MAZETHRAMYCIN, A NEW MEMBER OF ANTHRAMYCIN GROUP ANTIBIOTICS

Sir:

A new antitumor antibiotic named mazethramycin has been isolated from the culture broth of *Streptomyces thioluteus* ME561-L4. It was easily converted into its methyl or ethyl ether and anhydromazethramycin during extraction and purification. In this communication, the isolation, characterization and structural elucidation of mazethramycin and its derivatives are reported. The structure of mazethramycin has been confirmed to be an N-methyl derivative of anthramycin[1,2].

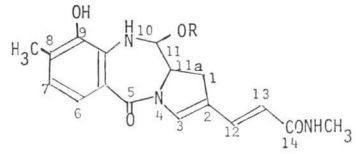
Mazethramycin (**I**) was produced in a shaking culture of the strain ME561-L4 at 27°C in a medium containing 2.5% glycerol, 0.5% meat extract, 0.5% Polypepton, 1.0% yeast extract, 0.2% NaCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% K_2HPO_4 and 0.32% $CaCO_3$ adjusted to pH 7.4. After 96-hour cultivation, the cultured broth (3.53 liters) was filtered. The antibiotic contained in the mycelial cake was extracted with methanol (2.5 liters) and the methanol extract was concentrated to an aqueous solution (500 ml). The culture filtrate and the mycelial extract were combined and extracted with *n*-butanol (4 liters) at pH 8.0. The butanol extract was concentrated to dryness. The residue was dissolved in 2 liters of water and the solution was adjusted to pH 7.4 with 1 N NaOH. The antibiotic in the aqueous solution was adsorbed on a column of Amberlite XAD-2 (400 ml) and eluted with 2 liters of 50% aqueous acetone. The eluate was concentrated to dryness, yielding a brownish crude powder (2.2 g). The crude powder was subjected to silica gel (Wakogel C-200, 100 g) column chromatography eluted with a mixture of chloroform and methanol (20: 1 in volume). The active eluate was concentrated to dryness (150 mg) and crystallization from hot methanol gave yellow needles (68 mg) of mazethramycin methyl ether (**II**), m.p. 245~279°C (decomp.); $[\alpha]_D^{23}+900°$ (*c* 0.2, dimethylformamide). Found: C 63.38, H 6.18, N 12.40%. Calcd. for $C_{18}H_{21}N_3O_4$: C 62.96, H 6.16, N 12.24%. UV $\lambda_{max}^{CH_3CN}$ nm(ε): 218 (34,600), 235 (sh. 30,700) and 335 (51,000); IR ν_{max}^{KBr} cm^{-1}: 3350, 3120, 2950, 1660, 1630, 1610, 1565, 1515, 1465, 1410, 1370, 1345, 1315, 1250, 1220, 1170, 1145, 1070, 1025, 990, 955, 940, 910, 880, 855, 820 and 760. The fragment peak (*m/e* 311.1254, calcd. for $C_{17}H_{17}N_3O_3$: *m/e* 311.1268) corresponding to the molecular ion peak of anhydromazethramycin (**IV**) was obtained by high resolution mass spectroscopy. The PMR spectrum of **II** was very similar to that of anthramycin methyl ether[2] except for NCH_3 and CONH signals in **II** instead of $CONH_2$ in the latter, as shown in Table 1. Acid hydrolysis of **II** by refluxing with 1 N HCl for 2 hours gave methylamine which was detected by gas chromatography (glass column 3 mm×1 m, Chromosorb 103,80/100, RT=2.2 minutes). Thus, the structure of **II** was elucidated to be 5, 10, 11, 11a-tetrahydro-9-hydroxy-11-methoxy-8-methyl-5-oxo-1*H*-pyrrolo-[2,1-*c*][1,4]-benzodiazepine-2-N-methylacrylamide. It is suggested that **II** has 11 *R* and 11a *S* configurations by comparison of the PMR spectrum and optical rotation values with those of anthramycin methyl ether[1,2].

Mazethramycin (**I**) was obtained as a light yellow amorphous powder by evaporation of a solution of **II** in 20 ml of 50% aqueous acetone, m.p. 181~193°C (decomp.); $[\alpha]_D^{21}+730°$ (*c* 0.062, dimethylformamide). Found: C 62.35, H 5.72, N 12.82%. Calcd. for $C_{17}H_{19}N_3O_4$: C 61.99, H 5.82, N 12.76%. UV $\lambda_{max}^{CH_3CN}$ nm(ε): 320 (sh. 34,600) and 335 (39,400); IR ν_{max}^{KBr} cm^{-1}: 3500,

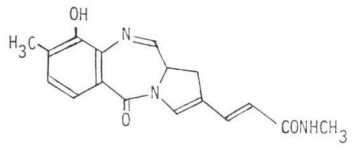
Table 1. PMR chemical shifts in mazethramycin methyl ether (**II**) and anthramycin methyl ether.

Proton	ppm (*J* Hz)	
	Mazethramycin methyl ether	Anthramycin methyl ether
1-H_2	2.67 (15, 6)	2.66 (16, 6)
	3.10 (15, 11)	3.12 (16, 11)
3-H	7.42	7.43
6-H	7.28 (8)	7.30 (8)
7-H	6.48 (8)	6.48 (8)
10-H	7.10 (6.4)	7.12 (6.4)
11-H	4.76 (6.4)	4.78 (6.4)
11a-H	4.21 (11, 6)	4.22 (11, 6)
CONH	7.82 (5)	—
$CONH_2$	—	6.9, 7.3
N-CH_3	2.66 (5)	—
12-H	7.27 (15)	7.29 (15)
13-H	5.72 (15)	5.74 (15)
8-CH_3	2.19	2.20
O-CH_3	3.25	3.25

Spectra were measured in DMSO-d_6 using TMS as the internal reference.



I: R=H, II: R=CH_3, III: R=CH_2CH_3



IV

3350, 3270, 3100, 2930, 1645, 1610, 1560, 1530, 1415, 1350, 1325, 1255, 1220, 1150, 1095, 1055, 980, 960, 945, 865, 840, 805, 755 and 760.

Anhydromazethramycin (**IV**) was obtained as a light yellow crystalline powder by refluxing **II** with acetonitrile in the presence of Amberlite CG-50 (H^+ form) for an hour, m.p. 252~262°C (decomp.); $[\alpha]_D^{22}+1940°$ (*c* 0.05, dimethylformamide). Found: C 65.04, H 6.10, N 13.04%. Calcd. for $C_{17}H_{17}N_3O_3$: C 65.58, H 5.50, N 13.50%. MS: *m/e* 311. UV $\lambda_{max}^{CH_3CN}$ nm(ε): 229 (16,100), 235 (sh. 15,800), 298 (sh. 19,300), 315 (21,800) and 352 (21,100); IR ν_{max}^{KBr} cm^{-1}: 3320, 3050, 2950, 1660, 1605, 1545, 1490, 1435, 1405, 1380, 1340, 1295, 1245, 1220, 1170, 1145, 1075, 1045, 970, 960, 920, 875, 835, 825, 800, 760 and 720.

Mazethramycin methyl ether (**II**) and ethyl ether (**III**) were easily obtained as stable crystals from either **I** or **IV** by treatment with anhydrous methanol or ethanol, respectively, but the *n*-butyl ether was not obtained. The ethyl ether (**III**) shows m.p. 216~223°C (decomp.); $[\alpha]_D^{21}+450°$ (*c* 0.067, dimethylformamide). Found: C 63.25, H 6.53, N 12.25%. Calcd. for $C_{19}H_{23}N_3O_4$: C 63.85, H 6.48, 11.76%. UV $\lambda_{max}^{CH_3CN}$ nm(ε): 217 (25,700), 235 (sh. 19,300) and 333 (43,600); IR ν_{max}^{KBr} cm^{-1}: 3300, 3060, 2900, 1650, 1605, 1550, 1515, 1410, 1350, 1330, 1305, 1250, 1220, 1150, 1080, 1055, 1025, 965, 875, 835, 800 and 760.

From these data, the structures of mazethramycin and its derivatives can be shown by **I**~**IV**.

The antimicrobial spectrum of **II** is shown in Table 2. A marked effect of **II** in prolonging of the survival period of mice inoculated with leukemia L-1210 was observed as shown in Table 3. The acute LD_{50} of **II** in mice was 0.8 mg/kg by intravenous injection.

Table 2. Antimicrobial spectrum of mazethramycin methyl ether.

Test organisms	Minimum inhibitory concentration (mcg/ml)
Staphylococcus aureus FDA 209P	3.12
Micrococcus flavus	3.12
Sarcina lutea PCI 1001	3.12
Bacillus subtilis PCI 219	1.56
Escherichia coli NIHJ	6.25
Shigella dysenteriae JS 11910	3.12
Salmonella typhi	50
Proteus vulgaris OX 19	25
Klebsiella pneumoniae PCI 602	3.12
Mycobacterium 607	100
Candida pseudotropicalis	6.25
Candida krusei	>50
Saccharomyces cerevisiae	>25
Pyricularia oryzae	6.25
Xanthomonas oryzae	1.56
Aspergillus niger	>50
Trichophyton asteroides	12.5

Bacteria were incubated on nutrient agar plates at 37°C for 17 hours and fungi were incubated on nutrient agar plates containing 1% glucose at 27°C for 40 hours.

Table 3. Effect of mazethramycin methyl ether against L-1210 in CDF_1 mice.

Dose mcg/kg/day	T/C×100*
62.5	205
31.3	240
15.6	164
7.8	164
3.9	123

* $T/C=\frac{\text{Survival period of treated mice}}{\text{Survival period of control mice}}\times 100$

Treatment was started 2 hours after the inoculation of 1×10^5 cells of L-1210 and continued daily for 10 days.

Acknowledgement

The authors wish to thank Hoffmann-La Roche,

Inc., Nutley, New Jersey, for a gift of anthramycin methyl ether.

This work was partly supported by a Grant for Cancer Research from the Ministry of Education, Science and Culture in Japan.

Setsuko Kunimoto
Toru Masuda
Nobuo Kanbayashi
Masa Hamada
Hiroshi Naganawa
Masashi Miyamoto
Tomio Takeuchi
Hamao Umezawa

Institute of Microbial Chemistry
Kamiosaki, Shinagawa-ku, Tokyo 141,
Japan

(Received March 18, 1980)

References

1) Leimgruber, W.; V. Stefanović, F. Schenker, A. Karr & J. Berger: Isolation and characterization of anthramycin, a new antitumor antibiotic. J. Am. Chem. Soc. 87: 5791~5793, 1965
2) Leimgruber, W.; A. D. Batcho & F. Schenker: The structure of anthramycin. J. Am. Chem. Soc. 87: 5793~5795, 1965