Benzoic Glycoside from *Euphorbia hirta*

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(Received on 23rd December 2010, accepted in revised form 10th October 2011)

Summary: A new benzoic glycoside was isolated from the ethyl acetate fraction of the ethanol extract from *Euphorbia hirta*. Its structure was elucidated as (1'*R*, 5'*R*)-5-(5-methylcarboxymethyl-2-oxocyclopentyl)-3*Z*-pentenyl-*β*-D-(6-*O*-*p*-hydroxybenzoyl) glucopyranoside (**1**) by 1D and 2D NMR spectroscopicanalysis. This is the first compound with this skeleton from *E. hirta*.

Introduction

Euphorbia hirta Linn, mainly distributed in the southern and southwestern district of China [1], has been used traditionally for gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis), bronchial, conjunctivitis, and respiratory diseases (asthma, bronchitis, hay fever) [2, 3]. Previous phytochemical studies have demonstrated the presence of some alkanes, diterpenoids, triterpenes, phytosterols, tannins, polyphenols, and flavanoids in this plant [4-6]. Diverse structure and siginificant biological activities of this plant have been attracting our considerable interest. Chemical investigation of this plant has led to the isolation of one new benzoic glycoside, (1'*R*,5'*R*)-5-(5-methylcarboxymethyl-2-oxocyclopentyl)-3*Z*-pentenyl-*β*-D-(6-*O*-*p*-hydroxybenzoyl) glucopyranoside (**1**), which is the first isolate with this skeleton from *Euphorbia hirta*. In this paper, we report the isolation and structural elucidation of this compound [7].

Results and Discussion

Compound **1**, obtained as a yellow amorphous powder, showed an $[M + Na]^+$ at *m*/*z* 545.1999 (calcd. 545.1998) corresponding to the molecular formula $C_{26}H_{34}O_{11}$ in the positive ion HR-ESI-MS, indicating ten degrees of unsaturation. The IR spectrum revealed absorption bands due to hydroxy (3452 cm-1), carboxylic hydroxy (2810 cm-1), carboxylic carbonyl (1715 cm-1), ketone (1710 cm-1), and aromatic ester (1696 cm-1) functions and an aromatic ring (1612 and 1517 cm-1). The presence of a *p*-hydroxybenzoyl group was supported by the characteristic signals of four methine protons at δ_H 8.19 and 7.09 (each 2H, d, J = 8.5 Hz) in the ^{1}H NMR spectrum and five carbon signals (δ_C 166.8, 163.6, 132.5, 121.7, and 116.1) in the ^{13}C NMR spectrum (Table-1). The ^{1}H and ^{13}C NMR spectra showed the presence of *β*-glucopyranose moiety in **1**. The 6″-methylene protons at δ_H 5.18 (br d, 1H, J = 11.6 Hz) and 5.00 (dd, 1H, J = 11.6, 5.5 Hz) showed HMBC correlations with the carboxylic carbonyl (δ_C 166.8) of the *p*-hydroxybenzoyl group, indicating the acylation of the C-6″ hydroxy function (Fig. 1). In the HSQC spectrum, the remaining 13 carbons corresponded to one ketone (δ_C 218.1), one carboxyl (δ_C 172.7), two olefinic methines (δ_C 128.3 × 2C), one oxymethylene (δ_C 69.4), two sp^3 methines (δ_C 54.0 and 38.2), five methylenes (δ_C 38.9, 37.8, 28.6, 27.4, and 25.9), and one methane (δ_C 51.5). Two olefinic proton signals at δ_H 5.58 (dt, 1H, J = 10.5, 7.1 Hz) and 5.46 (dt, 1H, J = 10.5, 7.1 Hz) supported the presence of a *cis* double bond. The proton sequence, observed in the ^{1}H-^{1}H COSY spectrum, showed the presence of a framework of -$OCH_2CH_2CHCHCH_2CHCH(CH_2$-)$CH_2CH_2$-(Fig. 2). Careful examination of NMR data of **1** as well as detailed comparison of its 1D, 2D NMR spectra with those of the known compound (1'*R*,5'*R*)-5-(5-carboxymethyl-2-oxocyclopentyl)-3*Z*-pentenyl-*β*-D-(6-*O*-galloyl) glucopyranoside [7] suggested that they are very similar to each other except that a galloyl group in known compound was replaced by a *p*-hydroxybenzoyl one in **1**, and there is one more methane (δ_C 51.5, δ_H 3.63) in **1**, which was confirmed by the HMBC correlations of OCH_3 (δ_H 3.63) with carboxylic carbonyl (δ_C 172.7). Furthermore, the HMBC correlations from the anomeric proton at H-1'' (δ_H 4.86) to the oxymethylene carbon at C-1 (δ_C 69.4) disclosed the glucose moiety was attached to C-1, together with the geminal methylene protons H-6' (δ_H 2.69 and 2.33) correlated with carboxylic carbon (δ_C 172.7), C-5' (δ_C 38.2), and C-1' (δ_C 54.0), H-5 (δ_H 2.37) correlated with C-2' (δ_C 218.1) and C-5' (δ_C 38.2), and H-3' (δ_H 2.29) and H-4' (δ_H 2.10) correlated with C-2'(δ_C 218.1) demonstrated the structure of compound **1** as shown in Fig. 1. Then, the gross structure of **1** was identified as

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5-(6-carboxymethyl-2-oxocyclopentyl)-3*Z*-pentenyl -*β*-D-(6-*O*-*p*-hydroxybenzoyl) glucopyranoside.

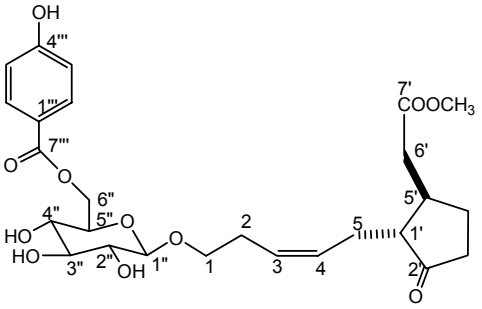

Fig. 1: The structure of **1**

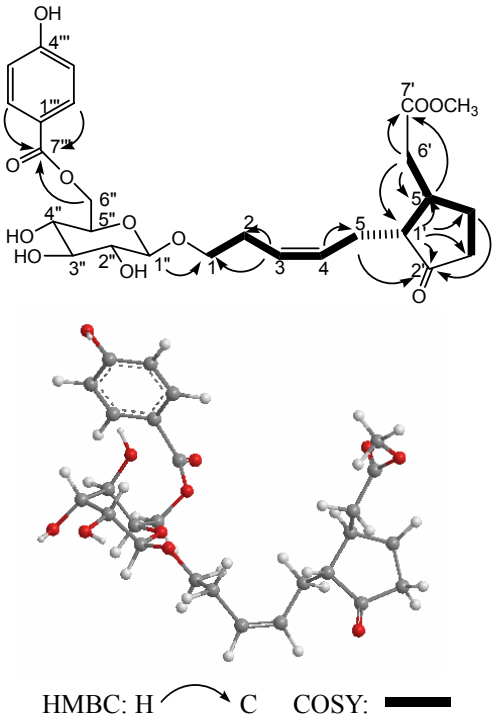

Fig. 2: Key HMBC and COSY correlations for **1**.

In the ^{1}H NMR spectrum, H-1' and H-5' resonated at δ_H 1.93 and 2.24, respectively, which was consistent with the resonances for H-1' (δ_H 1.91) and H-5' (δ_H 2.24) of the 1',5'-*trans* isomer [7], whereas the 1',5'-*cis* isomer showed chemical shifts of H-1'and H-5' at δ_H 2.35–2.45 and 2.80, respectively [8-11], which suggested that **1** possessed 1',5'-*trans* relative configuration as shown in Fig. 2. The 3D structure of **1** obtained using a molecular modeling program with MM2 force-field calculations for energy minimization, was in good agreement with above conclusion. Accordingly, compound **1** was elucidated as (1'*R*,5'*R*)-5-(6-carboxymethyl-2-oxo cyclopentyl)-3*Z*-pentenyl-*β*-D-(6-*O*-*p*-hydroxyben zoyl) glucopyranoside.

Due to the limited amount of material available, compound **1** was not tested for biological activity.

Experimental

General Experimental

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

Table-1: ^{1}H and ^{13}C NMR data of compound (**1**) (δ ppm).

C	δ_C [a,b]	δ_H [a,c]	C	δ_C [a,b]	δ_H [a,c]
1	69.4 t	4.08 (dd, 1H, *J* = 13.7, 8.1 Hz)	2"	75.2 d	4.09 (m, 1H)
		3.78 (m, 1H)			
2	28.6 t	4.08 (br d, 2H, *J* = 6.4 Hz)	3"	78.5 d	4.24 (overlapped, 1H)
3	128.3 d	5.58 (dt, 1H, *J* = 10.5, 7.1 Hz)	4"	71.6 d	4.23 (overlapped, 1H)
4	128.3 d	5.46 (dt, 1H, *J* = 10.5, 7.1 Hz)	5"	75.5 d	4.10 (m, 1H)
5	25.9 t	2.37 (m, 2H)	6"	64.8 t	5.18 (br d, 1H, *J* = 11.6 Hz)
					5.00 (dd, 1H, *J* = 11.6, 5.5 Hz)
1'	54.0 d	1.93 (m, 1H)	1"'	121.7 s	
2'	218.1 s		2"'	132.5 d	8.19 (d, 1H, *J* = 8.5 Hz)
3'	37.8 t	2.29 (m, 2H)	3"'	116.1 d	7.09 (d, 1H, *J* = 8.5 Hz)
4'	27.4 t	2.10 (m, 1H)	4"'	163.6 s	
		1.39 (m, 1H)			
5'	38.2 d	2.24 (m, 1H)	5"'	116.1 d	7.09 (d, 1H, *J* = 8.5 Hz)
6'	38.9 t	2.69 (d, 1H, *J* = 10.8 Hz)	6"'	132.5 d	8.19 (d, 1H, *J* = 8.5 Hz)
		2.33 (overlapped, 1H)			
7'	172.7 s		7"'	166.8 s	
1"	104.9 d	4.86 (d, 1H, *J* = 7.8 Hz)	OCH_3	51.5 q	3.63 (s, 3H)

[a] Recorded in C_5D_5N. [b] Recorded at 125 MHz. [c] Recorded at 500 MHz.

Plant Material

The materials of the herb (*Euphorbia hirta*) were collected in the Xishuang Banna prefecture, Yunnan Province, People's Republic of China, in September 2009. A voucher specimen (YTCM 20090909) was deposited at the College of Yunnan traditional Chinese medical, and was identified by Prof. Yao-wen Yang.

Isolation

Dry herbs of *Euphorbia hirta* (4.0 kg) was percolated with 95% ethanol (3 × 25 L) at room temperature overnight. The crude extract was evaporated under reduced pressure to yield a dark brown residue (174 g). The residue was suspended in H_2O and then partitioned with EtOAc. The EtOAc concentrated solution (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 repeated chromatographed over silica gel, eluting with a gradient of increasing acetone in petroleum ether, to give fractions A1–A5. Fraction A2 (3.5 g) was further separated by RP-18, eluting with a gradient of increasing MeOH in H_2O, to afford subfractions A2-1–A2-7. Subfraction A2-5 (43 mg) was purified repeatedly by normal-phase silica gel, using petroleum ether-EtOAc (2:1), to yield compound **1** (3 mg).

Acknowledgments

This work was supported financially by the Natural Science Foundation of Yunnan Province (No. 2009CD051) and the Project of the Backup Talented of the Leader of Academic and Technology of Yunnan Province (No. 2010CI040).

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