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TWO ACETOGENINS FROM *HEMSLEYA ELLIPSOIDEA*

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Key Word Index—*Hemsleya ellipsoidea*; Cucurbitaceae; acetogenin; cucurbitacin; NMR.

Abstract—Two new acetogenins, ellipsoidones A and B, were isolated from the tubers of *Hemsleya ellipsoidea* along with five known cucurbitacins, isocucurbitacin B, 23, 24-dihydroisocucurbitacin B, cucurbitacin F, 25-*O*-acetylcucurbitacin F, 25-*O*-acetyl-23, 24-dihydrocucurbitacin F, and two known compounds, siphonodin, (*E*)-2-methyl-2-butene-1,4-diol. The structures of the two new acetogenins were determined on the basis of the spectroscopic and photo-chemical evidence. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Plants of the genus *Hemsleya* (Cucurbitaceae) are distributed throughout the southwest region of China and particularly in the Sichuan and Yunnan provinces. More than 30 species of the plants grow in the southwest region of China [1–3]. The tubers of the plants have been used in folk medicine [4]. Triterpene saponins including cucurbitacins have been reported as the main components of the plants [5–14]. As part of our survey of Chinese medicinal resources, the components of the tuber of *H. ellipsoidea* L. T. Shen et W. J. Chang were examined. This paper describes the structure of two new acetogenins, the first examples from the genus *Hemsleya*, along with seven known compounds.

RESULTS AND DISCUSSION

Ellipsoidone A (**1**) was obtained as yellow needles, mp 141–142.5°. The molecular formula $C_{11}H_{10}O_5$ was determined by the high-resolution mass spectrum. The IR spectrum of **1** disclosed the absorption bands due to hydroxy and lactone carbonyl groups and the UV spectrum absorption maxima at 230, 257, and 368 nm. The ^{1}H NMR spectrum of **1** showed proton signals due to two hydroxymethyls at δ 4.57 (2H, *br d*, J = 6.0 Hz) and 4.73 (2H, *m*), four olefinic protons at δ 6.19 (1H, *dt*, J = 0.6 and 1.5 Hz), 6.30 (1H, *d*, J = 0.6 Hz), 6.48 (1H *dd*, J = 0.7 and 3.3 Hz), and 6.90 (1H, *d*, J = 3.3 Hz), two hydroxy protons at δ 4.37 (1H, *br t*, J = 6.0 Hz) and 4.70 (1H, *br t*, J = 5.9 Hz) (Table 1). The coupling constant (J = 3.3 Hz) between the proton signals at δ 6.48 and 6.90 led to the assumption that **1** has a 2,5-disubstituted furan ring system. The two-dimensional (2D) ^{1}H-^{1}H correlation spectroscopy (COSY) as well as the decoupling experiment revealed two spin systems. One consists of the protons at δ 4.37 (OH), 4.57 (CH_2), 6.48 (CH), and 6.90 (CH), and the other is of the protons at δ 4.70 (OH), 4.73 (CH_2), 6.19 (CH), and 6.30 (CH). In the former system, the olefinic proton at δ 6.48 showed a long-range allylic coupling with the methylene protons at δ 4.57, while in the latter system, the olefinic proton at δ 6.19 showed long-range allylic couplings with the methylene protons at δ 4.73 and the olefinic proton at δ 6.30. In order to define the connectivities, 2D ^{13}C-^{1}H

Table 1. ^{13}C NMR chemical shifts (ppm) of **1** and **2** (in acetone-d_6)

C	**1** CH connectivities		**2** CH connectivities	
C-2	169.12		168.47	
C-3	114.24	6.19	118.00	6.39
C-4	161.47		160.58	
C-5	145.73		146.14	
C-6	57.22	4.73	60.16	4.96
C-7	99.79	6.30	103.46	6.60
C-2′	149.34		147.86	
C-3′	116.41	6.90	117.84	6.72
C-4′	110.98	6.48	110.56	6.45
C-5′	158.50		159.06	
C-6′	57.37	4.57	57.37	4.63
		4.70		4.68
		4.37		4.47

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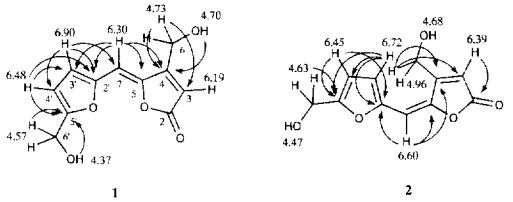

Fig 1. The HMBC spectra of **1** and **2** (J_{CCH} = 8 Hz).

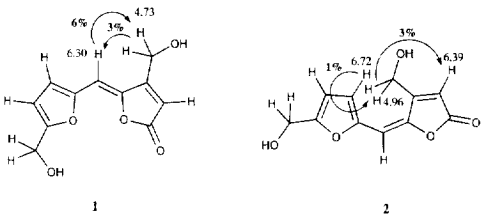

Fig 2. The NOE experiments of **1** and **2**.

COSY and long-range ^{1}H-^{13}C COSY (HMBC) spectra were measured and the connectivities among the ^{1}H and ^{13}C signals revealed **1** to be an acetogenin with a 5-hydroxymethyl furan group and a 4-hydroxymethyl-5-methylidene-2-furanone group (Fig. 1). Furthermore, difference NOE spectra by the irradiation of the olefinic proton at δ 6.30 as well as the methylene protons at δ 4.73 revealed the geometry of the double bond to be *Z*-form (Fig. 2). Thus, the structure of ellipsoidone A is represented by **1**.

Ellipsoidone B (**2**) was obtained as yellow needles, mp 159–161°. The molecular formula $C_{11}H_{10}O_5$ was determined by the high-resolution mass spectrum. The IR spectrum of **2** disclosed the absorption bands due to hydroxy and lactone carbonyl groups. The UV spectrum was closely similar to that of **1**, suggesting ellipsoidone B to be an analogue of **1**. The signal pattern of the ^{1}H NMR spectrum of **2** was also essentially the same as that of **1**. The protons due to two hydroxymethyls, four olefinic protons, and two hydroxy protons were observed in the ^{1}H NMR spectrum (Table 1). Comparison of the ^{13}C NMR spectrum of **2** with that of **1** suggested that **2** has the same carbon skeleton as **1** (Table 1). The HMBC spectrum of **2** supported the suggestion (Fig. 1). These results indicated **2** to be a stereoisomer of **1** with respect to the geometry of the double bond. The difference NOE spectrum by the irradiation of the methylene protons at the C-6 gave an evidence for the geometry of the double bond being (*E*)-form (Fig. 2). Further examination of the geometry was carried out by a photochemical method. Photo-irradiation of the methanol solution of **2** with 100 W high-pressure mercury lamp gave **1** in 30% yield. From the above results, the structure of ellipsoidone B is represented by **2**.

The other known compounds, siphonodin (**3**) [16], (*E*)-2-methyl-2-butene-1,4-diol (**4**), isocucurbitacin B [17], 23,24-dihydroisocucurbitacin B [17], cucurbitacin F [18], 25-*O*-acetylcucurbitacin F [19], 25-*O*-acetyl-23,24-dihydrocucurbitacin F [20], were identified by comparing the physical and NMR data with published data.

The acetogenins **1** and **2** represent the first isolation of this class of compound from the plants of genus *Hemsleya*. Siphonodin 6-*O*-β-D-glucopyranoside (siphonoside) has been reported as a cytotoxic compound against Walker-256-sarcoma cells [16, 21]. From the structural similarity of **1** and **2** with **3**, cytotoxity of **1** against P-388 cells was examined and it showed almost the same activity as **3** [**1**: IC_{50}47 mg ml^{-1}, **3**: IC_{50} 67 mg ml^{-1}.

EXPERIMENTAL

General. Mp are uncorr. ^{1}H and ^{13}C NMR spectra were recorded using tetramethylsilane (TMS) as an int. standard. HPLC was carried out on a SSC (Senshu Scientific Co. Ltd., Tokyo) equipped with UV and R_I (refractive index) detectors using a Senshu Pak Silica-4251-N column (10 ϕ × 250 mm). Wakogel C-200 and B-5F (Wako) were used for CC and prep. TLC, respectively.

Plant materials. The tubers of *Hemsleya ellipsoidea* were collected in Pengzhou city, Sichuan Province, China, in July 1994, and were identified by one of authors (Wenjin Chang).

Extraction and isolation. The fresh tubers of *Hemsleya ellipsoidea* (3.77 kg) were extracted with 95% EtOH and then the EtOH soln was concd *in vacuo* to give a residue (200 g). The residue was suspended in H_2O followed by the extraction with petrol and EtOAc to give a petrol portion and an EtOAc portion. The EtOAc extract (27 g) was chromatographed over silica gel using $CHCl_3$ increasing amount of Me_2CO (10:0.9:1.8:2 and 7:3) as an eluent to prepare frs 1–52. The combined fr 7–8 (8 mg) was fractionated by HPLC [*n*-hexane–EtOAc (1:1)] to give isocucurbitacin B (3 mg) and 23,24-dihydroisocucurbitacin B (1.5 mg). The combined fr. 25–27 (60 mg) was purified by prep. TLC [ether–EtOAc (1:1)] followed by HPLC [*n*-hexane–EtOAc (1:14)] to give ellipsoidone A (**1**, 18 mg) and ellipsoidone B (**2**, 12 mg). The combined fr. 33–36 (2.5 g) was rechromatographed over silica gel [$CHCl_3$–MeOH (9:1)] followed by prep. TLC [$CHCl_3$–MeOH (9:1)] to give **4** (30 mg). The combined fr. 48–49 (300 mg) was subjected to rechromatography over silica gel [$CHCl_3$–Me_2CO–EtOAc (5:3:2)] followed by HPLC [*n*-hexane–EtOAc (1:3)] to give cucurbitacin F (4 mg). The combined fr. 10–15 (20 g) was rechromatographed over silica gel using $CHCl_3$–Me_2CO (9:1.8:2) to give frs 1′–60′. The combined fr. 24′–40′ (12 g) was recrystallized from MeOH to give **3** (10 g). The fraction 44′–46′ (10 mg) was purified by HPLC [*n*-hexane–Me_2CO (1:1)] to give 25-*O*-acetyl-23,24-dihydrocucurbitacin F (3 mg) and 25-*O*-acetylcucurbitacin F (4 mg).

Ellipsoidone A (**1**). Yellow needles (Me_2CO), mp 141–142.5° UV λ_{max}^{MeOH} nm (log ε): 203 (3.5), 230 (3.6), 257 (3.3), 368 (4.1). EIMS *m/z* (rel. int.): 222 $[M]^+$ (100), 205 (33), 175 (17), 121 (50), 79 (32). HRFABMS: *m/z* 223.0411 ($[M+H]^+$, $C_{11}H_{11}O_5$, requires 223.0606).

Ellipsoidone B (**2**). Yellow needles (Me_2CO), mp 159–161° UV λ_{max}^{MeOH} nm (log ε): 203 (4.0), 229 (3.9), 260 (3.7), 367 (4.4). EIMS *m/z* (rel. int.): 222 ($[M]^+$, 100), 205 (25), 175 (13), 121 (42), 79 (28). HRFABMS: *m/z* 223.0446 ($[M+H]^+$, $C_{11}H_{11}O_5$, requires 223.0606).

Photo-isomerization reaction of ellipsoidone B. A MeOH soln (1 ml) of ellipsoidone B (3 mg) was irradiated for 3 hr with a 100 W high-press. mercury lamp. Ellipsoidone A (**1**) was obtained as a photo-reaction product in 30% yield.

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