Isolation and Structure Elucidation of I-851, an Antagonist of Chloramphenicol

Yoshitaka IMAGAWA, Shoji SHIMA,
Akira HIROTA and Heiichi SAKAI

Department of Agricultural Chemistry,
College of Agriculture, University of Osaka
Prefecture, Sakai, Osaka, Japan

Received October 20, 1977

In screening for antagonists of the antibiotics from microbial origins, a culture of *Streptomyces* sp. No. 851 isolated from soil was found to produce an antagonist of chloramphenicol. The substance tentatively named I-851 markedly diminishes the antibiotic action of chloramphenicol against *Escherichia coli*. The antagonist of antibiotic agent showed several interesting biological activities to counteract the toxicity of the antibiotic agent.¹⁾ In this paper we wish to report the isolation and structure elucidation of the substance.

The strain producing I-851 grew well on various media, and its aerial mycelium was straight and the size of spore was *ca.* $0.75 \times ca. 1.2 \mu$. The spore had smooth surface. The strain contained LL-diaminopimelic acid in the cell wall. As the carbon source, D-glucose, D-mannitol, L-arabinose, D-xylose, L-rhamnose, D-fructose, raffinose, inositol, salicin and sucrose were found to be well utilized, while sorbitol, sorbose and erythritol were not

utilized. The strain was found to belong to the species, *Streptomyces fulvoviolaceus*, according to the guide in Bergey's Manual.²⁾

Production of I-851 was carried out in a 20-liter jar fermentor containing 14 liters of a fermentation medium composed of 2% glycerin, 0.5% $(\text{NH}_4)_2\text{SO}_4$, 0.4% soybean meal, 0.1% yeast extract, 0.2% NaCl, 0.14% KH_2PO_4 , 0.36% $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (adjusted to pH 7.4). Fermentation was conducted with agitation (300 rpm) and aeration (8 liters/min) for 30 hr at 30°C. The cultured broth was adjusted to pH 6 with NaOH. The active substance was extracted with ethyl acetate, adsorbed on a column of silica gel and eluted with benzene-ethyl acetate (95:5). I-851 was obtained as yellow crystals, mp 153~155°C (dec.).

The molecular formula of I-851 was determined to be $\text{C}_7\text{H}_5\text{NO}_5$ by high resolution mass spectrometry (M^+ , m/e : 183.0146: calcd. for $\text{C}_7\text{H}_5\text{NO}_5$, 183.0167). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3350, 3250, 2730, 1700, 1590, 1500, 1440, 1370, 1250, 1050, 990, 880, 750, 570. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 244 (9150), 292 (15200), 349 (660). This substance was positive to 2,4-dinitrophenyl hydrazine. The IR spectrum of I-851 shows absorption bands at 3350 and 3250 cm^{-1} due to the amino group and 1700 cm^{-1} due to an α,β -unsaturated carbonyl group. The NMR spectrum is shown in Fig. 1. Signals at δ 4.02 and 4.22 ppm (each 1H, d, $J=4$ Hz) were assigned as two epoxy protons. The two epoxy protons were adjacent respectively to the carbonyl group as judged from the chemical shift at the lower field. Signals at δ 13.2, 10.0 and 8.6 ppm disappeared on treatment with D_2O . A

FIG. 1. ^1H -NMR Spectrum of I-851 (100 MHz, in Acetone d_6).

signal at δ 13.2 was assigned as the hydroxy proton of the carboxyl group, and amino protons were observed nonequivalently at δ 10.0 and 8.6 ppm, respectively. In the CMR spectrum of I-851, signals at δ 187.4 (s) and 192.8 (s) ppm indicated the presence of two carbonyl carbons. The carboxyl carbon was observed at δ 168.7 (s) ppm. The epoxy carbons were observed at δ 53.6 (d, $J=54.2$ Hz) and 55.4 (d, $J=52.7$ Hz) ppm, respectively. Moreover, two olefin carbons were observed at δ 96.4 (s) and 155 (s) ppm. The pK_a values obtained from the titration curve of I-851 were 6.1 and 9.7. These values suggested the presence of both carboxyl and amino groups. The UV absorption of I-851 at 244 and 349 nm indicated that it resembled phyllostine.³⁾ However, the UV absorption of I-851 at 292 nm was different from that of phyllostine. This absorption indicated the presence of an α,β -unsaturated carboxyl group. All the above-mentioned data suggested that the structure of I-851 is 2-amino-3-carboxy-1,4-benzoquinone 5, 6-epoxide (I). It is a new nitrogen-containing derivative of cyclohexene epoxide, *e.g.* terreic acid,⁴⁾ epoxydon⁵⁾ and phyllostine.

The structure of I-851 (I) was similar to that of antibiotic G7063-2 (II),⁶⁾ recently isolated as a nitrogen-containing derivative of

cyclohexene epoxide. Antibiotic G7063-2 was moderately active against bacteria, whereas I-851 was an antagonist of chloramphenicol. The biological activities of I-851 as antagonist of chloramphenicol are now under investigation.⁷⁾

Acknowledgements. The authors wish to express their thanks to Dr. A. Isogai, Department of Agricultural Chemistry, The University of Tokyo, for the measurement of the NMR spectra and helpful discussions. The authors are also indebted to Fujisawa Pharmaceutical Co., Ltd., for electron microscopic measurement and to Takeda Chemical Industries, Co., Ltd., for the measurement of high resolution mass spectrum.

REFERENCES

- 1) H. Yonehara, H. Seto, A. Shimazu, S. Aizawa, T. Hidaka, K. Kakinuma and N. Ōtake, *Agric. Biol. Chem.*, **37**, 2771 (1973).
- 2) T. G. Pridham and H. D. Tresner, Streptomycetaceae, in *Bergey's Manual of Determinative Bacteriology* (8th Edition), The Williams and Wilkins Co., 1974, pp. 747~845.
- 3) S. Sakamura, J. Ito and R. Sakai, *Agric. Biol. Chem.*, **34**, 153 (1970).
- 4) J. Sheehan, W. Lawson and R. Graul, *J. Am. Chem. Soc.*, **80**, 5536 (1958).
- 5) A. Closse, R. Mauli and H. P. Sigg, *Helv. Chim. Acta*, **49**, 204 (1966).
- 6) M. Noble and D. Noble, *J. Antibiot.*, **30**, 455 (1977).
- 7) Petri plates were prepared with bouillon medium inoculated with *E. coli* and containing an inhibitory concentration of 10 $\mu\text{g/ml}$ of chloramphenicol. The paper discs containing I-851 at the concentration of 0.625~5 $\mu\text{g/disc}$ were placed on the agar surface. Zones of growth appeared around each disc containing I-851.