Notes

OXANTHROMICIN, A NOVEL ANTIBIOTIC FROM *ACTINOMADURA*

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In the course of screening for novel antibiotics, a new solvent extractable antibiotic, oxanthromicin, 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 characterized 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 pigments. 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 *Actinomadura* designated *Actinomadura* sp. SCC 1646.

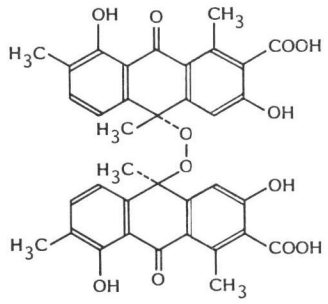
The inoculum for antibiotic production was prepared in a medium containing: 0.3% beef extract, 0.5% Tryptone, 0.5% yeast extract, 0.1% Cerelose, 2.4% potato starch and 0.2% $CaCO_3$, in tap water. A 250-ml Erlenmeyer flask containing 50 ml of this medium was inoculated with 5 ml of a stock suspension of the antibiotic producing 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 consisting of: 0.5% yeast extract, 0.5% NZ-Amine, 2.5% Cerelose, 2% soluble starch, 0.4% $CaCO_3$ and 0.1% $CoCl_2$ (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 production was monitored by bioassay using *C. albicans* C43 and *S. aureus* 209P.

The whole broth from seven, 10-liter fermentations (70 liters) was extracted twice with equal volumes of ethyl acetate. The extracts were concentrated to an oil, redissolved in a small volume of acetone, and precipitated with a mixture of ethyl ether - hexane (6: 4). The yellow precipitate that formed was filtered and dried *in vacuo*. The resulting antibiotic complex, a yellow amorphous material (6.3 g), was differentiated from most other known antibiotics by paper and thin layer chromatography in numerous solvent systems. 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 chromatography 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)[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, petroleum 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 $[\alpha]_D$ −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[1].

Oxanthromicin is similar to the anthraquinone fungal metabolites emodin[2], endocrocin[2] and cynodonthin[3]. In addition, dimeric anthraquinones, such as rugulosin[4] and skyrin[5], 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[6]. Oxanthromicin was

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

Organism	pH 5.6	pH 6.8[a]	pH 6.8 +lipids[b]	pH 6.8 +serum[c]
Trichophyton mentagrophytes ATCC 22839	32	4	16	>64
T. rubrum ATCC 10789	>64	—	16	>64
Microsporum canis ATCC 18615	64	4	4	>64
M. gypseum ATCC 10215	64	2	16	>64
Epidermophyton floccosum ATCC 15693	32	8	16	>64
Candida albicans (Burke) C40	>64	>64	32	>64
C. albicans (Collins) C41	>64	>64	8	>64
C. albicans (Sparks) C42	64	32	16	>64
C. albicans (Wisconsin) C43	64	32	16	>64
C. tropicalis ATCC 18526	64	32	16	>64
C. stellatoidea ATCC 11006	64	32	—	>64
C. parapsilosis ATCC 14054	32	>32	32	>64
Rhodotorula rubra C47	64	64	16	>64
Saccharomyces cerevisiae ATCC 9763	>64	>32	32	>64
Torulopsis glabrata ATCC 15545	64	>32	32	>64
Monosporium apiospermum ATCC 9258	64	32	32	>64
Aspergillus niger ATCC 16404	32	8	16	>64
Geotrichum candidum ATCC 18301	>64	32	32	>64

[a] Buffered with 0.1 M phosphate buffer.
[b] Lipids consisted of (μg/ml): 12 cholesterol, 50 tripalmitin, 100 tristearin, 60 squalene, 50 stearyl oleate.
[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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