Communications to the Editor

UCN-01, A SELECTIVE INHIBITOR OF PROTEIN KINASE C FROM *STREPTOMYCES*

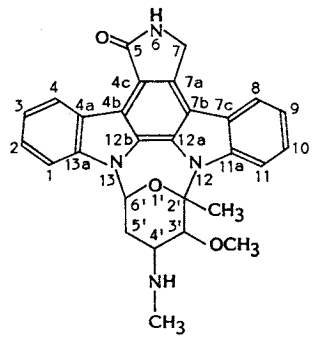
Sir:

The Ca^{2+}/phospholipid-dependent protein kinase (protein kinase C) is receiving increasing attention, since the enzyme is activated by diacylglycerol, a transient product of hormone-induced phosphatidyl inositol breakdown, and also activated by tumor-promoting phorbor esters.[1~5] In addition this enzyme has been identified as the main cellular receptor for the tumor-promoting phorbol esters.[6,7] A potent and selective inhibitor of protein kinase C should be useful to learn the mechanisms by which the enzyme influences cellular metabolism and cellular proliferation. In the course of screening of inhibitors of protein kinase C, we have found that staurosporine, a microbial alkaloide, is a potent inhibitor of protein kinase C with IC_{50} value of 2.7 nM.[8] Staurosporine has also been shown to inhibit other protein kinases such as cAMP dependent protein kinase (protein kinase A) and tyrosine specific-protein kinase of p60 transforming protein of Rous sarcoma virus with IC_{50} value of 8.2 nM and 6.4 nM, respectively.[9] Thus, we have focused our screening on selective inhibitors of protein kinase C. Protein kinase C and protein kinase A have been known as abundant kinases in a number of tissue, and histone H1 is phosphorylated at significant rate by both of those kinases. So we used histone H1 as the substrate of protein kinase C and protein kinase A, and compared the IC_{50} value for protein kinase C with that for protein kinase A. We have now isolated a selective inhibitor of protein kinase C, UCN-01, from the culture broth of strain No. 71 and have found that UCN-01 shows antitumor activity against murine lymphotic leukemia P388 *in vivo*.

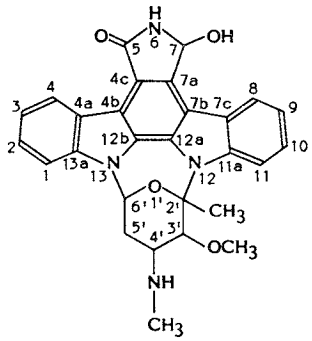
Strain No. 71 was isolated from a soil collected in Yamaguchi Prefecture in Japan and was assigned to the genus, *Streptomyces*. The fermentation medium consisted of glucose 20 g, soybean meal 15 g and $CaCO_3$ 4 g per liter of tap water. The pH of media was adjusted to 7.2 prior to sterilization. The production of UCN-01 was followed by the inhibition assay of protein kinase C and that of protein kinase A, and UCN-01 was later analyzed by HPLC with ODS column. The peak titers usually reached after 3 days incubation at 28°C. The culture broth (30 liters) was filtered and the filtrate was applied on a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited). The acetone eluate was evaporated to remove acetone. The aqueous solution was adjusted to pH 10 and extracted with ethyl acetate. The resulting organic phase was extracted with 0.1 N hydrochloric acid and after the separation the aqueous phase was immediately adjusted to pH 10 by addition of NaOH and extracted with ethyl acetate. The ethyl acetate extract was applied to silica gel column chromatography ($CHCl_3$ - MeOH, 9:1) followed by reversed phase HPLC (ODS 60% MeOH) to yield 5.0 mg of UCN-01.

UCN-01 is pale yellow needles, and exhibits mp 245~250°C (dec), $[\alpha]_D^{22}$ +132.0° (*c* 0.3, MeOH). The molecular formula of UCN-01 was determined as $C_{28}H_{26}N_4O_4$ by electron impact mass spectra (EI-MS) and elementary analysis. The IR spectrum (KBr) of UCN-01 indicates the existence of NH and OH (3500~3300 cm^{-1}), amide (1690 cm^{-1}) and aromatic (750 cm^{-1}). The UV absorption maxima of UCN-01 (in MeOH) are observed at 240 nm (ε 29,000), 264 (sh, 20,000), 274 (sh, 21,000), 300 (55,000), 326 (sh, 9,600), 338 (sh, 8,300), 358 (7,500) and 374 (8,500) showing that UCN-01 resembles closely to staurosporine which has an indolo[2,3-a]carbazole chromophore.[10,11] ^1H and ^{13}C NMR spectra of UCN-01 (in DMSO-d_6) indicates the presence of one amide group, eighteen sp^2 carbons, eight aromatic protons, one quaternary carbon, four methines, one methoxyl group, one *N*-methyl group, one methylene and one methyl group. ^{13}C NMR spectra of UCN-01 (in DMSO-d_6) are similar to those of staurosporine except that a 45.3-ppm (t, C-7) in staurosporine is replaced by a 78.4-ppm (d) in UCN-01. In ^1H NMR spectrum of UCN-01 (in DMSO-d_6), the signal at 4.95 ppm (2H, s) of the 7-methylene protons of staurosporine is not observed, the signals at

Fig. 1.



Staurosporine



UCN-01

6.44 ppm (1H, d, J=9.8 Hz) and at 6.39 ppm (1H, dd, J=9.8 and 1.1 Hz) are observed. The signals at 6.44 ppm are collapsed and the signals at 6.39 ppm are changed to singlet on addition of D_2O. Furthermore, in proton decoupling experiment, the signals at 6.39 ppm are changed to doublet (J=9.8 Hz) on irradiation of the 6-imino proton at 8.72 ppm (1H, d, J=1.1 Hz). Therefore the signals at 6.44 ppm and at 6.39 ppm are assigned the 7-hydroxyl proton and the 7-methine proton, respectively. Based on these results, the planar structure of UCN-01 has determined as shown in Fig. 1. This structure differs from staurosporine in that C-7 carbon bears a hydroxyl group.

UCN-01 has been shown to inhibit protein kinase C with IC_{50} value of 4.1 nM while IC_{50} value for protein kinase A and tyrosine specific protein kinase are 42 nM and 45 nM, respectively. Thus UCN-01 is a selective inhibitor of protein kinase C and will be a valuable research tool to study the role of protein kinase C in the regulation of cellular functions. Furthermore UCN-01 exhibited antitumor activity against murine lymphotic leukemia P388 *in vivo* and produced 24% increase of life span at a dose of 15 mg/kg by a single ip injection. Since staurosporine has been shown to lack the antitumor activity in P388 murine lymphotic leukemia assay, it will be of great interest to see whether antitumor activity of UCN-01 is due to a selective inhibition of protein kinase C.

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