Cairomycin B, a New Antibiotic

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Cairomycin B is a new cyclic peptide antibiotic that was isolated from *Streptomyces* As-C-19 obtained from the soil of Cairo. The antibiotic had the following empirical formula: $C_{10}H_{15}N_3O_3$; on acid hydrolysis, it yielded aspartic acid and lysine. Spectral analysis and its chemical characteristics indicated that it was a cyclic peptide. The antibiotic melted at 120 to 121°C and was freely soluble in chloroform, ethyl acetate, and acetone, slightly soluble in alcohols, and rather insoluble in water and petroleum ether. Cairomycin B was mainly active against gram-positive bacteria, with high toxicity to experimental animals and weak serum-binding properties.

During our screening program to evaluate members of *Streptomyces* from the soil of the Middle East area as producers of antimicrobial substances, an isolate, no. 19, obtained from the soil of Cairo, exerted interesting antimicrobial potentialities. It secretes, in its culture

filtrate, several antimicrobial agents; among these, cairomycin B was isolated and its chemical structure was identified.

This paper describes the culture conditions and isolation of the antibiotic, for which the chemical, physical, and biological properties

TABLE 1. Mass spectrum fragments of cairomycin B

<i>m/e</i>	% Abundance	Fragment
41	77	$\begin{array}{c} \text{O} \\ \parallel \\ (\text{C}-\text{CH})^+ \end{array}$
43	94	$\begin{array}{c} \text{H} \cdots \text{O} \\ \parallel \\ (\text{C}-\text{CH}_2)^+ \end{array}$
55	84	$\begin{array}{c} \text{O} \\ \parallel \\ (\text{C}-\text{CH}=\text{CH})^+ \end{array}$
56	34	$\begin{array}{c} \text{H} \cdots \text{O} \\ \parallel \\ (\text{C}-\text{CH}_2-\text{CH})^+ \end{array}$
57	100	$\begin{array}{c} \text{O} \\ \parallel \\ (\text{CH}_2-\text{NH}-\text{C})^+ \end{array}$
83	55	$\begin{array}{c} \text{O} \qquad \qquad \text{O} \\ \parallel \qquad \qquad \parallel \\ (\text{C}-\text{CH}-\text{CH}_2-\text{C})^+ \end{array}$
85	48	$\begin{array}{c} \text{H}-\text{O} \qquad \qquad \text{O}-\text{H} \\ \parallel \qquad \qquad \parallel \\ (\text{C}-\text{CH}-\text{CH}_2-\text{C})^+ \end{array}$
97	36	$(\text{N}-\text{CH}_2-(\text{CH}_2)_3-\text{CH}-\text{NH})^+$
99	23	$(\text{HN}-\text{CH}-(\text{CH}_2)_3-\text{CH}-\text{NH})^+$
225	M	$(C_{10}H_{15}N_3O_3)$
226	$10(m+1)$	$(C_{10}H_{16}N_3O_3)$

were determined. Finally, a suggested chemical structure was derived from the collected spectroscopic findings.

MATERIALS AND METHODS

Production, isolation and purification of cairomycin B. Microscopic examination of the cairomycin B-producing organism revealed narrow hyphae and straight chains of cylindrical spores, indicating that the organism is a streptomycete, designated as species AS-C-19.

Streptomyces AS-C-19 thrived at 28°C in shake flasks (220 rpm). The medium used for the production of cairomycin B contained the following ingredients (g/100 ml): glucose, 2.5; KNO₃, 0.1; K₂HPO₄, 0.1; MgSO₄·7H₂O, 0.05; and KCl, 0.05.

The pH of the medium was adjusted to 7.0 before sterilization. The antibiotic contents reached a maximum (7.25 mg/liter) after 5 days of incubation, and the broth was filtered and extracted with a mixture of ethyl acetate and chloroform (vol/vol, 1:1) at pH 7.0. The extract was evaporated in vacuum to dryness, and the residue was dissolved in a minute amount of dry chloroform to which petroleum ether (boiling point, 40 to 60°C) was then added till turbid-

ity appeared. The mixture was left for complete precipitation in a refrigerator and was subsequently separated by centrifugation as a light-brown powder.

The use of thin-layer Silica Gel G F254 and Whatman no. 1 cellulose strips revealed that the crude preparation was composed of three active principles, A, B, and C, contaminated with some inactive materials. For purification, a 200-mg portion of crude preparation was chromatographed on a cellulose CF11 column and eluted with a mixture of chloroform-petroleum ether (vol/vol, 1:1). Three colored, active substances were separated; fraction A (the fastest) was yellowish (1 mg), fraction B, which eluted next was greenish brown (70 mg), and fraction C, which eluted last, was reddish brown (15.4 mg). Fraction B was further purified with preparative Silica Gel G plates using a chloroform-petroleum ether mixture (vol/vol, 1:1) as a developing solvent. Twenty-three milligrams of pure cairomycin B was obtained.

RESULTS AND DISCUSSION

Physical properties. The pure antibiotic melts at 120 to 121°C. It is readily soluble in chloroform, ethyl acetate, and acetone but in-

TABLE 2. Antibacterial spectrum of cairomycin B using serial agar dilution method^a

Test organism ^b	Resistance to other antimicrobials ^c	MIC (μg/ml) after 24 h
<i>Staphylococcus aureus</i>		0.78
<i>S. aureus</i> 209.P		0.78
<i>S. aureus</i> (Terashima's strain)		0.78
<i>S. aureus</i> (Smith's strain)		0.78
<i>S. aureus</i> 252 R	PC, SM, TC, EM, LM, SPM	0.78
<i>S. aureus</i> 199 R	SM, KM, TC, EM, LM	0.78
<i>S. aureus</i> 644 R	EM, SPM	0.78
<i>S. epidermidis</i> 10131	TC, KM, CP, STH, SM	0.78
<i>S. epidermidis</i> (Kawamura's strain)		0.78
<i>Streptococcus faecalis</i>		0.78
<i>S. faecalis</i> (Urayama's strain)	TC, STH, SM, KM, LM, LIM	0.78
<i>Sarcina lutea</i> PCI-1001		0.78
<i>Bacillus subtilis</i> PCI-219		0.78
<i>Escherichia coli</i> NIHJ		100
<i>E. coli</i> K-12		100
<i>E. coli</i> 33-R	PC, SM, KM, TC, CP	100
<i>Salmonella typhimurium</i>		100
<i>Klebsiella pneumoniae</i> PCF602		100
<i>K. pneumoniae</i> R	PC, SM, KM, TC, CP, CI, PL	100
<i>Pseudomonas aeruginosa</i> A3		50
<i>P. aeruginosa</i> 35P	PC, CET, CER, SM, KM, CP	100
<i>Proteus mirabilis</i> 1698		100
<i>Mycobacterium tuberculosis</i> H37RV		50 (14 days)

^a Medium for all species but *Mycobacterium* was heart infusion agar; *Mycobacterium* was grown in Kirchner medium.

^b Test organisms were kindly supplied by Tomohoru Okuda, Director of Microbial Chemistry Research Laboratory, Tanabe Seiyaku Co. Ltd., Japan.

^c EM, Erythromycin; LM, leucomycin; LIM, lincomycin; SPM, spiramycin; CP, chloramphenicol; TC, tetracycline; PC, penicillin; SM, streptomycin; KM, kanamycin; STH, streptothricin; CET, cephalothin; CER, cephaloridine; CI, colistin; PL, polymixin.

TABLE 3. Antifungal spectrum of cairomycin B using serial agar dilution method

Fungus ^a	MIC (μg/ml) after 5 days
<i>Trichophyton asteroides</i>	50
<i>T. granulosis</i>	50
<i>T. violaceum</i>	50
<i>T. concentricum</i>	50
<i>Mucor canis</i>	50
<i>Aspergillus fumigatus</i>	50
<i>Candida albicans</i>	50 (2 days)

^a Fungi were grown in Sabouraud agar. Test organisms were kindly supplied by Tomohoru Okuda, Tanabe Seiyaku Co. Ltd., Japan.

soluble in petroleum and water. The antibiotic has a molar extinction coefficient of 228 at 290 nm. The infrared spectrum of cairomycin B showed absorption at wavelengths of 760, 850, 1,060, 1,243, 1,280, 1,385, 1,435, 1,475, 1,502, 1,625, and 1,695 nm.

Cairomycin B did not migrate on cellulose thin-layer chromatography when developed with methanol, petroleum ether, distilled water, acidified water, and ethyl acetate, whereas it did move with the solvent front of chloroform, ethyl acetate, and acetone. Chloroform-petroleum ether (vol/vol, 1:1) and water saturated with ethyl acetate move the spot of the antibiotic to R_f values of 0.5 and 0.49, respectively. Location of the zones occupied by the antibiotic was determined visually and bioautographically using *Staphylococcus aureus* as the test organism. One definite inhibition zone was always observed.

Chemical properties. Elemental analysis yielded the following data, which correspond to the empirical formula $C_{10}H_{15}N_3O_3$: calculated, C_{53.33}, H_{6.67}, N_{18.67}, and O_{21.33}; found, C_{53.26}, H_{6.70}, N_{18.72}, and O_{21.32}. Cairomycin B showed a negative response to the following chemical tests: Molisch, Fehling, Biuret, Millons, Sakaguchi, Tollens, and nitration. Also, it did not react with alkaline permanganate, ninhydrin reagent, ferric chloride, and iodine solutions and was reduced with cold acidic permanganate.

After hydrolysis of the pure antibiotic with 6 N HCl at 105°C for 24 h, aspartic acid and lysine were the only products that could be detected. The abundant peaks in mass spectrum analysis of the pure antibiotic are shown in Table 1.

In light of the elemental analysis, acid hydrolysis of the antibiotic and data collected

from mass, ultraviolet, and infrared spectra, the following structural formula is suggested.

Biological properties. The minimal inhibitory concentrations (MICs) of cairomycin B for a variety of microorganisms are given in Tables 2 and 3. Determination of the MIC was carried out using the following medium (g/100 ml): yeast extract, 0.15; beef extract, 0.15; peptone, 0.5; glucose, 0.1; K_2HPO_4 , 0.13; and NaCl, 0.35; the pH was adjusted to 7.0 before sterilization. The test organisms were kindly supplied by Tomohoru Okuda. The results shown in Tables 2 and 3 demonstrate that cairomycin B is mainly active against gram-positive bacteria, whereas very limited activities were exerted against a few of the gram-negative bacteria and fungi.

In vivo tests were then carried out using male Swiss mice, 4 weeks of age. The antibiotic was administered intraperitoneally, and the mice were observed for 7 days, when a delayed toxicity was noticed. The mean lethal dose was 12.5 mg/kg of body weight. The mean protective dose of cairomycin B was 3 mg/kg of body weight using the Swiss mice and *S. aureus*; the challenge inoculum was 2.2×10^4 cells per mouse. The infected animals were kept under observation for 10 days.

Cairomycin B is unique among other antibiotics of a similar nature in its structure, amino acid content, and biological properties. Although amphotycin (1) and aspartocin (2) contain, in their acid hydrolysates, aspartic acid and lysine, among other hydrolytic products, these antibiotics are still distinctly different from cairomycin B in other properties.

LITERATURE CITED

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