HENEICOMYCIN, A NEW ANTIBIOTIC (A21A): FERMENTATION, ISOLATION, AND ANTIBACTERIAL SPECTRUM

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In the course of our screening for new antibiotics, we have found heneicomycin (antibiotic A21A)[1] in culture broth of a newly isolated strain of *Streptomyces filipinensis*, designated NRRL #11044 (MA-4581 in the Merck & Co., Inc. culture collection).

From the physico-chemical and biological properties described in this communication, heneicomycin is considered to be equivalent to 3-deoxyaurodox (Fig. 1), a new antibiotic belonging to the aurodox (X5108)[2] - kirromycin[3] group.

Heneicomycin, which is active *in vitro vs* both Gram-positive and Gram-negative organisms (Table 1), is produced by cultivating *S. filipinensis* NRRL 11044 under submerged, aerobic conditions in aqueous medium containing 4% tomato paste and 1.5% ground oatmeal, with pH adjusted to 6.0 with NaOH. Production of the antibiotic reached a maximum after 5 days' incubation at 28°C on a rotary shaker at 220 rpm.

Fig. 1. Structure of heneicomycin.

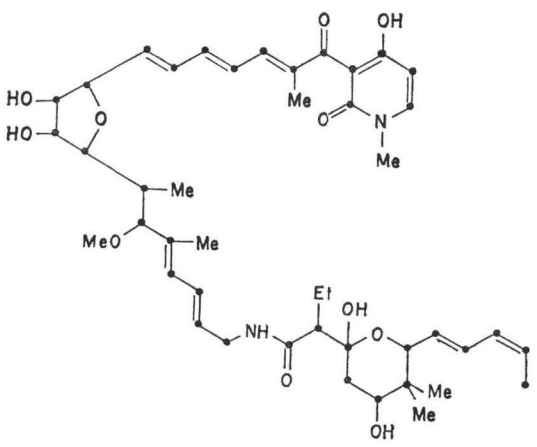


Table 1. Antibacterial spectrum of antibiotic A21A.

Organism, MB No. (ATCC)	Inhibition zone diameter, mm.
Bacillus sp. 633	16
Staphylococcus aureus 108 (6538P)	11
Bacillus subtilis 964 (6633)	12
Sarcina lutea 1101 (9341)	30
Streptococcus faecalis 753	0
Corynebacterium pseudodiphtheriticum 261 (9742)	20
Streptococcus faecium 2820	26
Streptococcus agalactiae 2875	27
Proteus vulgaris 1012	15
Pseudomonas aeruginosa 979	11
Serratia marcescens 252 (990)	16
Alcaligenes faecalis 10 (213)	17
Brucella bronchiseptica 965 (4617)	16
Salmonella gallinarum 1287	15
Vibrio percolans 1272 (8461)	21
Xanthomonas vesicatoria 815	12
Proteus vulgaris 838 (21100)	23
Pseudomonas stutzeri 1231 (11607)	13
Klebsiella pneumoniae 1264	18
Aerobacter aerogenes 835	12
Erwinia atroseptica (4446)	12
Pseudomonas aeruginosa 2824	0
Escherichia coli 60 (9637)	12
Proteus vulgaris 2112 (episome)	17
Proteus mirabilis 3126	14
Mycoplasma gallisepticum S-6	32

The culture broth containing heneicomycin was filtered through Celite and the filtrate was adjusted to pH 5.0 with hydrochloric acid before extraction with ethyl acetate. The ethyl acetate extract was triturated with *n*-hexane thereby precipitating heneicomycin. The resulting precipitate was subjected to preparative silica-gel thin-layer chromatography using chloroform, methanol, and concentrated aqueous NH_3 (80: 20: 1.5) as a developing solvent. The major band, which was bright yellow in color, was extracted with a mixture of chloroform, methanol, and distilled water (60: 30: 3). The solution was concentrated, redissolved in benzene - methanol (95: 5) and lyophilized, yielding heneicomycin as an amorphous bright yellow powder which was soluble in methanol and ethanol and slightly soluble in chloroform and acetone. Heneicomy-

Fig. 2. U.V. Spectrum of heneicomycin. (0.822 mg/100 ml MeOH)

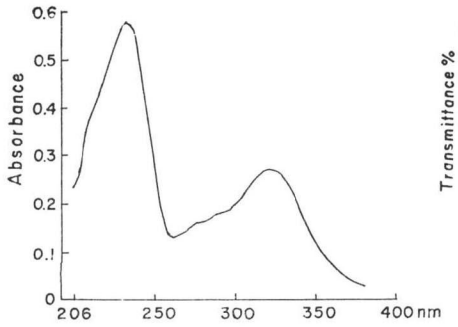


Fig. 3. Infrared spectrum of heneicomycin. (Film spectrum)

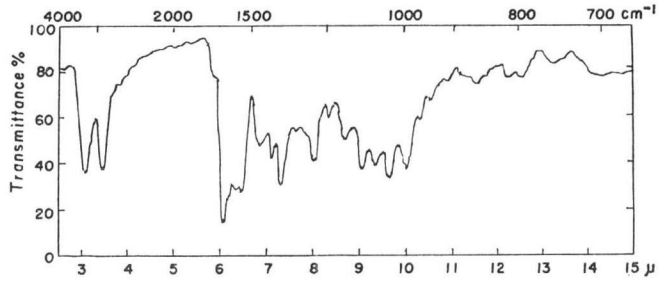
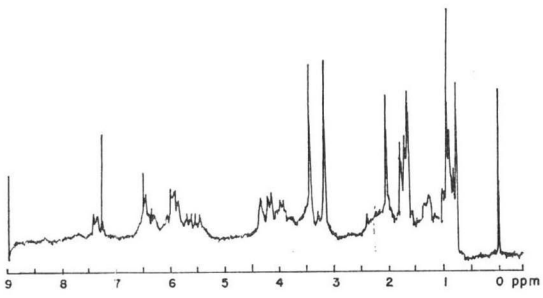


Fig. 4. NMR Spectrum of heneicomycin ($CDCl_3$-TMS internal).



cin has prominent characteristic absorption peaks at approximately λ (0.01 N HCl in methanol) = 322 nm, E% = 328; and 231 nm, E% = 722. Combustion analysis gave C 65.23%, H 7.78%, and N 3.47%, values consistent with $C_{44}H_{62}N_2O_{11} \cdot H_2O$. The UV, IR, and NMR spectra are shown in Figs. 2, 3, and 4, respectively. The NMR spectrum was taken in deuterated $CHCl_3$, with trimethylsilane as internal reference. The field desorption mass spectrum showed peaks at *m/e* 794 (M^+) as well as *m/e* 795 (M^+H^+) and 817 (M^+Na^+). The electron impact mass spectrum showed peaks (relative intensities in parentheses) at *m/e* 680 (0.6), 648 (2.1), 630 (0.8), 594 (1.0), 483 (1.7), 481 (1.1), 465 (1.1), 463 (0.8), 384 (6.7), 338 (6.3), 306 (4.6), 302 (9.4), 275 (10.5), 274 (9.2), 245 (33), 192 (36), 181 (23), 179 (21), 166 (33), 152 (100), 135 (21), 133 (13), 123 (92), 121 (19), 119 (25), 108 (35), 107 (36), 97 (94). The trimethylsilyl derivative showed peaks up to *m/e* 1226 ($M^+ + 6TMS$). The above physical properties together with a detailed spectral examination indicated that heneicomycin was a new antibiotic, namely the 3-deshydroxy pyran modification of aurodox.

A solution of 1,000 μg/ml of heneicomycin in 40% acetone-distilled water demonstrated a broad antibacterial spectrum (Table 1), using a sample droplet of 0.015 ml on the surface of seeded agar plates containing nutrient agar. The 40% acetone-water solution alone demonstrated no inhibition with any of the test organisms. Of all organisms tested, *Mycoplasma gallisepticum* S-6 was the most susceptible.

References

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2) LIU, C.; T. HERMANN & P. A. MILLER: Feedback inhibition of the synthesis of an antibiotic: Aurodox (X5108). J. Antibiotics 30: 244~251, 1977
3) WOLF, H. & H. ZAHNER: Stoffwechselprodukte von Mikroorganismen. 99. Mitteil. Kirromycin. Arch. Mikrobiol. 83: 147~154, 1972