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C-p-HYDROXYBENZOYLGLYCOFLAVONES FROM *CITRULLUS COLOCYNTHIS*

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Key Word Index—*Citrullus colocynthis*; Cucurbitaceae; *C*-linked flavone *C*-glucoside; *C-p*-hydroxybenzoylisoorientin; vitexin; isovitexin 4′-*O*-glucoside.

Abstract—In a flavonoid investigation of the fruits and aerial parts of *Citrullus colocynthis* six flavone *C*-glycosides were identified. The fruits contained isovitexin, iso-orientin and iso-orientin 3′-methyl ether, while the aerial parts contained three new *C-p*-hydroxybenzyl derivatives, viz. 8-*C-p*-hydroxybenzoylisovitexin, 6-*C-p*-hydroxybenzoylvitexin, and 8-*C-p*-hydroxybenzoylisovitexin 4′-*O*-glucoside. Their chemical identity was established by NMR spectroscopic methods including 2D-NMR, as well as UV and MS analyses. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The fruits of *Citrullus colocynthis* Schrad (Cucurbitaceae) are well known for their medicinal properties [1]. Several cucurbitacins have been reported [2, 3] but there are no records of the flavonoid constituents of *C. colocynthis*. However, flavone *C*-glycosides have been isolated from other members of the Cucurbitaceae [4–6].

The present paper describes the isolation, purification and structure eludication of the glycoflavones of the fruits and aerial parts of *C. colocynthis*.

RESULTS AND DISCUSSION

The methanolic extract of powdered *C. colocynthis* fruits was purified by column chromatography (CC) on a Sephadex LH20 to give two fractions rich in flavonoids. Further purification afforded isoorientin 3′-methyl ether (**1**), isoorientin (**2**), and isovitexin (**3**) [7]. The aerial parts were treated similarly on silica gel CC and further purification on preparative TLC resulted in three new flavone C-glycosides, viz. 8-*C-p*-hydroxybenzoylisovitexin (**4**), 6-*C-p*-hydroxybenzoylvitexin (**5**), and 8-*C-p*-hydroxybenzoylisovitexin 4′*O*-*β*-D-*glucoside* (**6**). The identity of the isolated flavonoids was achieved using extensive UV, MS and NMR spectroscopic analyses. The complete ^{13}C NMR data are given in Table 1, and additionally confirmed by 2D-COSY, HETCOR, Selective INEPT experiments for the first time.

Isoorientin 3′-methyl ether (**1**) from the fruits showed similar spectral data to isoorientin (**2**), [8] and its $[M]^+$ showed *m/z* 462 indicating the addition of a methyl group to **2**. Addition of $AlCl_3$ and $AlCl_3$/HCl reagents to **1** gave bathochromic shifts of 53 nm (from 337 to 390 nm) [9]. The absence of the catechol at ring B was confirmed by the NaOAc/H_3BO_3 spectrum which gave no bathochromic shift at 337 nm band while a 55 nm bathochromic shift in band-I of NaOAc spectrum showed the absence of a free 4′-hydroxyl and the 5.5 nm bathochromic shift of band-II indicated the presence of a free 7-hydroxyl. This was supported by the presence of 336 nm shoulder in the NaOAc spectrum [10, 11]. The ^1H NMR of **1** showed a doublet at δ_H 4.62 (1H, $J = 9.8$ Hz, $H_{1''}$) suggesting an anomeric proton of a *β*-linked sugar [4] and the presence of the methyl group was supported by δ_H 3.9 singlet and δ_C 55.84 signals. The signal at δ_C 108.72 ppm was assigned to a C_6-linked to the sugar (normally between 96–98 ppm) and its connectivity was confirmed by a selective INEPT experiment where irradiation of the anomeric proton at δ_H 4.62 enhanced the carbon signals at δ_C 108.72 (C_6) and δ_C (163.24 (C_7).

Compounds **2** and **3** were identified by comparison of their physical properties and spectral analyses with reported data [7, 8].

The EI-MS of **4**, from the aerial parts, gave a molecular ion at *m/z* 538 for $C_{28}H_{26}O_{11}$. The ^1H NMR spectral data (Table 1) showed four doublets (each of 2 protons) in two aromatic AB systems, viz. δ_H 7.8 and 6.92 ($J = 7.5$ Hz) corresponding to $H_{2',6'}$ and $H_{3',5'}$ of ring B, and δ_H 7.07 and 6.63 ($J = 7.8$ Hz) corresponding to $H_{2'',6''}$ and $H_{3'',5''}$ of ring D. The ^{13}C NMR data (Table 1) showed δ_C 127.99, 128.70, 115.81, and 114.77 shifts assigned to $C_{2',6'}$ and $C_{2'',6''}$ and $C_{3',5'}$,

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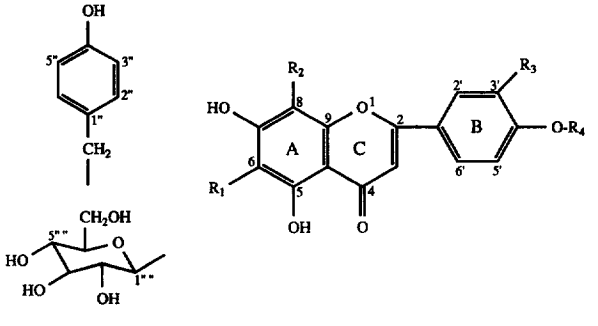
Table 1. ^{13}C NMR data of isolated flavonoids (90.56 MHz, DMSO-d_6, TMS as int. standard)*

C	1	2	3	4	5	6
2	163.32 *s*	163.48 *s*	163.71 *s*	162.91 *s*	163.00 *s*	162.80 *s*
3	103.03 *d*	102.88 *d*	102.85 *d*	103.20 *d*	102.13 *d*	103.45 *d*
4	181.85 *s*	181.85 *s*	182.07 *s*	181.46 *s*	181.72 *s*	182.12 *s*
5	160.48 *s*	160.61 *s*	160.82 *s*	157.25 *s*	157.13 *s*	157.25 *s*
6	108.72 *s*	108.87 *s*	108.87 *s*	107.90 *s*	106.95 *s*	107.73 *s*
7	163.24 *s*	163.03 *s*	163.97 *s*	160.97 *s*	160.80 *s*	160.76 *s*
8	93.64 *d*	93.45 *d*	94.00 *d*	106.87 *s*	108.10 *s*	106.76 *s*
9	156.21 *s*	156.06 *s*	156.46 *s*	153.86 *s*	152.07 *s*	153.69 *s*
10	103.29 *s*	103.36 *s*	103.43 *s*	103.20 *s*	107.10 *s*	103.56 *s*
1′	121.32 *s*	121.40 *s*	121.15 *s*	121.19 *s*	121.39 *s*	123.86 *s*
2′	109.99 *d*	113.23 *d*	128.54 *d*	127.99 *d*	127.87 *d*	127.90 *d*
3′	147.90 *s*	145.53 *s*	116.27 *s*	115.81 *d*	115.80 *d*	116.47 *d*
4′	150.59 *s*	149.54 *s*	161.52 *s*	160.94 *s*	160.80 *s*	160.08 *s*
5′	115.68 *d*	115.96 *d*	116.27 *d*	115.81 *d*	115.80 *d*	116.47 *d*
6′	120.23 *d*	18.93 *d*	128.54 *d*	127.99 *d*	127.87 *d*	127.90 *d*
1″	—	—	—	130.83 *s*	130.92 *s*	130.21 *s*
2″,6″	—	—	—	128.70 *d*	128.77 *d*	128.87 *d*
3″,5″	—	—	—	114.77 *d*	114.66 *d*	114.87 *d*
4″	—	—	—	155.07 *s*	152.07 *s*	155.16 *s*
CH_2-bridge	—	—	—	26.90 *t*	27.02 *t*	26.77 t
1‴	72.98 *d*	72.96 *d*	73.33 *d*	74.26 *d*	74.38 *d*	74.03 *d*
2‴	70.48 *d*	70.52 *d*	70.76 *d*	71.42 *d*	71.43 *d*	71.74 *d*
3‴	78.83 *d*	78.88 *d*	79.17 *d*	77.98 *d*	78.00 *d*	77.61 *d*
4‴	70.16 *d*	70.14 *d*	70.44 *d*	68.93 *d*	68.94 *d*	68.96 *d*
5‴	81.43 *d*	81.47 *d*	81.86 *d*	80.78 *d*	80.69 *d*	80.92 *d*
6‴	61.36 *t*	61.32 *t*	61.60 *t*	59.65 *t*	59.66 *t*	59.72 *t*
1⁗	—	—	—	—	—	99.64 *d*
2⁗	—	—	—	—	—	72.86 *d*
3⁗	—	—	—	—	—	76.88 *d*
4⁗	—	—	—	—	—	69.41 *d*
5⁗	—	—	—	—	—	76.29 *d*
6⁗	—	—	—	—	—	60.42 *t*
O-CH_3	55.84 *q*	—	—	—	—	—

*Multiplicities were determined by DEPT pulse sequence.



Compound	R_1	R_2	R_3	R_4
1	glucose	H	OMe	H
2	glucose	H	OH	H
3	glucose	H	H	H
4	glucose	*p*-hydroxybenzyl	H	H
5	*p*-hydroxybenzyl	glucose	H	H
6	glucose	*p*-hydroxybenzyl	H	glucose

$C_{3'',5''}$, respectively. The two singlets at δ_H 6.71 and 4.02 were assigned to H_3 and CH_2-bridge protons and the δ_H 4.79 ($J = 7.5$ Hz) doublet was assigned to the anomeric proton of the β-linked sugar [4]. DEPT experiment confirmed the resonance at δ_C 26.90 and 59.65 for the CH_2-benzyl and C_6-sugar, respectively. The COSY-45° spectrum showed that the doublet at δ_H 7.80 ($H_{2',6'}$) was coupled to that at δ_H 6.92 ($H_{3',5'}$) forming an AB system in ring-B. The doublet at δ_H 7.07 ($H_{2'',6''}$) was also coupled to δ_H 6.63 ($H_{3'',5''}$) forming the second AB-system in the *p*-hydroxybenzyl moiety. The CH_2-bridge protons at δ_H 4.02 was weakly coupled to the doublet at δ_H 7.07 ($H_{2'',6''}$) which confirmed the presence of a *p*-hydroxybenzyl moiety. The selective INEPT experiment showed that, irradiation (7 Hz) of the CH_2-bridge proton signal at δ_H 4.02 enhanced δ_C 160.98 (C_7), 153.90 (C_9), 106.87 (C_9) and 103.20 (C_{10}) indicating the flavonoid-C_8 connection to the *p*-hydroxybenzyl moiety.

From the previous discussion, it can be concluded that the β-D-glucose is *C*-linked to the flavonoid skeleton at C_6 and **4** is 8-*C*-*p*-hydroxybenzoylisovitexin, which is isolated from this plant for the first time.

The ^1H, ^{13}C NMR (Table 1), DEPT, HETCOR and COSY-45°, spectra of **5** are similar to those of **4**. Compound **5** was identified as 6-*C*-*p*-hydroxybenzoylvitexin.

The mass spectrum of **6** showed a $[M]^+$ of *M*/*Z* 700 for $C_{34}H_{36}O_{16}$. Both ^1H and ^{13}C NMR data (Table 1) displayed similar spectral patterns to **4**; an extra doublet at δ_H 5.02 ($J = 7.2$ Hz) was assigned to the anomeric proton of a β-linked sugar ($H_{1'''}$) [10]. The carbon spectrum showed 11 aliphatic hydroxy carbons between 59 and 81 ppm, six for the *C*-linked glucose (as in **4**) and the remaining five signals coincided with chemical shifts for *O*-β-D-glucose [10] and the sixth signal resonated at δ_C 99.64 ($C_{1'''}$). The COSY-45° experiment indicated that the doublets at δ_H 7.95 ($H_{2',6'}$) and δ_H 7.07 ($H_{2'',6''}$) were coupled to δ_H 7.19 ($H_{3',5'}$), and δ_H 6.64 ($H_{3'',5''}$) doublets, respectively. The singlet at δ_H 4.06 (CH_2 bridge) was weakly coupled to the δ_H 7.07 doublet. A selective INEPT (9 Hz) irradiation of the δ_H 4.06 singlet enhanced δ_C 106.76 (C_8), 128.27 ($C_{2'',6''}$), 130.21 ($C_{1''}$) and 153.69 (C_9) indicating that the CH_2-bridge of the *p*-hydroxybenzyl moiety is linked to the flavonoid skeleton at C_8. Irradiation (9 Hz) of δ_H 4.81 ($H_{1'''}$) of the anomeric doublet of the *C*-linked sugar enhanced δ_C 107.82 (C_6). Attempts to irradiate δ_H 5.03 ($H_{1'''}$) of the *O*-linked sugar was unsuccessful; however, a ROESY experiment demonstrated that this anomeric signal was coupled to a δ_H 7.19 ($H_{3',5'}$) doublet and suggested its location in close vicinity to $H_{3',5'}$. Thus, the *O*-linkage must be at C_4 and the singlet at δ_H 6.92 (H_3) was coupled to the doublets at δ_H 7.95 ($H_{2',6'}$), and δ_H 7.19 ($H_{3',5'}$). Acid and enzymic hydrolysis of **6** gave D-glucose (comparative TLC) and **4** (co-TLC, UV, MS). These results confirm the structure of **6** as 8-*C*-*p*-hydroxybenzoylisovitexin 4′-*O*-β-D-glucoside.

The new compounds **4**–**6** represent a new class of flavone *C*-glycosides, in which the *p*-hydroxybenzoic acid moeity is attached through a carbon atom to the flavonoid skeleton. Glycoflavones acylated with *p*-hydroxybenzoic acid through an oxygen atom to the *C*-sugar are already known from families other than Cucurbitaceae, e.g. 2″-*O*-*p*-hydroxybenzoylvitexin from *Vitex lucens* (Verbenaceae) [12] and 2″-*O*-*p*-hydroxybenzoyliso-orientin and its 4′-*O*-glucoside from *Gentiana asclepiadea* (Gentianaceae) [13].

EXPERIMENTAL

Plant material. Aerial parts and fruits of *C. colocynthis* Schrad were collected early October, 1989 during the flowering and fruiting stage from Gabel EI-Magharba, Sinai, Egypt. The plants were identified and authenticated by Professor I. Mashaly, of the Botany Department, University of Mansoura, Egypt, and a voucher specimen has been deposited at the Department of Pharmacognosy (# 12.10.89), University of Mansoura Herbarium.

Extraction and isolation. Powdered fruits (300 g) and aerial parts (600 g), of *C. colocynthis* were extracted with 70% MeOH (5 l). The concd extract was purified on an Amberlite Column (500 g), using H_2O and MeOH as eluants. The concd MeOH eluants gave 4.35 g and 10.1 g, respectively. The MeOH fruit extract was sepd by CC Sephadex LH 20 (Pharmacia) with 50% MeOH. Similar frs were pooled to afford 38 mg of **1**, and 232 mg of a mixt. of two compounds which upon further purification on Rp-C18 CC and eluting with (MeOH–H_2O–HCOOH, 20:80:1) gave 52 mg of **2**, and 78 mg of **3**. The aerial parts extract was purified on silica gel CC (MeOH–CH_2Cl_2, 1:4). Collected frs were concd and further purified on prep. TLC on silica gel containing 10% $ZnSO_4$ using MeOH–EtOAc (9:1) to afford 25 mg of **4**, 12 mg of **5**, and 15 mg of **6**, in addition to isovitexin.

Mps are uncorr. MS: Kratos MS-50 triple analyser using xenon as a carrier gas and 3-nitrobenzyl alcohol (3-NBA). HR-EI-MS: VG ZAB HF (70 eV). ^1H NMR 360 and 600 MHz, ^{13}C NMR 90.56 and 150 MHz, DMSO-d_6. TMS as int. standard, chemical shifts (ppm) and *J* (Hz).

Compound **1**. Isoorientin 3′-methyl ether. Yellow amorphous powder, mp 212–213°, UV λ_{max}^{MeOH} nm: 273 and 337; +MeONa: 260 sh, 278, 336 sh, 408; +$AlCl_3$: 262 sh, 280, 363, 390; +$AlCl_3$–HCl: 276, 352, 389; +NaOAc: 278, 319, 392; and +NaOAc–H_3BO_3: 274, 333. MS: *m*/*z* (rel. int.) 491 $[M + 29]^+$ (3), 463 $[M + 1]^+$ (36), 445 $[M - H_2O]^+$ (26), 427 $[M - 2H_2O]^+$ (1.7), 314 $[M - 148]^+$ (3), and 313 $[M - 149]^+$ (5), [165] (12), [151] (11), and [147] (31). ^1H NMR: δ_H 7.57 (*d*, 1H, $J = 8.6$ Hz, $H_{6'}$) 7.56 (*s*, 1H, $H_{2'}$), 6.96 (*d*, 1H, $J = 8.6$ Hz, H_5), 6.55 (*s*, 1H, H_3), 6.91 (*s*, 1H, H_8), 4.62 (*d*, 1H, $J = 9.7$ Hz, $H_{1'''}$), and 3.90 (*s*, 3H, OCH_3). ^{13}C NMR (Table 1).

Compound **4**. 8-*C*-*p*-Hydroxybenzoylisovitexin. Yellow needles, mp 232–235°. UV λ_{max}^{MeOH} nm: 277, 327;

+MeONa: 286, 335, 404; +$AlCl_3$: 283, 310, 354, 399; +$AlCl_3$/HCl: 284, 309, 350, 388 sh; +NaOAc: 285, 305 sh, 394; and +NaOAc–H_3BO_3: 278, 323 sh, 360 sh. MS: *m/z* (rel. int.) 512 $[M + 1\text{-}H_2O]^+$ (1.5), and 121 $[C_7H_5O_2]$ (100); FAB-MS (3-NBA) *m/z* 561 [M + Na], and 539 $[M + 1]^+$ HR-FAB-MS: *m/z* 539. 1544 $[M + H]^+$, calculated for $C_{28}H_{27}C_{11}$, 539.1545. ^1H NMR: δ_H 7.80 (*d*, 2H, $J = 7.50$ Hz, $H_{2',6'}$), 7.07 (*d*, 2H, $J = 7.8$ Hz, $H_{2'',6''}$), 6.92 (*d*, 2H, $J = 7.50$ Hz, $H_{3',5'}$), 6.63 (*d*, 1H, $J = 7.70$ Hz, $H_{3'',5''}$), 6.71 (*s*, 1H, H_3), 4.79 (*d*, 1H, $J = 7.50$ Hz, $H_{1'''}$), and 4.02 (*s*, 2H, CH_2-bridge), HETCOR demonstrated that δ_H 4.02 (CH_2) is correlated to δ_C 26.90, δ_H 3.60 ($H_{6'''}$) to δ_C 59.65 ($C_{6'''}$), δ_H 3.45 ($H_{2'''}$) to δ_C 71.42 ($C_{2'''}$), δ_H 4.79 ($H_{1'''}$) to δ_C 74.26 ($C_{1'''}$), δ_H 3.30 ($H_{3'''}$) to δ_C 77.90 ($C_{3'''}$), δ_H 3.20 ($H_{5'''}$) to δ_C 80.78 ($C_{5'''}$), δ_H 6.71 (H_3) to δ_C 103.20 (C_3), δ_H 6.63 ($H_{3'',5''}$) to δ_C 114.77 ($C_{3'',5''}$), δ_H 6.92 ($H_{3',5'}$) to δ_C 115.81 ($C_{3',5'}$), δ_H 7.80 ($H_{2',6'}$) to δ_C 127.99 ($C_{2',6'}$), and δ_H 7.07 ($H_{2'',6''}$) to δ_C 128.70 ($C_{2'',6''}$). ^{13}C NMR (Table 1).

Compound **5**. 6-*C*-*p*-Hydroxybenzoylvitexin. Amorphous powder, mp 242–243°, UV spectral data were identical to **4**. FAB-MS. *m/z* 539 $[M + 1]^+$ and 561 $[M + Na]^+$, and HR-FAB-MS: *m/z* $[M]^+$ 538.1544 (calc. $C_{28}H_{26}O_{11}$, 538.1545). ^1H NMR: δ_H 7.77 (*d*, 2H, $J = 7.30$ Hz, $H_{2'',6''}$), 7.07 (*d*, 2H, $J = 7.0$ Hz, $H_{2'',6''}$), 6.90 (*d*, 2H, $J = 7.30$ Hz, $H_{3',5'}$), 6.61 (*d*, 1H, $J = 7.70$ Hz, $H_{3'',5''}$), 6.62 (*s*, 1H, H_3), 4.75 (*d*, 1H, $J = 9.20$ Hz, $H_{1'''}$), and. 3.98 (*s*, 2H, CH_2-bridge), DEPT, HETCOR and COSY-45° spectra are similar to those of **4**. ^{13}C NMR (Table 1).

Compound **6**. 8-*C*-*p*-Hydroxybenzoylisovitexin 4′-*O*-β-D-glucoside. Yellow needles, mp 224–224°. UV λ_{max}^{MeOH} nm: 279, 322; +MeONa: 289, 333, 393; +$AlCl_3$: 267, 288, 344, 378 sh; +AlCl3–HCl: 265, 287, 343, 372, +NaOAc: 284, 301 sh, 280 sh; +NaOAc–H_3BO_3: 278, 326 sh. MS (rel. int.) *m/z* 683 $[M - H_2O + 1]^+$ (0.3), and 520 $[M - O - glc - H_2O]^+$ (0.6) indicating $[M]^+$ of 700. HR-FAB-MS, *m/z* 701.2082, 100% (calcd 701.2070 for $C_{34}H_{36}O_{16}$ + 1). ^1H NMR: δ_H 7.95 (*d*, 2H, $J + 6.80$ Hz, $H_{2',6'}$), 7.19 (*d*, 2H, $J = 6.80$, $H_{3',5'}$), 7.07 (*d*, 2H, $J = 7.70$ Hz, $H_{2'',6''}$), 6.92 (*s*, 1H, H_3), 6.64 (*d*, 1H, $J = 7.90$ Hz, $H_{3'',5''}$), 5.03 (*d*, 1H, $J = 7.20$ Hz, $H_{1'''}$) 4.81 (*d*, 1H, $J = 9.70$ Hz, $H_{1'''}$), and 4.06 (*s*, 2H, CH_2-bridge), DEPT, HETCOR and COSY-45° spectra are identical to those of **4**. ^{13}C NMR (Table 1).

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