New Antibacterial Agent (U-24,544) Isolated from
Streptomyces griseus
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Antibiotic U-24,544 is a new agent isolated from the culture broth of a streptomy-cete strain. The antibiotic inhibits a variety of gram-positive and gram-negative bacteria in vitro, but is ineffective in treatment of experimental bacterial infections in mice. It is fairly cytotoxic in mammalian cell cultures and remarkably nontoxic in mice.

Antibiotic U-24,544 is a new antibacterial agent synthesized by a new variety of Strepto-myces griseus to be described elsewhere. This paper describes the preparation, isolation, and characterization of this new agent.

MATERIALS AND METHODS

Fermentations were conducted under submerged culture conditions in 500-ml shaken flasks in a medium containing: sucrose, 20 g/liter; soybean meal, 20 g/liter; CaCO3, 5 g/liter; tap water to 1 liter; pH adjusted to 7.2 with NaOH before sterilization; 100-ml portions of medium were added per flask.

Antibiotic production was measured by a microbiological disc-plate assay procedure (1) with Bacillus cereus as the test organism. A log dose-response curve was linear over a concentration range of 1 to 20 μg/ml. Peak titers were usually obtained after 2 days of incubation at 28 C.

For extraction purposes, larger quantities of fer-mentation broth were prepared in a fermentation tank with a capacity of 5,000 liters. The medium used was the same as described for shaken flasks. The tank was operated at an impeller speed of 166 rev/min, aeration was 2,000 liters/min, and temperature was maintained at 25 C during the fermentation cycle. Peak titers were attained after 2 days of incubation.

In vitro and in vivo antibacterial activities were assessed by the methods of Lewis et al. (2).

Antifungal tests were done on agar plates. Antibiotic was dissolved in fungal spectrum agar to give concentrations of 1,000, 100, 10, or 1 μg/ml. Plates were inoculated by a cross-streak technique. Results are expressed as minimal inhibitory concentrations of antibiotic yielding total inhibition of fungal growth.

Inhibition of KB cell growth was tested by the method of Smith et al. (3).

A partition column was prepared by suspending 120 g of buffered Dicalite in sufficient toluene half-saturated with propylene glycol (see below) to form a thick slurry. Propylene glycol (40 ml) was added with stirring. The mixture was then poured into a glass column (2.45 cm diameter and packed under pressure (4 psi). A layer of sea sand (0.635 cm thick) was applied on top of the column bed. Toluene, approximately half-saturated with propylene glycol, was prepared by mixing 4 ml of propylene glycol with 3,785 ml of toluene. This mixture was used as eluent for the partition column. Buffered Dicalite as used for the preparation of the column bed was prepared by adding 100 ml of an aqueous solution of KH2PO4 (27.2 g/liter) and 100 ml of a NaH2PO4 solution (28.4 g/liter) to 100 g of Dicalite while stirring vigorously. The mixture was subsequently dried thoroughly at 120 C.

RESULTS AND DISCUSSION

Isolation of antibiotic U-24,544. Antibiotic U-24,544 was isolated by the following proce-dure. A fermentation broth (5,000 liters) was filtered at harvest pH, and the filtrate was extracted with an equal volume of ethyl acetate. The extract was washed with a small amount of water and concentrated under reduced pressure (< 40 C) to a volume of 5,000 ml. A 700-g portion of this oily concentrate was poured into 7,000 ml of cyclohexane with good mixing. Precipitated material was collected by filtration and dissolved in 2,100 ml of ethyl acetate. The solution was filtered, solids were discarded, and filtrate was evaporated to dryness under reduced pressure at < 40 C. This residue was dissolved in 420 ml of methylene chloride, the solution was filtered and the solids were discarded. The filtrate was again evaporated to dryness under reduced pressure to give 146 g of dry material.

Further purification was achieved by sub-jecting this material to column partition chromatography. A 2.2-g sample was dissolved in 7.3 ml of propylene glycol by heating on a steam bath while stirring. This solution was then mixed with 15 g of buffered Dicalite, diluted with a minimal amount of toluene half-saturated with propylene glycol, and applied onto the partition
column prepared as described in Materials and Methods. A layer of sea sand (0.635 cm thick) was placed on top of the column, and elution was started with the toluene-propylene glycol eluent at a flow rate of 2 ml/min. Fractions of 20 ml were collected. Portions (0.1 ml) of each third fraction were diluted with 9.9 ml of ethyl alcohol. Filter paper discs (Schleicher and Schuell, no. 740-E) were dipped into these solutions, and discs were dried in warm air. These discs were then placed on an agar tray seeded with *B. cereus*. The tray was incubated overnight, and resulting zones of inhibition served as a rough measure to localize antibacterial activity within fractions.

*Chemical characterization of antibiotic U-24,544.* Crystalline antibiotic U-24,544 is colorless. The ultraviolet spectrum determined in ethyl alcohol showed inflections at 221 (ε = 22,706) and 255 mε (ε = 5,107) and absorption maxima at 262 (ε = 4,626), 269 (ε = 4,064), 303 (ε = 8,853), and ∼350 mε (ε = 173) (Fig. 2). The infrared spectrum of antibiotic U-24,544 in mineral oil suspension is shown in Fig. 3. The antibiotic has a specific rotation (α) _D_ of −82° (ε = 1.35 in ethyl alcohol) and melts at 214 to 217°C. Its solubility is greater than 100 mg/ml in methylene chloride; greater than 10 mg/ml in ethyl acetate, and is less than 100 μg/ml in methanol.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Minimal inhibitory concn</th>
<th>μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 26</td>
<td></td>
<td>125</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> PCI 602</td>
<td></td>
<td>125</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> ATCC 8427</td>
<td></td>
<td>250</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 9027</td>
<td></td>
<td>1,000</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> UC-263</td>
<td></td>
<td>250</td>
</tr>
<tr>
<td><em>S. pullorum</em> MSDH 75</td>
<td></td>
<td>62.5</td>
</tr>
<tr>
<td><em>S. typhosa</em> MSDH TG3</td>
<td></td>
<td>125</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> UC-76</td>
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<td>250</td>
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<tr>
<td><em>S. aureus</em> UC-70</td>
<td></td>
<td>250</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em> ATCC 6057</td>
<td></td>
<td>125</td>
</tr>
<tr>
<td><em>S. hemolyticus</em> C-203</td>
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<td>250</td>
</tr>
<tr>
<td><em>S. viridans</em> UC-155</td>
<td></td>
<td>250</td>
</tr>
</tbody>
</table>

**Table 1. In vitro antibacterial activity of antibiotic U-24,544 in Brain Heart Infusion broth**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Minimal inhibitory concn</th>
<th>μg/ml</th>
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<tbody>
<tr>
<td><em>Nocardia asteroides</em> UC-2052</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Blastomyces dermatitidis</em> UC-1911</td>
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<tr>
<td><em>Coccidioides immitis</em> UC-1119</td>
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<td>&gt;1,000</td>
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<tr>
<td><em>Geotrichum</em> sp. UC-1207</td>
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<td>&gt;1,000</td>
</tr>
<tr>
<td><em>Hormodendrum compactum</em> UC-1222</td>
<td></td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em> UC-1139</td>
<td></td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em> UC-1220</td>
<td></td>
<td>1,000</td>
</tr>
<tr>
<td><em>Sporotrichum schenckii</em> UC-1364</td>
<td></td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><em>Monosporium apiospermum</em> UC-1248.</td>
<td></td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em> UC-1458</td>
<td></td>
<td>&gt;1,000</td>
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<tr>
<td><em>T. interdigitale</em> UC-1399</td>
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<td>&gt;1,000</td>
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<tr>
<td><em>Candida albicans</em> UC-1077</td>
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</tr>
<tr>
<td><em>T. violaceum</em> UC-1459</td>
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<td><em>T. asteroides</em> UC-4775</td>
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</tr>
<tr>
<td><em>T. mentagrophytes</em> UC-4797</td>
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<tr>
<td><em>T. mentagrophytes</em> UC-4860</td>
<td></td>
<td>&gt;1,000</td>
</tr>
</tbody>
</table>

**Table 2. In vitro antifungal activity of antibiotic U-24,544 in agar dilution test**
glacial acetic acid, or ethyl alcohol; approximately 5 mg/ml in 1-butanol; 2 mg/ml in benzene; and less than 1 mg/ml in water and Skellysolve B. The molecular weight was found to be 540 by mass spectrometry. Analysis calculated for C_{27}H_{32}N_{4}O_{8} (mol wt: 540.56) was C, 59.99; H, 5.96; N, 10.37; O, 23.68. Found: C, 59.97; H, 6.05; N, 10.46; O, 22.88.

**Biological characterization of antibiotic U-24,544.** Antibiotic U-24,544 inhibits gram-positive and gram-negative bacteria in vitro when assayed in a twofold broth dilution test (Table 1). No antibacterial activity was observed in mice experimentally infected with *Klebsiella pneumoniae* when U-24,544 was administered subcutaneously at 600 mg/kg. Antibiotic U-24,544 inhibits the growth of KB cells yielding an ID_{50} (50% inhibition of protein synthesis) of 4.4 μg/ml. The antibiotic is, therefore, fairly toxic in mammalian cells grown in vitro. The compound is remarkably nontoxic in mice; the acute LD_{50} (intraperitoneally) was >1,000 mg/kg in mice.

Of 14 pathogenic fungi tested *in vitro*, antibiotic U-24,544 inhibited only *Nocardia asteroides* at a concentration of 1 μg/ml and *Histoplasma capsulatum* at a concentration of 1,000 μg/ml (Table 2).

**Acknowledgments**
Performance of *in vitro* antifungal tests by A. Dietz and *in vivo* studies by C. Lewis is gratefully acknowledged.

**Literature Cited**