ABBEYMYCIN, A NEW ANTHRAMYCIN-TYPE ANTIBIOTIC PRODUCED BY A STREPTOMYCETE

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A new antibiotic, abbeymycin, has been isolated from *Streptomyces* sp. AB-999F-52. The structure of abbeymycin was assigned on the basis of NMR, mass spectrometric and UV spectral data. Abbeymycin has weak activity against a limited number of anaerobic bacteria.

In the course of screening for antibiotics with selective activity against obligate anaerobes we have frequently encountered compounds of the anthramycin class. Although this class is known to interact with DNA and to exhibit a wide variety of cytotoxic manifestations, individual members frequently exhibit only weak activity against common, aerobically-grown, Gram-positive and Gram-negative pathogens. In contrast they exhibit moderate activity against most pathogenic obligate anaerobes.

We report here the discovery, isolation and structural identification of a new member of this class, abbeymycin.

Producing Organism

Abbeymycin is produced by strain AB-999F-52, an actinomycete isolated from a soil sample collected at Mount Angel, Oregon. The organism forms aerial mycelium with long, flexuous, branched spore chains. The L-isomer of diaminopimelic acid was found in whole cell hydrolysates indicating cell walls of Type I.[1] The morphology and Type I cell wall composition place this organism in the genus *Streptomyces*. Mature sporulated aerial mycelium is yellowish gray or pale yellow-green on most media but is light gray and greenish yellow on inorganic salts - starch agar. The spore surface is smooth (Fig. 1). Melanoid pigments are not produced.

Fermentation

Agar slant cultures or −80°C frozen seed stocks of *Streptomyces* sp. AB-999F-52 were used to inoculate seed tubes (25×150 mm, glass) containing 10 ml of a medium consisting of glucose monohydrate 1.5%, soybean flour 1.5%, yeast extract (Difco) 0.1%, NaCl 0.1% and $CaCO_3$ 0.1% in distilled water. Seed tubes were incubated at 28°C for 96 hours on a rotary shaker (250 rpm, 3.2 cm stroke). Five percent vegetative inoculum was then transferred to 500 ml second passage seed flasks, containing 100 ml of the same medium, which were also incubated under the same conditions for 72 hours. Five percent inoculum from the seed flasks was used to inoculate a number of 500-ml Erlenmeyer flasks closed with rayon plugs and each containing 100 ml of the fermentation medium consisting of glucose monohydrate (added post sterilization) 2%, Lexein F-152 liquid peptone (Inolex) 1%, Brer Rabbit green label molasses (Del Monte) 0.5%, yeast extract (Difco) 0.1% and $CaCO_3$ 0.2% in distilled water. Fermentation flasks were incubated at 28°C on a rotary shaker (250 rpm, 3.2 cm stroke) and were harvested after 96 hours.

Fig. 1. Scanning electron micrograph of aerial mycelium of strain AB-999F-52 (inorganic salts-starch agar, 8 days, 28°C).

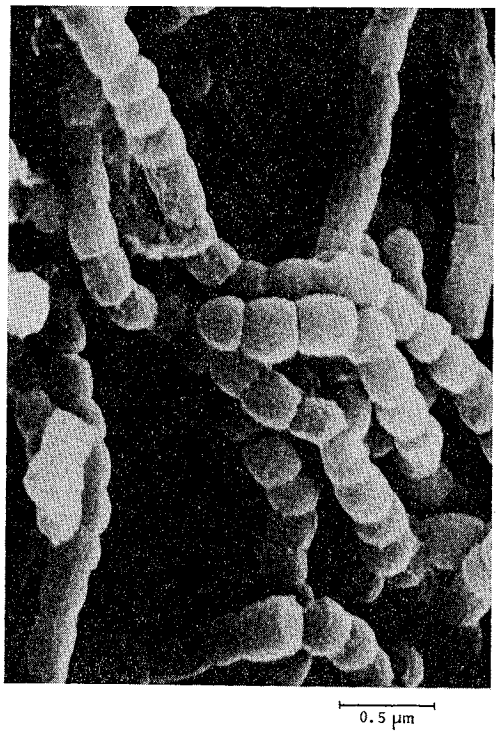


Table 1. ^{1}H NMR of abbeymycin (DMSO).

H at C No.	Chemical shift (δ)	Multiplicity	Coupling constants (J, Hz)
1α	1.90	ddd	13.6, 1.6, 1.3
1β	2.20	ddd	13.6, 9.2, 4.5
2	4.35	br	
2-OH	5.08	d	2.2
3α	3.46	br d	12.6
3β	3.56	dd	12.6, 4.1
6	7.60	dd	7.7, 1.5
7	6.97	td	7.7, 1.5
8	7.29	td	7.7, 1.5
9	6.94	dd	7.7, 1.5
10-NH	6.31	br s	
11	4.77	dd	8.8, 1.4
11-OCH_3	3.35	s	
11a	3.39	ddd	9.2, 8.8, 1.3

Table 2. ^{13}C NMR of abbeymycin (DMSO).

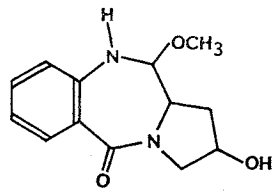
Carbon No.	Chemical shift (δ)
1	36.8
2	68.3
3	55.2
5	167.2
5a	128.3
6	129.4
7	120.8
8	131.3
9	122.5
9a	143.4
11	94.7
11-OCH_3	54.2
11a	57.7

Table 3. Antibacterial activity of abbeymycin.

Organism	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	>100
S. epidermidis 3519	>100
Streptococcus pyogenes EES61	>100
Escherichia coli Juhl	>100
Klebsiella pneumoniae ATCC 8045	>100
Pseudomonas aeruginosa BMH 10	>100
Bacteroides fragilis ATCC 25285	16
B. thetaiotaomicron ATCC 29741	32
B. loescheii ATCC 15930	16
Veillonella parvula ATCC 10790	>64
Clostridium perfringens ATCC 13124	>64
C. difficile ATCC 9689	>64
Peptococcus asaccharolyticus ATCC 14963	64
Peptostreptococcus micros ATCC 33270	32

Isolation

At harvest, the contents of 200 fermentation flasks were combined and centrifuged at pH *ca.* 6.5 to give 12.5 liters of decanted clarified supernate. The antibiotic was adsorbed from the supernate onto a column of Amberlite XAD-4 (1,000 ml) and eluted with methanol. The active eluate (1,500 ml) was concentrated under vacuum to leave 19.5 g of crude residue. This was further purified by chromatography on a Sephadex LH-20 column eluted with methanol - chloroform (3 : 2). Active fractions were pooled and concentrated under vacuum to a small volume. After chilling a semi-crystalline material deposited. This was removed by filtration and recrystallized from a chloroform - methanol mixture to yield 3.5 g of colorless platelets of pure abbeymycin.



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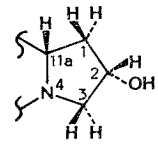

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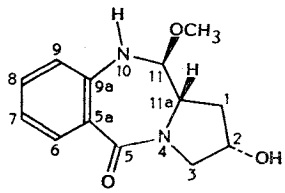

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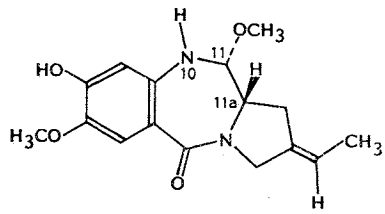

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Characterization and Structural Elucidation

The antibiotic is soluble in water, dimethyl sulfoxide and methanol but insoluble in acetone, acetonitrile, benzene and chloroform. It exhibits a melting point of 142~144°C (dec) and is optically active with a rotation of $[\alpha]_D^{25}$ +303° (*c* 0.741, H_2O).

An electron impact mass spectrum of abbeymycin gave a parent peak at *m/z* 248. As this peak was too weak to mass match, the fragment ion at *m/z* 216, corresponding to $M^+ - CH_3OH$, was used to obtain high resolution data. The *m/z* of 216.0897 observed corresponded to a formula of $C_{12}H_{12}N_2O_2$, indicating a molecular formula of $C_{13}H_{16}N_2O_3$ for abbeymycin.

The UV spectrum with bands at: λ_{max} nm (ε) 216 (37,200), 236 (18,100) and 316 (3,600) in H_2O, together with an ABCD set of aromatic protons (see Table 1) suggested that abbeymycin might be a member of the anthramycin group of antibiotics.

Single frequency decoupling experiments, together with a chemical shift correlation map, supported an anthramycin-type structure with planar configuration **1** (see Tables 1 and 2).

Nuclear Overhauser enhancement (NOE) experiments established the relative configurations at carbon numbers 2 and 11a. Strong NOE's were observed between hydrogen pairs; 3β–2, 3α–2-OH, 2–1β, 2-OH–1α and 1β–11a, indicating that the methine protons at carbon atoms 11a and 3 were on the same side of the 5-membered ring as in **2**.

The stereochemistry at C-11 was deduced by consideration of the coupling constants for 11-H. A value of J_{10-11}=1.3 Hz indicates a dihedral angle for H-N(10)–C(11)-H of close to 90°. Further, J_{11-11a}=8.8 Hz suggests a dihedral angle for H-C(11)–C(11a)-H of close to 0° or close to 180°. Examination of Dreiding models indicates a stereochemistry at C(11) in which the C(11)-OCH_3 is *syn* to 11a-H as in structure **3**. These data contrast well with reported coupling constant information for tomaymycin (**4**), the structure of which has been determined by X-ray diffraction.[2] Tomaymycin, with the opposite configuration at 11-H, has coupling constants of J_{10-11}=6 Hz and J_{11-11a}=0 Hz.[3]

Biological Data

Abbeymycin exhibited weak antibacterial activity against a limited number of anaerobic bacteria

(see Table 3). MICs were determined by the agar dilution method using brain-heart infusion agar for aerobic bacteria and Wilkins - Chalgren agar for anaerobic bacteria.

Acute toxicity was determined when graded doses of abbeymycin were administered as a single injection intraperitoneally to groups of five mice. An LD_{50} value of 36.2 mg/kg was calculated from the cumulative mortalities on the sixth day.

Acknowledgments

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References

1) BECKER, B.; M. P. LECHEVALIER & H. A. LECHEVALIER: Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl. Microbiology 13: 236~243, 1965
2) ARORA, S. K.: Structure of tomaymycin, a DNA binding antitumor antibiotic. J. Antibiotics 34: 462~464, 1981
3) TOZUKA, Z. & T. TAKAYA: Studies on tomaymycin. I. The structure determination of tomaymycin on the basis of NMR spectra. J. Antibiotics 36: 142~146, 1983