Acremolactone A, a Novel Herbicidal Epoxydihydropyranyl γ-Lactone from *Acremonium roseum* I4267

Sir:

Only a few herbicidal substances are found from microorganisms: some of these are isolated from phytopathogenic fungi and others by screening from other microorganisms, mainly *Streptomyces*. In addition, a large number of them include nitrogen atom(s) in the molecule regardless of their structural diversity[1]. In our search of new plant growth regulators from fungi, we found a potent herbicidal substance in a culture broth of *Acremonium roseum* I4267[2]. It had no nitrogen atoms in the molecule and showed non-selective strong herbicidal activity against harmful weeds: particularly its pre-emergence herbicidal activity against crabgrass (*Digitaria adscendens* Henr.) and smartweed (*Polygonum blumei* Meisn.) was 9 to 10 on evaluating by a zero to 10 (complete killing) rating system at the dosage of 1.0 kg/ha, herbicidal activity which was higher than that of synthetic herbicides such as alachlor[3]. The present paper reports the isolation and structural determination of an unprecedented herbicidal compound from the culture filtrate of this fungus. This compound named acremolactone A (**1**, Fig. 1) possesses a novel epoxydihydropyran nucleus.

The fungus was shake-cultured in a medium containing glucose 4%, corn starch 2%, soybean meal (Nissin) 2%, Pharmamedia (Traders Protein) 2% and $CaCO_3$ 1% at 25°C for 2 days and successively surface-cultured for 14 days. The culture filtrate (*ca.* 3 liters) obtained by filtration was extracted with EtOAc at pH 9.0, and the extract (*ca.* 2 g) was immediately separated by flash column chromatography using Wako gel FC-40 silica gel and *n*-hexane - EtOAc (1 : 1, v/v) to give colorless crystalline **1** (90 mg). **1** had mp 123.5~124.0°C (benzene-EtOAc), $[\alpha]_D^{20}$ +68° (*c* 0.13, MeOH) and molecular formula $C_{26}H_{34}O_8$ [HR-FAB-MS *m/z* 497.2151 $(M+Na)^+$; calcd for $C_{26}H_{34}O_8Na$, 497.2149]. It gave a triacetate on acetylation with acetic anhydride and pyridine, which showed no hydroxyl IR bands. Three ^1H NMR signals in the spectrum of the triacetate had acetylation shifts [δ ($CDCl_3$, *J*Hz) 5.661 (H-11′, 4.5, 2, 2), 5.592 (H-3, br s) and 4.925 (H-5′, 12.5, 4.4), *cf.* Table 1]. These data indicated the presence of three secondary hydroxyls and the absence of tertiary hydroxyls. Its IR band (1757 cm^{-1} in a $CHCl_3$ solution) and ^{13}C NMR signal [δ 179.19 (C-1)] due to a carbonyl function (Table 1) suggested the presence of a γ-lactone ring in the molecule. Accordingly three oxygen atoms remaining unassigned in **1** were assignable to ether oxygen(s) and/or epoxy one(s). ^1H-^1H and ^1H-^{13}C COSY and decoupling experiments (long-range couplings from H-5 to H-3 and H-9) on **1** revealed three partial structures of C-3′~C-2~C-4 (C-9)~C-8 (**A**), C-5′~C-8′ (**B**) and C-11′~C-14′ (**C**) (Fig. 2), which included three secondary hydroxyl groups. Correlations among ^1H and ^{13}C NMR signals of **1** in one-bond relationship were determined by DEPT and ^1H-^{13}C COSY (Table 1).

The partial structure **A** had a trisubstituted double bond in the side chain and its configuration was assigned as *E* on the basis of chemical shift of the methyl (C-9) and an NOE observation between H-6 and H-9. HMBC cross peaks from H-2 to C-1 and C-3, from H-3 to C-1, from H-3′ to C-2, C-3, C-4′, C-5′, C-7′ and C-17′ and from H-5′ to C-4′ and C-17′, and COLOC ones from H-17′ to C-3′, C-4′ and C-5′, which connected the partial structures **A** to **B**, clearly showed that 1) the carbon C-4′

Fig. 1. Structures of acremolactone A (**1**) and its H_5IO_6-oxidation product (**2**), and H_5IO_6-oxidation mechanisms of **1** in H_2O/EtOH.

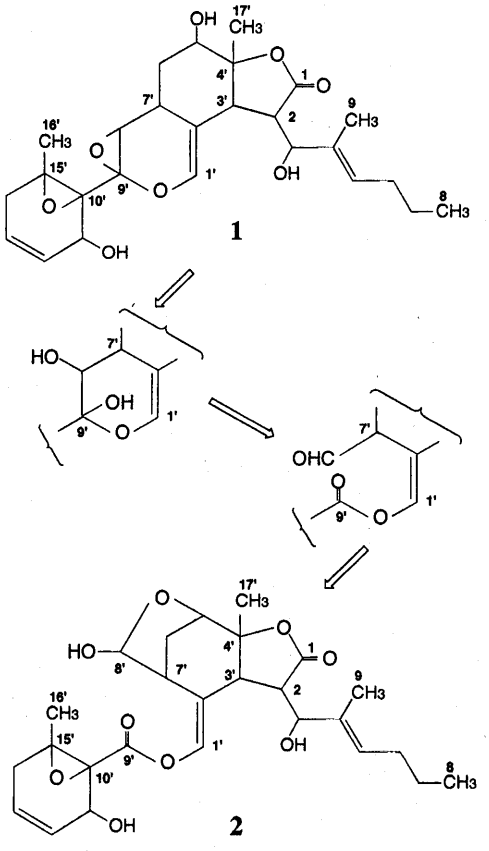
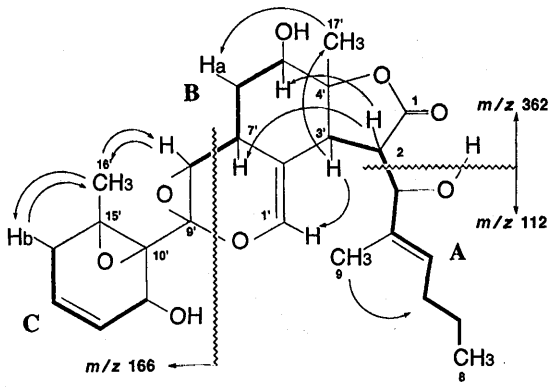


Fig. 2. Partial structures (——) **A**, **B** and **C** of acremolactone A (**1**), and NOE observations (↷) on **1** in NMR spectrometry and its characteristic fragment ions (〰) in MS.



(δ_C 88.06) had both a lactonic oxygen atom and the third methyl (C-17′) of **1** and 2) carbons of C-1 (=O), C-2, C-3′ and C-4′ and an oxygen of (C-4′)–O– formed the γ-lactone ring described above. In addition, HMBC cross peaks from H-1′ to C-2′, C-3′ and C-7′, from H-3′ to C-1′ and C-2′, from H-8′ to C-2′ and C-7′, and COLOC ones from H-7′ to C-2′ and C-6′ revealed the presence of a trisubstituted vinyl ether group [–O–C-1′ = C-2′ (C-3′)–C-7′] between the partial structures **A** and **B**. This vinyl ether moiety, which constructed the first six carbocyclic ring (C-2′ ~ C-7′) in **1**, was well interpreted from not only chemical shifts of H-1′ (δ 5.877), C-1′ (δ 138.58) and C-2′ (δ 107.36)[4] but also a long-range coupling from H-1′ to H-7′ (J = 2 Hz) and an NOE observation between H-1′ and H-3′.

The partial structure **C** was extended to an epoxycyclohexenol residue [C-10′ ~ C-15′ (C-16′)] as the second carbocyclic ring moiety of **1** from HMBC cross peaks from H-11′ to C-10′, C-13′ and C-15′ and from H-14′ to C-12′, C-13′ and C-15′. C-10′ and C-15′ were assigned to epoxy carbons from their chemical shifts (δ 64.01 and 62.99, respectively). Further DIF-NOE observations between Hb-14′ and H-16′ indicated that the fourth methyl (H-16′) was attached at C-15′ and located at the side of C-14′ but not C-11′. Finally the last carbon C-9′ (δ 86.0) remaining unassigned in **1**, which gave HMBC correlations with H-1′ and H-8′, had to connect directly to C-10′ of the second carbocyclic ring to give the novel epoxydihydropyran ring of **1**. Chemical shifts of C-8′ and C-9′ (δ 58.51 and 86.0) were accountable for an epoxy and an epoxy ethereal carbon, respectively[5]. Direct connectivity of the second carbocyclic ring (partial structure **C**) to the epoxydihydropyran one was confirmed by an observation of DIF-NOE between H-16′ and H-8′. The structure of **1**, therefore, was elucidated as **1** shown in Fig. 1.

Table 1. Spectral data and NMR assignments (in CD_3OD) of acremolactone A.

UV λ_{max} (MeOH) nm	End absorption
IR ν_{max} (KBr) cm^{-1}	3430, 1745, 1668, 1235, 1163, 1093, 973, 904, 864, 807, 767, 685
EI-MS m/z (%)	474 (M^+, 0.4), 456 (2.0), 362 (1.2), 301 (4.5), 166 (45), 137 (53), 119 (63), 112 (24), 109 (71), 97 (71), 55 (100)

Carbon No.	δ_C[a] (DEPT)	δ_H[b] (mult., J Hz)
1	179.19 (C)	
2	47.12 (CH)	3.093 (dd, 12, 2.4)
3	72.94 (CH)	4.504 (br s)
4	134.84 (C)	
5	125.26 (CH)	5.60 (br t, 7)
6	30.91 (CH_2)	2.07 ~ 1.90 (2H, m)
7	23.85 (CH_2)	1.56 ~ 1.35 (2H, m)
8	14.43 (CH_3)	0.933 (t, 7.3)
9	14.36 (CH_3)	1.443 (s)
1′	138.58 (CH)	5.877 (d, 2)
2′	107.36 (C)	
3′	45.82 (CH)	3.003 (d, 12)
4′	88.06 (C)	
5′	74.13 (CH)	3.875 (dd, 12, 4.4)
6′	33.73 (CH_2) a	1.860 (ddd, 12, 4.4, 4)
	b	1.44 (m)[c]
7′	29.89 (CH)	2.833 (br d, 12)
8′	58.51 (CH)	3.435 (d, 3.4)
9′	86.0 (C)	
10′	64.01 (C)	
11′	64.86 (CH)	4.382 (ddd, 4.5, 2, 2)
12′	126.58 (CH)	5.644 (dddd, 10, 4.5, 2.5, 2)
13′	125.04 (CH)	5.531 (ddd, 10, 4.5, 2.5)
14′	32.25 (CH_2) a	2.517 (dddd, 19, 2.5, 2.5, 2)
	b	2.439 (dddd, 19, 4.5, 2, 2)
15′	62.99 (C)	
16′	21.06 (CH_3)	1.313 (s)
17′	17.37 (CH_3)	1.404 (s)

[a] 100 MHz, [b] 400 MHz, [c] δ ($CDCl_3$) 1.627 (ddd, 12, 12, 12).

This novel structure of **1** was also supported by (HR-)EI-MS and DIF-NOE experiments. Characteristic fragment ions of **1** at m/z 362 and 112 ($C_7H_{12}O$), and 166 ($C_9H_{10}O_3$) in the MS spectrum were well interpreted by MacLafferty rearrangement in the side chain and

retro-Diels-Alder fragmentation in the epoxydihydropyran ring, respectively (Fig. 2). These fragment ions of *m/z* 362 and 166, further, were well explained by their shift of two mass unit in the EI-MS spectrum of 12′,13′-dihydro-**1** [*m/z* 364 (0.4%) and 168 (54%)] which was prepared by catalytic hydrogenation of **1**. Several NOE observations between partial structures **A** and **B** diagnostic for the structure of **1** were displayed in Fig. 2.

In order to chemically characterize the novel epoxydihydropyran nucleus of **1**, its selective cleavage was tried and carried out satisfactorily by H_5IO_6-oxidation. The oxidation product from **1** (10 mg) was purified by silica gel column chromatography to afford a colorless solid [**2**; 7 mg, FAB-MS *m/z* 491 $(M+H)^+$]. It gave a new ^{13}C NMR signal of an additional carbonyl at δ 166.14 along with a strong IR-carbonyl band at ν_{max} ($CHCl_3$) 1754 cm^{-1}, suggesting the formation of a vinyl ester group in the molecule. Its NMR spectra, further, demonstrated characteristic signals of a hemiacetal group at δ_C ($CDCl_3$) 102.6 and δ_H 4.95 (d, $J=3$ Hz). Thus its structure including a vinyl ester group and a hemiacetal group was elucidated as **2** shown in Fig. 1 by ^1H-^{13}C COSY and HMBC: cross peaks in the latter were observed from H-1′ (δ 6.759, s) to C-2′ (δ 123.89), C-3′ (δ 137.90), C-7′ (δ 40.64) and C-9′ (δ 166.14), from H-5′ (δ 4.168, d, 6) to C-3′ and C-7′ and from H-8′ (δ 4.953, d, 3) to C-5′ (δ 79.62), C-6′ (δ 24.48) and C-7′. The other NMR signals of the carbocyclic ring moiety having the hemiacetal ring were also assigned as H-3′ [δ 2.792 (d, $J=7$ Hz)], Ha-6′ [δ 2.250 (ddd, 13, 6, 4)], Hb-6′ [δ 1.590 (d, 13)] and H-7′ [δ 3.243 (d, 4)], and C′-1 (δ 134.50), C-4′ (δ 85.07) and C-8′ (δ 102.57). This structure was also supported from a characteristic intense ion at *m/z* 153 (27%) in the EI-MS spectrum which was generated from the acyl moiety (C-9′ ~ C-16′). Reaction mechanism of the H_5IO_6-oxidation of **1** is shown in Fig. 1††. Acremolactone A, therefore, was determined to be the structure **1** as shown in Fig. 1. This is a new type of compound unprecedented for microbial metabolites[6].

2 showed no hypocotyl growth-inhibitory activity against Chinese cabbage seedlings at concentrations less than 300 ppm, whereas **1** showed complete inhibition at a concentration of 1 ppm. Accordingly the epoxydihydropyran nucleus in **1** is considered to play an important role for its herbicidal activity.

We have recently reported that this fungus produces acremoauxin A (**3**), a novel arabitol derivative of 2-(indolyl-3)propionic acid, having potent auxin activity[7]. The fungus produced **3** but not **1** on shake-culturing in a glucose - malt extract medium. A few minor metabolites structurally related to **1** were also detected by TLC from the filtrate of the surface culture using the medium described above.

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†† Charactrization and stereochemistry of degradation products derived from **2** will be reported elsewhere.