Isolation of Cairomycins A and C

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Cairomycin B in the fermentation broths of *Streptomyces* sp. strain AS-C-19 accompanied cairomycin A and cairomycin C. The cairomycins are peptides with potent activity against gram-positive bacteria. On acid hydrolysis, cairomycin A yielded valine and aspartic acid, whereas cairomycin C yielded lysine, glycine, valine, leucine, and aspartic acid, as identified by paper and gas chromatography. These amino acids were found to exist in their α-L form. Cairomycin A was tentatively assigned a 6-isopropyl-2,5-diketopiperazine-3-acetic acid structure. The three cairomycins were distinct from each other in their ultraviolet, infrared, and mass spectra; elemental analyses; and their chromatographic behavior in different developing solvents.

*Streptomyces* sp. strain AS-C-19 was isolated from a soil sample collected from the Cairo area. Culture broths of this organism were found to contain three antibiotics which were designated cairomycins A, B, (14), and C.

The present paper describes the isolation, purification, and physicochemical and biological properties of both cairomycin A and C.

MATERIALS AND METHODS

Production and isolation of cairomycins A and C. *Streptomyces* sp. strain AS-C-19 was grown in 14-liter fermentors (New Brunswick Scientific Co.) on starch-nitrate medium of the following composition (g/100 ml): soluble starch, 2.0; NaNO₃, 0.2; KH₂PO₄, 0.1; MgSO₄·7H₂O, 0.05; KCl, 0.05; and FeSO₄·5H₂O, 0.00075 at neutral pH. Aeration was at the rate of 1 volume of air per 1 volume of medium per min, the temperature was maintained at 28°C, and the agitator speed was 600 rpm. The antimicrobial activities of the broth were followed by the serial dilution agar method, using *Staphylococcus aureus* ATCC 6538 as the test organism. Maximum antimicrobial activity was achieved after 72 h of fermentation. The broth was then filtered, and the filtrate was extracted with chloroform-ethyl acetate (1:1, vol/vol) at pH 7.0. The extract was evaporated under vacuum to dryness, and the residue was washed repeatedly with petroleum ether (40 to 60°C). The brown residue was harvested by centrifugation and was then chromatographed on preparative thin-layer chromatography plates (Silica Gel GF₂₅₄₄) with chloroform-methanol (9:1, vol/vol) as the developing mixture. Three biologically active components (A, B, and C) with *R*₅₀ values of 0.9, 0.65, and 0.0, respectively, were separated in relative proportions of 1:3:5:8. Each of the components was eluted from silica gel plates with aceton

Further purification was carried out by repeating the above thin-layer chromatography technique with aceton-benzene (1:1, vol/vol) as the developing solvent. The ultraviolet, infrared, nuclear magnetic resonance, and mass spectral analyses were carried out as usual. Antimicrobial properties are shown in Table 1.

RESULTS

Physicochemical properties. Cairomycin A was obtained as a yellowish brown powder which melted at 110 to 112°C. It was freely soluble in chloroform, ethyl acetate, acetone, and benzene; fairly soluble in higher alcohols; and rather insoluble in petroleum ether and water. Cairomycin C was obtained as a reddish brown powder that melted at 138 to 140°C and was soluble in chloroform, ethyl acetate, and acetone.

Migration of the pure antibiotics on thin-layer chromatography with different developing solvents is represented in Fig. 1. Zones containing the antibiotics were allocated by their colors and also bioautographically by using *S. aureus* ATCC 6538 as the test organism. One definite inhibition zone was always observed for each of the antibiotics.

Pure cairomycin A had no characteristic absorption bands in the ultraviolet range, whereas cairomycin C had characteristic peak at 235 nm and a broad one at 275 nm. The infrared spectra of cairomycins A and C were very similar and exhibited absorption bands at the following wavelengths (cm⁻¹): 760, 1,030, 1,250, 1,280, 1,385, 1,435, 1,475, 1,625, and 1,735. Absorption of cairomycin A was strong at 1,030, 1,625, and 1,735 nm but very weak at 1,280 nm, whereas that of cairomycin C showed strong absorption at 1,280 nm but almost nil at 1,030, 1,625 and 1,735 nm. The proton magnetic resonance spectrum of cairomycin A when run in CDCl₃ with a Varian T₆₀ spectrometer showed the following signals: δ 1.02 ppm (doublet) for methyl protons;
were from acids the individual PYE GCV of chromatography (4:1:5). with mycin C, mycin and aspartic liberated follows: found: C, measured by and 0, 19.43%. Oxygen difference. The amino acids existed in equimolecular ratios. The absolute configuration of the amino acids in cairomycin A as determined by the methods of Crumpler (4) and Meister (10) showed α-L configuration for both valine and aspartic acid. Cairomycin A responded negatively to the Molisch, Fehling, biuret, ninhydrin, Millons, Saka-guchi, ferric chloride, iodine, nitration, and KCNS tests and positively to the dimethyl amino benzaldehyde test, whereas cairomycin C showed a positive response to the biuret, ninhydrin, and dimethyl amino benzaldehyde tests.

**Biological activities of cairomycins A and C.** The antimicrobial spectra (with a serial dilution agar procedure) of cairomycins A and C for a variety of bacteria are presented in Table 1. Bacteria resistant to medically important antibiotics were included among the tests organisms.

The 50% lethal doses (intraperitoneal, male Swiss mice) for both cairomycins A and C were 10 mg/kg of body weight.

**DISCUSSION**

Cairomycins A and C were isolated from *Streptomyces* sp. strain AS-C-19, which also produced cairomycin B (14). The three cairomycins differ in their amino acid contents as follows: Cairomycin A contains α-L-valine and α-L-aspartic acid; cairomycin B contains lysine and aspartic acid, and cairomycin C contains lysine, glycine, valine, leucine, and aspartic acid. Tests for the presence of organic acids and carbohydrates in the acid hydrolysates of the three cairomycins were negative.
On the basis of mass and nuclear magnetic resonance spectra, analytical values, empirical formula, and hydrolytic studies the structure of cairomycin A has been tentatively assigned as 6-isopropyl-2,5-diketopiperazine-3-acetic acid, whereas the structure of cairomycin C is still to be elucidated. The piperazine nucleus in cairomycin C may be connected to a straight-chain oligopeptide or to a cyclic one.

For comparative purposes, cairomycin C is quite distinct from amphomycin (7), aspartocin (13), cephalomycin (9), cinnamycin (2), duramycin (16), and thioestreptone (12), which have one or more of its constituent amino acids. Cairomycins A, B, and C differ from other diketopiperazine antibiotics that contain sulfur in their
TABLE 1. Antimicrobial spectrum of cairomycins A and C

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum inhibitory concn (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cairomycin A</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 6538</td>
<td>0.30 1.5</td>
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<tr>
<td>S. aureus 209 P</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>S. aureus Terashima</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>S. aureus Smith</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>S. aureus 252 R (PC, SM, TC, EM, LM, and SPM resistant)</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>S. aureus 199 R (SM, KM, TC, EM, and LM resistant)</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>S. aureus 644 R (EM and SPM resistant)</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>E. coli 10131</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>E. coli 33-3 R (PC, SM, KM, TC, and CP resistant)</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>K. pneumoniae R (PC, SM, KM, TC, CP, CI, and PL resistant)</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa A3</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>P. aeruginosa 35P (PC, CET, CER, SM, KM, and CP resistant)</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Proteus mirabilis 1698</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis H3 7RV</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td>Trichophyton asteroides</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>T. granulosis</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>T. violaceum</td>
<td>0.30 1.5</td>
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<tr>
<td>T. concentric</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Mucor canis</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Aspergillus lumigates</td>
<td>0.30 1.5</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0.30 1.5</td>
</tr>
</tbody>
</table>

*Medium for bacteria was 1-22 heart infusion agar; that for mycobacteria was 23 Kirchner medium; and that for fungi was Sabouraud agar (1+7). Abbreviations: EM, erythromycin; LIM, lincomycin; CP, chloramphenicol; PC, penicillin; KM, kanamycin; CET, cephalothin; CI, colistin; LM, leucmycin; SPM, spiramycin; TC, tetracycline; SM, streptomycin; STH, streptothricin; CER, cephaloridine; and PI, polymyxin. Test organisms were kindly supplied by Tomoharu Okuda, Tanabe Seiyaku Co., Ltd., City, Japan. Minimum inhibitory concentrations were determined after 24 h for bacteria and 5 days for fungi, except those of M. tuberculosis (14 days) and C. albicans (2 days)."

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molecules, e.g., aranotin (11), sporidesmins (5), and gliotoxin (1). Also, tryptophan-dehydrobutyryl-diketopiperazine (8) differs from the cairomycins in having an indole structure, whereas albonoursin (3) includes an aromatic configuration in its structure.

**LITERATURE CITED**


