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TWO ALKALOIDS FROM *ZEPHYRANTHES CARINATA*

KEISUKE KOJIMA,* MOTOH MUTSUGA, MAKOTO INOUE and YUKIO OGIHARA

Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe Dori, Mizuho-ku, Nagoya 467, Japan

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Key Word Index—*Zephyranthes carinata*; Amaryllidaceae; alkaloid; pancratistatin; cytotoxicity.

Abstract—Two new Amaryllidaceae alkaloids, 1-*O*-(3-hydroxybutyryl)pancratistatin and 1-*O*-(3-*O*-β-D-glucopyranosylbutyryl)pancratistatin, were isolated from the bulbs of *Zephyranthes carinata*. The structures of the two alkaloids were established by comprehensive spectral analysis. The cytotoxicity of these two alkaloids and pancratistatin is appraised.

INTRODUCTION

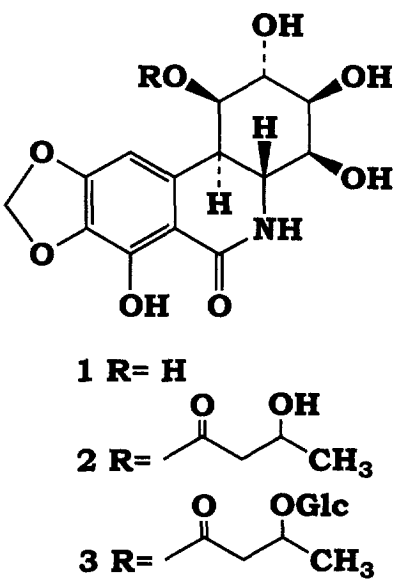
Some plants of the Amaryllidaceae have been used in the primitive treatment of human cancer, and the family have yielded over 100 different alkaloids [1–3]. Amaryllidaceae alkaloids have aroused great interest among researchers in a wide range biological field. Several workers have reported cytotoxic and antineoplastic activities of certain Amaryllidaceae alkaloids [4, 5].

Pancratistatin (**1**), isolated from the roots of *Pancratium littorale* Jacq., has been found to exhibit a highly characteristic differential cytotoxicity profile against the U.S. National Cancer Institute's (NCI) panel, and pancratistatin is one of the Amaryllidaceae alkaloids with the strongest cytotoxicity [6–11]. Its total synthesis was reported by some groups [12–14].

We have investigated *Zephyranthes carinata* Herb. which occurs in Mexico [15]. In the genus *Zephyranthes*, *Z. parulla* appears in a history of Peru for treating tumours [16], *Z. candida* has been employed in Africa as a treatment for diabetes mellitus [16], *Z. rosea* and *Z. flava* are used for variety of therapeutic purposes in India [17–20]. The present paper deals with the isolation, the structural elucidation and cytotoxicity of two new Amaryllidaceae alkaloids.

RESULTS AND DISCUSSION

The bulbs were extracted with $CHCl_3$, Me_2CO and MeOH successively, and the MeOH extract furnished pancratistatin (**1**) and two new alkaloids (**2**, **3**). Compound **2**, $C_{18}H_{21}NO_{10}$ (HRMS), showed ^1H, ^{13}C NMR spectral signals for one OCH_2O group (δ_H 5.95, δ_C 102.4), one singlet Ar—H (δ_H 6.61), one amidic NH (δ_H 9.23), one amidic CO (δ_C 171.0) and six protons all interacting with each other (COSY), which have been attributed to H-1 (δ_H 6.22), H-2 (δ_H 4.94), H-3 (δ_H 4.76), H-4 (δ_H 4.67), H-4a (δ_H 4.72) and H-10b (δ_H 3.96) on the same ring, suggesting that **2** is a pancratistatin-type alkaloid including the stereochemistry (Tables 1, 2).



The substituent was identified as a 3-hydroxybutyryl group on the basis of the chemical shifts in the ^{13}C NMR spectral signals at δ_C 22.9 (*q*), 45.7 (*t*), 63.8 (*d*) and 171.5 (*s*). The confirmation of the position of the 3-hydroxybutyryl group was possible by application of the HMBC technique, the correlation peak was observed from the proton at C-1 and CO of 3-

* Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data in pyridine-d_5

	2	**3**
1	6.22 (1H, *br s*)	6.16 (1H, *br s*)
2	4.94 (1H, *br s*)	5.05 (1H, *br s*)
3	4.76 (1H, *br s*)	4.79 (1H, *br s*)
4	4.67 (1H, *dd*, *J*3, 10 Hz)	4.65 (1H, *dd*, *J*3, 10 Hz)
4a	4.72*	4.72*
5(NH)	9.23 (1H, *s*)	9.22 (1H, *s*)
10	6.61 (1H, *br s*)	6.57 (1H, *br s*)
10b	3.96 (1H, *br d*, *J* 15 Hz)	3.89*
—CH_2—	5.95 (2H, *br s*)	5.99 (2H, *br s*)
Ester		
2′	2.66 (1H, *dd*, *J*9, 15Hz)	2.75 (1H, *dd*, *J*5, 15 Hz)
	2.74 (1H, *dd*, *J*4, 15 Hz)	2.91 (1H, *dd*, *J*6, 15 Hz)
3′	4.44*	4.72*
4′	1.22 (1H, *d*, *J*7 Hz)	1.40 (1H, *d*, *J*6 Hz)
Glucose		
1		5.02 (1H, *d*, *J*8 Hz)
2		3.96*
3		4.18*
4		4.20*
5		3.90*
6		4.31 (1H, *dd*, *J*5, 12Hz)
		4.47 (1H, *br d*, *J*12 Hz)

* Overlapped with other signals.

Table 2. ^{13}C NMR spectral data in pyridine-d_5

	2	**3**
1	71.0	71.4
2	69.8	69.9
3	74.0	73.8
4	72.0	72.0
4a	51.9	51.9
6	171.0	170.9
6a	108.8	108.8
7	147.6	147.6
8	133.4	133.3
9	153.1	153.1
10	96.7	96.6
10a	134.9	135.0
10b	39.1	39.2
—CH_2—	102.4	104.4
Ester		
1′	171.5	171.6
2′	45.7	42.8
3′	63.8	73.3
4′	22.9	22.2
Glucose		
1		103.6
2		75.2
3		78.5
4		71.5
5		78.4
6		62.7

hydroxybutyryl group. Therefore, **2** is 1-*O*-(3-hydroxybutyryl)pancratistatin.

Compound **3**, $C_{24}H_{31}NO_{15}$ (HRMS), gave rise to ^{1}H and ^{13}C NMR spectra that were similar to those of **2**, except for the presence of signals due to one glucose residue. A cross-peak between the $H_{G\text{-}1}$ and C-3′ in the HMBC spectrum suggested that the glucose residue was linked to 3-hydroxybutyryl group. When the ^{13}C NMR data of **3** was compared with that of **2**, glycosylation shift was observed at C-3′. On the other hand, the mass spectrum exihibits a $[M+H]^+$ at *m/z* 574 and the fragments at 412 and 326 confirm the presence of the glucose and 3-hydroxybutyryl groups. Thus **3** is 1-*O*-(3-*O*-*β*-D-glucopyranosylbutyryl) pancratistatin.

Treatment of **2** and **3** with NaOMe yielded pancratistatin, the ^{1}H NMR spectrum of which indicated the absence of the 3-hydroxybutyryl group and 3-*O*-*β*-D-glucopyranosylbutyryl group, respectively.

The isolated alkaloids, **1**–**3** were evaluated for their cytotoxicities by the MTT method. Each compound was tested at four different concentration ranges (0.1, 1, 10 and 50 μg/ml) (Fig. 1). The concentration-response curves thus generated were used to calculate LD_{50} (−50% growth, or 50% cell kill) (Table 3) [21, 22].

Compound **1**, **2**, and **3** showed potent cytotoxicity against KB, HeLa and P388-D1 cells. The cytotoxicity of **2**, compared with pancratistatin, was approximately 3-fold higher on these four cells. Significant

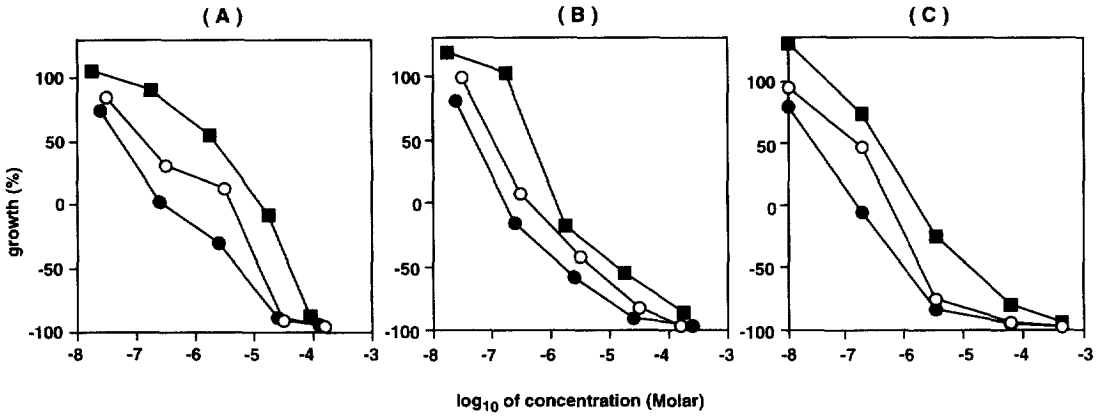


Fig. 1. Dose-response curves for three cell lines incubated for 48 h. (A) HeLa (4×10^4 cells/ml), (B) KB (5×10^4 cells/ml), (C) P388-D1 (10^5 cells/ml). ○; 1, ●; 2, ■; 3.

differences of activity related to structure could be observed.

EXPERIMENTAL

General

NMR spectra were recorded in pyridine-d_5 with TMS as int. standard.

Plant material

The bulbs of *Zephyranthes carinata* Herb were collected in Morelos, Mexico in 1987. It was identified by Dr G. S. Ortega in the Jardin de Etno-botanico, Instituto Nacional de Antropologia e Historia, Mexico, where voucher specimens of the plant are deposited.

Extraction and isolation

The air-dried bulbs of *Zephyranthes carinata* (800 g) were extracted successively with $CHCl_3$, Me_2CO and MeOH (31×2, 6 h in each) under reflux. After evapn of the MeOH, a part of this mixt. (20 g) was subjected to repeated CC on silica gel with $CHCl_3$–MeOH (10:1–1:1). Three frs were afforded. Fr. I was subjected to Lober RP-18 using MeCN–HOAc–H_2O (20:1:79), followed by further prep. HPLC, eluting with MeCN–HOAc–H_2O (15:1:84), **2** (19 mg) was isolated. Fr. II was subjected to CC using a $CHCl_3$–MeOH step gradient; after final purification on Sephadex LH-20, **3** (35 mg) was isolated. Fr. III was subjected to prep. HPLC, eluting with MeCN–HOAc–H_2O (10:1:89), pancratistatin (6 mg) was isolated.

Table 3. Evaluation of the cytotoxicity

	LD_{50} (M)		
	HeLa	KB	P388-D1
1	1.2×10^{-5}	5.2×10^{-6}	2.2×10^{-6}
2	4.5×10^{-6}	1.8×10^{-6}	9.1×10^{-7}
3	4.1×10^{-5}	1.1×10^{-5}	5.8×10^{-6}

Deacylation of **2** *and* **3**

A sample of each compound (ca 10 mg), 90% aq. MeOH (1 ml) and a freshly prepared soln (0.1 ml) of NaOMe (0.1 g Na dissolved in 100 ml of MeOH) were mixed and stirred for 1 h at room temp. Na^+ removed by treatment with Amberlite IR-120 (H^+). The soln was processed in the usual way to give pancratistatin (^{1}H NMR).

Cell culture

HeLa cells (human epithelial carcinoma), KB cells (human epidermoid carcinoma) and P388-D1 cells (mouse lymphoid neoplasma) were provided from Health Science Research Resources Bank. At the beginning of the experiments, the cells were adjusted to a concentration of 4×10^4/ml for HeLa, 5×10^4/ml for KB and 10^5/ml for P388-D1 cells. For MTT assay, 100 μl/well of these cell suspensions were seeded in 96-well culture plates and incubated over 24 h at 37° in a 5% CO_2/air atmosphere. Cytotoxicity was determined using the MTT tetrazolium salt colorimetric assay. The culture medium was changed to the stock solution of the alkaloids which were diluted in culture medium at 4 different concentrations (0.1, 1, 10 and 50 μg/ml), and 100 μl/well. The cells were then incubated (37°, 5% CO_2) for 48 h. At this time, 10 μl of 10 mg/ml MTT stock solution was added to the wells and incubation was continued for 6 h at 37°. During this period purple-coloured formazan crystals were formed, and the absorbance was determined following solubilization of formazan with 100 μl of 10% SDS by usual manners.

1-*O*-(3-Hydroxybutyryl)pancratistatin (**2**). $[\alpha]_D^{25}$ +19.2° (MeOH; *c* 0.18). λ_{max} (MeOH) 231 (4.05), 279 (3.70). FAB-MS *m/z*: 412 $[M+H]^+$, 326 $[M-C_4H_5O_2]^+$. High-resolution FAB-MS *m/z*: 412.1243, $C_{18}H_{22}NO_{10}$ requires $[M+H]^+$, 412.1220.

1-*O*-(3-*O*-β-D-Glucopyranosylbutyryl)pancratistatin (**3**). $[\alpha]_D^{25}$ +20.7° (MeOH; *c* 0.5). λ_{max} (MeOH) 235 (3.84), 272 (3.57). FAB-MS *m/z*: 574 $[M+H]^+$, 412 $[M-C_6H_9O_5]^+$, 326 $[M-C_{10}H_{15}O_7]^+$. High-resolution FAB-MS *m/z*: 574.1772, $C_{24}H_{32}NO_{15}$ requires $[M+H]^+$, 574.1791.

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