UCN-1028A, A NOVEL AND SPECIFIC INHIBITOR OF PROTEIN KINASE C, FROM *CLADOSPORIUM*

Sir:

Protein kinase C (PKC) plays crucial roles in signal transduction following receptor activation by hormones, in cellular proliferation and in differentiation[1,2]. To understand and characterize the biochemical role of PKC, specific and potent inhibitors of this enzyme have been expected to be very useful.

In the course of screening for PKC inhibitors, we have found staurosporine which is the most potent inhibitor of PKC[3], cAMP dependent protein kinase (PKA) and $P60^{src}$ tyrosine kinase[4], and UCN-01 which is a selective inhibitor of PKC[5].

These findings prompted us to continue further screening for specific inhibitors of PKC, and we have now isolated a novel and specific inhibitor of PKC, UCN-1028A, from the culture broth of *Cladosporium cladosporioides*. In this communication, we report the production, isolation and physico-chemical properties of UCN-1028A.

The producing organism was isolated from a block fence in Osaka, Japan, and has been identified as *C. cladosporioides* FERM BP-1285.

The seed medium contained peptone (Kyokuto) 5 g, yeast extract 5 g, glucose 10 g, vegetable juice (V8) 50 ml, $CaCO_3$ 3 g and malt extract 2 g per liter of deionized water. It was inoculated with spores from an agar slant culture and incubated at 25°C for 3 days. The seed culture was added at the rate of 5% to the production medium consisting of soluble starch 50 g, dry yeast 15 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.5 g and $CaCO_3$ 5 g (pH 6.0) per liter of deionized water. The jar fermentor was stirred at 300 rpm and

Table 1. Physico-chemical properties of UCN-1028A.

Appearance	Dark red amorphous solid
Molecular formula	$C_{44}H_{38}O_{12}$
EI-MS	758.2362 (calcd 758.2361)
UV λ_{max}^{MeOH} nm	224, 256 (sh), 344, 476, 540 (sh), 586
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	2970, 2880, 1710, 1610, 1260, 830, 700
Rf value[a]	0.6
Solubility	
Soluble	DMSO, $CHCl_3$, Me_2CO, EtOAc
Slightly soluble	MeOH, hexane
Insoluble	H_2O

[a] Silica gel TLC (Merck 5715), $CHCl_3$ - MeOH (97 : 3).

Table 2. ^1H and ^{13}C NMR data of UCN-1028A.
The diminution in the number of the signals is ascribed to the symmetry of the molecule.

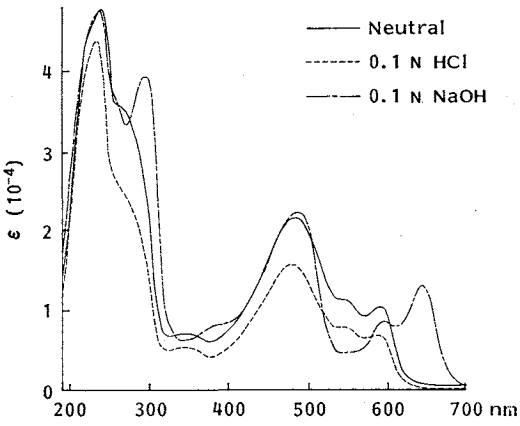
Proton No.	^1H NMR ($CDCl_3$, ppm, J in Hz)
4-OH	15.84 s
5-H	6.22 s
13-H	3.18 d,d (J=10.0, 13.4)
13-H	3.67 d,d (J=2.0, 13.4)
14-H	5.03 d,d,q (J=2.0, 10.0, 6.3)
15-H_3	1.29 d (J=6.3)
19-H_3	4.33 s
20-H_3	3.79 s
3′-H, 7′-H	6.84 d,d (J=4.1, 8.2)
4′-H, 6′-H	6.90 d,d (J=7.4, 8.2)
5′-H	7.22 t,t (J=1.4, 7.4)

Carbon No.	^{13}C NMR ($CDCl_3$, ppm)
C-1	134.6
C-2	151.5
C-3	177.9
C-3a	106.3
C-3b	125.6
C-4	172.5
C-5	101.2
C-6	166.2
C-6a	116.6
C-12b	127.4
C-13	39.1
C-14	72.3
C-15	21.1
C-19	61.2
C-20	55.8
C-1′	164.6
C-2′	129.1
C-3′, C-7′	128.4
C-4′, C-6′	127.5
C-5′	132.0

aerated with 1 v/v/minute. The product was accumulated in mycelium with a maximum after 3 days incubation at 25°C.

The mycelial cake obtained by filtration was extracted 3 times with acetone. The acetone extract (30 liters) was evaporated to remove acetone, followed by the extraction with ethyl acetate at pH 2.0. The organic layer was evaporated to dryness. The residue was subjected to silica gel chromatography with chloroform-methanol to obtain UCN-1028 complex. It was further purified by Sephadex LH-20 chromatography with acetone to yield 2 mg of UCN-1028A, the most hydrophobic component, as a red powder.

Fig. 1. UV spectrum of UCN-1028A.



Physico-chemical properties of UCN-1028A are summarized in Table 1. The molecular formula of UCN-1028A was determined as $C_{44}H_{38}O_{12}$ by electron impact mass spectrum (EI-MS) and elementary analysis. The UV spectrum (Fig. 1) suggests the presence of a perylene quinone ring structure. The IR spectrum (Fig. 2) indicates the presence of carbonyl (1710, 1610) and aromatic (830, 700) groups. The ^{1}H and ^{13}C NMR spectra indicate a similarity to phleichrome, a phytotoxic compound[6] as shown in Table 2. From an analysis of ^{1}H and ^{13}C NMR data, the structure of UCN-1028A was identified as shown in Fig. 3 by IIDA, details of which will be reported in a separate paper.

As shown in Table 3 UCN-1028A inhibited PKC with an IC_{50} value of 0.19 μg/ml, while it scarcely inhibited PKA even at 40 μg/ml. Thus, UCN-1028A is a specific inhibitor of PKC, and has much higher specificity for PKC than other known inhibitors. Furthermore UCN-1028A showed strong cytotoxic activity against tumor cells, HeLa S_3 (IC_{50} 0.29 μg/ml) and MCF-7 (IC_{50} 0.21 μg/ml), whereas it did not show antimicrobial activity against the following bacteria and fungi;

Fig. 2. IR spectrum of UCN-1028A ($CHCl_3$).

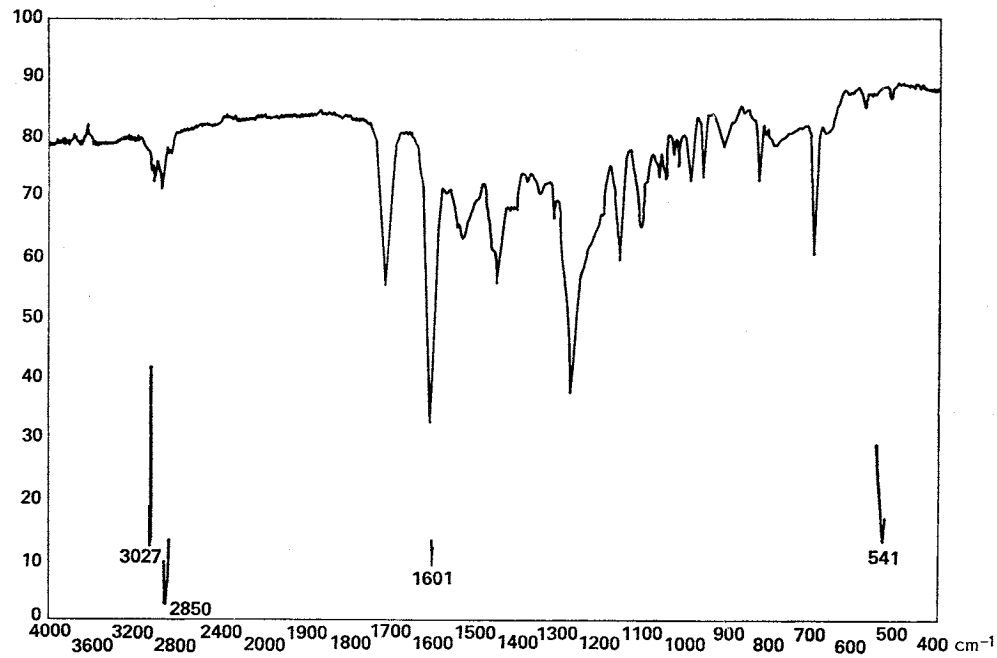


Fig. 3. Chemical structure of UCN-1028A.

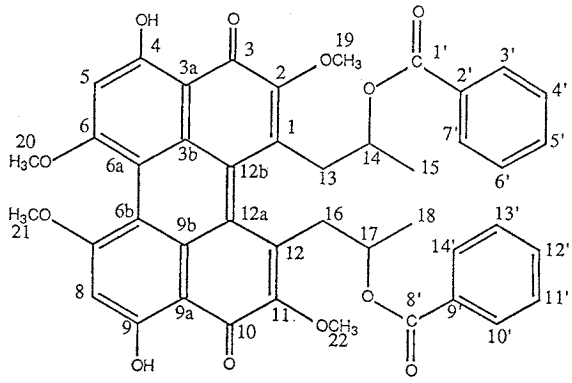


Table 3. Effects of UCN-1028A and phleichrome on activities of PKC and PKA.

Compound	IC_{50} (μg/ml)	
	PKC	PKA
Phleichrome	5.5	33
UCN-1028A	0.19	>40 (30[a])

[a] Inhibition activity (%) at 40 μg/ml.

Enterococcus faecalis, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Shigella sonnei*, *Salmonella typhosa*, *Klebsiella pneumoniae* and *Candida albicans*.

Further studies on the effects of UCN-1028A on other protein kinases, mode of inhibitory action and antitumor activity are now in progress.

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