OXANTHROMICIN, A NOVEL 
ANTIBIOTIC 
FROM ACTINOMADURA 

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In the course of screening for novel antibiotics, 
a new solvent extractable antibiotic, oxanthromi-
cin, was isolated from an unusual Actinomadura 
sp. SCC 1646. Oxanthromicin exhibited good 
in vitro activity against dermatophytic fungi, as 
well as moderate activity against Candida albicans 
and Staphylococcus aureus.

The producing culture, SCC 1646, was chara-
terized by the formation of tan to light orange 
vegetative mycelia and abundant white aerial 
mycelia composed of long filaments with side 
branches fragmenting into non-motile spores. 
Good growth was observed on most rich organic 
media without the production of diffusible pig-
ments. Maximum growth occurred between 
28°C and 35°C. Glucose, sucrose and trehalose 
were utilized; hypoxanthine, tyrosine, casein and 
hippurate were hydrolyzed, while starch was not. 
Good growth occurred in the presence of 50 µg/ 
ml of benzylpenicillin, cephalothin, tetracycline 
and rifamycin. Hydrolyzed whole cells contained 
meso-diaminopimelic acid and madurose. 
The strain was identified as a species of Actino-
madura designated Actinomadura sp. SCC 1646.

The inoculum for antibiotic production was 
prepared in a medium containing: 0.3% beef 
extract, 0.5% Tryptone, 0.5% yeast extract, 0.1% 
Cerelose, 2.4% potato starch and 0.2% CaCO₃, 
in tap water. A 250-ml Erlenmeyer flask con-
taining 50 ml of this medium was inoculated with 
5 ml of a stock suspension of the antibiotic pro-
ducing culture. The flask was incubated at 35°C 
on a rotary shaker at 350 rpm for 96 hours. 

Twenty-five ml of this seed culture was transferred 
to a 2-liter Erlenmeyer flask containing 350 ml of 
the above medium and incubated as above. The 
entire contents were used to inoculate a 14-liter fermentor containing 10 liters of a medium con-
sisting of: 0.5% yeast extract, 0.5% NZ-Amine, 
2.5% Cerelose, 2% soluble starch, 0.4% CaCO₃, 
and 0.1% CoCl₂ (10⁻³ M), in tap water. The 
fermentation was carried out for 90 hours at 
35°C, with an air flow of 3.5 liters per minute, and 
an agitation rate of 350 rpm. Antibiotic pro-
duction was monitored by bioassay using C. 
albicans C43 and S. aureus 209P.

The whole broth from seven, 10-liter fermenta-
tions (70 liters) was extracted twice with equal 
 volumes of ethyl acetate. The extracts were con-
centrated to an oil, redissolved in a small volume 
of acetone, and precipitated with a mixture of 
ethyl ether - hexane (6: 4). The yellow precipi-
tate that formed was filtered and dried in vacuo. 
The resulting antibiotic complex, a yellow amor-
phous material (6.3 g), was differentiated from 
most other known antibiotics by paper and thin 
layer chromatography in numerous solvent sys-
tems. Silica gel thin-layer chromatography in a 
chloroform - methanol - water mixture (2: 2: 1, 
lower phase), followed by bioautography (against 
S. aureus and Trichophyton mentagrophytes), 
demonstrated that this crude complex consisted of 
at least three biologically active components. 
Component 3, oxanthromicin, whose Rf was 
closest to the origin, was the major component.

Isolation of oxanthromicin was accomplished 
by preparative high performance liquid chromat-
ography using a Waters PREP LC system 500A 
fitted with two 300 g silica gel cartridges. The 
columns were eluted with a chloroform - methanol - water mixture (2: 2: 1, lower phase). 
The fractions containing oxanthromicin were 
combined and precipitated as described above. 
From the 6.3 g of crude antibiotic complex, 
700 mg of pure oxanthromicin was isolated. 

Oxanthromicin (Fig. 1) is a yellow amorphous 
compound which decomposes between 211 ~ 
213°C and is soluble in methanol, ethanol, ethyl 
acetate and acetone but insoluble in ether, petro-
leum ether and water. Oxanthromicin has the 
following spectroscopic properties: UV maxima
Fig. 1. Structure of oxanthromicin.

(MeOH, HCl) 272 (ε 5,560), 312 (7,060), 358 nm (4,710) which in base (MeOH, NaOH) shifts to 257 (ε 8,630), 374 nm (9,810); IR (KBr) 3700 - 2800 (OH's), 1650 - 1620 (carbonyls), 1568 cm⁻¹ (C=C's); optical rotation [α]D -172.1° (c 0.3, EtOH). Additional spectroscopic data for oxanthromicin and several synthetic derivatives will be reported elsewhere along with X-ray diffraction studies on the tetra-O-methyl derivative.

Oxanthromicin is similar to the anthraquinone fungal metabolites emodin, endocrocin and eynodonthin. In addition, dimeric anthraquinones, such as rugulosin and skyrin, have been reported, but in these compounds dimerization is through a carbon-carbon bond. Oxanthromicin is the first dimeric anthrone peroxide reported.

In vitro broth dilution tests for fungi (Sabouraud dextrose broth, pH 5.6), or microtiter dilution tests for S. aureus (Mueller Hinton broth, pH 7.4), were performed to determine MICs. The results, shown in Table 1, indicate that oxanthromicin has broad-spectrum antifungal activity in vitro. The compound was more active at pH 6.8 than at pH 5.6. At pH 6.8 the geometric mean MICs against dermatophytes and Candida were 4.0 µg/ml (range 2 ~ 8 µg/ml) and 52.5 µg/ml (range 32 ~ 64 µg/ml), respectively. The addition of lipids increased activity against Candida (geometric mean MIC 18.0 µg/ml) but generally decreased activity against dermatophytes (geometric mean MIC 12.1 µg/ml). The addition of serum significantly reduced activity (MICs > 64 µg/ml), indicating high protein binding. The geometric mean MIC against 32 strains of S. aureus was 6.2 µg/ml (range 0.125 ~ 32 µg/ml). The addition of 4% bovine serum albumin increased the MICs against S. aureus to greater than 512 µg/ml, another indication of the high protein binding of oxanthromicin.

The activity of oxanthromicin in vivo was determined in male guinea pigs infected topically with T. mentagrophytes D-24.

Table 1. In vitro MICs (µg/ml) of oxanthromicin against various fungi.

<table>
<thead>
<tr>
<th>Organism</th>
<th>pH 5.6</th>
<th>pH 6.8a</th>
<th>pH 6.8 +lipidsb</th>
<th>pH 6.8 +serumc</th>
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<tbody>
<tr>
<td>Trichophyton mentagrophytes ATCC 22839</td>
<td>32</td>
<td>4</td>
<td>16</td>
<td>&gt;64</td>
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<tr>
<td>T. rubrum ATCC 10789</td>
<td>&gt;64</td>
<td>—</td>
<td>16</td>
<td>&gt;64</td>
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<tr>
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<td>4</td>
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<tr>
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<td>&gt;64</td>
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<td>8</td>
<td>16</td>
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<tr>
<td>Candida albicans (Burke) C40</td>
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<td>&gt;64</td>
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<td>&gt;64</td>
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<td>&gt;64</td>
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<td>C. albicans (Sparks) C42</td>
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<td>32</td>
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<td>&gt;64</td>
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</table>

a Buffered with 0.1 M phosphate buffer.
b Lipids consisted of (µg/ml): 12 cholesterol, 50 tripalmitin, 100 tristearin, 60 squalene, 60 stearyl olate.
c 10% horse serum/ml.
applied topically, as a 2% solution in 10% EtOH - 45% glycerol - 45% polyethylene glycol 400 twice daily for 10 days. Animals were cultured and lesions were scored every other day for 16 days. No improvement was noted in animals treated with oxanthromicin.

The intravenous LD$_{50}$ of oxanthromicin in male mice was 150 mg/kg.

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References


