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INHIBITION OF DNA TOPOISOMERASES BY MICROBIAL INHIBITORS

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New inhibitors of DNA topoisomerase named 2280-DTI and 2890-DTI have been discovered in the culture filtrates of *Micromonospora* sp. strain No. 2280 and *Streptomyces* sp. strain No. 2890, respectively. Both inhibitors were purified from each culture filtrate by column chromatography on Diaion, Dowex and gel filtration. Both inhibitors were thermostable acidic substances with high molecular weight and inhibited topoisomerase I in a non-competitive manner. They differed from well-known inhibitors of topoisomerases such as camptothecin and doxorubicin, which inhibit the DNA rejoining reaction of the enzyme by intercalation into DNA strands or stabilizing the cleavable complex (enzyme–DNA reaction intermediate). 2280-DTI and 2890-DTI did not intercalate into DNA strands and also had no ability to stabilize the cleavable complex. It is suggested that 2280-DTI and 2890-DTI inhibit the DNA breaking and rejoining reactions of topoisomerase by direct action on the enzyme molecule.

Keywords: DNA topoisomerase inhibitor; *Micromonospora*; *Streptomyces*; 2280-DTI; 2890-DTI

INTRODUCTION

DNA topoisomerases are nuclear enzymes that catalyze the concerted breaking and rejoining of DNA strands and are involved in producing the necessary topological and conformational changes in DNA which are critical to many cellular processes such as replication, recombination and transcription.[1] There are two types of DNA topoisomerases, topoisomerase I[2]

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and topoisomerase II,[3] which have been isolated from mammalian cells. Topoisomerase I catalyzes the passage of the DNA strands through a transient single-strand break, while topoisomerase II catalyzes the passage of DNA double strands through a transient double-strand break. In addition to their normal cellular functions, both enzyme have recently emerged as important cellular targets for chemical intervention in the development of anticancer agents.[4,5] Several of the anticancer agents in clinical use have been shown to be potent inhibitors of topoisomerases. Adriamycin (doxorubicin),[6] amsacrine (m-AMSA),[7] ellipticine,[8] saintopin,[9] streptonigrin[10] and terpentecin[11] have been demonstrated to possess significant activity as inhibitors of topoisomerase II. The plant alkaloid camptothecin[12] and its synthetic derivatives such as CPT-11[13] and topotecans[14] are the most extensively studied topoisomerase I inhibitors. All of these inhibitors inhibit the rejoining reaction of topoisomerases by stabilizing a tight topoisomerase-DNA complex termed "cleavable complex".[15] However, there are no known inhibitors which inhibit topoisomerase itself before it binds to DNA.

In order to find new inhibitors of topoisomerases, we have screened microorganisms having the ability to inhibit the breaking and rejoining reactions of DNA by the enzymes. Recently, we have found two inhibitors in the culture filtrates of *Micromonospora* sp. strain No. 2280 and *Streptomyces* sp. strain No. 2890 isolated from soil samples. These inhibitors, termed 2280-DTI and 2890-DTI, are high molecular weight substances and show different inhibitory spectra. As far as we can ascertain, these inhibitors are the first reported examples of high molecular inhibitors of microbial origin with inhibitory activity against topoisomerases. This report describes the purification procedures and some properties of 2280-DTI and 2890-DTI.

MATERIALS AND METHODS

Enzymes and Substrates

Bam HI (EC 3.1.23.6) from *Bacillus amyloliquefaciens* H, *Eco* RI (EC 3.1.23.13) from *Escherichia coli* RY13, *Hin* dIII (EC 3.1.23.21) from *Haemophilus influenzae* Rd, T4 DNA ligase (EC 6.5.1.1) from *Escherichia coli* and DNA topoisomerase I (EC 5.99.1.2) from calf thymus gland were obtained from MBI Fermentas. *Alu* I (EC 3.1.23.1) from *Arthrobacter luteus*, *Pst* I (EC 3.1.23.31) from *Providencia stuartii* and *Sca* I (EC 3.1.21.4) from *Streptomyces caespitosus* were obtained from Gibco BRL.

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DNase I (EC 3.1.21.1) from bovine pancreas, DNase II (EC 3.1.22.1) from porcine spleen and RNase A (EC 3.1.27.5) from bovine pancreas were obtained from Sigma Chemicals. DNA topoisomerase II (EC 5.99.1.3) from human placenta and proteinase K (EC. 3.4.21.14) from *Tritirachium album* were obtained from TopoGEN Inc. and Boehringer Mannheim GmbH, respectively. Supercoiled pBR322 DNA and RNA from yeast used as substrates were obtained from MBI Fermentas and Kohjin Co. Ltd., respectively.

Doxorubicin and camptothecin used as control topoisomerase inhibitors were purchased from Sigma Chemicals and Aldrich, respectively.

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Measurement of Topoisomerases Activities

Relaxation activities of topoisomerases were determined by detecting the conversion of supercoiled pBR322 DNA to its relaxed form.[16,17] Topoisomerase I reaction was performed in 20 µl of reaction mixture containing 50 mM Tris-HCl buffer (pH 7.5), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM dithiothreitol, bovine serum albumin (0.6 µg), supercoiled pBR322 DNA (0.2 µg) and 1 unit of topoisomerase I (25 units for DNA cleavage reaction). The reaction mixture was incubated at 37°C for 40 min and terminated by adding 10 µl of loading buffer consisting of 200 mM Tris, 200 mM boric acid, 5 mM EDTA, 50% glycerin and 10% bromphenol blue. The mixture (12 µl) was subjected to 1% agarose gel electrophoresis at 100 V for 60 min in 100 mM Tris-borate buffer (pH 8.5) containing 2.5 mM EDTA. The agarose gel was stained with ethidium bromide and washed thoroughly with deionized water, and the supercoiled pBR322 DNA on the gel was measured by a densitometer (Atto Co., AE-6900M). One unit of inhibitory activity was defined as the amount of inhibitor causing a decrease of relaxed pBR322 DNA concentration by 50%. For DNA cleavage reaction,[18,19] the reaction mixture was terminated by the addition of 2 µl of a solution containing 5% SDS and proteinase K (2.5 mg/ml) and incubated for an additional 30 min at 37°C. After agarose gel electrophoresis, the nicked pBR322 DNA on the gel was measured by the densitometer. The increase of nicked pBR322 DNA was estimated as the stabilizing of cleavable complex by an inhibitor.

Relaxation activity of topoisomerase II[3,20] was measured in 20 µl of reaction mixture containing 5 mM Tris-HCl buffer (pH 8.0), 12 mM KCl, 1 mM $MgCl_2$, 0.5 mM ATP, 0.05 mM dithiothreitol, bovine serum albumin (0.06 µg), supercoiled pBR322 DNA (0.15 µg) and topoisomerase II (1 unit) at 37°C for 40 min. After addition of 4 µl of the loading buffer, the

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supercoiled pBR322 DNA in the reaction mixture was analyzed by agarose gel electrophoresis as described above.

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Measurement of Other Enzyme Activities

Activities of restriction enzymes (*Alu* I,[21] *Bam* HI,[22] *Eco* RI,[23] *Hin* dIII,[24] *Pst* I[25] and *Sca* I[26]) and nucleases (DNase I,[27] DNase II[28] and RNase A[29]) were determined by measuring the concentration of undigested pBR322 DNA or RNA after enzymatic reactions. The reaction buffer for *Alu* I, *Eco* RI and *Hin* dIII consisted of 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl and 1 mM dithiothreitol. The buffer for *Bam* HI, *Pst* I and *Sca* I consisted of 5 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 10 mM NaCl and 0.1 mM dithiothreitol. The buffers for DNase I, DNase II and RNase A consisted of 50 mM Tris-HCl (pH 7.5) and 10 mM $MgCl_2$, 50 mM acetate (pH 5.7), 10 mM EDTA and 20 mM KCl, and 50 mM Tris-HCl (pH 8.0) and 10 mM EDTA, respectively. The reaction mixture (20 µl) consisting of the buffer, enzyme (4 unit) and supercoiled pBR322 DNA (0.25 µg) or RNA (1.2 µg) was incubated at 37°C for 40 min. After incubation, the concentration of undigested pBR322 DNA or RNA in the reaction mixture was measured using a densitometer after 1% agarose gel electrophoresis containing ethidium bromide (0.5 µg/ml). The assay of T4 DNA ligase was based on its ability to rejoin linear pBR322 DNA which was cleaved by *Hin* dIII.[30] The reaction mixture (20 µl) consisting of 66 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, linear pBR322 DNA (0.3 µg) and T4 DNA ligase (4 unit) was incubated at 15°C for 40 min, and the reaction was terminated by adding 10 µl of 60 mM EDTA. The concentration of remaining linear pBR322 DNA in the reaction mixture was measured using a densitometer after 1% agarose gel electrophoresis containing ethidium bromide. The inhibitory activity (IC_{50}) was defined as the amount of inhibitor which reduced the enzyme activity by 50%.

Competition of DNA Binding with Ethidium Bromide

DNA intercalation (DNA binding) of the inhibitor was analyzed by an ethidium bromide competition assay.[31] The inhibitor solution (100 µl) was mixed with 100 µl of 5 µM ethidium bromide and 40 µM herring sperm DNA (Boehringer Mannheim GmbH) in a buffer consisting of 50 mM Tris-HCl (pH 7.5), 100 mM NaCl and 1 mM EDTA. The fluorescence intensity of the mixture was measured with a spectrofluorometer (Hitachi

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F-4010). Emission wavelength was 575 nm and excitation wavelength was 545 nm or 300 nm for competition between ethidium bromide and inhibitor.

Cultural Conditions for the Production of 2280-DTI and 2890-DTI

Micromonospora sp. strain No. 2280 and *Streptomyces* sp. strain No. 2890 were isolated from soil samples collected in Japan by us. Each strain was grown aerobically with 50 ml of S medium in a 200 ml Erlenmeyer flask at 28°C for 2 days on a rotary shaker (180 rpm, 5 cm radius) for producing seed culture and then 2 ml of seed culture was inoculated into the 50 ml of S medium and cultivated at 28°C for production of inhibitors. S medium consisted of 2% glucose, 3% starch, 1% corn steep liquor, 1% soybean flour, 0.5% peptone and 0.3% NaCl, prepared in tap water, and adjusted to pH 7 prior to addition of 0.5% $CaCO_3$.

Purification Procedure of 2280-DTI and 2890-DTI

The strain No. 2280 was cultivated for 12 days, and the mycelia and residual matter were removed by centrifugation at 5000 rpm for 15 min. Two volumes of acetone was added to the culture filtrate and left for overnight at 5°C. The resulting precipitate was collected by centrifugation at 10,000 rpm for 15 min and dissolved in deionized water. This solution was then heated to inactivate nucleases in a boiling water bath for 10 min. The solution was applied to a Diaion HP-10 column (2.6 × 20 cm, Mitsubishi Chemical Industries Inc.) and eluted with deionized water. The eluate (crude solution) was applied to a Dowex 50 × 2 column (2.6 × 15 cm, H^+ form, Dow Chemical Co.) and eluted with deionized water. The eluate (active fraction I) was concentrated and dialyzed against 0.02 M Tris-HCl buffer (pH 8.0) containing 0.1 M KCl. The dialysate was applied to a Sepharose 2B column (1.6 × 70 cm, Pharmacia Biotech) previously equilibrated with the above dialyzing buffer, and eluted with the same buffer. The eluate (active fraction II) was concentrated *in vacuo* and applied to a Avicel column (1.6 × 30 cm, Merck) and eluted with deionized water. The accumulated active fractions were dialyzed against deionized water, followed by lyophilization (purified 2280-DTI).

To obtain 2890-DTI, the strain No. 2890 was cultivated for 4 days. The culture filtrate was heated in a boiling water bath for 10 min and two volumes of acetone was added to the filtrate. After standing overnight at 5°C, the resulting precipitate was collected and dissolved in 0.05 N NaOH, followed by dialysis against deionized water for 2 days. The dialysate

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(crude solution) was acidified slowly with 1 N HCl until a turbidity appeared (about pH 2.5) and allowed to stand overnight at 5°C. The precipitate was suspended in deionized water and dialyzed against 0.02 M Tris-HCl buffer (pH 8.0). The dialysate was applied to a Sephadex G-75 column (1.6 × 90 cm, Pharmacia Biotech) previously equilibrated with the above dialyzing buffer, and eluted with the same buffer. The eluate was applied to a Dowex 50 × 2 column (2.6 × 15 cm) and eluted with a linear gradient of NaCl (0–1.0 M). The eluate (active fraction 1) eluted with 0.7 M NaCl was concentrated *in vacuo* and two volumes of acetone was added. The resulting precipitate was dissolved in 0.02 M Tris-HCl buffer (pH 8) containing 0.1 M KCl and applied to a Sephacryl S-300 column (1.6 × 70 cm, Pharmacia Biotech). The eluate (active fraction 2) was applied to a Avicel column (1.6 × 30 cm) and the active fraction was dialyzed against deionized water, followed by lyophilization (purified 2890-DTI).

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RESULTS AND DISCUSSION

Results of Screening Test

Among 500 strains of actinomycetes tested, 3 strains produced topoisomerase inhibitors in their culture filtrates. The two inhibitors produced by strain No. 2280 and 2890 were high molecular weight substances, and another strain produced thermolabile inhibitors of low molecular weight. Taxonomical characteristics of strain No. 2280 and 2890 were examined and are summarized in Table I.

Strain No. 2280 formed well-developed and branched mycelium without aerial mycelium, and a single sessile spore was produced on substrate mycelium. On most media, raised, folded and orange-colored growth developed well or moderately. In some cases, the orange color of growth later turned to black, but no melanoid pigments were produced. Strain No. 2890 produced spiral spore chains on aerial mycelium. Aerial mass color was white or grey, and melanoid pigments were produced in tyrosine agar and peptone-yeast-iron agar. From these properties, strain No. 2280 and No. 2890 were concluded to be *Micromonospora* species and *Streptomyces* species, respectively.[32]

Time Courses of 2280-DTI and 2890-DTI Production

Cultivation of *Micromonospora* sp. strain No. 2280 and *Streptomyces* sp. strain No. 2890 were carried out under the cultural conditions described in

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TABLE I Cultural characteristics of strain No. 2280 and No. 2890

Medium	*Strain No.* 2280	*Strain No.* 2890
	Growth *Aerial mycelium* *Reverse side of colony* *Soluble pigment*	*Growth* *Aerial mycelium* *Reverse side of colony* *Soluble pigment*
Yeast malt ext. agar (ISP No. 2)	Moderate None Dark greyish olive green Brown	Abundant Abundant, greyish brown Light brown Brownish orange
Oatmeal agar (ISP No. 3)	Good None Moderate brown None	Moderate Scant Light olive brown Light brown
Sucrose-nitrate agar (Czapek's soln. agar)	Moderate None Greyish brown Brown	Moderate Moderate, white Colorless None
Inorganic salts-starch agar (ISP No. 4)	Abundant None Moderate olive brown Light brown	Abundant Scant, reddish brown Dark reddish brown Brown
Glycerol-asparagine agar (ISP No. 5)	Scant None Colorless None	Moderate Scant, pinkish grey Dark reddish brown Brown
Peptone-yeast ext. iron agar (ISP No. 6)	Moderate None Pale yellowish pink None	Moderate None Colorless Dark brown
Tyrosine agar (ISP No. 7)	Moderate None None Cream colored	Abundant Abundant, greyish brown Dark reddish brown Brown
Nutrient agar	Scant None Light brown Colorless	Moderate None Colorless Brown
Formation of melanoid pigment	Negative	Positive
Liquefaction of gelatin	Negative	Negative
Coagulation of milk	Positive	Negative
Peptonization of milk	Negative	Positive
Hydrolysis of starch	Positive	Positive
Decomposition of cellulose	Positive	Negative
Carbon.utilization: Positive	D-glucose, L-arabinose, sucrose, raffinose, starch, cellulose	D-glucose, L-arabinose, I-inositol, D-mannitol, D-fructose, rhamnose, salicin, starch
Negative	D-xylose, I-inositol, D-mannitol, D-fructose, rhamnose	sucrose, D-xylose, raffinose, cellulose

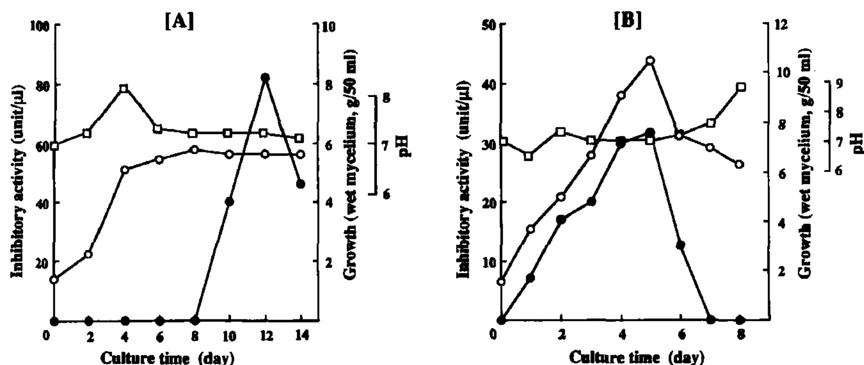

FIGURE 1 Time courses of *Micromonospora* sp. strain No. 2280 (A) and *Streptomyces* sp. strain No. 2890 (B) cultures. ●, inhibitory activity; ○, growth; □, pH.

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MATERIALS AND METHODS. Changes in the inhibitory activity against topoisomerase I, growth of mycelium and pH of the broth with cultivation time are shown in Figure 1. For the measurement of topoisomerase I inhibitory activity, the culture filtrates were boiled at 100°C for 10 min to eliminate the effect of nucleases in the filtrates.

The growth of strain No. 2280 reached the stationary phase around 6 days of cultivation and remained almost stable for a further 8 days. The inhibitory activity appeared in the late phase of cultivation and reached a maximum after 12 days, and then rapidly decreased. The inhibitory activity of strain No. 2890 culture increased with the growth of the mycelium and reached a maximum after 5 days of cultivation, and then rapidly decreased.

Purification of 2280-DTI and 2890-DTI

Table II gives a summary of the purification of 2280-DTI and 2890-DTI from each crude solution. The yields of 2280-DTI and 2890-DTI obtained from 1000 ml of the culture filtrates were 0.77 mg and 0.79 mg, respectively. As shown in Figure 2, 1.04 ng of purified 2280-DTI gave 1 unit (50% inhibition) of inhibitory activity in the assay system for topoisomerase I activity, but topoisomerase II activity was not inhibited by addition of as much as 80 ng of 2280-DTI. On the other hand, 0.13 ng and 0.59 ng of purified 2890-DTI gave 1 unit of inhibitory activity in the assay systems for topoisomerase I and topoisomerase II activities, respectively.

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TABLE II Summary of purification steps of 2280-DTI and 2890-DTI

2280-DTI			2890-DTI		
Step	*Total activity* (*unit* × 10^{-3})	*Yield* (%)	*Step*	*Total activity* (*unit* × 10^{-3})	*Yield* (%)
Crude 2280-DTI	129,900	100.0	Crude 2890-DTI	1,638	100.0
Active fraction I	108,770	83.7	Active fraction 1	648	39.6
Active fraction II	10,140	7.8	Active fraction 2	717	15.1
Purified 2280-DTI	7,020	5.4	Purified 2890-DTI	598	12.6

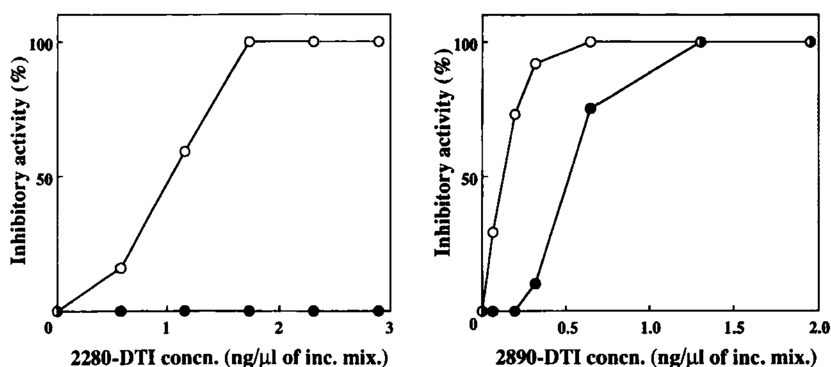

FIGURE 2 Inhibitory activities of 2280-DTI and 2890-DTI against topoisomerase I (○) and topoisomerase II (●).

Some Properties of 2280-DTI and 2890-DTI

Some properties of 2280-DTI and 2890-DTI are summarized in Table III. Both inhibitors were obtained as white powders which were soluble in water but insoluble in acidic water, methanol, acetone and chloroform. The molecular weights of 2280-DTI and 2890-DTI were estimated to be about 3.0×10^6 dalton and 3.7×10^5 dalton by gel filtration with Sepharose 2B and Sephacryl S-300 in 0.02 M Tris-HCl buffer (pH 8.0) containing 0.1 KCl, respectively. Thermostability was tested at 100°C for 15 min, and both inhibitors retained about 80% of initial inhibitory activity. As shown in Figure 3, the UV spectrum of 2280-DTI in deionized water (25 mg/ml) showed end absorption, and that of 2890-DTI (0.11 mg/ml) showed a peak with λ_{max} 225 nm (E value, 85.5) and λ_{min} at 214 nm. 2280-DTI was positive, in bromcresol green (detection of acidic substance) and sulfuric acid (detection of carbohydrate) reactions, and negative in Fiske-SubbaRow[33] (detection of inorganic phosphorus), rhodizonate[34] (detection of sulfur) and phenol–sulfuric acid[35] (detection of glucoside) reactions. These results suggested that 2280-DTI was an acidic carbohydrate without phosphoryl,

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TABLE III Some properties of 2280-DTI and 2890-DTI

	2280-DTI	2890-DTI
Producer	*Micromonospora* sp.	*Streptomyces* sp.
Appearance	white powder	white powder
Solubility		
soluble	water	water
insoluble	acidic water, methanol, acetone, chloroform	acidic water, methanol, acetone, chloroform
Molecular weight	3,000 kD	370 kD
Thermostability (100°C, 15 min)	79% (remaining activity)	84% (remaining activity)
UV λ_{max} ($E^{1\%}_{1cm}$)	end absorption	225 nm (85.5)
Color reaction		
sulfuric acid	positive	positive
bromcresol green	positive	positive
ninhydrin	positive	negative
anisaldehyde	negative	positive
phenol–sulfuric acid	negative	positive (sugar content; 2.1% as glucose)
Fiske-SubbaRow	negative	negative
rhodizonate	negative	negative

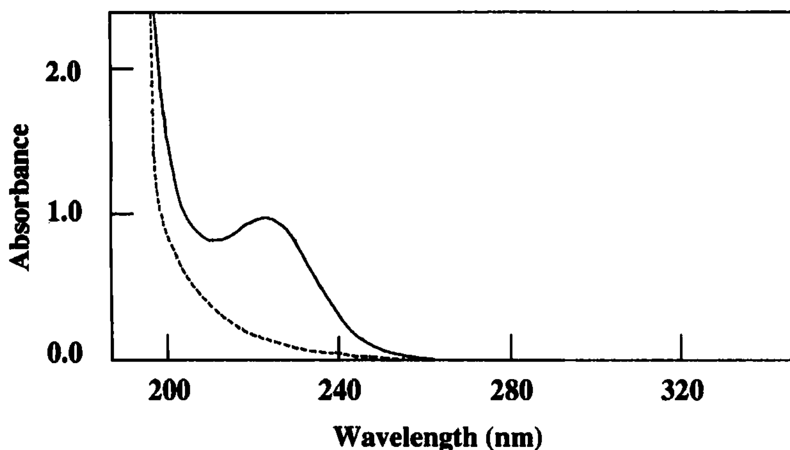

FIGURE 3 UV spectra of 2280-DTI (----, 25 mg/ml) and 2890-DTI (—, 0.11 mg/ml) in deionized water.

sulfonyl and glucoside groups, and it may contain a carboxyl group. 2890-DTI contained a glucoside group, and its content was estimated to be approximately 2.1% (w/w) as glucose by the phenol–sulfuric acid reaction. Further investigations will be required for the structural elucidation of both inhibitors.

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K_i Values of 2280-DTI and 2890-DTI Against Topoisomerase I

The type of inhibition exhibited by 2280-DTI and 2890-DTI were determined by a Lineweaver–Burk plot[36] of substrate (supercoiled pBR322 DNA) concentration against rate of relaxation by topoisomerase I in the presence and absence of the inhibitor. As shown in Figure 4, both inhibitors inhibited in a non-competitive manner. The K_m value of the enzyme against supercoiled pBR322 DNA was 5.00 nM, and the K_i values of 2280-DTI and 2890-DTI were 0.27 nM and 0.37 nM, respectively. From these results, 2280-DTI and 2890-DTI were highly potent inhibitors for topoisomerase I and the both were considered to bind strongly to a different site from the binding site of the substrate DNA on the enzyme molecule.

Effects of 2280-DTI and 2890-DTI on DNA

Most topoisomerase inhibitors such as doxorubicin and camptotecin inhibit the enzyme by intercalation into DNA strands or by stabilizing the

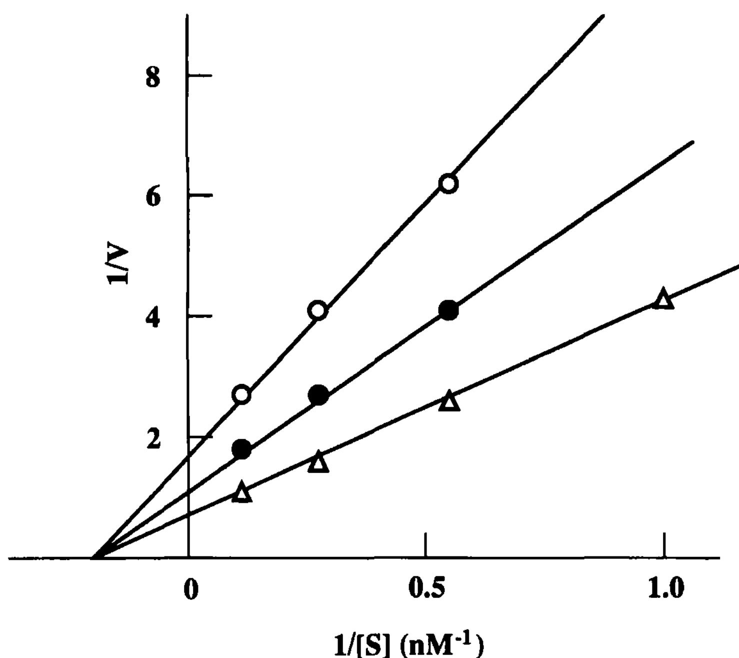

FIGURE 4 Lineweaver–Burk plots of substrate (supercoiled pBR322 DNA) concentration against rate of relaxation by topoisomerase I with 2280-DTI (○), 2890-DTI (●) and without inhibitors (△). The concentrations of 2280-DTI and 2890-DTI in the reaction mixture were 3.7×10^{-10} M and 2.4×10^{-10} M, respectively.

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cleavable complex (enzyme–DNA reaction intermediate). To determine whether 2280-DTI and 2890-DTI had the ability to intercalate into DNA strands, an ethidium bromide competition assay was carried out using herring sperm DNA. Doxorubicin and camptothecin were used as controls at similar concentrations. As shown in Figure 5, doxorubicin, an intercalator competed with ethidium bromide for DNA and decreased the intensity of fluorescence. On the other hand, 2280-DTI, 2890-DTI and camptothecin did not affect the intensity of fluorescence since they did not intercalate into DNA strands.

Camptothecin stabilizes the cleavable complex and inhibits DNA rejoining reaction of topoisomerase I; therefore, the inhibitor induced the nicked pBR322 DNA in the cleavage reaction using topoisomerase I and supercoiled pBR322 DNA. As shown in Figure 6, camptothecin induced nicked pBR322 DNA with increasing concentration. Contrary to that, nicked pBR322 DNA was not induced by 2280-DTI and 2890-DTI, i.e. both inhibitors did not stabilize the cleavable complex. The results suggest that 2280-DTI and 2890-DTI do not inhibit topoisomerase by stabilizing the complex.

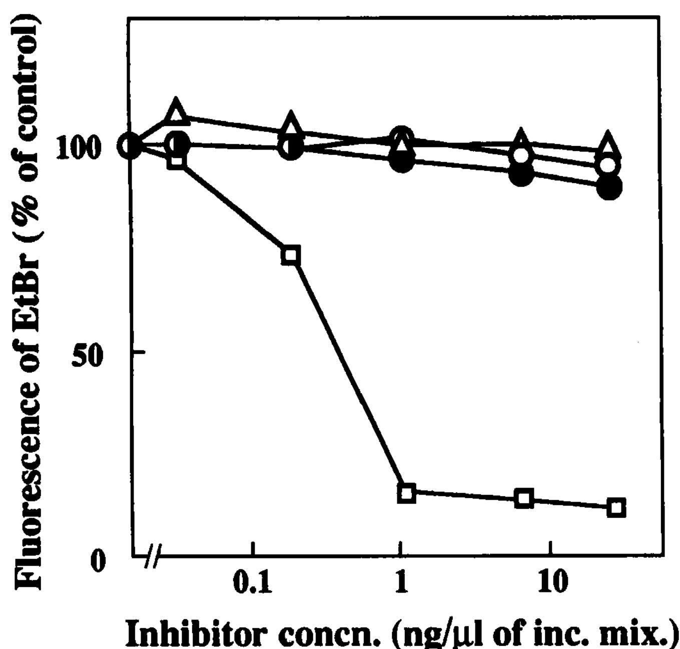

FIGURE 5 Effects of 2280-DTI (○), 2890-DTI (●), camptothecin (△) and doxorubicin (□) on DNA binding competition with ethidium bromide.

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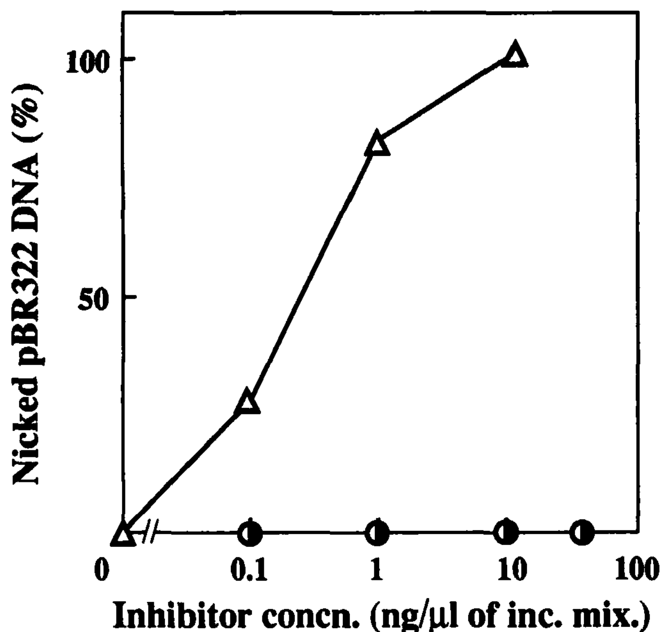

FIGURE 6 Topoisomerase I-mediated DNA cleavage by 2280-DTI (○), 2890-DTI (●) and camptothecin (△).

The inhibition mechanism of 2280-DTI and 2890-DTI against topoisomerase differed from that of doxorubicin and camptothecin. Both inhibitors may directly act on the enzyme molecule in the earlier step before the formation of the cleavable complex and inhibit DNA breaking and rejoining reactions by the enzyme.

Inhibitory Spectra of 2280-DTI and 2890-DTI

The effects of 2280-DTI and 2890-DTI on various enzymes were examined and are summarized in Table IV. 2280-DTI inhibited only topoisomerase I, and did not inhibit the other enzymes tested. Therefore, 2280-DTI was considered to be a specific inhibitor for topoisomerase I. In contrast, 2890-DTI inhibited topoisomerase I with an IC_{50} (0.35 nM) about equal to that of 2280-DTI, but also inhibited topoisomerase II and some restriction endonucleases. Both inhibitors showed quite different inhibitory spectra and neither inhibited DNases, RNase and ligase.

The known topoisomerase inhibitors inhibit the DNA rejoining reaction of the enzyme by stabilizing cleavable complex. However, 2280-DTI and

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TABLE IV Inhibitory spectra of 2280-DTI and 2890-DTI

Enzyme	IC_{50} (ng/μl *of incubation mixture and* (nM))			
	2280-DTI		2890-DTI	
Topoisomerase I	1.04	(0.35)	0.13	(0.35)
Topoisomerase II	>80.00	(>26.67)	0.59	(1.51)
Eco RI	>80.00	(>26.67)	0.01	(0.03)
Hin dIII	>80.00	(>26.67)	0.01	(0.03)
Sca I	>80.00	(>26.67)	0.01	(0.03)
Pst I	>80.00	(>26.67)	0.05	(0.03)
Bam HI	>80.00	(>26.67)	0.19	(0.51)
Alu I	>80.00	(>26.67)	1.26	(3.41)
DNase I	>80.00	(>26.67)	>10.00	(>27.03)
DNase II	>80.00	(>26.67)	>10.00	(>27.03)
RNase A	>80.00	(>26.67)	>10.00	(>27.03)
T4 ligase	>80.00	(>26.67)	>10.00	(>27.03)

2890-DTI acted directly on the enzyme molecule and inhibited the DNA breaking and rejoining reactions of the enzyme. 2280-DTI and 2890-DTI are high molecular weight substances and show different inhibitory spectra. These properties prove that both inhibitors are completely different from the other topoisomerase inhibitors so far reported. As far as we are aware, both inhibitors are the first reported examples of high molecular inhibitors of microbial origin with inhibitory activity against topoisomerases.

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