INTERACTION OF NAPHTHOMYCIN A WITH SULFHYDRYL COMPOUNDS

Sir:

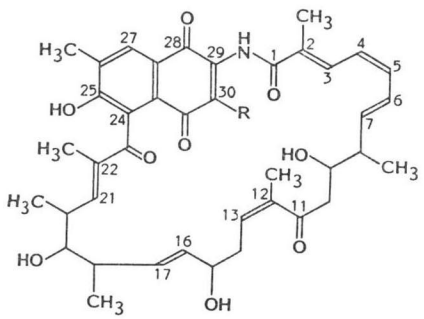
We have reported that naphthomycin A exhibits antitumor activity in mice and the mechanism of cytotoxicity is the inhibition of various SH enzymes[1]. We have further found that naphthomycin A interacts with a variety of SH compounds by replacing the Cl group by an SR group. These results are presented in this communication.

Materials and Methods

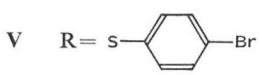
Naphthomycin A (**I**) was prepared as described previously[1]. **I**: $C_{40}H_{46}ClNO_9$; FD-MS *m/z* 719 (M^+); shows UV λ_{max}^{MeOH} nm (ε) 231 (38,900) and 275 (33,400). The elemental analysis revealed that **I** contains 4.49% Cl but no S. The retention time of **I** was 4.20 minutes by HPLC.

Methanethiol, ethanethiol and propanethiol were purchased from Nakarai Chemical Co., Tokyo. *p*-Bromothiophenol was a product of Aldrich Chemical Co., Wisconsin, U.S.A. HPLC was performed on a reverse phase silica gel column (8NVC18 5 μ, 10 cm×8 mm, diameter, Waters Associates) developing with MeOH - H_2O - AcOH (80: 20: 1). The column was eluted with a flow rate of 2.0 ml/minute and the eluate was monitored with a UV detector at 280 nm.

Fig. 1. The structure of naphthomycin A and its derivatives.



I R=Cl (Naphthomycin A)
II R=SCH_3
III R=SCH_2CH_3
IV R=$SCH_2CH_2CH_3$



V R= S—Br

Table 1. Cytotoxicity of naphthomycin A and its derivatives.

The cytotoxicity was assayed by the inhibition of [^{3}H]thymidine uptake into DNA of L5178Y murine lymphoblastoma. The cells were grown to a density of 2×10^5/ml and incubated with the drug for 2 hours at 37°C in RPMI 1640 medium supplemented with 10% horse serum in a humidified atmosphere of 95% air and 5% CO_2. Then 0.2 μCi of [^{3}H]thymidine was added to 200 μl of the culture, and the TCA-insoluble radioactivity was determined.

Antibiotic	IC_{50} (μM)
I	1.2
II	1.2
III	3.8
IV	3.0

Experimental

Methanethiol Derivative (**II**) of Naphthomycin A

A mixture of naphthomycin A (8.9 mg) dissolved in 1/15 M phosphate buffer (7 ml, pH 7.4) and methanethiol (4.7 mg) dissolved in MeOH (0.5 ml) was kept at 37°C for 10 minutes. The reaction product was extracted with EtOAc and purified by preparative silica gel TLC developed with $CHCl_3$ - EtOAc (1: 2) to yield 4.9 mg of **II**. The retention time of **II** was 3.54 minutes by HPLC. **II**: $C_{41}H_{49}NO_9S$; MS *m/z* 731 (M^+); shows UV λ_{max}^{MeOH} nm (ε) 230 (57,500) and 275 (33,200). The elemental analysis indicated that **II** contains 4.42% S but no Cl. The ^{1}H NMR spectrum of **II** was similar to that of **I**, except additional SCH_3.

Ethanethiol (**III**) and Propanethiol (**IV**) Derivatives of Naphthomycin A

Naphthomycin A (11.1 mg) and ethanethiol or propanethiol were treated as described above to yield 7.3 mg of **III** or 10.9 mg of **IV**. MS *m/z* 745 (M^+) for **III** and 759 (M^+) for **IV**.

p-Bromothiophenol Derivative (**V**) of Naphthomycin A

Naphthomycin A (5.2 mg) and *p*-bromothiophenol (1 mg) were treated as described above to yield 2.8 mg of **V**. The retention time was 7.13 minutes by HPLC. MS *m/z* 871 (M^+).

Cytotoxicity of Naphthomycin A Derivatives

Although the cytotoxicity of naphthomycin A (**I**) was reversed by SH compounds such as 2-

mercaptoethanol, dithiothreitol and glutathione[1], the alkanethiol derivatives **II**, **III** and **IV** showed cytotoxicity for L5178Y murine lymphoma. As presented in Table 1, **II** exhibited the same degree of inhibition of DNA synthesis as **I**, and **III** and **IV** less degree of inhibition.

The present result together with the previous studies[1] gave a molecular basis for the action of naphthomycin A.

Acknowledgments

The current work was supported in part by a Grant-in-Aid for cancer research from the Ministry of Education, Science and Culture, Japan. The authors express their deep thanks to Dr. HAMAO UMEZAWA, Institute of Microbial Chemistry, Tokyo, for his generous advice and cooperation in the present study.

TAKAYOSHI OKABE
KOKICHI SUZUKI
HIDEO SUZUKI
YOSHIO INOUYE†
SHOSHIRO NAKAMURA†
NOBUO TANAKA*

Institute of Applied Microbiology
University of Tokyo
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan
† Institute of Pharmaceutical Sciences
Hiroshima University School of Medicine
1-2-3 Kasumi, Minami-ku,
Hiroshima 734, Japan

(Received October 1, 1985)

Reference

1) OKABE, T.; B. D. YUAN, F. ISONO, I. SATO, H. FUKAZAWA, T. NISHIMURA & N. TANAKA: Studies on antineoplastic activity of naphthomycin, a naphthalenic ansamycin, and its mode of action. J. Antibiotics 38: 230~235, 1985