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A NEW CYCLOPENTANONE DERIVATIVE FROM *Euphorbia hirta*

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*A new cyclopentanone derivative, (1′R,5′R)-5-(5′-carboxymethyl-2′-oxocyclopentyl)-3Z-pentenyl acetate (**1**), was isolated from* Euphorbia hirta*. Its structure was elucidated on the basis of spectroscopic analysis including 1D and 2D NMR techniques. The EtOAc extract of* Euphorbia hirta *was evaluated against K562 and A549 cancer cell lines.*

Keywords: *Euphorbia hirta*, cyclopentanone, cytotoxicity.

Euphorbia plants exhibit a broad range of biological activities, such as antianaphylactic, antioxidant, antiproliferative, antiarthritic, antimicrobial, antinociceptive, and cytotoxic activities [1–5]. *Euphorbia hirta* L., distributed mainly in the southern and southwestern districts of China [6], has been used traditionally for the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis) and bronchial disorders, conjunctivitis, and respiratory diseases (asthma, bronchitis, hay fever) [7, 8]. Previous phytochemical studies have demonstrated the presence of some alkanes, diterpenoids, triterpenes, phytosterols, tannins, polyphenols, and flavonoids in this plant [9–13]. Due to the diverse structure and siginificant biological activities of this plant, we have investigated its chemical constituents and isolated a new cyclopentanone derivative, (1′*R*,5′*R*)-5-(5′-carboxymethyl-2′-oxocyclopentyl)-3*Z*-pentenyl acetate (**1**), from *Euphorbia hirta*. In this paper, we describe the isolation and structural elucidation of this compound as well as the cytotoxicity activities of the EtOAc extract.

Compound **1** was obtained as a white, amorphous powder from the EtOH extract of *E. hirta*. The molecular formula was determined as $C_{14}H_{20}O_5Na$ on the basis of the sodiated HR-ESI-MS data (291.1236 $[M + Na]^+$, calcd 291.1208), which corresponded to five degrees of unsaturation. The IR spectrum showed specific absorption bands for carboxylic hydroxy (2798 cm^{-1}), carboxylic carbonyl (1725 cm^{-1}), ketone (1710 cm^{-1}), and an olefinic (1643 cm^{-1}) groups. The ^{13}C NMR data (Table 1) showed that compound **1** possesses one carboxyl group (δ_C 173.6), acetyl group (δ_C 171.0, 20.7; δ_H 1.98), carbonyl (δ_C 218.3) and olefinic groups (δ_C 129.8, 127.5; δ_H 5.48, 5.43), which disclosed the presence of a ring to meet the degrees of unsaturation.

The COSY spectrum exhibited the presence of one proton sequence as shown in Fig. 1. The correlations of H-1′ (δ_H 2.07), H-4′ (δ_H 2.24), and H-5′ (δ_H 2.27) with C-2′ (δ_C 218.3) in the HMBC spectrum, together with the proton sequence of -CH_2 (3′)-CH_2 (4′)-CH (5′)-CH_2 (1′)- observed in the ^1H–^1H COSY correlations, established the 2′-oxocyclopentyl moiety. The correlation of H-1′ (δ_H 2.07) to C-5 (δ_C 26.0), as well as H-5 (δ_H 2.40) to C-2′ (δ_C 218.3) and C-5′ (δ_C 38.6), observed in the HMBC spectrum indicated that the 2′-oxocyclopentyl moiety was located at C-5. The fact that the acetyl group was connected with C-1 through the O atom and carboxymethyl group attached to C-5′ were confirmed by the correlations of H-1 (δ_H 4.02) with acetyl carbon (δ_C 171.0), of H-1′ (δ_H 2.07), H-4′ (δ_H 2.24) and H-5′ (δ_H 2.27) with C-6′ (δ_C 39.0), as well as of H-5′ (δ_H 2.27) and H-6′ (δ_H 2.71) with C-7′ (δ_C 173.6) in the HMBC spectrum. The HMBC correlations from H-3 (δ_H 5.43) to C-1 (δ_C 64.1) and from H-4 (δ_H 5.48) to C-1′ (δ_C 54.3) disclosed the position of the double bond, as shown in Fig. 1. Thus, the planar structure of compound **1** was assigned as 5-(5′-carboxymethyl-2′-oxocyclopentyl)-3-pentenyl acetate.

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TABLE 1. NMR Data (400 MHz, acetone-d_6) of **1**

C atom	δ_C (DEPT)	δ_H, J/Hz	HMBC	^1H–^1H COSY
1	64.1 (CH_2)	4.02 (t, J = 6.9)	C-2, 3, OAc	H-2
2	27.7 (CH_2)	2.41 (m)	C-1, 4	H-1, 3
3	127.5 (CH)	5.43 (dt, J = 11.2, 5.7)	C-1, 2, 5	H-2, 4
4	129.8 (CH)	5.48 (dt, J = 11.2, 5.8)	C-2, 5, 1′	H-3, 5
5	26.0 (CH_2)	2.40 (m)	C-3, 1′, 2′, 5′	H-4, 1′
1′	54.3 (CH)	2.07 (m)	C-5, 3′, 4′, 6′	H-5, 5′
2′	218.3 (C)			
3′	37.9 (CH_2)	2.35 (m)	C-2′, 5′	H-4′
		2.05 (m)		
4′	27.5 (CH_2)	2.24 (m)	C-1′, 2′, 5′, 6′	H-3′, 5′
		1.56 (m)		
5′	38.6 (CH)	2.27 (m)	C-1′, 2′, 3′, 7′	H-1′, 4′, 6′
6′	39.0 (CH_2)	2.71 (dd, J = 14.9, 3.2)	C-1′, 4′, 5′, 7′	H-5′
		2.39 (m)		
7′	173.6 (C)			
OAc	20.7 (CH_3)	1.98 (s)	C=O	
	171.0 (C)			

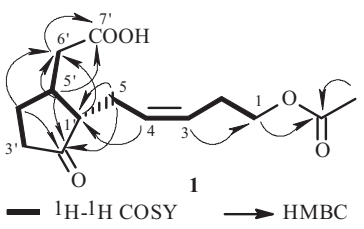

Fig. 1. Key ^1H-^1H COSY and HMBC correlations of **1**.

In the ^1H NMR spectrum, two olefinic proton signals at δ 5.48 (dt, J = 11.2, 5.8 Hz) and 5.43 (dt, J = 11.2, 5.7 Hz) indicated the presence of a *cis* double bond. H-1′ and H-5′ resonated at δ 2.07 and 2.27, respectively. Resonances for H-1′ and H-5′ of the 1′,5′-*cis* isomer were reported at δ 2.35–2.45 and 2.80 [14–17], whereas the *trans* isomer showed chemical shifts of H-1′ and H-5′ at δ 1.91 and 2.24, respectively [18]. Thus **1** has the 1′,5′-*trans* relative configuration.

On the basis of the evidence above, the structure of compound **1** was eventually elucidated as (1′*R*,5′*R*)-5-(5′-carboxymethyl-2′-oxocyclopentyl)-3*Z*-pentenyl acetate.

Due to the limited amount of material, compound **1** was not tested for its cytotoxicity; however, the EtOAc extract of *E. hirta* was evaluated for cytotoxicity in K562 (human leukemia) and A549 (lung cancer) cell lines using the sulforhodamine B (SRB) method, as reported previously [19], with adriamycin as the positive control. The extract exhibited weak activity against A549 cells (inhibition ratio 15.02 ± 11.60 % at 20 μg/mL) and inactivity against K562 cells.

EXPERIMENTAL

General Experimental Procedures. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR spectra were measured on a Bruker DRX-500 instrument with TMS as internal standard. Mass spectra were obtained on a VG Auto Spec-3000 spectrometer or on a Finnigan MAT 90 instrument. Column chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, People′s Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} on glass plates (Qingdao Marine Chemical Inc.) using various solvent systems.

Extraction and Isolation. Dry herbs of *Euphorbia hirta* (4.0 kg) were extracted with ethanol (3 × 25 L) at room temperature overnight. The extract was partitioned between H_2O and EtOAc, and the EtOAc layer (58 g) was chromatographed on MCI-gel CHP 20P (eluted with 90% CH_3OH–H_2O, then 100% CH_3OH). The 90% CH_3OH fraction (32 g) was repeatedly

chromatographed over silica gel, eluting with a gradient of petroleum ether to acetone, followed by RP-18 column chromatography (52% MeOH–H_2O) to yield **1** (2 mg).

(**1′*R*,5′*R*)-5-(5′-Carboxymethyl-2′-oxocyclopentyl)-3*Z*-pentenyl Acetate (1)**. White amorphous powder. FT-IR (KBr, ν_{max}, cm^{-1}): 2798, 1725, 1710, 1643, 1520, 1376, 1254, 1033, 811, 635. ESI-MS *m/z* (ESI + Na^+, %): 561 (24), 292 (100). HR-ESI-MS *m/z* (HR-ESI + Na^+, %): 291.1236 (calcd for $C_{14}H_{20}O_5Na$, 291.1208). For ^1H NMR, ^{13}C NMR, and 2D NMR data, see Table 1.

Cytotoxicity Bioassay. The cytotoxicity of the extract against suspended tumor cells was determined by the trypan blue exclusion method, and against adherent cells by the sulforhodamine B (SRB) assay. Cells were plated in a 96-well plate 24 h before treatment and continuously exposed to different concentrations of extract for 72. After extract treatment, cells were counted (suspended cells) or fixed and stained with SRB (adherent cells). Adriamycin was used as positive control.

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