79 (3)	1.91 (3)	2.617 (2)	149 (3)
O4—H4···O6^{i}	0.83 (3)	1.89 (3)	2.717 (2)	176 (3)
O6—H6···O1	0.76 (3)	1.96 (3)	2.717 (2)	172 (2)

Symmetry code: (i) $-x, y-\frac{1}{2}, \frac{3}{2}-z$.

H atoms attached to O3, O4 and O6 were located in Fourier difference maps and were allowed to refine with isotropic displacement parameters. All other H atoms were included at calculated positions (C—H = 0.94–0.99 Å) and refined as riding, with U_{iso}(H) set a 1.2 or 1.5 times U_{eq}(parent atom).

Data collection: *SMART* (Bruker, 1997); cell refinement: *SAINT* (Bruker, 1997); data reduction: *SAINT*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997*a*); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997*a*); molecular graphics: *SHELXTL* (Sheldrick, 1997*b*); software used to prepare material for publication: *SHELXTL*.

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