NOTE

FACTUMYCIN, A NEW ANTIBIOTIC (A40A): FERMENTATION, ISOLATION AND ANTIBACTERIAL SPECTRUM

VINCENT P. GULLO, SHELDON B. ZIMMERMAN, RAY S. DEWEY, OTTO HENSENS and PATRICK J. CASSIDY

Merck Sharp and Dohme Research Laboratories
Rahway, New Jersey 07065, U.S.A.

RUIKO ŌIWA and SATOSHI ŌMURA

Kitasato University
Shirokane, Minato-ku, Tokyo 108, Japan

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In the course of our screening for new antibiotics, we have discovered factumycin (antibiotic, A40A)[1], a unique antibiotic belonging to the aurodox (X-5108)[2], kirromycin[3], efrotomycin[4], mocimycin[5], heneicomycin (A21A)[6] group. Factumycin is found in the culture broth of *Streptomyces lavendulae*, designated ATCC 31312 (MA4758 in the Merck & Co., Inc., Culture Collection) and is active *in vitro vs.* both Gram-positive and Gram-negative organisms. In addition, factumycin can be used as a growth-permitting agent for animals[1].

Factumycin is produced by cultivating *Streptomyces lavendulae* under submerged, aerobic conditions in aqueous medium containing 2% tomato paste, 1% primary yeast NF, 2% CPC modified starch, 5 ppm $CoCl_2 \cdot 6H_2O$ and 0.1% polyglycol 2000 with a pH 7.2~7.4 before sterilization. Production of the antibiotic reached a maximum after 5 days incubation at 28°C using an airflow of 283 liters/minute and an agitation rate of 130 rpm.

Fig. 1. Structure of factumycin.

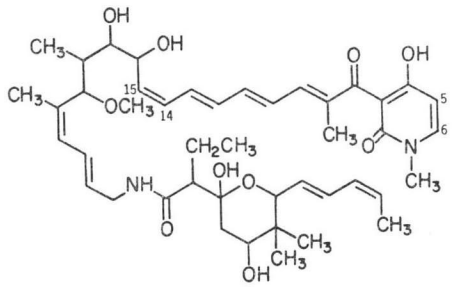

Fig. 2. UV spectrum of factumycin (MeOH).

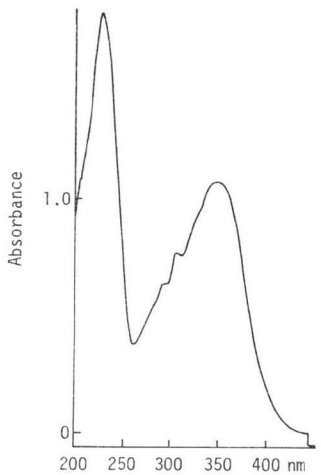

Fig. 3. Infrared spectrum of factumycin ($CHCl_3$ solution).

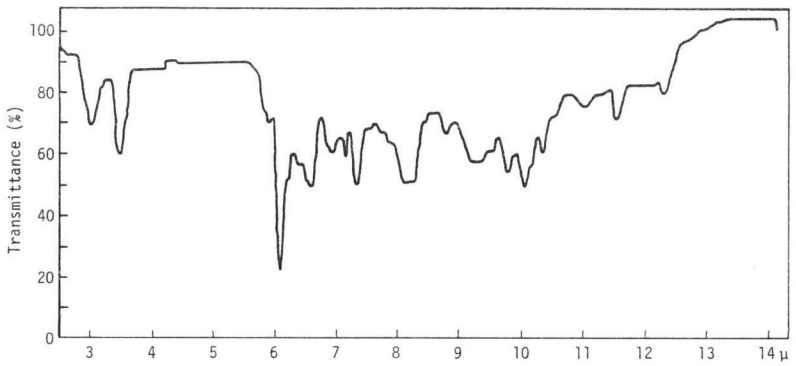

Fig. 4. 300 MHz NMR spectrum of factumycin on Varian SC 300 (perdeuteromethanol).

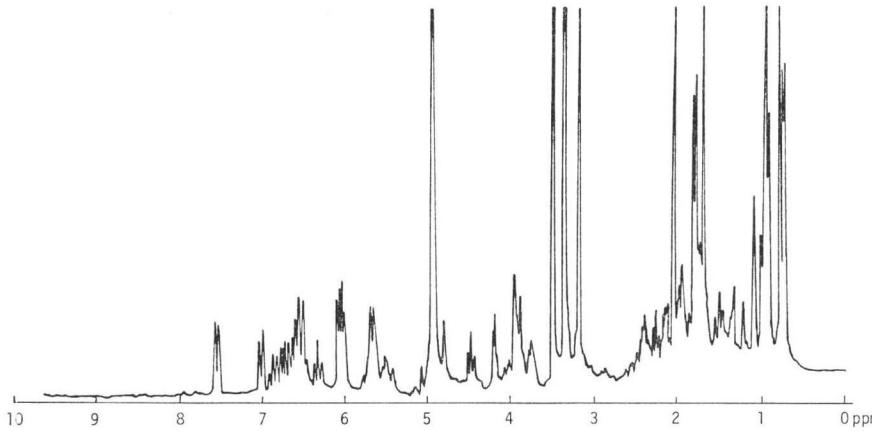

Table 1. Antibacterial spectrum of factumycin.

Organism, MB No. (ATCC)	Inhibition zone diameter (mm)
Bacillus sp. 633	14
Proteus vulgaris 1012	18
Pseudomonas aeruginosa 979	9
Serratia marcescens 252 (990)	16
Staphylococcus aureus 108 (6538P)	12
Bacillus subtilis 964 (6633)	20
Micrococcus luteus 1101 (9341)	26
Brucella bronchiseptica 965 (4617)	20
Salmonella gallinarum 1287	18
Vibrio percolans 1272 (8461)	22
Xanthomonas vesicatoria 815	13
Proteus vulgaris 838 (21100)	22
Escherichia coli 1418	20
Pseudomonas stutzeri 1231 (11607)	13
Klebsiella pneumoniae 1264	21
Enterobacter aerogenes 835	16
Erwinia atroseptica 1159 (4446)	16
Escherichia coli 605	18
Pseudomonas aeruginosa 2824	12
Escherichia coli 60 (9637)	23
Streptococcus faecium 2820	26
Bacillus subtilis 9645	16
Proteus mirabilis 3126	19

In order to isolate factumycin, the whole broth (16 liters) is adjusted from pH 6.7 to pH 9.0 with 50% NaOH and filtered through Supercel. The filtrate is adsorbed onto an XAD-2 column (1 liter). The column is washed with deionized H_2O (1 liter) and factumycin is eluted with 75% acetone - water (2 liters). The eluate is concentrated *in vacuo* to 625 ml. The pH of the aqueous concentrate is adjusted from 7.2 to 4.0 and extracted with ethyl acetate (2×625 ml). The extracts are combined and evaporated to an oily residue. The residue is redissolved in ethyl acetate (200 ml) and this solution is added dropwise to hexanes (500 ml) with vigorous stirring. The yellow precipitate formed is chromatographed on preparative TLC plates (E. Merck, silica gel 60 F-254) using 75% acetone - hexane as the developing solvent. The major dark yellow band is extracted to yield 290 mg of oily factumycin.

The physico-chemical properties of factumycin allowed the structure in Fig. 1 to be assigned. The mass spectrum of the trimethylsilyl derivative exhibited peaks up to *m/z* 1,210 which corresponds to $C_{44}H_{62}N_2O_{10}+6C_3H_8Si$. The combustion analysis gave C 65.32, H 7.92, N 3.14 consistent with $C_{44}H_{62}N_2O_{10}\cdot 2H_2O$. The ultraviolet spectrum of factumycin was unique for this family of compound: UV(CH_3OH) λ 355 nm ($E^{1\%}_{1cm}$ 420) and 231 ($E^{1\%}_{1cm}$ 648) (Fig. 2). The characteristic longer wavelength absorption for this family of compounds is approximately 322 nm. A simple explanation for the bathochromic shift is the presence of an additional double bond extending the conjugation of the usual trienone. The IR ($CHCl_3$) and ^1H NMR (CD_3OD) are shown in Figs. 3 and 4, respectively. The above physical properties together with detailed spectral examination as compared with other members in this family indicate that factumycin is a new anti-

biotic as shown in Fig. 1. Factumycin differs from heneicomycin and all other members of this family by containing an open tetrahydrofuran ring and an additional double bond in conjugation with the ketone.

Recently, a new member of this group, kirrothricin, was reported[7,8]. This compound is quite similar to factumycin differing from factumycin in two respects. The first variation is in the pyridone ring. The pyridone ring in kirrothricin is hydrogenated at the 5,6 position which is similar to dihydromocimycin[9] in this respect. In addition, the 14,15 double bond in kirrothricin is depicted in the *E* configuration. For factumycin, the ^{1}H NMR for this double bond, although partly obscured, was best interpreted as the *Z* configuration*. If the 14,15 double bond in kirrothricin is also *Z*, kirrothricin can be described as dihydrofactumycin.

The antibacterial spectrum of factumycin at 1,000 μg/ml is shown in Table 1. The spectrum was obtained by dissolving factumycin in 40% acetone - distilled water and placing a sample droplet of 0.015 ml on the surface of seeded agar plates containing nutrient media. The 40% acetone - water solution demonstrated no inhibition with any of the test organisms. Factumycin is active *in vitro vs.* both Gram-positive and Gram-negative organisms.

* The coupling constant for the 14, 15 hydrogens is 11.3 Hz. The ^{1}H NMR will be described in a forthcoming paper.

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