INHIBITORS OF DIACYLGLYCEROL KINASE FROM *Drechslera sacchari*

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Cells respond to a variety of signals by activation of a phospholipase C which catalyzes the phosphodiesteric cleavage of phosphatidylinositol 4,5-bisphosphate to generate intracellular second messengers, inositol 1,4,5-trisphosphate and 1,2-diacylglycerol. Previously, we described the isolation of an inhibitor for inositol-specific phospholipase C from *Actinomadura* sp. This inhibitor therefore may be a valuable tool for elucidating the mechanism of the signal transduction pathway of various systems as well as a hopeful candidate for cancer chemotherapy[1,2]. On the other hand, diacylglycerol kinase phosphorylates diacylglycerol to form phosphatidic acid. This enzyme is known to be elevated by extracellular stimuli to accumulate phosphatidic acid by phosphorylating diacylglycerol, and to be involved in the attenuation of protein kinase C by decreasing the concentration of diacylglycerol in the cells[3]. However, the detailed function of this enzyme remains to be clarified. Thus, an inhibitor of diacylglycerol kinase would be a useful reagent to explore the role of this enzyme in the signal transduction pathways in various systems and a possible candidate for cancer chemotherapy. This report describes the isolation of D2597 substances from *Drechslera sacchari*, which are inhibitors of diacylglycerol kinase from bovine thymus.

The microorganism producing the inhibitors was isolated from a fallen leaf sample. Based on the cultural and physiological properties, the producing strain D2597 was identified as *Drechslera sacchari*.

Diacylglycerol kinase used for the screening was partially purified from bovine thymus by the method of Sakane *et al.*[4] with a slight modification. Diacyglycerol kinase activity was determined in a final volume of 50 μl containing 219 μM 1,2-dilauroylglycerol (C12 : 1), 100 μM [γ-^{32}P]ATP (0.5 ~ 1.0 μCi), 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1% bovine serum albumin, 0.5 mM ethyleneglycol bis(β-aminoethylether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA), 5 mM $MgCl_2$, 0.04% sodium deoxycholate, 40 ng partially purified diacylglycerol kinase and with or without the inhibitor. After incubation for 20 minutes at 37°C, the reaction was terminated by the addition of 500 μl of a mixture composed of chloroform, methanol and concentrated HCl (200 : 100 : 1, v/v/v). Then, 125 μl of 1 N HCl was added to the mixture, mixed and was centrifuged for 10 seconds at 16,000 rpm. The lower phase was collected and concentrated to dryness. The dried solid was dissolved in 10 μl of chloroform and the solution was applied to a silica gel thin-layer plate. After development with chloroform, methanol, acetic acid and water (170 : 25 : 25 : 6, v/v/v/v), a spot corresponding to phosphatidic acid was cut out and the radioactivity in this area was measured by a liquid scintillation counter after dissolving in methanol.

Five loopful of cells of the strain D2597 from an agar slant were inoculated into each of 20 bottles of a 200-ml Erlenmeyer flask containing 40 ml of potato - glucose agar. The medium was incubated at 26°C for 14 days. The inhibitors were purified as shown in Fig. 1. To each of the bottles 80 ml of 50% acetone in water were added and the inhibitors were extracted. The acetone solution was concentrated and 500 ml each of water and ethylacetate were added. After the pH was adjusted to 2.0 with HCl and the inhibitors were extracted into the ethylacetate layer, the organic solvent layer was concentrated to dryness under reduced pressure to give 400 mg of crude solid. The solid thus obtained was dissolved in a small amount of dichloromethane and purified by passing through a column of 100 g silica gel. The active fraction was eluted with 200 ml of methanol-dichloromethane 1 : 19 (v/v) and 240 mg of crude active fractions were obtained after concentration under reduced pressure. The crude fractions were further fractionated into two active components by the CAPCELL PAK C18 column (30 mm × 250 mm) chromatography by developing with a linear gradient of acetonitrile from 50 to 90% in water at a speed of 9 ml per minute. After 30 minutes from the beginning of the elution, the fractions were collected at 1 minute interval and the bioactive Fractions 1 (fraction 36 to 38) and 2 (fractions 45 to 52) were collected. From the Fraction 2, 70 mg of yellow needle crystals were obtained after recrystallization from *n*-hexane. The melting point was 130 ~ 132°C. From the physico-chemical prop-

Fig. 1. Purification procedure of cochlioquinone A and stemphone.

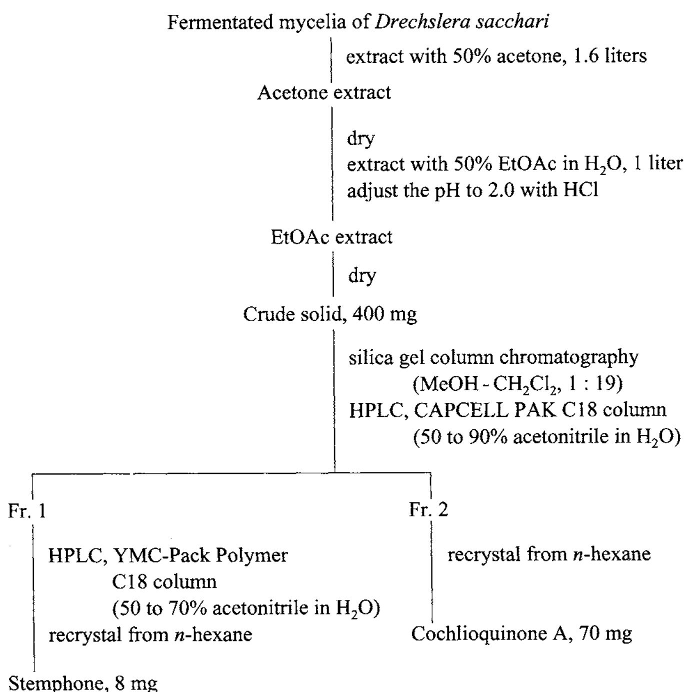
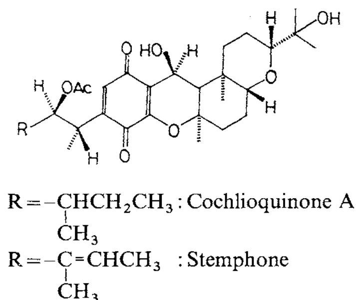


Fig. 2. Chemical structures of cochlioquinone and stemphone.



erties like mass spectrum, ^{1}H and ^{13}C NMR spectra, the crystals were identified as cochlioquinone A[5] (Fig. 2). On the other hand, from the Fraction 1, 8 mg of yellow needle crystals were obtained after HPLC on YMC-Pack Polymer C18 column chromatography and recrystallization from *n*-hexane. From the physico-chemical properties including melting point (160~162°C), the mass spectrum and ^{1}H and ^{13}C NMR spectra, the crystals were identified as stemphone[6] (Fig. 2).

Cochlioquinone A and stemphone showed IC_{50} values for diacylglycerol kinase from bovine thymus at 2.3 and 3.3 μM, respectively under the condition of no additional ATP. However, they showed no inhibitory activity against phospholipase C from bovine brain. Only one compound R59022 has been shown to be an inhibitor of diacylglycerol kinase[3] so far, however, a detailed report on its activity has not been described. In addition, although cochlioquinone A and stemphone were reported to show antimicrobial activity against *Bacillus megaterium* and toxicity to chick embryo[6], and to be nematocidal agents[7], no report has been published concerning their activities as inhibitors of signal transduction systems. Therefore, cochlioquinone A and stemphone should be useful reagents for the analysis of the detailed mechanism of signal transduction and may be helpful drugs for the diseases caused by disorders of signal transduction systems including cancer. Cochlioquinone A was reported to compete for the specific ivermectin binding site in the membrane preparation from *Caenorhabditis elegans*[7]. However, ivermectin did not show inhibitory activity against diacylglycerol kinase (unpublished data).

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