HYDROHEPTIN: A WATER-SOLUBLE POLYENE MACROLIDE

II. CHEMICAL AND BIOLOGICAL PROPERTIES

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Hydroheptin, a new polyene macrolide antifungal antibiotic, is co-produced with the antibiotic, chartreusin, by a strain of Streptomyces chartreusis designated as IMRU 3962 isolated in our laboratory. The unique water-solubility of this antibiotic at neutrality, revealing in aqueous solution molecular dispersion and an ultraviolet-visible absorption spectrum characteristic of an all-trans heptaene chromophore, clearly distinguishes it from all previously-described and naturally-occurring heptaene macrolides. The isolation and identification of the amino sugar, mycosamine (3-amino-3,6-dideoxy-D-mannose), in acid hydrolysates of hydroheptin and the absence of an aromatic amine upon retrograde alkaline dealdolization of the molecule certainly characterize the antibiotic as a member of the non-aromatic heptaene macrolide group. Chromatographic and countercurrent distribution studies likewise support its novelty. With little or no demonstrable activity against bacteria, hydroheptin as compared to other non-aromatic heptaene macrolides exhibits excellent but somewhat less activity against a wide variety of yeasts and fungi. Likewise, its parenteral toxicity appears to be less than that of other heptaene macrolides.

In the previous report1 the production and isolation of a new water-soluble heptaene macrolide antibiotic, hydroheptin, by a strain of Streptomyces chartreusis was described. This report describes spectrophotometric, stability, solubility, chromatographic and countercurrent distribution studies as well as hydrolytic procedures to determine the presence of structural moieties often encountered in polyene macrolide molecules. Biological studies dealing with the in vitro antifungal activity and acute intravenous toxicity of hydroheptin are also described.

Materials and Methods

Antibiotic Preparations

The reference polyene macrolide samples came from the Waksman Institute of Microbiology collection.

Hydroheptin Solubility and Precipitation

The solubility of hydroheptin, as the free acid and its ammonium salt, was determined in protic (water, 0.05 M phosphate buffer-pH 7, methanol, ethanol, and water-saturated 1-butanol) and aprotic (acetone, carbon tetrachloride, ether, hexane, and dimethylsulfoxide) solvents. Saturated antibiotic solutions were centrifuged at 5,000 rpm for 15 minutes in order to determine the weight of undissolved residue. Solubility was calculated by difference in weights. The precipitation of hydroheptin prepara-

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tions was determined in various aqueous buffers. A stock solution consisting of 0.5 mg/ml of hydroheptin in distilled water was prepared. The precipitation of hydroheptin in 2 ml of stock solution was tested at different pH levels by adding 8 ml of the following 0.02 M buffers: KCl-HCl, pH 2; succinic acid - NaOH, pH 4; KH$_2$PO$_4$ - KOH, pH 6; and boric acid - NaOH, pH 9. Ammonium sulfate at a final concentration of 10% was then added to each tube. The resultant precipitate was separated by centrifugation at 5,000 rpm for 15 minutes. The supernatant and precipitate were analyzed spectrophotometrically for hydroheptin content.

Spectrophotometric Measurements

A purified ammonium salt of hydroheptin$^{17}$ ($E_{280}^{1%}$, 980, in methanol) was employed as a standard. Antibiotic preparations were prepared as solutions in water, methanol, or dimethyl sulfoxide at a concentration of 10 $\mu$g/ml. A Cary recording spectrophotometer (Model 14 M, Applied Physics Corp., Pasadena, Calif.) was employed for the spectrophotometric analysis in the ultraviolet-visible spectral region. For the determination of the infrared spectra of various hydroheptin preparations a Beckman infrared spectrophotometer (Model IR-8, Beckman Instruments, Fullerton, Calif.) was employed. The infrared spectra of different antibiotic preparations were determined in potassium bromide tablets.

Chemical Studies

(1) Elemental Analysis: Hydroheptin as the free acid ($E_{280}^{1%}$, 980) was used for the elemental analysis carried out by Schwartzkopf Microanalytical Laboratory, Woodside, New York.

(2) Alkaline Retrograde Dealdolization: The procedure$^{17}$ involved adding hydroheptin (5.0 mg) to 2 ml of 10% aqueous sodium hydroxide followed by heating the mixture in a boiling water bath for 15 minutes. After cooling, the reaction mixture was extracted twice with 2-ml aliquots of chloroform. The combined chloroform extracts were washed with distilled water. The presence of absorption near 318 nm was determined spectrophotometrically. The aromatic heptaene macrolide, candicidin, was used as a control.

(3) Acid Hydrolysis: A purified preparation of hydroheptin (5.0 mg) was dissolved in 5 ml of 1 N methanolic hydrochloric acid and the solution was refluxed for 20 minutes. After cooling, the reaction mixture was filtered, and the precipitate was washed with 5 ml of distilled water. The filtrate and washing were concentrated in vacuo to a syrupy liquid residue. Thin-layer chromatography of the residue on silica gel 60 plates (F-254, E. Merck, Darmstadt, Germany) was carried out with the developing solvent system consisting of 1-butanol - acetic acid - water (3: 1: 1, v/v/v). Spray reagents employed for the detection of amino and sugar-reducing groups were ninhydrin and anisaldehyde, respectively.

(4) Acetolysis: A procedure$^{17}$ was employed whereby the antibiotic (0.47 g) was dissolved in 3 ml of acetic acid with constant stirring with a magnetic stirrer. To the resultant solution 7 ml of acetic anhydride and 0.2 ml of concentrated sulfuric acid were added, and the reaction mixture was stirred for 4 days at room temperature. After subsequent completion of acetolysis the reaction mixture was diluted in ice water and stirred for approximately 15 minutes to hydrolyze excess acetic anhydride. The sugar peracetate was extracted from the reaction mixture by two volumes of chloroform, and the chloroform extract was washed with sodium bicarbonate to neutralize excess acetic acid. The washed chloroform solution was dried with anhydrous sodium sulfate and then concentrated to a small volume. Thin-layer chromatography of the sugar peracetate was carried out on silica gel G plates (Analtech, Inc., Newark, Del.) developed with ethyl acetate. An authentic sample of mycosamine peracetate was used as a control. The sugar peracetates were detected by spraying the plates with sulfuric acid - methanol (1: 1, v/v).

Liquid-Liquid Partition

Hydroheptin liquid-liquid partition studies were carried out with the solvent system, chloroform - methanol - 0.05 M buffer (2: 2: 1, v/v/v), adjusted to pH values from 2 to 10. Four ml of the upper phase and 5 ml of the lower phase of the solvent system were placed into test tubes whereupon 1 ml of hydroheptin stock solution was added. The antibiotic stock solution contained 6 $\mu$g/ml of hydroheptin ($E_{280}^{1%}$, 875) dissolved in the upper phase of the solvent system, chloroform - methanol - water (2: 2: 1, v/v/v). Another solvent system, consisting of pyridine - ethyl acetate - water (3.5: 6.5: 8.3,
v/v/v), was also employed in the comparisons of different heptaene macrolides with hydroheptin. Spectrophotometric analyses of the respective solvent phases after equilibration permitted the calculation of the partition (distribution) coefficient, $K$, expressed as the concentration of antibiotic in the upper phase divided by the concentration in the lower phase.

**Countercurrent Distribution**

For hydroheptin countercurrent distribution (CCD) studies the solvent system, chloroform - methanol - 0.01 m borate buffer, pH 8.3 (2: 2: 1, v/v/v) was employed. Two Craig countercurrent distribution apparatus (H. O. Post, Spectrum Medical Industries, New York, N.Y.) were employed. A 30-cell manual Craig distribution apparatus of 10 ml fixed lower phase volume and a 200-cell automatic apparatus of 40 ml fixed lower phase volume were used in these studies. A crude hydroheptin preparation containing chartreusin was dissolved in the upper phase of the solvent system. After filtration through Whatman #1 filter paper, 0.5 g of antibiotic was added to the first tube and 25 g were added to the first five tubes of the 30-cell and 200-cell apparatus, respectively. In the 30-cell unit each transfer cycle consisted of a 50-stroke equilibration phase and a 2-minute settling period whereas in the 200-cell unit the transfer cycle consisted of 20-strokes and a 20-minute settling period. After a total of 30 and 195 transfers were applied, respectively, aliquots from the upper and lower phases were obtained for the spectrophotometric analysis of hydroheptin and chartreusin content for the determination of the partition (distribution) coefficient, $K$. Recoveries of hydroheptin were made by pooling the contents of tubes 19~25 and tubes 132~149 in the 30-cell and 200-cell apparatus, respectively. The solvents were evaporated in vacuo to a slurry which was dissolved in water-saturated 1-butanol. After two washes with water the butanol phase was recovered and evaporated in vacuo to a second slurry from which hydroheptin was precipitated with the addition of an excess of diethyl ether. The precipitate was further washed with diethyl ether and dried under vacuum.

**Thin-Layer Chromatography**

Hydroheptin preparations were compared with related antibiotics by thin-layer chromatography on silica gel G plates, 250 nm thickness, (Analtech, Inc., Newark, Del.). The lower phase of the solvent system, chloroform - methanol - 2% aqueous ammonium hydroxide (2: 2: 1, v/v/v), was used to develop the plates at 50°C. Antibiotic components were detected spectrophotometrically.

**Biological Studies**

1. **Minimal Inhibitory Concentration (MIC):** The streak dilution method employing SABOURAUD-glucose agar was adapted for the determination of antimicrobial activity. The assay plates were incubated at 28°C for 24 hours. The concentration of antibiotic that visually gave no growth or rudimentary growth was taken as the minimal inhibitory concentration.

2. **Acute Toxicity:** The toxicity of hydroheptin was determined by the intravenous route with CF-1 male albino mice of 20-g body weight (Carworth Farms, New York, N.Y.). Hydroheptin as the ammonium salt ($E_{1\text{max}}$ 980) dissolved in sterile 5% aqueous glucose was employed in these studies. The test animals were injected in the lateral tail veins with 0.5 ml of the antibiotic solutions. The animals were observed for a 10-day period, and the LD$_{50}$ value was calculated.

3. **Cholesterol Reversal of Antifungal Activity:** The interference of hydroheptin antifungal activity by cholesterol was tested by the method of ZYGUMONT and TAVORMINA. $Saccharomyces cerevisiae$ ATCC 9763, was grown in SABOURAUD-glucose broth, adjusted to pH 6.5. Different levels of cholesterol dissolved in ethanol were added to the culture media along with 1 $\mu$g/ml of hydroheptin before inoculation with the test organism. The resultant broths were incubated for 18 hours with continuous shaking at 28°C. Turbidity was measured with a Klett-Summerson colorimeter (filter 66) and the amount of cholesterol which restored growth was determined.

**Results and Discussion**

**Characterization of the Antibiotic**

1. **Solubility and Precipitation**

The unique water solubility of hydroheptin at neutral pH clearly differentiates it from other
naturally-occurring polyene macrolide antifungal antibiotics described to date. As an acidic compound hydroheptin is relatively insoluble in water but readily forms water-soluble salts upon interaction with bases. The ammonium salt of hydroheptin exhibits solubility in water at 100 mg/ml. The free acid is soluble in lower alcohols and dimethyl sulfoxide whereas it is insoluble in most aprotic solvents. In aqueous solution but not in alcoholic solution hydroheptin is readily precipitated by ammonium sulfate at all pH levels tested, pH 2–9, but it is not precipitated by sodium chloride or sodium sulfate. Hydroheptin free acid is readily precipitated from aqueous solutions of hydroheptin salts by acidification to pH 2.

(2) Spectroscopy

Based on a most characteristic ultraviolet-visible absorption spectrum, hydroheptin was initially clearly identified as a heptaene macrolide belonging to the large candicidin (aromatic)-amphotericin B (non-aromatic) group of antifungal antibiotics. The ultraviolet-visible spectra of hydroheptin ammonium salt obtained in methanol, water, and dimethyl sulfoxide are given in Fig. 1. The comparable spectra in the three solvents indicate that the antibiotic as a salt indeed forms a true solution in water with molecular dispersion. Although the N-acylated and esterified derivatives of the polyene macrolides also exhibit improved solubility in water as salts, their ultraviolet-visible absorption spectra reflect poor and moderate molecular dispersion, respectively, indicating that in water the salts of these biologically-active derivatives still exist as colloidal micelles rather than as true solutions. Amphotericin B methyl ester hydrochloride has been reported to exhibit water solubility in excess of 75 mg/ml but a comparison of $E_{\text{max}}$ values of this derivative in water and in 90% ethanol reveals a 50% difference in magnitude.

The ultraviolet-visible spectrum of hydroheptin, like that of amphotericin B, is characteristically that of an all-trans heptaenic chromophore. It is evident that the peak of the highest wave length (405 nm) exhibits maximal absorption as compared to the second absorption peak (381 nm) characteristic of heptaene macrolide molecular dispersion in water. The ultraviolet-visible absorption spectrum of hydroheptin ammonium salt in aqueous solution changes significantly with time as is shown in Fig. 2. With incubation in the dark of the antibiotic solution at 29°C the characteristic all-trans heptaene chromophore gradually changes to a cis-trans chromophore by isomerization of the conjugated double bonds. This is shown by the reduced absorption of the first peak of maximum intensity at 405 nm and the relative increased absorption of the adjacent second peak at 381 nm which then
becomes maximum in intensity. The overall degradation of the antibiotic is indicated by the general decrease in the heights of each of the absorption peaks of the ultraviolet-visible spectrum.

For structural information and for an analysis of the functional groups associated with hydroheptin the infrared spectra of the ammonium salt and free acid of hydroheptin were examined in KBr tablets (Fig. 3). The polyene macrolide nature of hydroheptin is confirmed by an absorption band at 2900 cm\(^{-1}\). This indicates symmetric stretching characteristic of olefinic methylene groups. The presence of lactone or ether groups is suggested by the bands at 1050 cm\(^{-1}\) and 1260 cm\(^{-1}\). Although comparatively lower than expected of a free hydroxyl group (3600 cm\(^{-1}\)) the observed band at 3400 cm\(^{-1}\) indicates O-H stretching. The N-H stretching vibrations also may give rise to the observed absorption in the same region as OH, but the band of medium intensity at 300 cm\(^{-1}\) and the broad, diffused absorption at 840 cm\(^{-1}\) are distinguishing features for the presence of an amino group. An interesting absorption band at 1700 cm\(^{-1}\) is typical of that of a carboxylic group, particularly a carboxylic dimer. Only the acid form of the antibiotic revealed this band whereas the antibiotic salt produced absorption bands characteristic of the symmetric stretching vibration of a carboxylate. Both the acid and the salt form of the antibiotic revealed absorption bands at 1010 cm\(^{-1}\) indicating trans molecular geometry. Degraded antibiotic revealing a cis-trans ultraviolet-visible absorption spectrum also revealed on infrared analysis rather diffuse bands at this wave length, confirming cis stereo-chemical configuration.

(3) Chemical Studies

The elemental analysis of hydroheptin free acid (Found: C, 58.84; H, 7.82; N, 1.14; O, 29.82) reveals carbon, hydrogen and oxygen levels suggestive of the polyketide nature of polyene macrolides.
The low nitrogen content also suggests that hydroheptin lacks the aromatic amine characteristic of the aromatic heptaene macrolides. This is further confirmed by the alkaline retrograde dealdolization studies where no release of an aromatic amine, either p-aminoacetophenone or N-methyl-p-aminoacetophenone, could be detected.

Acid hydrolysis of hydroheptin followed by thin-layer chromatographic analysis of the hydrolysate clearly revealed the presence in small quantities of a ninhydrin-positive, anisaldehyde-positive component suggestive of an amino sugar. Acetolysis studies with hydroheptin clearly revealed the presence of the amino sugar, mycosamine (3-amino-3, 6-dideoxy-D-mannose), in the molecule. The product isolated from acetolysates of hydroheptin was identified as mycosamine peracetate based on thin-layer chromatography in comparison with an authentic sample. Both were superimposable in thin-layer chromatograms.

(4) Liquid-Liquid Partition and Countercurrent Distribution Studies

The results of the spectrophotometric, alkaline retrograde dealdolization and acetolysis studies clearly place hydroheptin in the non-aromatic group of heptaene macrolide antifungal antibiotics, including amphotericin B, candidin, mycoheptin, and tbilimycin. Considering the need for differentiation, liquid-liquid partition studies employing the solvent system, pyridine - ethyl acetate - water (3.5: 6.5: 8.3, v/v/v) revealed the following partition coefficients for non-aromatic heptaene macrolides: hydroheptin, 0.92; amphotericin B, 0.57 and candidin, 0.48. Mycoheptin and tbilimycin were not available for comparison.

In preparation for countercurrent distribution studies the partition coefficient of hydroheptin was determined in the solvent system composed of chloroform - methanol - 0.05 M buffer (2: 2: 1, v/v/v) adjusted to pH values of 2~10. The curve representing antibiotic partition coefficient, $K$, versus pH is given in Fig. 4. The partition coefficients were low at acid pH, increasing with higher pH levels to a maximum value of 5.3 at pH 9. Hydroheptin thus exhibited greater solubility in the lower organic phase at acidic pH whereas solubility in the upper aqueous phases increased with increasing pH.

With the solvent system, chloroform - methanol - 0.01 M borate buffer, pH 8.3 (2: 2: 1, v/v/v), a preliminary countercurrent distribution study with a crude hydroheptin preparation containing chartreusin was carried out in a 30-cell apparatus followed by a study in a 200-cell automatic apparatus. The partition distribution coefficients, $K$, for hydroheptin in the 30-cell and 200-cell study, were 2.75 and 2.70, respectively, whereas those of chartreusin were 0.27 and 0.28, respectively. The distribution curve for crude hydroheptin after 195 transfers is presented in Fig. 5. The theoretical curve of an ideal solute has been fitted to the experimental curve for hydroheptin. It is evident that the fit is imperfect.

It is interesting to note that the hydroheptin recovered from the 30-cell countercurrent distribution study (6 hours) retained the all-trans stereochemistry in the heptaene chromophore whereas that isolated from the study (4 days) in the 200-cell apparatus exhibited ultraviolet-visible absorption characteristic of the cis-trans configuration of the chromophore of degraded hydroheptin.

The non-aromatic heptaene macrolide antifungal antibiotic, tbilimycin, is also produced by a chartreusin-producing streptomycete. The reported partition coefficients for tbilimycin and chartreusin in the chloroform - methanol - 0.01 M borate buffer, pH 8.3 (2: 2: 1, v/v/v) solvent system are 1.93 and 0.30, respectively. Hence, the partition coefficients for hydroheptin and tbilimycin are significantly different whereas those of chartreusin are similar in the two independent studies. Thus chartreusin, serving as an internal standard, gives credence to the differences in the partition coefficients of
both antibiotics although these values were determined independently. Hydroheptin and tbilimycin also differ in their solubility in water and their ultraviolet-visible absorption spectra. Tbilimycin has been reported to be insoluble in water and to exhibit ultraviolet-visible absorption at 450 nm.

The partition coefficients of the other non-aromatic heptaene macrolides in the same solvent system were reported to be as follows: candidin, 3.4; mycoheptin, 4.2; and amphotericin B, 2.6. Although the partition coefficients of hydroheptin and amphotericin B are very similar in this solvent system, their partition coefficient in the solvent system, pyridine - ethyl acetate - water (3.5: 6.5: 8.3, v/v/v) are 0.92 and 0.57, respectively. Hydroheptin and amphotericin B significantly differ also in their water solubility at neutrality.

(5) Thin-Layer Chromatography

The solvent system employed for thin-layer chromatography of most polyene macrolides consists of the lower phase of the solvent system, chloroform - methanol - water (2: 2: 1, v/v/v). With hydroheptin this solvent system produced on silica gel G plates pronounced tailing and streaking. The addition of 2% ammonium hydroxide eliminated the problem. Purified hydroheptin preparations thus revealed two major components of Rf values 0.19 and 0.22 on the silica gel G plates. Spectrophotometric analysis of both components revealed that the former exhibited all-trans chromophore stereochemistry whereas the latter exhibited a cis-trans configuration seen in degraded hydroheptin solutions. It is interesting to note that thin-layer chromatographic analysis of hydroheptin recovered from the countercurrent distribution study in the 200-cell apparatus revealed the presence of 4 components, Rf values 0.19, 0.20, 0.22 and 0.25. The first component of Rf 0.19 exhibited an ultraviolet-visible
spectrum characteristic of an all-trans chromophore, whereas the remaining three components exhibited cis-trans spectra. It is evident that the prolonged exposure of hydroheptin to the solvent system led to degradation. With this thin-layer chromatographic procedure the RF values for amphotericin B and chartreusin were 0.17 and 0.63, respectively. Candidin produced two spots of RF values 0.14 and 0.18.

Biological Studies

(1) In Vitro Antimicrobial Activity

The minimal inhibitory concentration (MIC) of hydroheptin was determined by streak dilution assay employing a variety of yeasts, fungi and bacteria. The results of these studies are presented in Table 1. Hydroheptin was found to be primarily antifungal with little or no observed activity against the bacteria. Hydroheptin exhibits minor activity against Mycobacterium smegmatis. It also appeared from these preliminary studies that the yeasts are more sensitive to hydroheptin than are the filamentous fungi. In comparing the in vitro activity of hydroheptin with other polyene macrolides against the yeast, Saccharomyces cerevisiae, it is seen in the results presented in Table 2 that the activity of hydroheptin is comparable to that of the tetraene macrolide nystatin but not to that of the heptaene macrolides, amphotericin B or candididin, or of the hexaene macrolide, mediocidin. It appears that as a non-aromatic heptaene macrolide, hydroheptin exhibits lower in vitro activity than other members of this group. It is interesting to observe that the activity of hydroheptin is comparable to that reported \(^{10}\) for N-acylated heptaene macrolides which also exhibit reduced activity when compared to the parent compound and increased water solubility as salts. Nevertheless, the observed true solubility of hydroheptin salts in water would suggest some other modification of heptaene macrolide structure.

(2) Acute Toxicity

The acute intravenous toxicity of hydroheptin, expressed as LD\(_{50}\), was found to be 17.5 mg/kg. Hence, hydroheptin appears to be less toxic than reported \(^{11}\) for amphotericin B, LD\(_{50}\) = 4.5 mg/kg, and nystatin, LD\(_{50}\) = 3 mg/kg.

Table 1. Antimicrobial activity of hydroheptin.

<table>
<thead>
<tr>
<th>Organism*</th>
<th>Minimal inhibitory concentration (MIC) (\mu g/ml)</th>
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<tbody>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
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<tr>
<td>Saccharomyces cerevisiae ATCC 9763</td>
<td>1.0</td>
</tr>
<tr>
<td>Candida albicans IMRU 204</td>
<td>1.0</td>
</tr>
<tr>
<td>Rhodotorula sp. PP 41</td>
<td>0.5</td>
</tr>
<tr>
<td>Aspergillus niger ATCC 10254</td>
<td>4.0</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
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<td>Mycobacterium smegmatis IMRU 24</td>
<td>80</td>
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<tr>
<td>Serratia marcescens IMRU 70</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bacillus cereus ATCC 12480</td>
<td>&gt;100</td>
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<tr>
<td>Sarcina lutea IMRU 14</td>
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<tr>
<td>Bacillus subtilis ATCC 7972</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 6538P</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Escherichia coli PP 01</td>
<td>&gt;100</td>
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</tbody>
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* ATCC indicates American Type Culture Collection; IMRU, Waksman Institute of Microbiology, Rutgers University; PP, Pilot Plant, Waksman Institute of Microbiology, Rutgers University.

Table 2. Antimicrobial activity against Saccharomyces cerevisiae ATCC 9763 of hydroheptin as compared with other known polyene macrolides.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Minimal inhibitory concentration (MIC) (\mu g/ml)</th>
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</thead>
<tbody>
<tr>
<td>Hydroheptin</td>
<td>1.0</td>
</tr>
<tr>
<td>Candididin</td>
<td>0.06</td>
</tr>
<tr>
<td>Mediocidin</td>
<td>0.06</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.18</td>
</tr>
<tr>
<td>Nystatin</td>
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</table>
(3) Cholesterol Reversal of Antifungal Activity

As with most polyene macrolides, the antifungal activity of hydroheptin could be related to its affinity for sterol components in the cell membrane of susceptible microorganisms. This is supported in part by the reversal of hydroheptin antifungal activity by the addition of cholesterol to the medium. The addition of 50 μg cholesterol was needed to neutralize the activity of 1 μg of hydroheptin, corresponding to a molar ratio of 120 to 1, respectively.

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References