· Letters ·

A New Flavone *C*-glycoside from *Citrullus colocynthis*

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Abstract: **Objective** To study the chemical constituents of *Citrullus colocynthis*. **Methods** The chemical constituents were isolated and purified by column chromatography on silica gel and Sephadex LH-20 and recrystallization as well. NMR spectra and physicochemical property were characterized for structural identification. **Results** Eleven compounds were isolated and identified as β-sitosterol (**1**), α-spinasterol-3-*O*-β-*D*-glucopyranoside (**2**), α-spinasterone (**3**), bis (2-ethylhexyl) phthalate (**4**), *p*-hydroxybenzoic acid (**5**), 6-*C*-*p*-methylbenzoylvitexin (**6**), dihydrocucurbitacin E (**7**), cucurbitacin E (**8**), dihydro-*epi*-iso-cucurbitacin D (**9**), dihydroisocucurbitacin B-25-acaetate (**10**), and cucurbitacin E 2-*O*-β-*D*-glucopyranoside (**11**). **Conclusion** Compound **6** is a novel compound. Compounds **1**—**5**, **7**, **9**, and **10** are isolated from *C. colocynthis* for the first time.

Key words: citrullus; *Citrullus colocynthis*; 6-*C*-*p*-methylbenzoylvitexin; cucurbitacin; steride
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Introduction

Citrullus colocynthis (L.) Schrad. (Cucurbitaceae), commonly known as “bitter apple” or “wild gourd”, has been used as a traditional Uigur medicine. It is the fruit of cucurbitaceous plant *C. colocynthis*, distributing in desert and semiarid areas of Africa, Western Asia, and the Mediterranean region. And now it could be cultivated in Hetian region and Yecheng county of Xinjiang in China. Since *C. colocynthis* was introduced to Xinjiang via “the Silk Road” as medicinal material in the Song Dynasty, it has been used to treat astriction, indigestion, phlegm plug, paralysis, epilepsia, and arthritis. In the present paper, we described the isolation and identification of a new flavone *C*-glycoside, named 6-*C*-*p*-methylbenzoylvitexin, and ten known compounds as well.

Materials and methods

Equipments

^{1}H-NMR, ^{13}C-NMR, and DEPT spectra were recorded on a Bruker ZAB-HS Spectrometer. Melting points were determined on a Chinese X—5 Melting Point Apparatus. HR-ESI-MS and ESI-MS data were obtained on QSTAR Elite Instrument. Optical rotation was determined by WXG—4 Disc Spectrometer. Sephadex LH-20 was purchased from GC company. Silica gel (100 － 200 and 200 － 300 mesh) was purchased from Qingdao Marine Chemical Factory.

Plant material

The dried fruits of *Citrullus colocynthis* (L.) Schrad. were purchased from Urumqi (Xinjiang Uygur Autonomous Region, China) in November, 2009 and were verified by Prof. YAN Ping of Shihezi University. A voucher specimen was deposited at Key Laboratory for Green Processing of Chemical Engineering of Xinjiang Bingtuan.

Extraction and isolation

The dried fruits of *C. colocynthis* (9.5 kg) were crushed and then extracted with 90% ethanol (10 times volume) continuously. The combined ethanol extracts were evaporated under reduced pressure. A suspension of the crude extract in distilled water was partitioned

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into petroleum ether, $CHCl_3$, and EtOAc. Removal of the solvent from each phase gave the petroleum ether fraction (51.6 g), $CHCl_3$ fraction (220.5 g), EtOAc fraction (13.9 g), and water-soluble fraction (56.7 g). The petroleum ether extract was eluted with a step gradient of petroleum ether-EtOAc (100:0→0:100) to yield six fractions which were chromatographed to afford compounds **1** (1500 mg), **2** (300 mg), and **3** (150 mg). The EtOAc extract was separated by column chromatography on silica gel ($CHCl_3$-MeOH 100:0→0:100) and Sephadex LH-20 ($CHCl_3$-MeOH 1:1) to obtain compounds **4** (45 mg), **5** (40 mg), and **6** (25 mg). The $CHCl_3$ extract was subjected to silica gel column, eluting with a step gradient of $CHCl_3$-MeOH (100:0→0:100) and purified by Sephadex LH-20 gel column repeatedly to yield compounds **7** (300 mg), **8** (40 mg), **9** (35 mg), **10 (**43 mg), and **11** (35 mg).

Results and discussion

Compound **6**: yellow granulated solid ($CHCl_3$-MeOH), $[\alpha]_D^{20}$ +71.43° (*c* 0.0056, MeOH), mp 260.8－262.6 ℃. Its molecular formula was assigned as $C_{29}H_{28}O_{10}$ by HR-ESI-MS at *m/z*: 537.1753 $[M + H]^+$ (calcd 536.1755 for $C_{29}H_{28}O_{10}$), so its degree of unsaturation is 16. ^{1}H-NMR and ^{13}C-NMR data were shown in Table 1.

Table 1 ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of compound 6 in DMSO-*d*$_6$

No.	δ_H	δ_C	No.	δ_H	δ_C
2		163.9	1″		130.6
3	6.81 (1H, s)	102.9	2″	7.06 (1H, d, *J* = 8.4 Hz)	129.1
4		182.5	3″	6.62 (1H, d, *J* = 8.4 Hz)	115.2
5		157.7	4″		154.1
6		107.0	5″	6.62 (1H, d, *J* = 8.4 Hz)	115.2
7		161.5	6″	7.06 (1H, d, *J* = 8.4 Hz)	129.1
8		108.1	1‴	4.79 (1H, d, *J* = 10.0 Hz)	74.4
9		155.6	2‴		72.2
10		107.0	3‴		78.1
1′		121.4	4‴		69.3
2′	7.83 (1H, d, *J* = 8.8 Hz)	128.6	5‴		81.4
3′	6.92 (1H, d, *J* = 8.8 Hz)	116.2	6‴		60.1
4′		161.5	bridged-CH_2	4.04 (2H, s)	27.2
5′	6.92 (1H, d, *J* = 8.8 Hz)	116.2	-CH_3	2.50 (3H, brs)	18.5
6′	7.83 (1H, d, *J* = 8.8 Hz)	128.6			

Its HCl-Mg reaction is positive, so compound **6** may be a flavone. The ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) spectrum showed characteristic signals of flavone at δ_H 6.81 (1H, s) and two benzene rings at δ_H 7.83 (2H, d, *J* = 8.8 Hz), 7.06 (2H, d, *J* = 8.4 Hz), 6.92 (2H, d, *J* = 8.8 Hz), and 6.62 (2H, d, *J* = 8.4 Hz), and a proton of a β-linked sugar at δ_H 4.79 (1H, d, *J* = 10.0 Hz). The ^{13}C-NMR (100 MHz, DMSO-*d*$_6$) spectrum (Table 1) including DEPT spectra exhibited 24 carbon signals, consisting of a methyl group, two methylene groups, 10 methyne groups, and 11 quaternary carbon atoms, and six of them were attributed to the existence of glycosidic moieties while C-2 (163.9), C-3 (102.9), and C-4 (182.5) were attributed to the C ring of flavone.

From the proton δ_H 4.79 (1H, d, *J* = 10.0 Hz), the secondary carbon (δ 60.1), and other five tertiary carbon atoms (δ 74.4, 72.2, 78.1, 69.3, 81.4), the sugar unit was identified as β-*D*-glucopyranose. The sugar sequence and its linkage site were derived from the HMBC signals at H-1‴ (δ_H 4.79), C-7 (δ_C 161.5), and C-9 (δ_C 155.6) in its HMBC spectrum, suggesting that the β-*D*-glucopyranosyl group was attached to C-8 (δ 108.1). In addition, the HMBC spectrum showed that H-bridged-CH_2 (δ_H 4.04) was correlated with C-2″, 6″ (δ_C 129.1), C-5 (δ_C 157.7), and C-7 (δ_C 161.5), H-CH_3 (δ_H 2.50) was correlated with C-3″, 5″ (δ_C 115.2) and C-4″ (δ_C 154.1), and H-3 (δ_H 6.81) was correlated with C-1′ (δ_C 121.4) and C-2 (δ_C 163.9). Therefore, bridged-CH_2 was attached to C-1″ (δ_C 130.6) and C-6 (δ_C 107.0), -CH_3 to C-4″ (δ_C 154.1), and C-1′ (δ_C 121.4) to C-2 (δ_C 163.9). From the above evidences, compound **6** was identified as 6-*C*-*p*-methyl-benzoylvitexin (Fig. 1).

The ^{1}H-NMR, ^{13}C-NMR, and MS data of β-sitosterol (**1**), α-spinasterol-3-*O*-β-*D*-glucopyranoside (**2**, Luo, He, and Kong, 2006), α-spinasterone (**3**, Gong *et al*, 2010), bis (2-ethylhexyl) phthalate (**4**, Gong *et al*, 2004), *p*-hydroxybenzoic acid (**5**, Wang, Yang, and Zhang, 2009), dihydrocucurbitacin E (**7**, Wu *et al*, 2004),

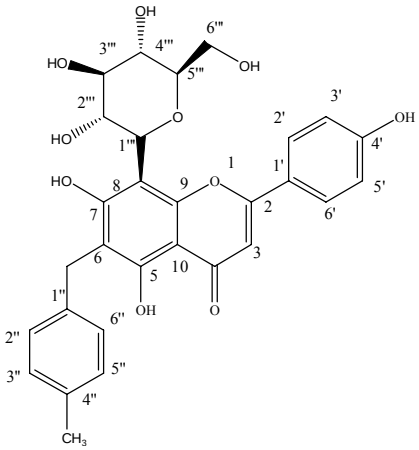

Fig. 1 Chemical structure of compound 6

cucurbitacin E (**8**, Liu *et al*, 2004), dihydro-*epi*-iso-cucurbitacin D (**9**, Peter *et al*, 1986), dihydro-isocucurbitacin B-25-acaetate (**10**, Li *et al*, 1998), and cucurbitacin E 2-*O*-β-*D*-glucopyranoside (**11**, Natiq, Donald, and Nahia, 1989) were consistent with those of physicochemical constants and so identified, respectively.

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Captions of Cover Photo

Commelina communis L., commonly known as the Asiatic dayflower, is a herbaceous annual plant in the dayflower family. It is native throughout much of East Asia and northern parts of Southeast Asia. In China, the plant is known as Yazhicao, roughly translating to “duckfoot herb”. It has also been introduced to parts of central and southeastern Europe and much of eastern North America, where it has spread to become a noxious weed. It is common in disturbed sites and in moist soil. The flowers emerge from summer through fall and are distinctive with two relatively large blue petals and one very reduced white petal.

In China, it is used as a medicinal herb with febrifugal, antipyretic, anti-inflammatory, and diuretic effects. Additionally, it is also used for treating sore throats and tonsillitis. Recent pharmacological investigations revealed that the Asiatic dayflower contains at least five active compounds. One is *p*-hydroxycinnamic acid showing antibacterial activity, while another, *D*-mannitol, has an antitussive effect. In China and India the plant is also used as a vegetable and fodder crop.

C. communis is also used as a model organism in plant physiology and plant development to a limited extent, especially in relation to stomatal physiology and the biology of pigmentation development.