NOTES

Gelastatins A and B, New Inhibitors of Gelatinase A from *Westerdykella multispora* F50733

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Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases which include collagenases, stromelysins and gelatinases[1]. In the MMP family, gelatinase A (72-kDa type IV collagenase, EC 3.4.24.24) is unique in its ability to cleave type IV collagen, a principle structural component of basement membrane. This enzyme has thus been implicated in tumor cell invasion and metastasis, as well as angiogenesis and other connective tissue diseases[2]. Consequently gelatinase inhibitors may be of value in the therapy of cancers as well as other disease states involving tissue remodeling. Matlystatins[3] and BE16627B[4] have been isolated from *Actinomadura altramentaria* and *Streptomyces* sp., respectively, as gelatinase inhibitors.

In our screening program for inhibitors of gelatinase A from fungi, we have found that *Westerdykella multispora* F50733 produced novel non-peptidic inhibitors of gelatinase A, designated gelastatins A and B (Fig. 1). In this report the isolation and structural elucidation of gelastatins A and B are discussed.

The producing organism, *W. multispora* F50733, was isolated from a soil sample in Korea, and deposited at the Korea Research Institute of Bioscience and Biotechnology, Korean Collection for Type Cultures, under the accession number KCTC 0265BP.

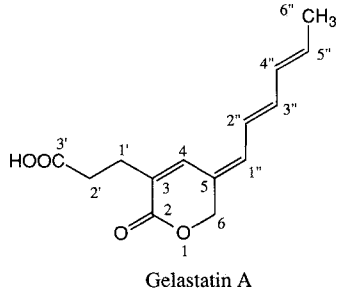
Seed culture was prepared by transferring a loopful of surface growth from an agar slant culture of *W. multispora* F50733 into a 250-ml Erlenmeyer flask containing 50 ml of sterilized medium. The inoculated flask was shaken on a rotary shaker at 25°C for 3 days. This seed culture (50 ml) was transferred to a 5-liter fermenter containing 3 liters of the medium. The seed and production medium consisted of glucose 1%, tryptone 0.5%, yeast extract 0.3% and malt extract 0.3%. The pH of the medium was adjusted to 6.5 before autoclaving. The fermentation was continued for 5 days at 25°C with agitation at a rate of 180 rpm and aeration of 1.0 vvm.

The culture broth was filtered to remove the mycelium and the filtrate was applied on a column of Diaion HP-20. After washing with water and 50% MeOH, the active principles were eluted with 80% MeOH. The active fractions were evaporated, dissolved in water and extracted with equal volume of EtOAc. The separated organic layer was evaporated to dryness *in vacuo* and the remaining oily residue was applied to a column of silica gel with $CHCl_3$-MeOH (10:1). The active eluate was concentrated and subjected to a Sephadex LH-20 column chromatography with MeOH and further purified by HPLC using a reversed phase column (YMC pack ODS-AM, i.d. 4.6 mm × 250 mm) eluting isocratically with 35% CH_3CN containing 0.05% TFA. The active fractions were concentrated *in vacuo* to give a mixture of gelastatins A and B (4.6 mg) as a pale yellow oil. As their further chromatographic separation was unsuccessful, the physico-chemical properties and the structural analysis proceeded with the mixture of gelastatins A and B.

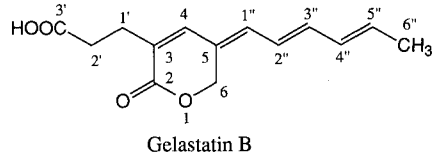
The physico-chemical properties of the mixture of gelastatins A and B are summarized in Table 1. Gelastatins A and B showed a molecular ion peak at *m/z* 249 ($[M+H]^+$) in the FAB-MS, and the molecular formula, $C_{14}H_{16}O_4$, was established by the HRFAB-MS (*m/z* 249.1099, $[M+H]^+$). The IR absorption at 1708 cm^{-1} was attributed to α,β-unsaturated carbonyl group and the UV absorption at 263 nm (ε 9500) suggested the presence of α,β-unsaturated δ-lactone.

The ^{13}C and ^1H NMR spectral data of gelastatins A and B are summarized in Table 2. From the ^{13}C and ^1H NMR spectra, the compounds were found to be a mixture of two stereoisomers (gelastatins A and B) in a ratio of approximately 2:1. The ^{13}C NMR and DEPT spectra showed a pair of signals due to two carbonyl, eight sp^2 (two quarternary and six methine) and four sp^3 (three methylene and a methyl) carbons. The ^1H-^1H COSY experiment revealed the presence of two partial structures as shown in Fig. 2. The structure of gelastatin A was further assigned by HMBC experiment, which showed the long-range coupling from the protons at 1'-H and

Fig. 1. Structures of gelastatins A and B.



Gelastatin A



Gelastatin B

Table 1. Physico-chemical properties of gelastatins*.

Appearance	Pale yellow oil
Molecular formula	$C_{14}H_{16}O_4$
HRFAB-MS $(M+H)^+$	
Found :	249.1099
Calcd :	249.1127
UV λ_{max} nm(ε) in MeOH	263 (br. 9,500), 338 (18,900)
IR ν_{max} (cm^{-1}) in KBr	2940, 1708, 1600, 1407, 1209, 1106, 1037, 991
R_f value on silica gel TLC	0.35 (in $CHCl_3$-MeOH, 10:1)

* The physico-chemical properties were determined with the 2:1 mixture of gelastatins A and B.

Table 2. ^{13}C and ^1H NMR spectral data for gelastatins A and B in CD_3OD.

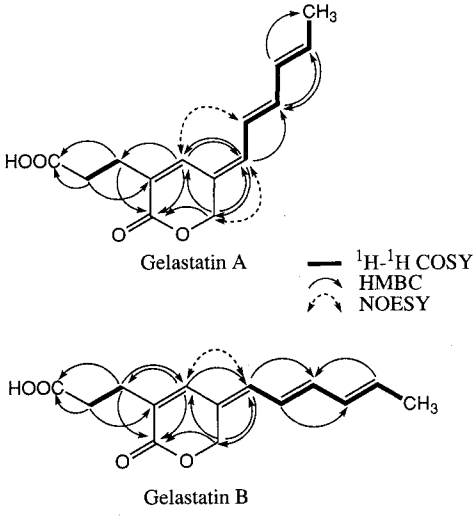
Carbon No.	Gelastatin A		Gelastatin B	
	^{13}C	^1H	^{13}C	^1H
2	167.2		167.0	
3	126.9		127.7	
4	137.0	7.54 s	143.4	7.00 s
5	129.6		128.0	
6	72.2	4.89 s	68.0	5.20 s
1'	27.9	2.65 dd (7.4)	27.6	2.60 dd (7.4)
2'	34.0	2.54 dd (7.4)	34.0	2.51 dd (7.4)
3'	176.6		176.5	
1"	133.0	6.27 d (11.4)	134.1	6.32 d (11.4)
2"	125.1	6.59 dd (14.4, 11.4)	125.4	6.59 dd (14.4, 11.4)
3"	139.7	6.42 dd (14.4, 10.5)	140.7	6.45 dd (14.4, 10.5)
4"	132.5	6.22 ddd (14.7, 10.5, 1.5)	133.1	6.23 ddd (14.7, 10.5, 1.5)
5"	134.5	5.91 dq (14.7, 7.2)	135.0	5.91 dq (14.7, 7.2)
6"	18.6	1.81 br.d (7.2)	18.7	1.81 br.d (7.2)

^{13}C : 125 MHz, ^1H : 300 MHz; δ in ppm
( ) : Coupling constants in Hz.

2'-H to a carboxyl carbon at C-3' implying the presence of a propanoic acid moiety. The structure of gelastatin A together with the presence of an α,β-unsaturated δ-lactone moiety was unambiguously established by the long-range correlations from the proton at 1''-H to the carbons at C-4 and C-6, from the proton at 4-H to the carbons at C-2, C-1' and C-1'', and from the protons at 6-H to the sp^2 carbons at C-2, C-4 and C-1''. The *E* configurations of 2''- and 4''-methine were deduced by ^1H-^1H coupling constants (J=14.4 and 14.7 Hz) and the *E* configuration of C-5 was determined by the NOE between 2''-H and 4-H. Thus the chemical structure of gelastatin A was elucidated to be 3-(5*E*-hexa-2*E*,4*E*-dienylidene-2-oxo-5,6-dihydro-2H-pyran-3yl)-propanoic acid. The structure of gelastatin B was determined by the comparison of ^1H and ^{13}C NMR chemical shifts with those of gelastatin A and the NOESY data (Table 2 and Fig. 2). The chemical shift of C-4 in gelastatin A shifted toward high field as compared to that of C-4 in gelastatin B, while the chemical shift of C-6 in gelastatin B shifted toward high field as that of C-6 in gelastatin A (γ-effects)[5]. These γ-effects established the configuration of C-5 of gelastatin B as *Z* and this result was also supported by the NOE between 1''-H and 4-H. Thus the structure of gelastatin B was determined as 3-(5*Z*-hexa-2*E*,4*E*-dienylidene-2-oxo-5,6-dihydro-2H-pyran-3yl)-propanoic acid, a stereoisomer of gelastatin A.

The activity of gelatinase A was assayed with a fluorescent peptide, Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg amide (10 μM) as a substrate and *p*-aminophenylmercuric acetate (APMA) activated gelatinase A (1 nM). Progelatinase A was purified from T98G human glio-

Fig. 2. ^{1}H-^{1}H COSY, HMBC and NOESY experiments of gelastatins A and B.



blastoma cells to nearly homogenous form as described elsewhere[6]. For the measurement of the inhibitory activity, the activity of gelatinase A in the presence of the inhibitor was assayed according to KNIGHT *et al.*[7] with some modifications. The 2:1 mixture of gelastatins A and B inhibited activated gelatinase A with an IC_{50} value of 0.63 μM but did not inhibit other metalloproteinases including aminopeptidase M and thermolysin with 100 μM. In addition, the methyl esters of the mixture of gelastatins A and B with diazomethane had no inhibitory activity against gelatinase A with 100 μM.

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