COMMUNICATIONS TO THE EDITOR

CRM-51005, a New Phospholipase C Inhibitor Produced by Unidentified Fungal Strain MT51005

Sir:

Phospholipase C (PLC)-mediated phosphoinositide (PI)-turnover (PI-turnover) has been recognized to play an important role in intracellular signalling induced by a variety of cellular stimuli such as growth factor, hormones and neurotransmitters. PLC is a key enzyme of PI-turnover and hydrolyze PI to inositol phosphates (IPs) and diacylglycerol (DAG) as second messengers. The activation of PLC and PI-turnover ultimately lead to DNA synthesis and cell proliferation[1]. Therefore, PLC and PI-turnover inhibitors would be expected to be cell growth inhibitors[2]. In the course of our screening programs for PLC and PI-turnover inhibitors from microbial metabolites, we discovered a novel inhibitor, CRM-51005 (**1**), from the fermentation broth of unidentified fungal strain MT51005. In this communication, we describe the fermentation, isolation, structure elucidation and biological activities of **1**.

PLC was purified to homogeneity (over 95% purity of PLCγ1) from bovine cerebellum through DE-52, matrix green gel affinity, phenyl 5-PW and Mono Q column chromatography. The enzyme activity of PLC was assayed using [^{3}H]PIP$_2$ as a substrate according to the method of RHEE *et al.*[3]. PI-turnover was examined by measuring the formation of total inositol phosphates (IP$_t$) in response to platelet-derived growth factor (PDGF) in PLCγ1 overexpressing NIH3T3 (NIH3T3γ1) cells[4]. For the measurement of IP$_t$ formation, NIH3T3γ1 cells were prelabelled with 1 μCi/ml of myo-[2-^{3}H]-inositol in inositol-free DMEM for 24 hours and preincubated in 20 mM LiCl for 15 minutes. Test samples were treated for 10 minutes, and then PDGF was added. After 30 minutes, the cells were treated with ice-cold 5% $HClO_4$ for the extraction of IP$_t$. The measurement of IP$_t$ was performed by the method of FLEISCHMAN *et al.*[5], using Biorad AG 1-X8 anion exchange column.

The producing organism, a fungal strain MT51005 was isolated from a soil sample collected in Jeju island, Korea. The seed culture was incubated in a medium consisting of 0.4% yeast extract, 0.4% malt extract, 0.4% soytone and 1.0% glucose (adjusted to pH 7.0 before sterilization) at 25°C for 3 days, and then, added to a jar fermentor containing 10 liters of the same medium. The production of **1** reached the maximum at 10 days culture. Active materials were extracted with EtOAc from the filtered broth and then with acetone from the mycelium. After the combined organic layers were concentrated *in vacuo*, its residue (5.4 g) was chromatographed on silica gel (Kiesegel 60, 0.063~0.2 mm, Merck) using a mixture of $CHCl_3$/MeOH = 10/1 as an eluting solvent. The active fractions were combined and evaporated *in vacuo* and rechromatographed on ODS RP-18 (70~230 mesh, YMC Co.) with MeOH/H_2O (7/3~9/1, stepwise). The concentrated active fractions were purified by Sephadex LH-20 (Sigma Co.) column chromatography with $CHCl_3$/MeOH/*n*-hexane = 2/1/3. Finally, the biologically active compounds were purified by preparative HPLC (YMC-Pack ODS-AM, i.d. 6.0 × 250 mm, 254 nm, Shimadzu LC-6AD) using a solvent system of MeOH/H_2O (8/2) to give **1** (132 mg) together with the known compound, anguillosporal[6] (**2**) (430 mg) (Fig. 1).

The molecular formula of **1** was established as $C_{15}H_{20}O_3$ by HREI-MS *m/z* 248.1423 (248.1412 calcd. for $C_{15}H_{20}O_3$). The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of the coisolated **2** except the peaks of C-1′ and C-2′ (Table 1). The peak at 3.33 (1H, br sextet,

Fig. 1. Chemical structures of CRM-51005 (**1**) and anguillosporal (**2**).

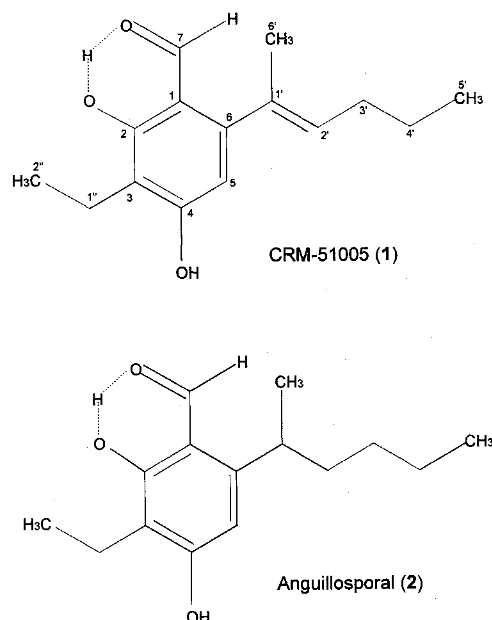


Table 1. ^{1}H- and ^{13}C-NMR data of **1** and **2** in $CDCl_3$.

Carbon No.	CRM-51005 (**1**)		Anguillosporal (**2**)	
	^{1}H (δ)[a]	^{13}C (δ)[a]	^{1}H (δ)	^{13}C (δ)
1	—	112.7	—	112.4
2	—	163.1	—	164.0
3	—	115.4	—	114.5
4	—	159.9	—	160.8
5	6.19 (s)	107.5	6.28 (s)	105.4
6	—	150.9	—	152.1
7	9.83 (s)	195.1	10.17 (s)	192.6
2-OH	12.47 (s)	—	12.89 (s)	—
4-OH	5.30 (s)	—	5.51 (s)	—
1′	—	131.5	3.33 (sextet, 6.9)	32.4
2′	5.39 (t, 7.2)[b]	134.0	1.60 (m)	38.1
3′	2.18 (q, 7.2)	30.6	1.27 (m)	29.8
4′	1.54 (m)	22.5	1.27 (m)	22.7
5′	0.95 (t, 6.9)	14.0	0.87 (t, 6.9)	14.0
6′	1.98 (s)	19.2	1.26 (s)	22.2
1″	2.62 (q, 7.5)	15.4	2.63 (q, 7.5)	15.3
2″	1.15 (q, 7.5)	13.9	1.15 (t, 7.5)	13.0

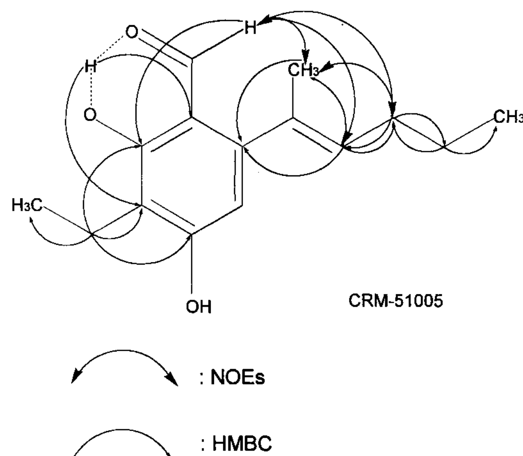
[a] The spectral data of **1** and **2** were measured at 300 MHz (^{1}H) and 75 MHz (^{13}C), using the $CDCl_3$.
[b] Coupling constants (*J* values in Hz) are given in parentheses.

J=6.9 Hz, H-1′) observed in ^{1}H-NMR spectrum of **2** was absent in that of **1**. On the other hand, a new peak at δ 5.39 (1H, t, J=7.2 Hz, H-2′), which did not exist in the ^{1}H-NMR spectrum of **2** appeared in that of **1**. Also, the ^{13}C-NMR data supported the structure of **1** (**1**, δ ^{13}C-1′=131.5, ^{13}C-2′=134.0; **2**, δ ^{13}C-1′=32.4, ^{13}C-2′=38.1). Furthermore, the long-range couplings from H-2′ to C-6, C-3′ and C-6′, from H-3′ to C-2′ and C-4′, from H-4′ to C-3′ and C-5′ indicated the presence of 1′-methylpentenyl group at C-6. Judging from these data, it was concluded that **1** contained a double bond between C-1′ and C-2′ position.

In the NOESY spectrum of **1**, the obvious NOEs between H-7 (δ 9.83) and H-6′ (δ 1.98), H-2′ (δ 5.39), H-3′ (δ 2.18) and also between H-6′ and H-3′ were observed. But, the NOE between H-2′ and H-6′ was very weakly observed. The geometry of the trisubstituted double bond (C-1′ and C-2′) of **1** was determined to be *E* isomer. These results also suggested that the plane of benzene ring and alken plane were located perpendicular to each other. When **1** was kept in $CDCl_3$ for 21 days at the room temperature, complicated signals were appeared. The change of spectral data in ^{1}H-NMR and HMBC experiments suggested that an unusual conversion from *E* form to *Z* form occurred. Generally, the geometry of double bonds favors *E* form than *Z* form except for α,β-unsaturated carbonyl compounds[7].

Although **1** contained double bond between C-1′ and C-2′ position of **2**, **1** and **2** showed almost same inhibitory potency against PLC enzyme (IC_{50} of 13.0 μg/ml) and PI-turnover in response to PDGF stimulation in NIH3T3γ1 cells (IC_{50} of 0.8 μg/ml). The inhibitory activity of these compounds on PLC enzyme and PDGF induced PI-turnover in NIH3T3γ1 cells has not been reported[8,9].

Fig. 2. Significant correlations observed in the HMBC and NOESY spectra of CRM-51005 (**1**).



Thus, we will report a more detailed effect of **1** and **2** against PLC-mediated intracellular signalling in various cell lines and proofs on conversion from *E* form to *Z* form in other papers.

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