CATACANDINS, NOVEL ANTICANDIDAL ANTIBIOTICS OF BACTERIAL ORIGIN

EDWARD MEYERS*, RAYMOND COOPER†, LORETTA DEAN, JANICE H. JOHNSON, DOROTHY S. SLUSARCHYK, WILLIAM H. TREJO and PUSHPA D. SINGH

The Squibb Institute for Medical Research,
Princeton, New Jersey 08540, U.S.A.

(Received for publication August 5, 1985)

Two novel antibiotics, catacandin A and catacandin B, were isolated from the fermentation broth of the bacterium, *Lysobacter gummosus*, by extraction and adsorption, reverse-phase and gel filtration chromatography. On the basis of their physico-chemical properties, they are acyltetramic acids that are easily distinguishable from others in this class. Catacandin A and catacandin B possess good anticandidal activity.

It is becoming increasingly evident that bacteria can produce a wide variety of antibiotic types, a biosynthetic ability once thought to be the domain of the *Actinomycetales*. In recent years, bacteria have been shown to produce β-lactam antibiotics[1], a nucleoside antibiotic[2], aminoglycosides[3], amino acid analogs[4], *etc.* In addition to structural diversity, bacterially-produced antibiotics encompass a wide variety of antibiotic activities, from narrow to broad spectrum, to those with antifungal properties.

These considerations have led us to examine bacteria for the production of novel compounds with anticandidal activity. In the course of this screening effort, we found two novel antifungal agents. One, catacandin, is the subject of this report; the other, xylocandin, will be reported elsewhere[5].

Taxonomy

The strain producing catacandin was originally isolated from a soil sample taken on the slopes of Mt. Etna, Sicily. The taxonomic characteristics of this strain are as follows:

Morphology

The organism is a long, slender Gram-negative rod with rounded rather than tapered ends. Some chain formation is evident. It is motile by means of gliding, leaving slime trails on the agar surface. No fruiting bodies are formed.

Culture Characteristics

Liquid cultures are viscous and agar cultures are mucilaginous. Colonies on sucrose 0.25%, yeast extract 0.5% agar are yellow and transparent with scalloped, colorless edges that spread in an irregular manner.

Biochemical Reactions

The organism is capable of lysing eukaryotic cells (*e.g., Saccharomyces cerevisiae*) and is proteolytic, as evidenced by clearing of the opaqueness of a skim-milk acetate agar medium. It produces catalase, oxidase and phosphatase and is able to utilize citrate as the sole source of carbon for growth.

† Present address: Schering Corporation, 60 Orange Street, Bloomfield, New Jersey 07003, USA.

It does not hydrolyze starch; it does produce acid from glucose, cellobiose, sucrose (delayed) and lactose, but not from glycerol or mannitol. The organism is lipolytic on Tween 20 and Tween 80. It does not produce water-soluble pigments and it grows well on eosin-methylene blue agar. The G+C content of the DNA is 66.9 mol %, as determined by the thermal denaturation method.

Based upon morphological, cultural and biochemical properties, the organism was identified as *Lysobacter gummosus*, in accordance with the description by CHRISTENSEN and COOK[6]. The culture has been deposited in the American Type Culture Collection, Rockeville, Maryland and has the accession number, ATCC 39472.

Production

L. gummosus ATCC 39472 was maintained by storage in a mechanical freezer at −90°C. When needed, working stock cultures were prepared on agar slants composed of yeast extract 1%, peptone 2%, glucose 2% and agar 1.5%. The slants were incubated at 25°C for 16 to 18 hours and used to inoculate germinator flasks containing 100 ml of medium in 500-ml Erlenmeyer flasks. This medium consisted of yeast extract 0.4%, malt extract 1% and dextrose 0.4%. The germinators were incubated overnight (18 hours) at 25°C on a rotary shaker operating at 300 rpm with a 5-cm stroke, and then used to inoculate (1%) the same medium (50 liters) in a 75-liter Fermatron fermentor (New Brunswick Scientific, Edison, New Jersey, U.S.A.). The fermentation was run for 30 hours at 25°C, with an air flow of 50 liters/minute and an agitation rate of 200 rpm. The fermentation and the subsequent isolation steps were monitored by paper-disc, agar-diffusion assay with *Candida albicans* SC5314 as the assay organism.

Isolation Procedure

The isolation and purification procedures for catacandins A and B are outlined in Fig. 1. The

Fig. 1. Isolation of catacandins A and B.

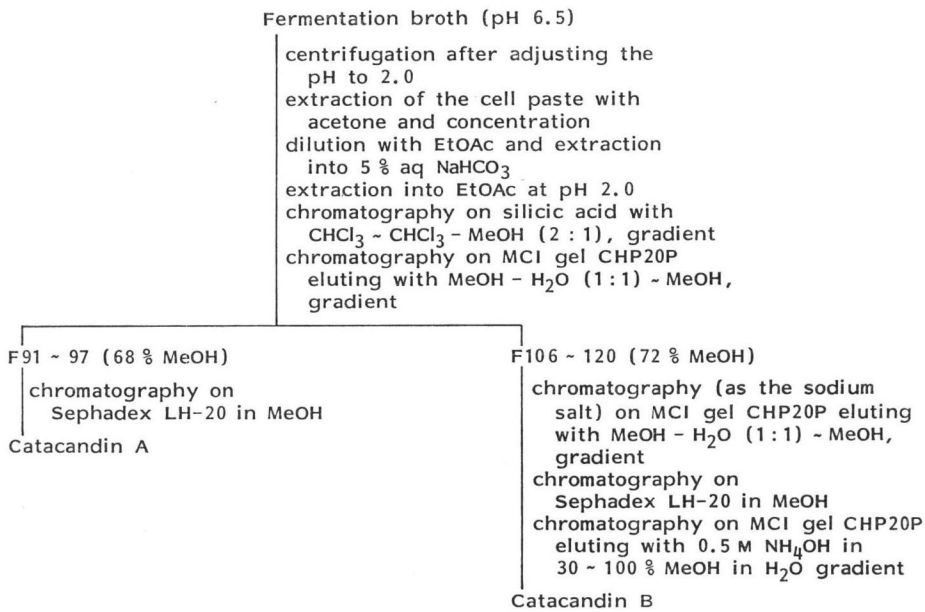


Table 1. Physico-chemical properties of catacandins A and B.

	Catacandin A	Catacandin B
TLC[a]	Rf 0.31	Rf 0.42
IR (KBr)[b] cm^{-1}	3360, 2940, 2870, 2375, 1720, 1705, 1655, 1610, 1460	3320, 2955, 2860, 2375, 1720, 1705, 1655, 1600, 1460
UV nm ($E^{1\%}_{1cm}$) MeOH	204 (17,500), 258 (30,500), 343 (8,500)	205 (16,100), 236 (15,600), 320 (7,800)
0.01 M HCl in MeOH	205 (14,000), 268 (25,500), 358 (13,500), 375 (11,500)	215 (17,500), 324 (10,500)
0.01 M NaOH in MeOH	257 (33,000), 340 (8,500)	236 (15,800), 317 (7,800)
FAB-MS ($(M-H)^-$, m/z)	509.267	509.261
Molecular formula[c]	$C_{29}H_{38}N_2O_6$	$C_{29}H_{38}N_2O_6$

[a] Silica gel, $CHCl_3$ - MeOH - NH_4OH, 6: 4: 1.
[b] The IR spectral data for catacandins A and B were obtained on their sodium and ammonium salts, respectively.
[c] Molecular formulae were determined by FAB mass spectrometry.

fermentation broth, acidified to pH 2 with hydrochloric acid, was centrifuged to separate solids from the supernate. Since most of the activity was associated with the solids, they were extracted with acetone and the resulting acetone extract concentrated *in vacuo*. The concentrate, diluted with ethyl acetate, was then extracted with 5% sodium bicarbonate. The bicarbonate solution was acidified to pH 2 and the activity extracted into fresh ethyl acetate. The active components were further purified by a combination of normal-phase, reversed-phase and size-exclusion chromatography.

Physico-chemical Properties

The major component of the fermentation, catacandin A, is a methanol soluble, acidic antibiotic. Physico-chemical data are given in Table 1. The IR spectrum indicates the presence of ester (1720 cm^{-1}) and amide (1655 cm^{-1}) functions. The hypsochromic shift of the UV maximum at 375 nm to 340 nm in going from acidic to alkaline pH is characteristic of dienoyltetramic acid antibiotics, *e.g.*, tirandamycin[7]. The molecular weight and empirical formula of catacandin A were determined by FAB[8] mass spectrometry. Both positive and negative ion spectra indicated a molecular weight of 510 for the free acid. The exact mass observed for $C_{29}H_{37}N_2O_6$ $(M-H)^-$ was 509.267 (theory 509.265). Catacandin A gave a major fragment at m/z 98 in the negative ion FAB mass spectrum. This ion ($C_4H_4NO_2$ by high resolution FAB-MS) fragments from the parent as shown by MS-MS[9,10] data and is further evidence to support the presence of an acyltetramic acid in catacandin A (Fig. 3). The ion, m/z 98, could arise from a combination of an α-cleavage of the C2-C13 bond and a McLafferty rearrangement involving the C4-C5 bond cleavage with the hydrogen migration. Under identical conditions, tirandamycin also gave a major fragment at m/z 98 ($C_4H_4NO_2$), arising from the negative ion FAB mass spectrum. This type of fragmentation may be characteristic of tetramic acid antibiotics. Unfortunately, samples of ikarugamycin and capsimycin were not available to test this hypothesis.

The ^1H NMR spectrum and a partial structure, **1**, for catacandin A are shown in Figs. 2 and 3, respectively. The two four-proton systems, consisting of the protons on C-9 (δ 5.90), C-10 (δ 7.15), C-11 (δ 6.13) and C-12 (δ 5.86), and on C-14 (δ 6.90), C-15 (δ 7.35), C-16 (δ 6.13) and C-17 (δ 6.03), were established by decoupling experiments. The assignments of the protons on C-14, 15, 16 and 17 are based on a comparison of their chemical shifts with those of analogous protons in tirandamycin[7].

Fig. 2. 400 MHz ^{1}H NMR spectrum of catacandin A in CD_3OD.

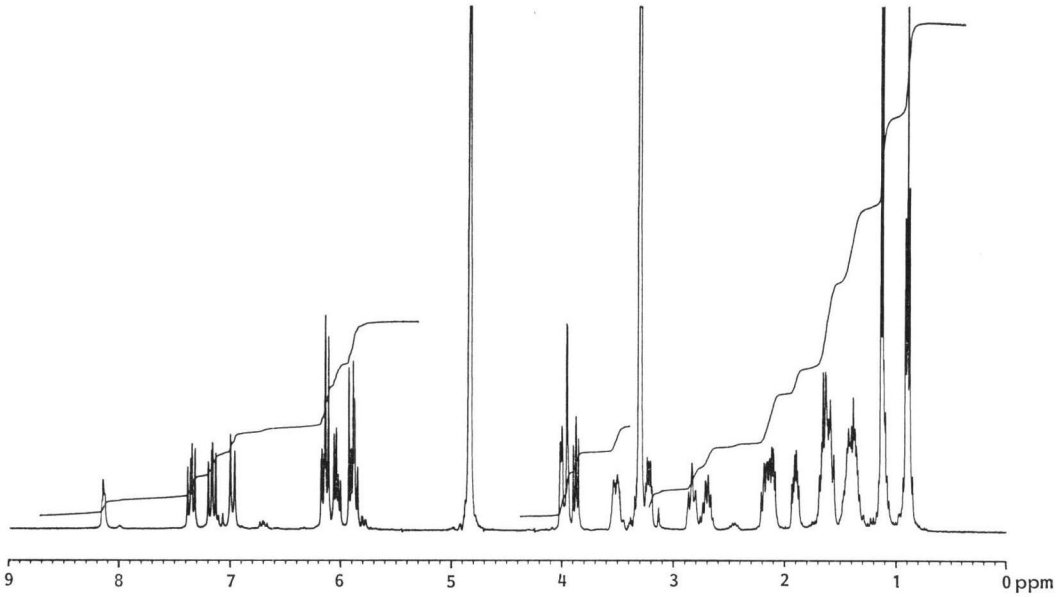
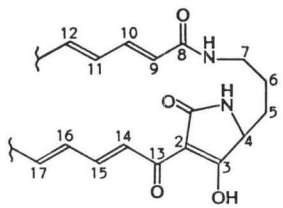


Fig. 3. Partial structure of catacandin A.



Catacandin A (**1**)

^{13}C NMR data and partial assignments are presented in Table 2. The multiplicities of the carbons were determined using the Inept[11] technique. Assignments of carbons at positions 1 through 4 and 13 through 17 are based on comparison of their chemical shifts with those of analogous carbons in tirandamycin[7].

Ozonolysis of catacandin A followed by hydrolysis[12] yields ornithine and oxalic acid as

Table 2. ^{13}C NMR data for catacandin A.

Chemical shifts (δ)[a]	Carbon type[b]	Assignment	Chemical shifts (δ)[a]	Carbon type[b]	Assignment
13.1	CH_3		76.4	CH	
19.3	CH_3		101.6	C	C-2
27.2	CH_2		122.7	C=C	C-12
32.2	CH_2		125.2	C=C	C-9
38.2	CH_2		126.6	C=C	C-17
41.6	CH_2		130.3	C=C	C-11
42.1	CH_2		135.9	C=C	C-16
43.8	CH		141.0	C=C	C-14
46.6	CH		144.5	C=C	C-10
48.2	CH or CH_2		147.0	C=C	C-15
55.6	CH		167.8	C=O	C-8
57.3	CH		174.7	C=O	C-13
57.4	CH		176.8	C=O	C-1
69.8	CH		194.1	C	C-3
71.7	CH				

[a] ppm Downfield from TMS, using dioxane (67.6 ppm) as an internal standard.
[b] Assignments are made by the Inept technique.

Fig. 4. 400 MHz ^1H NMR spectrum of catacandin B (ammonium salt) in CD_3OD.

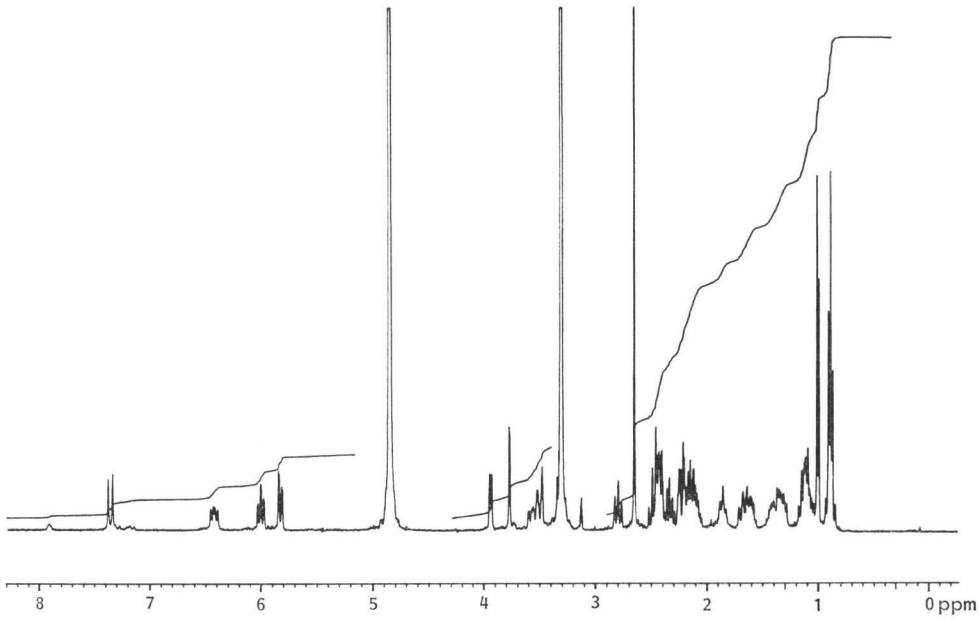


Table 3. *In vitro* potency of catacandin A and catacandin B.

Organism	MIC (μg/ml)	
	Catacandin A	Catacandin B
Candida albicans SC*5314	6.3	6.3
C. albicans SC9177	6.3	6.3
C. albicans SC9721	6.3	6.3
C. albicans SC10102	6.3	12.5
C. tropicalis SC9861	3.1	6.3
C. krusei SC2967	6.3	6.3
C. parakrusei SC2621	3.1	6.3
C. pseudotropicalis SC11241	3.1	6.3
C. guilliermondii SC2210	6.3	12.5
C. glabra SC9342	12.5	25
Staphylococcus aureus SC1276	50	
S. aureus SC2399	100	
Escherichia coli SC2927	>100	

* Squibb culture.

well as an unidentified fragment from the remaining portion of catacandin A.

A minor component of the fermentation, catacandin B, is also a methanol soluble, acidic antibiotic. Physico-chemical data are given in Table 1. The IR spectrum indicates the presence of ester (1720 cm^{-1}) and amide (1655 cm^{-1}) functions. A hypsochromic shift of the UV maximum from 324 nm to 317 nm in going from acidic to alkaline pH is indicative of an enoyltetramic acid system as in ikarugamycin[12]. Comparison of the UV of catacandins A and B indicates a higher degree of conjugated unsaturation for catacandin A. The molecular weight and empirical formula of catacandin B were determined by FAB mass spectrometry. Both positive and negative ion spectra indicated a molecular weight of 510 for the free acid. The exact mass observed for $C_{29}H_{37}N_2O_6$ $(M-H)^-$ was 509.261 (theory 509.265) and for $C_{29}H_{39}N_2O_6$ $(M+H)^+$ was 511.277 (theory 511.280). Thus, catacandins A and B are isomeric. The ^1H NMR spectrum is shown in Fig. 4.

Biological Properties

The antimicrobial spectrum of catacandin A and catacandin B is shown in Table 3. Assays were done by a two-fold agar dilution technique, with a medium containing Tryptone 0.5%, malt ex-

tract 0.3%, glucose 1.0%, yeast extract 0.3% and agar 1.5%. From the data in Table 3, it is evident that both catacandins A and B are potent anticandidal antibiotics with no significant difference in activity. Although not shown in Table 3, the presence of 50% calf serum decreased the activity approximately 33-fold.

Discussion

The catacandins are acidic antibiotics produced by a bacterium with good anticandidal activity but without appreciable antibacterial activity. It is evident from our studies that they belong to the acyltetramic acid class of antibiotics, but can be unequivocally differentiated from others in this group[7,12~23] by their empirical formula and spectroscopic properties.

Acknowledgments

We wish to acknowledge Mr. P. A. PRINCIPE for supplying fermentation broths, Dr. S. K. TANAKA for *in vitro* studies, Dr. M. A. PORUBCAN and Ms. A. KAHLE for NMR studies and Dr. S. E. UNGER and Mr. T. MCCORMICK for mass spectral data.

References

1) SYKES, R. B.; W. L. PARKER & J. S. WELLS: β-Lactam antibiotics produced by bacteria. *In* Trends in Antibiotic Research. Genetics, Biosyntheses, Actions & New Substances. *Ed.*, H. UMEZAWA *et al.*, pp. 115~124, Japan Antibiotics Res. Assoc., Tokyo, 1982
2) TYMIAK, A. A.; C. A. CULVER, J. F. GOODMAN, V. S. SEINER & R. B. SYKES: Oxazinomycin produced by a *Pseudomonas* species. J. Antibiotics 37: 416~418, 1984
3) TOMITA, K.; Y. HOSHINO, Y. UENOYAMA, K. FUJISAWA, H. TSUKIURA & H. KAWAGUCHI: Sorbistin, a new aminoglycoside antibiotic complex of bacterial origin. II. Isolation and taxonomy of sorbistin-producing organism. J. Antibiotics 29: 1147~1151, 1976
4) SHOJI, J.; H. HINOO, R. MASUNAGA, T. HATTORI, Y. WAKISAKA & E. KONDO: Isolation of L-cycloserine from *Erwinia uredovora*. J. Antibiotics 37: 1198~1203, 1984
5) MEYERS, E.; G. S. BISACCHI, L. DEAN, W. C. LIU, D. S. SLUSARCHYK, R. B. SYKES, S. K. TANAKA & W. H. TREJO: Xylocandin, a new complex of antifungal peptides. Taxonomy, isolation and biological activity. Program and Abstracts of 25th Intersci. Conf. Antimicrob. Agents Chemother., No. 808, Minneapolis, 1985
6) CHRISTENSEN, P. & F. D. COOK: *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. Int. J. Syst. Bacteriol. 28: 367~393, 1978
7) MACKELLAR, F. A.; M. F. GROSTIC, E. C. OLSON, R. J. WNUK, A. R. BRANFMAN & K. L. RINEHART, Jr.: Tirandamycin. I. Structure assignment. J. Am. Chem. Soc. 93: 4943~4945, 1971
8) BARBER, M.; R. S. BORDOLI, R. D. SEDGWICK & A. N. TYLER: Fast atom bombardment of solids (F.A.B.): A new ion source for mass spectrometry. J. Chem. Soc. Chem. Commun. 1981: 325~327, 1981
9) MCLAFFERTY, F. W., *Ed.*: Tandem Mass Spectrometry. John Wiley & Sons, New York, 1983
10) TOMER, K. B.; F. W. CROW & M. L. GROSS: Location of double bond position in unsaturated fatty acids by negative ion MS/MS. J. Am. Chem. Soc. 105: 5487~5488, 1983
11) DODDRELL, D. M. & D. T. PEGG: Assignment of proton-decoupled carbon-13 spectra of complex molecules by using polarization transfer spectroscopy. A superior method to off-resonance decoupling. J. Am. Chem. Soc. 102: 6388~6390, 1980
12) ITO, S. & Y. HIRATA: The structure of ikarugamycin, an acyltetramic acid antibiotic possessing a unique *as*-hydrindacene skeleton. Bull. Chem. Soc. Jpn. 50: 1813~1820, 1977
13) STICKINGS, C. E.: Studies in the biochemistry in microorganisms. 106. Metabolites of *Alternaria tenuis*. Auct. The structure of tenuazonic acid. Biochem. J. 72: 332~340, 1959
14) RINEHART, K. L., Jr.; J. R. BECK, D. B. BORDERS, W. W. EPSTEIN, T. H. KINSTLE, L. D. SPICER, D. KRAUSS & A. C. BUTTON: Structure of streptolydigin. Antimicrob. Agents Chemother. -1963: 346~348, 1964
15) BATELAAN, J. G.; J. W. F. K. BARNICK, J. L. VAN DER BAAN & F. BICKELHAUPT: The structure of the antibiotic K16. II. Chromophore and total structure. Tetrahedron Lett. 1972: 3107~3110, 1972

16) SCHABACHER, K. & A. ZEECK: Lipomycine, II. Die konstitution von α- und β-Lipomycin. Tetrahedron Lett. 1973: 2691~2694, 1973
17) GANDHI, N. M.; J. NAZARETH, P. V. DIVEKAR, H. KOHL & N. J. DE SOUZA: Magnesidin, a novel magnesium-containing antibiotic. J. Antibiotics 26: 797~798, 1973
18) GYIMESI, J.; ZS. MÉHESFALVI-VAJNA & GY. HORVÁTH: Reinvestigation of structure of the polyenic antibiotic, oleficin. J. Antibiotics 31: 626~627, 1978
19) AIZAWA, S.; H. AKUTSU, T. SATOMI, T. NAGATSU, R. TAGUCHI & A. SEINO: Capsimycin, a new antibiotic. I. Production, isolation and properties. J. Antibiotics 32: 193~196, 1979
20) HORVÁTH, G.; M. G. BRAZHNIKOVA, N. V. KONSTANTINOVA, I. V. TOLSTYKH & N. P. POTAPOVA: The structure of nocamycin, a new antitumor antibiotic. J. Antibiotics 32: 555~558, 1979
21) VESONDER, R. F.; L. W. TJARKS, W. K. ROHWEDDER, H. R. BURMEISTER & J. A. LAUGAL: Equisetin, an antibiotic from *Fusarium equiseti* NRRL 5537, identified as a derivative of N-methyl-2,4-pyrollidone. J. Antibiotics 32: 759~761, 1979
22) TSUNAKAWA, M.; S. TODA, T. OKITA, M. HANADA, S. NAKAGAWA, H. TSUKIURA, T. NAITO & H. KAWAGUCHI: Bu-2313, a new antibiotic complex active against anaerobes. II. Structure determination of Bu-2313 A and B. J. Antibiotics 33: 166~172, 1980
23) YOKOI, K.; H. HASEGAWA, J. SATOH, M. MATSUMOTO, J. OHNO & T. NAKAJIMA (SS Pharmaceutical KK): New antibiotic SS8201D. Japan Kokai 84-170,092, Sept. 26, 1984 [World Patents Index 84-278337/45]