We identified a new radical scavenger, 10T024A (C_{18}H_{12}N_{2}O_{2}), from a culture of the *Streptomyces* sp. Spectroscopic elucidation indicated that this compound is a new phenazine derivative. 10T024A showed radical-scavenging activity with an ED_{50} of 125 μM. Moreover, it showed prostaglandin D_2 (PGD_2) and leukotriene B_4 (LTB_4) release suppressive activity in rat basophilic leukemia (RBL-2H3) cells, at IC_{50}: 8 μM and 10 μM respectively.

**Key words:** phenazine; 2,2-diphenyl-1-picrylhydrazyl (DPPH); *Streptomyces* sp.; prostaglandin D_2; leukotriene B_4

Recently we identified several bioactive compounds that act as radical scavengers from a culture of microorganism and from a vegetable.^{1,2} Free radicals present in the human body cause oxidative stress in various organs and induce inflammatory diseases, while those present in food can cause deterioration of the nutrient factor via lipid peroxidation.\(^3\)\(^4\) Hence we focused on the identification of a new bioactive compound by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, and identified a new radical scavenger (10T024A, 1) in the culture broth of *Streptomyces* sp. 10T024. Here we describe the cultivation of the strain, and the extraction, isolation, and structural elucidation of it and measurement of its radical-scavenging activity and prostaglandin and leukotriene-release suppressive activity.

In the course of our screening for radical scavengers, we found that they were produced in *Streptomyces* sp. 10T024. This strain was inoculated into 9.6 L of a medium (glucose, 10 g/L; seafood extract, 10 g/L; yeast extract, 1 g/L; pH 7.3) in an Erlenmeyer flask and cultivated for 7 d at 30 °C. The active fractions were further purified by Sephadex LH-20 chromatography (eluent, MeOH) and preparative high-performance liquid chromatography (HPLC). Finally, radical scavenger 1 (10 mg) was isolated as a yellow amorphous powder from this culture broth (9.6 L). The details of the isolation of 1 are given in the experimental section below.

The chemical structure of 1 was elucidated by spectroscopic measurements. The result of high-resolution fast atom bombardment mass spectroscopy (HRFABMS) measurements indicated that the molecular formula of 1 was C_{18}H_{12}N_{2}O_{2}. Based on the IR spectrum of this compound, the presence of carbonyl and hydroxyl groups was confirmed. The \(^{1}\)H nuclear magnetic resonance (NMR) spectrum of 1 (400 MHz, CD_{3}OD) showed seven olefinic signals in the range δ_{H} 7.0–9.0 and one singlet methyl signal at δ_{H} 4.28 (s, 2H). \(^{13}\)C NMR (100 MHz, CD_{3}OD) and distortionless enhancement by polarization transfer (DEPT) spectra showed two carbon signals in the range 170–180 ppm, seven methine and five quaternary signals in an olefinic range of 110–150 ppm, and one methylene peak at 61.55 ppm. The \(^{1}\)H−\(^{1}\)H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra of 1 indicated the presence of three partial structures: A, B, and C (Fig. 1). The partial structures A and B were confirmed to be six-membered rings. The partial structure C was determined to be a short chain including a carboxyl group. The chemical formula of 1 (C_{18}H_{12}N_{2}O_{2}) and the nuclear Overhauser effect (NOE) correlations at 14-H/5-H and 14-H/8-H suggested that C-14, C-5, and C-8 were connected through one nitrogen atom. The chemical shift of 14-H (δ_{H} 4.28) and C-14 (δ_{C} 61.55) confirmed this. Another nitrogen atom was located between C-1 and C-12, and formed an NH group. Finally, two protons were assigned to form two COOH groups at the 13 and 15 positions. The proposed structure of 10T024A (1) is shown in Fig. 1. Compound 1 was deduced to be a new phenazine derivative having an N-carboxymethyl side chain.

We obtained compound 2, a trimethylsilyl diazomethane-treated product of 1, to confirm the chemical structure of 1. HRFABMS showed the molecular formula of 2, C_{18}H_{12}N_{2}O_{2}. In the \(^{1}\)H NMR spectrum...
(400 MHz, acetone-\textit{d}_6) of 2, we observed one methoxy proton (\textit{\delta}_H: 4.04) and seven olefinic protons, but we did not see the methylene signal in 2. This indicates that not only the methylation for the acidic group but also another chemical reaction occurred on 1. In the \textsuperscript{13}C NMR spectrum (100 MHz, acetone-\textit{d}_6) of 2, we observed one carbonyl carbon (\textit{\delta}_C: 167.84), four heteroatom neighboring olefinic quaternary carbons in the range of \textit{\delta}_C 141–145, seven olefinic methines in the range of 130–134 ppm, one olefinic quaternary carbon (\textit{\delta}_C: 133.93), and one methoxy carbon (\textit{\delta}_C: 52.64). These olefinic signals in the \textit{\textsuperscript{1}H} and \textit{\textsuperscript{13}C} NMR spectra overlapped with each other, but we proposed a chemical structure for 2 using 1D and 2D NMR spectra and molecular formula C\textsubscript{13}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}. As shown in Fig. 1, 2 had a phenazine skeleton like 1. The 11-carboxyl group in 1 was derived to form methyl ester. We assigned four heteroatom neighboring olefinic quaternary carbons for C-1, 5, 6, 7, and 12 in 2. The chemical shifts on C-6 (\textit{\delta}_C: 115.75) and C-7 (\textit{\delta}_C: 127.38) in 1 were affected to move in the range of 130–134 ppm, seven olefinic methines in the range of 130–134 ppm, one olefinic quaternary carbon (\textit{\delta}_C: 133.93), and one methoxy carbon (\textit{\delta}_C: 52.64). These olefinic signals in the \textit{\textsuperscript{1}H} and \textit{\textsuperscript{13}C} NMR spectra overlapped with each other, but we proposed a chemical structure for 2 using 1D and 2D NMR spectra and molecular formula C\textsubscript{13}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}. As shown in Fig. 1, 2 had a phenazine skeleton like 1. The 11-carboxyl group in 1 was derived to form methyl ester. We assigned four heteroatom neighboring olefinic quaternary carbons for C-1, 5, 6, 7, and 12 in 2. The chemical shifts on C-6 (\textit{\delta}_C: 115.75) and C-7 (\textit{\delta}_C: 127.38) in 1 were affected to move toward the typical nitrogen neighbouring olefinic carbon range (140–145 ppm) in 2, and suggests that the N-carboxymethyl side chain in 1 was removed during trimethylsilyl diazomethane treatment. These results confirmed the presence of an N-carboxymethyl portion between C-6 and C-7 in 1.

The results of our DPPH radical assay indicated that 1 shows radical scavenging activity with an ED\textsubscript{50} of 125 \textmu m. We used two known antioxidative compounds, \textalpha-tocopherol (ED\textsubscript{50} 20 \textmu m) and quercetin (ED\textsubscript{50} 10 \textmu m), as positive controls in this assay. Moreover, we assessed the bioactivity of 1 by prostaglandin and leukotriene release suppressive activity. Thus 1 indicated potent activity for prostaglandin D\textsubscript{2} (PGD\textsubscript{2}) release with IC\textsubscript{50} at 8 \textmu m and leukotriene B\textsubscript{4} (LTB\textsubscript{4}) release with IC\textsubscript{50} at 10 \textmu m.

Recently the biosynthesis and bioactivity of several phenazine-related compounds have been investigated. Parsons et al. carried out studies on phenazine-related compounds isolated from the human pathogen Pseudo- monas aeruginosa.\textsuperscript{31} Isnansetyo reported bioactive phe-
Cultivation of Streptomyces sp. 10T024. The 10T024 strain was inoculated into 800 mL of a medium containing 1.0% glucose, 1.0% seaweed extract, and 0.1% yeast extract at pH 7.3 in a 2-L Erlenmeyer flask and was cultivated at 30 °C for 10 d on a rotary shaker (150 rpm).

Isolation of 10T024A (1). The filtrate of a culture broth (9.6 L) of Streptomyces sp. 10T024 was acidified to pH 3.0 using HCl and extracted using an equal volume of EtOAc. After evaporation under reduced pressure, the crude extract was purified by silica gel column chromatography by stepwise elution using an n-hexane/acetone mixture. The 3:7 n-hexane/acetone fractions showed DPPH radical-scavenging activity. Radical scavenging activity was confirmed from a decrease in the color intensity on a TLC plate. After development in a scavenging activity. Radical scavenging activity was confirmed from a TLC plate. After development in a 23% CHCl3/C6H6 (95:15) system, the TLC plate was sprayed with a 2% DPPH/EtOH solution. The radical scavenger caused reduction of the absorbance of pure MeOH was measured. The DPPH radical-scavenging activity of each sample was recorded against the blank and expressed in percentage units. The ED50 values were taken to be the concentrations required for 50% DPPH radical scavenging activity.

Measurement of prostaglandin D2 (PGD2) and leukotriene B4 (LTB4) release suppressing activity. The effects of 10T024A (1) on prostaglandin D2 and leukotriene B4 release were investigated using rat basophilic leukemia (RBL-2H3) cells. The RBL-2H3 cells were seeded into a 12-well plate at a density of 1 x 10^6 cells/well. The experiment was performed 1 d after cell seeding. The cells were pretreated with 1 for 24 h. Subsequently, they were stimulated by 100 mM of ionophore, A23187. After standing for 15 min, the media were harvested. To each culture medium, PGD2-d6 and LTB4-d10 (1 ng each) were added as internal standards. The medium was adjusted to pH 4.0 with 1 M HCl, and was applied to an Empore C18 HD disk cartridge. The cartridge was washed with water (1 mL) and hexane (1 mL). PGD2 was eluted with hexane/ethyl acetate (1/2, v/v, 1 mL) and LTB4 with methanol (1 mL). After they were evaporated to dryness, the PGD2 and LTB4 contents were determined by LC/MS/MS system.9)

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References