Bioactive Saponins and Glycosides. XXVII.[1] Structures of New Cucurbitane-Type Triterpene Glycosides and Antiallergic Constituents from *Citrullus colocynthis*

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The methanolic extract from the fruit of *Citrullus colocynthis* showed an inhibitory effect on ear passive cutaneous anaphylaxis reactions as a type I allergic model in mice. From the methanolic extract, two new cucurbitane-type triterpene glycosides, colocynthosides A and B, were isolated together with 17 known constituents. The structures of colocynthosides A and B were elucidated on the basis of chemical and physicochemical evidence. In addition, the principal cucurbitane-type triterpene glycoside, cucurbitacin E 2-*O*-β-D-glucopyranoside, and its aglycon, cucurbitacin E, exhibited the antiallergic activity at a dose of 100 and 1.25 mg/kg, *p.o.*, respectively.

Key words *Citrullus colocynthis*; colocynthoside; colocynthol; cucurbitacin E 2-*O*-β-D-glucopyranoside; cucurbitane-type triterpene; antiallergic activity

The Cucurbitaceae plant *Citrullus colocynthis* (L.) SCHRAD., a climbing annual medicinal herb in the desert area, is distributed in African and Arabian countries and India. The fruit of *C. colocynthis* have been commonly used as an catharsis and antidiabetic agents in traditional Egyptian and Indian Ayurvedic medicines.[2—9] Previously, several triterpene,[3—5] flavonoid[7] and aliphatic compounds[6] have been isolated from the fruit and roots of this medicinal plant and the pharmacological activities such as anti-cancer[1] and insulinotropic effects[9] were reported. In the course of our characterization studies on Egyptian herbal medicines such as *Cyperus longus*,[10,11] *Anastatica hierochuntica*,[12,13] *Nigella sativa*,[14,15] *Crinum yemense*,[16] and *Dichrocephala integrifolia*,[17] the methanolic extract from the fruit of *C. colocynthis* was found to show an antiallergic effect on ear passive cutaneous anaphylaxis (PCA) reaction as a type I allergic model in mice. From the methanolic extract, two new cucurbitane-type triterpene glycosides, colocynthosides A (**1**) and B (**2**), were isolated from this herbal medicine together with 17 known compounds (**3**—**19**). This paper deals with the structure elucidation of new compounds (**1**, **2**) from the fruit of *C. colocynthis* as well as the antiallergic activities of the methanolic extract and the principal constituent, cucurbitacin E 2-*O*-β-D-glucopyranoside (**3**), and the aglycon, cucurbitacin E (**3a**), on ear PCA reaction in mice as type I allergic model.

The methanolic extract (13.9% from the dried fruit of *C. colocynthis* cultivated in Egypt) was found to show an inhibitory effect on ear PCA reaction in mice as shown in Table 1. The methanolic extract was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction and aqueous layer. The aqueous layer was extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH and H_2O-soluble fractions. The EtOAc-soluble fraction showed potent antiallergic activity, but the *n*-BuOH and H_2O-soluble fractions showed no activity (Table 1). The antiallergic activity of the methanolic extract was found to be concentrated upon the EtOAc-soluble fraction. From the EtOAc-soluble fraction, cucurbitacin E 2-*O*-β-D-glucopyranoside[5] (**3**, 3.08%) was isolated as a principal component together with 3,4′-dihydroxypropiophenone[18,19] (**18**, 0.0010%) and 3,4′-dihydroxy-3′-methoxypropiophenone[20] (**19**, 0.0008%). From the *n*-BuOH-soluble fraction, colocynthosides A (**1**, 0.0036%) and B (**2**, 0.018%) were isolated together with **3** (0.0077%), cucurbitacin I 2-*O*-β-D-glucopyranoside[5] (**4**, 0.15%), **5**[21] (0.0015%), cucurbitacin J 2-*O*-β-D-glucopyranoside[22] (**6**, 0.0015%), cucurbitacin K 2-*O*-β-D-glucopyranoside[22] (**7**, 0.0007%), cucurbitacin L 2-*O*-β-D-glucopyranoside[5] (**8**, 0.032%), khekadaengoside E[22] (**9**, 0.0014%), (22—27)-hexanocucurbitacin I 2-*O*-β-D-glucopyranoside[5] (**10**, 0.0020%), isovitexin[7,23] (**11**, 0.0039%), isoorientin 3′-methyl ether[7] (**12**, 0.0032%), isosaponarin[23] (**13**, 0.0005%), 4-(β-D-glucopyranosyloxy)benzaldehyde[24] (**14**=helicid, 0.0009%), 4-hydroxybenzyl β-D-glucopyranoside[25,26] (**15**, 0.0009%), benzyl β-D-glucopyranoside[27] (**16**, 0.0006%), and 4-(β-D-glucopyranosyloxy)benzyl alcohol[28] (**17**, 0.015%).

Structures of Colocynthosides A (1) and B (2) Colocynthoside A (**1**) was obtained as a white powder and exhibited a negative optical rotation ($[\alpha]_D^{27}$ −13.5° in MeOH). The IR spectrum of **1** showed absorption bands at 1718, 1686, 1680, and 1650 cm^{-1} ascribable to ketone, enone, and olefin functions, and broad bands at 3440 and 1078 cm^{-1}, suggestive of a glycoside structure. The UV spectrum of **1** indicated the presence of an enone moiety with absorption maxmum at 236 ($\log \varepsilon$ 4.04) nm. In the positive- and negative-ion fast atom bombardment (FAB)-MS of **1**, quasimolecular ion peaks were observed at *m/z* 757 $(M+Na)^+$ and 733 $(M-H)^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **1** to be $C_{38}H_{54}O_{14}$. The acid hydrolysis of **1** with 1.0 M hydrochloric acid (HCl) liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[29] Enzymatic hydrolysis of **1** with hesperidinase gave an aglycon named colocynthol A (**1a**) (Chart 1). The proton and carbon signals in the ^1H- and ^{13}C-NMR spectra of **1** and **1a** were similar to those of **3** and cucurbitacin E (**3a**), respectively, except for the signals due to the 7-hydroxyl group. The ^1H- (Table 2, CD_3OD) and ^{13}C-NMR (Table 3) spectra of **1**, which were assigned by various NMR

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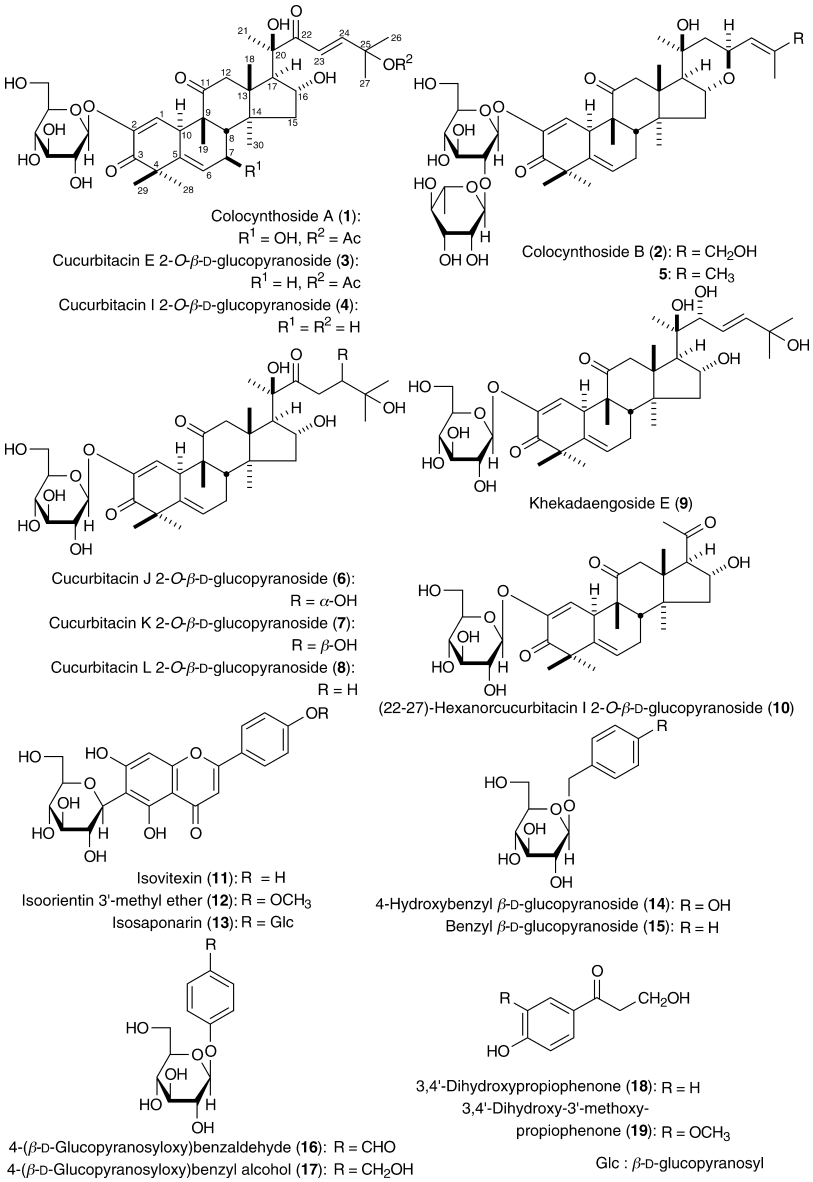

Table 1. Effects of MeOH Extract and EtOAc-, *n*-BuOH-, and H_2O-Soluble Fractions from *C. colocynthis* on Ear PCA Reaction in Mice

Treatment	Dose (mg/kg, *p.o.*)	*N*	Leakage of dye (O.D. at 620 nm)	Inhibition (%)
Normal (PBS)	—	5	0.034±0.002**	—
Control	—	9	0.334±0.036	—
MeOH ext.	25	8	0.279±0.034	18.5
	50	9	0.226±0.021*	35.9
	100	7	0.157±0.019**	59.0
	200	7	0.116±0.014**	72.5
Normal (PBS)	—	4	0.024±0.002**	—
Control	—	8	0.284±0.048	—
Etoac-soluble farction	100	4	0.109±0.030**	67.2
n-BuOH-soluble fraction	100	7	0.311±0.033	
H_2O-soluble fraction	100	8	0.354±0.055	

Values represent the means±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

experiments,[30] showed signals assignable to eight methyls [δ 0.93, 1.11, 1.29, 1.32, 1.36, 1.41, 1.54, 1.57 (all s, 18, 19, 29, 30, 28, 21, 27, 26-H_3)], two methines bearing an oxygen function [δ 4.12 (br d, J=*ca.* 3 Hz, 7α-H), 4.61 (br dd, J=*ca.* 7, 8 Hz, 16β-H)], and two trisubstituted olefins [δ 5.96 (br s, H-6), 6.11 (d, J=2.7 Hz, 1-H)], an *trans*-olefin pair [δ 6.84, 6.98 (both d, J=15.9 Hz, 23, 24-H)], and a β-glucopyranosyl moiety [δ 4.66 (d, J=7.6 Hz, 1′-H)] together with an acetyl group [δ 2.00 (s)]. As shown in Fig. 1, the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) experiment on **1** indicated the

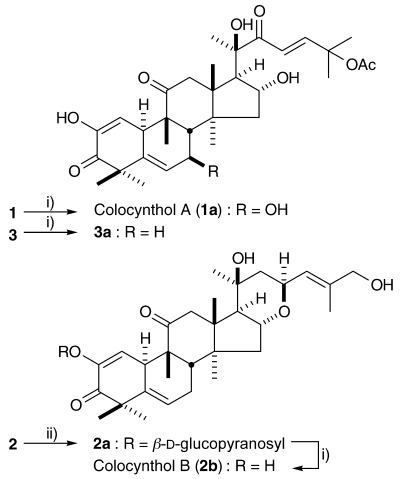

Chart 1

presence of partial structures written in bold lines, and the carbon skeleton and the positions of functional groups are characterized by the heteronuclear multiple-bond correlations (HMBC) experiment, which showed long-range correlations between the following protons and carbons (1-H and 2, 3-C; 6-H and 5-C; 7-H and 6, 8-C; 8-H and 9, 11, 14-C; 10-H and 5, 9-C; 12-H_2 and 11, 13-C; 15-H_2 and 14-C; 16-H and 17-C; 17-H and 13, 14, 20, 21-C; 18-H_3 and 12, 13, 14, 17-C; 19-H_3 and 9, 10, 11-C; 21-H_3 and 17, 20, 22-C; 23-H and 22, 25-C; 24-H and 22, 25-C; 26-H_3 and 24, 25, 27-C; 27-H_3 and 24, 25, 26-C; 28-H_3 and 3, 4, 5, 29-C; 29-H_3 and 3, 4, 5, 28-C; 30-H_3 and 8, 13, 14-C; acetyl proton and 25-C, acetyl carbon; 1′-H and 2-C). Next, the stereostructure of cucurbitane skeleton in **1** was characterized by nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed the NOE correlations between the following proton pairs (7-H and 10, 15α-H, 30-H_3; 8-H and 18-H_3; 10-H and 28-H_3, 30-H_3; 12α-H and 30-H_3; 15α-H and 17-H; 15β-H and 16-H; 16-H and 18-H_3; 17-H and 30-H_3). The stereostructure of the 20-position in **1** was deduced by comparison of the ^{13}C-NMR data of **1** and **1a** with those of **3**, **3a**, **4**, and **6**—**9** to be *R* orientation (Fig. 1). On the basis of the

Table 2. ^{1}H-NMR (500 MHz) Data of Colocynthosides A (**1**) and B (**2**) and Related Compounds (**1a**, **2a**, **2b**)

Proton	**1**[a] δ (*J* Hz)	**1a**[a] δ (*J* Hz)	**2**[b] δ (*J* Hz)	**2a**[b] δ (*J* Hz)	**2b**[a] δ (*J* Hz)
1	6.11 (d, 2.7)	5.93 (br d, *ca.* 2)	6.41 (d, 2.4)	6.41 (d, 2.5)	5.78 (d, 2.4)
6	5.96 (br s)	5.74 (br s)	5.64 (dd, 2.4, 2.4)	5.66 (dd, 2.5, 2.5)	5.80 (br dd, *ca.* 2, 2)
7α	4.12 (br d, *ca.* 3)	4.10 (br d, *ca.* 3)	1.91 (m)	1.91 (m)	2.08 (m)
7β			2.07 (m)	2.13 (m)	2.40 (m)
8	2.14 (br s)	2.11 (br s)	1.97 (br d, *ca.* 8)	1.97 (br d, *ca.* 8)	2.12 (br d, *ca.* 7)
10	3.68 (br s)	3.63 (br s)	3.65 (br s)	3.67 (br s)	3.59 (br s)
12	2.64 (d, 15.3)	2.60 (d, 15.3)	2.82 (d, 15.0)	2.78 (d, 15.0)	2.47 (d, 14.6)
	3.31 (d, 15.3)	3.34 (d, 15.3)	3.35 (d, 15.0)	3.27 (d, 15.0)	3.26 (d, 14.6)
15α	2.10 (m)	2.08 (m)	1.97 (m)	1.97 (m)	1.88 (m)
15β	1.56 (d, 11.0)	1.55 (d, 12.8)	1.65 (dd. 3.1,13.2)	1.66 (m)	1.40 (dd. 3.4, 13.5)
16	4.61 (br dd, *ca.* 7, 8)	4.61 (br dd, *ca.* 8, 9)	5.10 (ddd, 3.1, 9.5, 10.1)	5.10 (ddd, 3.1, 9.5, 10.1)	4.65 (ddd, 3.4, 9.8, 10.4)
17	2.53 (d, 7.3)	2.54 (d, 8.2)	2.19 (d, 9.5)	2.20 (d, 9.5)	2.04 (d, 9.8)
18	0.93 (s)	0.91 (s)	1.25 (s)	1.25 (s)	0.98 (s)
19	1.11 (s)	1.07 (s)	0.98 (s)	1.03 (s)	0.99 (s)
21	1.41 (s)	1.41 (s)	1.51 (s)	1.51 (s)	1.30 (s)
22			1.80 (m)	1.80 (m)	1.57 (br d, *ca.* 14)
			2.02 (m)	2.03 (dd, 6.8, 13.8)	1.88 (m)
23	6.84 (d, 15.9)	6.84 (d, 15.8)	5.02 (br dd, *ca.* 7, 8)	5.03 (br dd, *ca.* 7, 8)	4.72 (br dd, *ca.* 7, 8)
24	6.98 (d, 15.9)	6.98 (d, 15.8)	6.97 (dd, 1.2, 8.2)	6.98 (dd, 1.2, 8.2)	6.21 (dd, 1.5, 8.2)
26	1.57 (s)	1.57 (s)	4.29 (2H, br s)	4.30 (2H, br s)	3.94 (2H, br s)
27	1.54 (s)	1.54 (s)	1.86 (s)	1.86 (s)	1.68 (d, 1.5)
28	1.36 (s)	1.36 (s)	1.40 (s)	1.42 (s)	1.29 (s)
29	1.29 (s)	1.27 (s)	1.36 (s)	1.27 (s)	1.24 (s)
30	1.32 (s)	1.32 (s)	1.43 (s)	1.44 (s)	1.35 (s)
25-Ac	2.00 (s)	2.00 (s)			
2-*O*-Glc-1′	4.66 (d, 7.6)		5.52 (d, 7.9)	5.47 (d, 7.6)	
2′	3.39 (m)		4.26 (dd, 7.9, 8.9)	4.26 (dd, 7.6, 9.2)	
3′	3.42 (m)		4.44 (m)	4.20 (dd, 9.2, 9.5)	
4′	3.53 (m)		4.33 (dd, 9.2, 9.5)	4.44 (dd, 9.2, 9.2)	
5′	3.35 (m)		3.94 (m)	4.05 (m)	
6′	3.86 (dd, 3.7, 12.2)		4.42 (dd, 4.6, 11.9)	4.55 (dd, 3.7, 11.9)	
	4.05 (dd, 2.4, 12.2)		4.56 (dd, 2.5, 11.9)	4.67 (dd, 2.5, 11.9)	
2′-*O*-Rha-1″			6.29 (d, 1.5)		
2″			4.74 (dd, 1.5, 3.4)		
3″			4.47 (dd, 3.4, 9.2)		
4″			4.28 (m)		
5″			4.70 (m)		
6″			1.80 (d, 6.1)		

Measured in *a*) CD_3OD or *b*) pyridine-d_5.

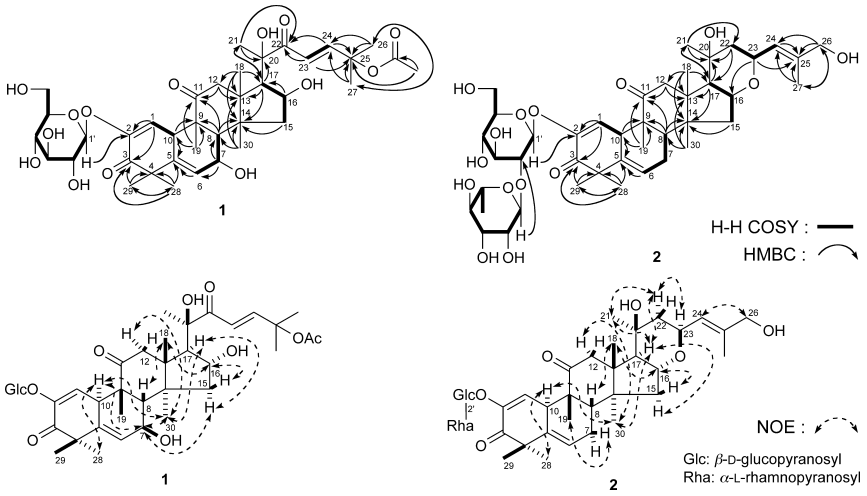

Fig. 1

Table 3. ^{13}C-NMR (125 MHz) Data of Colocynthosides A (**1**) and B (**2**) and Related Compounds (**1a**, **2a**, **2b**)

Carbon	**1**[a] δ_C (mult.)	**1a**[a] δ_C (mult.)	**2**[b] δ_C (mult.)	**2a**[b] δ_C (mult.)	**2b**[a] δ_C (mult.)
1	123.2 (d)	116.7 (d)	120.9 (d)	121.0 (d)	116.9 (d)
2	147.1 (s)	147.0 (s)	147.1 (s)	146.9 (s)	147.0 (s)
3	199.2 (s)	199.5 (s)	196.5 (s)	197.0 (s)	200.0 (s)
4	48.1 (s)	48.2 (s)	49.6 (s)	49.5 (s)	50.0 (s)
5	140.8 (s)	142.1 (s)	137.2 (s)	137.2 (s)	138.5 (s)
6	124.3 (d)	123.7 (d)	119.9 (d)	120.9 (d)	121.8 (d)
7	65.8 (d)	65.9 (d)	24.0 (t)	24.0 (t)	24.8 (t)
8	53.3 (d)	53.5 (d)	41.7 (d)	41.7 (d)	43.0 (d)
9	50.1 (s)	50.0 (s)	49.7 (s)	49.8 (s)	50.6 (s)
10	36.9 (d)	36.6 (d)	35.4 (d)	35.5 (d)	35.8 (d)
11	216.4 (s)	215.8 (s)	213.8 (s)	214.4 (s)	216.0 (s)
12	50.2 (t)	50.9 (t)	49.4 (t)	49.3 (t)	49.7 (t)
13	49.5 (s)	49.5 (s)	48.8 (s)	48.8 (s)	49.4 (s)
14	50.8 (s)	51.0 (s)	48.7 (s)	48.7 (s)	49.2 (s)
15	46.4 (t)	46.6 (t)	42.1 (t)	42.1 (t)	42.3 (t)
16	71.7 (d)	71.9 (d)	71.0 (d)	70.9 (d)	71.8 (d)
17	60.2 (d)	60.1 (d)	56.5 (d)	56.4 (d)	56.7 (d)
18	20.8 (q)	20.9 (q)	20.1 (q)	20.1 (q)	20.1 (q)
19	21.7 (q)	21.5 (q)	20.3 (q)	20.4 (q)	20.9 (q)
20	80.2 (s)	80.3 (s)	72.4 (s)	72.4 (s)	73.5 (q)
21	25.5 (q)	25.5 (q)	30.2 (q)	30.2 (q)	29.8 (q)
22	205.3 (s)	205.2 (s)	46.5 (t)	46.5 (t)	46.7 (t)
23	122.6 (d)	122.8 (d)	71.5 (d)	71.5 (d)	72.6 (d)
24	151.6 (d)	151.8 (d)	126.3 (d)	126.3 (d)	127.2 (d)
25	81.0 (s)	81.2 (s)	138.2 (s)	138.2 (s)	138.7 (s)
26	26.4 (q)	26.4 (q)	67.7 (t)	67.7 (t)	68.7 (t)
27	26.8 (q)	26.9 (q)	14.0 (q)	14.0 (q)	13.8 (q)
28	20.7 (q)	20.8 (q)	20.8 (q)	20.8 (q)	20.8 (q)
29	28.7 (q)	28.8 (q)	27.3 (q)	27.4 (q)	28.4 (q)
30	19.3 (q)	19.5 (q)	20.5 (q)	20.5 (q)	20.5 (q)
25-*O*Ac	171.9 (s)	171.9 (s)			
	21.9 (q)	21.9 (q)			
2-*O*-Glc-1′	101.1 (d)		98.7 (d)	100.8 (d)	
2′	74.2 (d)		79.4 (d)	74.5 (d)	
3′	77.5 (d)		78.1 (d)	78.5 (d)	
4′	70.6 (d)		70.6 (d)	70.6 (d)	
5′	78.1 (d)		78.6 (d)	78.7 (d)	
6′	61.9 (t)		62.0 (t)	62.2 (t)	
2′-*O*-Rha-1″			102.3 (d)		
2″			72.5 (d)		
3″			72.9 (d)		
4″			74.4 (d)		
5″			69.8 (d)		
6″			18.8 (q)		

Measured in *a*) CD_3OD or *b*) pyridine-d_5.

above-mentioned evidence, the structure of colocynthoside A was determined to be the 7-hydroxyl analogue (**1**) of cucurbitacin E 2-*O*-β-D-glucopyranoside (**3**).

Colocynthoside B (**2**) was also obtained a white powder with negative optical rotation ($[\alpha]_D^{27}$ −26.7° in MeOH). The IR spectrum of **2** showed absorption bands at 3569, 1686, 1655, 1637, 1078, and 1037 cm^{-1}, ascribable to hydroxyl, ketone, enone, olefin, and ether functions. The molecular formula, $C_{42}H_{62}O_{15}$, of **2** was determined from the positive- and negative-ion FAB-MS [*m/z* 829 $(M+Na)^+$ and 805 $(M-H)^-$] and by high-resolution positive-ion FAB-MS measurement. The acid hydrolysis of **2** with 1.0 M HCl liberated L-rhamnose and D-glucose, which were identified by HPLC analysis using an optical rotation detector.[29] Treatment of **2** with cellulase liberated a prosapogenin (**2a**), which gave its aglycon, colocynthol B (**2b**), by treatment with hesperidinase (Chart 1). The proton and carbon signals in the ^{1}H- (Table 2, pyridine-d_5) and ^{13}C-NMR (Table 3) spectra[30] of **2** were superimposable on those of **5**, except for the signals due to the 26-hydroxyl group {seven methyls [δ 0.98, 1.25, 1.36, 1.40, 1.43, 1.51, 1.86 (all s, 19, 18, 29, 28, 30, 21, 27-H_3)], a methylene and two methines bearing an oxygen function [δ 4.29 (2H, br s, 26-H_2), 5.02 (br dd, *J*=*ca.* 7, 8, 23-H), 5.10 (ddd, *J*=3.1, 9.5, 10.1 Hz, 16-H)], and three trisubstituted olefins [δ 5.64 (dd, *J*=2.4, 2.4 Hz, 6-H), 6.41 (d, *J*=2.4 Hz, 1-H), 6.97 (dd, *J*=1.2, 8.2 Hz, 24-H)] together with a β-glucopyranosyl group [δ 5.52 (d, *J*=7.9 Hz, 1′-H)] and a α-rhamnopyranosyl group [δ 1.80 (d, *J*=6.1 Hz, 6″-H), 6.29 (d, *J*=1.5 Hz, 1″-H)]}. The planar structure of **2** was confirmed by ^{1}H–^{1}H COSY and HMBC experiments. As shown in Fig. 1, the ^{1}H–^{1}H COSY experiment on **2** indicated the presence of the partial structures written in bold lines. In the HMBC experiment of **2**, long-range correlations were observed between the following proton and carbon pairs (1-H and 2, 3-C; 6-H and 5-C; 8-H and 9, 11, 14-C; 10-H and 5, 9-C; 12-H_2 and 11, 13-C; 15-H_2 and 14-C; 17-H and 13, 14, 20-C; 18-H_3 and 12, 13, 14, 17-C; 19-H_3 and 9, 10, 11-C; 21-H_3 and 17, 20, 22-C; 22-H_2 and 17, 20-C; 23-H and 16, 25-C; 24-H and 25-C; 26-H_3 and 24, 25, 27-C; 27-H_3 and 24, 25, 26-C; 28-H_3 and 3, 4, 5, 29-C; 29-H_3 and 3, 4, 5, 28-C; 30-H_3 and 8, 13, 14-C; 1′-H and 2-C; 1″-H and 2′-C), so that the connectivities of oligoglycoside to the aglycon in **2** were characterized.

Table 4. Effects of Cucurbitacin E 2-*O*-*β*-D-glucopyranoside (**3**) from *C. colocynthis* and Cucurbitacin E (**3a**) on Ear PCA Reaction in Mice

Treatment	Dose (mg/kg, *p.o.*)	*N*	Leakage of dye (O.D. at 620 nm)	Inhibition (%)
Normal (PBS)	—	5	0.033±0.007**	—
Control	—	8	0.325±0.038	—
Cucurbitacin E 2-*O*-*β*-D-glucopyranoside (**3**)	50	6	0.271±0.067	18.3
	100	7	0.206±0.035*	40.8
	200	6	0.180±0.017*	49.7
Normal (PBS)	—	5	0.040±0.004**	—
Control	—	9	0.388±0.043	—
Cucurbitacin E (**3a**)	1.25	7	0.254±0.043*	38.4
	2.5	4	0.135±0.027**	72.6
Normal (PBS)	—	5	0.050±0.009**	—
Control	—	8	0.309±0.054	—
Tranilast	100	8	0.171±0.016**	53.0
	200	8	0.125±0.017**	71.2

Values represent the means±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

The stereostructure of **2** was characterized by NOESY experiment, which showed the NOE correlations between the following proton pairs (7*β*-H and 19-H_3; 8-H and 18-H_3; 10-H and 28, 30-H_3; 12*α*-H and 30-H_3; 15*α*-H and 17-H; 15*β*-H and 16-H; 16-H and 18-H_3; 17-H and 21, 30-H_3, 22*α*-H; 21-H_3 and 22*α*-H; 22*α*-H and 23-H; 24-H and 26-H_2) as shown in Fig. 1. Consequently, the structure of colocynthoside B (**2**) was characterized to be as shown.

Effects of Cucurbitacin E 2-*O*-*β*-D-glucopyranoside (3) and Cucurbitacin E (3a) on Ear Passive Cutaneous Anaphylaxis (PCA) Reactions in Mice Previously, we reported that several isocoumarin[31—35] and phanylpropanoids[36] constituents showed the antiallergic activity in PCA reactions. As a continuing study of the antiallergic constituents from the herbal medicines, effects of the principal constituent of *C. colocynthis*, cucurbitacin E 2-*O*-*β*-D-glucopyranoside (**3**) and its aglycon (**3a**) on PCA reactions in mice were examined. As shown in Table 4, compound **3** significantly inhibited this model at the dose of 100 and 200 mg/kg, *p.o.*, whose activity was equivalent for that of a reference compound, tranilast.[37,38] Furthermore, the aglycon (**3a**) dramatically enhanced the antiallergic effect at 1.25 mg/kg, *p.o.* [inhibition (%): 53.0]. Those findings suggested that cucurbitane-triterpenes such as **3a** were useful for the treatment of type I allergy.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, YMC-Pack ODS-A (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The fruit of *C. colocynthis* was purchased from Egypt in August 2001, and was identified by one of authors (M.Y.). A voucher specimen of this herbal medicine is on file in our laboratory (2001.08. Egypt-04).

Extraction and Isolation The dried fruit of *C. colocynthis* (2.5 kg) was powdered and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (347 g, 13.9% from the dried fruit), and an aliquot (289 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (131 g, 6.3%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (58 g, 2.8%) and an H_2O-soluble fraction (100 g, 4.8%). The EtOAc-soluble fraction (85.8 g) was subjected to normal-phase silica gel column chromatography [3.0 kg, *n*-hexane–EtOAc (20 : 1→15 : 1→10 : 1→5 : 1→2 : 1→1 : 1→1 : 2, v/v)→EtOAc→MeOH] to give eight fractions {Fr. 1 (18.3 g), Fr. 2 (1.9 g), Fr. 3 (2.0 g), Fr. 4 (10.5 g), Fr. 5 (2.3 g), Fr. 6 (2.4 g), Fr. 7 (2.3 g), and Fr. 8 [=cucurbitacin E 2-*O*-*β*-D-glucopyranoside (**3**, 41.9 g, 3.08%)]}. Fraction 7 (2.3 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH–H_2O (30 : 70→40 : 60→60 : 40, v/v)→MeOH] to afford eight fractions [Fr. 7-1 (108 mg), Fr. 7-2 (33 mg), Fr. 7-3 (67 mg), Fr. 7-4 (364 mg), Fr. 7-5 (375 mg), and Fr. 7-6 (454 mg), Fr. 7-7 (134 mg), and Fr. 7-8 (817 mg)]. Fraction 7-2 (33 mg) was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to furnish 3,4′-dihydroxypropiophenone (**18**, 15 mg, 0.0010%). Fraction 7-3 (67 mg) was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to give 3,4′-dihydroxy-3′-methoxypropiophenone (**19**, 14 mg, 0.0008%). The *n*-BuOH-soluble fraction (41.5 g) was subjected to normal-phase silica gel column chromatography {2.5 kg, $CHCl_3$–MeOH–H_2O [(20 : 3 : 1→15 : 3 : 1→10 : 3 : 1→7 : 3 : 1, lower layer, v/v/v)→6 : 4 : 1, v/v/v]→MeOH} to give nine fractions [Fr. 1 (0.6 g), Fr. 2 (2.2 g), Fr. 3 (12.1 g), Fr. 4 (2.6 g), Fr. 5 (4.1 g), Fr. 6 (6.7 g), Fr. 7 (10.3 g), Fr. 8 (2.7 g), and Fr. 9 (0.2 g)]. Fraction 2 (2.2 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to afford seven fractions {Fr. 2-1 (125 mg), Fr. 2-2 (71 mg), Fr. 2-3 [=cucurbitacin L 2-*O*-*β*-D-glucopyranoside (**8**, 364 mg, 0.032%)], Fr. 2-4 (620 mg), Fr. 2-5 (=**3**, 83 mg, 0.0077%), Fr. 2-6 (11 mg), and Fr. 2-7 (38 mg)}. Fraction 3 (12.1 g) was subjected to reversed-phase silica gel column chromatography [350 g, MeOH–H_2O (30 : 70→50 : 50, v/v)→MeOH] to afford four fractions [Fr. 3-1 (859 mg), Fr. 3-2 (366 mg), Fr. 3-3 (10.4 g), and Fr. 3-4 (468 mg)]. Fraction 3-1 (859 mg) was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to furnish benzyl *β*-D-glucopyranoside (**15**, 14 mg, 0.0006%). Fraction 3-2 (366 mg) was further separated by HPLC [MeOH–H_2O (50 : 50, v/v)] to afford (22—27)-hexanorcucurbitacin I 2-*O*-*β*-D-glucopyranoside (**10**, 57 mg, 0.0020%). Fraction 3-3 (950 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to furnish cucurbitacin I 2-*O*-*β*-D-glucopyranoside (**4**, 364 mg, 0.15%). Fraction 3-4 (463 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give **10** (11 mg, 0.0004%). Fraction 4 (2.6 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH–H_2O (15 : 85→30 : 70→50 : 50, v/v)→MeOH] to afford six fractions [Fr. 4-1 (176 mg), Fr. 4-2 (347 mg), Fr. 4-3 (149 mg), Fr. 4-4 (555 mg), Fr. 4-5 (1300 mg), and Fr. 4-6 (218 mg)]. Fraction 4-4 (555 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give seven fractions [Fr. 4-4-1 (34 mg), Fr. 4-4-2 [=colocynthoside A (**1**, 71 mg, 0.0036%)], Fr.

4-4-3 (33 mg), Fr. 4-4-4 (35 mg), Fr. 4-4-5 (26 mg), Fr. 4-4-6 (70 mg), and Fr. 4-4-7 (271 mg)]. Fraction 4-4-6 (70 mg) was further separated by HPLC [CH_3CN–H_2O (90 : 10, v/v)] to furnish cucurbitacin J 2-*O*-*β*-D-glucopyranoside (**6**, 30 mg, 0.0015%) and cucurbitacin K 2-*O*-*β*-D-glucopyranoside (**7**, 15 mg, 0.0007%). Fraction 5 (4.1 g) was subjected to reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (15 : 85→30 : 70→50 : 50, v/v)→MeOH] to afford five fractions {Fr. 5-1 (734 mg), Fr. 5-2 (765 mg), Fr. 5-3 (733 mg), Fr. 5-4 (150 mg), and Fr. 5-5 (942 mg). Fraction 5-3 (733 mg) was purified by HPLC [MeOH–H_2O (50 : 50, v/v)] to give isoorientin 3′-methyl ether (**12**, 69 mg, 0.0032%). Fraction 5-4 (485 mg) was separated by HPLC [CH_3CN–H_2O (35 : 65, v/v)] to give three fractions [Fr. 5-4-1 (144 mg), Fr. 5-4-2 [=**4** (25 mg, 0.0035%)], and Fr. 5-4-3 (317 mg)]. Fraction 5-4-1 (144 mg) was further separated by HPLC [MeOH–H_2O (55 : 45, v/v)] to furnish khekadaengoside E (**9**, 10 mg, 0.0014%). Fraction 5-5 (942 mg) was purified by HPLC [MeOH–H_2O (60 : 40, v/v)] to furnish **5** (33 mg, 0.0015%). Fraction 6 (4.0 g) was further separated by reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (30 : 70→40 : 60→60 : 40, v/v)→MeOH] to afford eight fractions [Fr. 6-1 (65 mg), Fr. 6-2 (256 mg), Fr. 6-3 (811 mg), Fr. 6-4 [=isovitetin (**11**, 735 mg, 0.039%), Fr. 6-5 (189 mg), Fr. 6-6 (1200 mg), Fr. 6-7 (416 mg), and Fr. 6-8 (328 mg)]. Fraction 6-2 (256 mg) was purified by HPLC [CH_3CN–H_2O (10 : 90, v/v)] to give colocynthoside B (**2**, 78 mg, 0.0095%). Fraction 7 (10.3 g) was subjected to reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (15 : 85→30 : 70→50 : 50, v/v)→MeOH] to afford seven fractions [Fr. 7-1 (159 mg), Fr. 7-2 (1.80 g), Fr. 7-3 (730 mg), Fr. 7-4 (4.30, 218 mg), Fr. 7-5 (4.30 g), Fr. 7-6 (2.10 g), and Fr. 7-7 (13 mg)]. Fraction 7-2 (510 mg) was purified by HPLC [MeOH–H_2O (15 : 85, v/v)] to give 4-(*β*-D-glucopyranosyloxy)benzyl alcohol (**17**, 70 mg, 0.0015%). Fraction 7-5 (870 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give **2** (29 mg, 0.0085%). Fraction 8 (2.7 g) was subjected to reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (15 : 85→30 : 70→50 : 50, v/v)→MeOH] to afford eight fractions [Fr. 8-1 (814 mg), Fr. 8-2 (453 mg), Fr. 8-3 (56 mg), Fr. 8-4 (200 mg), Fr. 8-5 (81 mg), Fr. 8-6 (604 mg), Fr. 8-7 (397 mg), and Fr. 8-8 (157 mg)]. Fraction 8-4 (200 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give isosaponarin (**13**, 9 mg, 0.0005%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values[7,18—28]

Colocynthoside A (**1**): A white powder, $[\alpha]_D^{27}$ −13.5° (c=0.58, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{38}H_{54}O_{14}Na$ $(M+Na)^+$ 757.3411; Found 757.3418. CD [MeOH, nm ($\Delta\varepsilon$)]: 229 (−1.12), 279 (+0.63), 330 (−0.64). UV [MeOH, nm (log ε)]: 236 (4.04). IR (KBr, cm^{-1}): 3440, 1718, 1686, 1650, 1637, 1078. ^{1}H-NMR δ: given in Table 2. ^{13}C-NMR δ_C: given in Table 3. Positive-ion FAB-MS m/z 757 $(M+Na)^+$. Negative-ion FAB-MS m/z 733 $(M-H)^-$.

Colocynthoside B (**2**): A white powder, $[\alpha]_D^{27}$ −26.7° (c=0.58, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{62}O_{15}Na$ $(M+Na)^+$ 829.3986; Found 829.3978. CD [MeOH, nm ($\Delta\varepsilon$)]: 236 (−0.34), 271 (+0.41), 338 (−0.36). UV [MeOH, nm (log ε)]: 255 (3.82). IR (KBr, cm^{-1}): 3569, 1686, 1655, 1637, 1078, 1037. ^{1}H-NMR δ: given in Table 2. ^{13}C-NMR δ_C: given in Table 3. Positive-ion FAB-MS m/z 829 $(M+Na)^+$. Negative-ion FAB-MS m/z 805 $(M-H)^-$.

Acid Hydrolysis of 1 and 2 A solution of colocynthosides (**1** and **2**, 2.0 mg each) in 1 M HCl (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH_3CN–H_2O (85 : 15, v/v); flow rate 0.8 ml/min; column temperature, room temperature. Identification of L-rhamnose (i) from **2** and D-glucose (ii) from **1** and **2** present in the aqueous layer were carried out by comparison of their retention time and optical rotation with those of an authentic samples. t_R: (i) 7.8 min (negative optical rotation); (ii) 13.9 min (positive optical rotation).

Enzymatic Hydrolysis of 1 with Hesperidinase A solution of **1** (6.5 mg) in 0.2 M acetate buffer (pH 3.8, 2.0 ml) was treated with hesperidinase (20 mg, from *Aspergillus niger*, Sigma) and the solution was stirred at 40 °C for 48 h. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by reversed-phase silica gel column chromatography [1.0 g, MeOH–H_2O (50 : 50, v/v)] to give colocynthol A (**1a**) (4.2 mg, 83%). Through the similar procedure, a solution of **3** (50.0 mg) in 0.2 M acetate buffer (pH 3.8, 10.0 ml) was treated with hesperidinase (75 mg) and the solution was stirred at 40 °C for 48 h. Work-up of the reaction mixture as described above gave **3a** (31.0 mg, 80%).

Colocynthol A (**1a**): A white powder, $[\alpha]_D^{27}$ +12.7° (c=0.05, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{32}H_{44}O_9Na$ $(M+Na)^+$ 595.2883; Found 595.2891. CD [MeOH, nm ($\Delta\varepsilon$)]: 227 (−0.80), 290 (+1.37), 328 (−1.32). UV [MeOH, nm (log ε)]: 257 (3.58). IR (KBr, cm^{-1}): 3432, 1736, 1701, 1664, 1648, 1086. ^{1}H-NMR δ: given in Table 2. ^{13}C-NMR δ_C: given in Table 3. Positive-ion FAB-MS m/z 595 $(M+Na)^+$. Negative-ion FAB-MS m/z 571 $(M-H)^-$.

Enzymatic Hydrolysis of 2 with Cellulase A solution of **2** (10.0 mg) in 0.2 M acetate buffer (pH 5.0, 2.0 ml) was treated with cellulase (20 mg, from *Aspergillus niger*, Sigma) and the solution was stirred at 37 °C for 15 h. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by normal-phase silica gel column chromatography [1.5 g, $CHCl_3$–MeOH (4 : 1, v/v)] to give colocynthol B 2-*O*-*β*-D-glucopyranoside (**2a**, 6.2 mg, 76%).

Colocynthol B 2-*O*-*β*-D-Glucopyranoside (**2a**): A white powder, $[\alpha]_D^{27}$ +1.4° (c=0.36, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{36}H_{54}O_{11}Na$ $(M+Na)^+$ 683.3407; Found 683.3398. CD [MeOH, nm ($\Delta\varepsilon$)]: 240 (−0.23), 274 (+0.31), 332 (−017). UV [MeOH, nm (log ε)]: 257 (3.76). IR (KBr, cm^{-1}): 3569, 1686, 1637, 1078, 1032. ^{1}H-NMR δ: given in Table 2. ^{13}C-NMR δ_C: given in Table 3. Positive-ion FAB-MS m/z 683 $(M+Na)^+$. Negative-ion FAB-MS m/z 659 $(M-H)^-$.

Enzymatic Hydrolysis of 2a with Hesperidinase A solution of **2a** (6.0 mg) in 0.2 M acetate buffer (pH 3.8, 2.0 ml) was treated with hesperidinase (20 mg) and the solution was stirred at 40 °C for 48 h. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by reversed-phase silica gel column chromatography [1.0 g, MeOH–H_2O (50 : 50, v/v)] to give colocynthol B (**2b**, 4.0 mg, 89%).

Colocynthol B (**2b**): A white powder, $[\alpha]_D^{27}$ +45.2° (c=0.20, MeOH). High-resolution EI-MS: Calcd for $C_{30}H_{42}O_6$ (M^+) 498.2981; Found 498.2986. CD [MeOH, nm ($\Delta\varepsilon$)]: 245 (−0.95), 285 (+2.28), 330 (−1.78). UV [MeOH, nm (log ε)]: 268 (3.67). IR (KBr, cm^{-1}): 3440, 1686, 1655, 1078, 1040. ^{1}H-NMR δ: given in Table 2. ^{13}C-NMR δ_C: given in Table 3. EI-MS m/z (%): 498 (M^+, 9), 164 (100).

Bioassay Method. Animals Male ddY mice weighing about 25—30 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 24—26 h prior to the beginning of the experiment, but were allowed free access to tap water. All of the experiments were performed with conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Effects on Ear Passive Cutaneous Anaphylaxis (PCA) Reactions in Mice Experiments on the effects of the methanolic extract and its fractions from the fruit of *C. colocynthis* on ear PCA reactions were performed according to the method reported previously[36] with slight modification. Briefly, 10 μl of anti-DNP IgE diluted in PBS (20 μg/ml), or PBS alone (normal group) was injected intradermally into both ears of male ddY mice (5—6 weeks old). Forty-seven hours later, test compounds suspended in 5% acacia solution were administrated orally. After 1 h, 0.25 ml of PBS which contained 2% Evans blue and 0.25 mg of DNP-BSA was injected into the vein. Thirty minutes later, mice were killed by cervical dislocation and the both ears were removed and incubated with 1 M KOH solution overnight at 37 °C to dissolve them. The solution was then mixed with 4.5 ml of a mixture of acetone–0.2 M H_3PO_4 (13 : 5, v/v). After centrifugation at 4000 rpm for 10 min, absorbance was measured at 620 nm using a spectrophotometer (Beckmann DU 530). An antiallergic agent, tranilast, was used as a reference compound.[37,38]

Statistics Values were expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis.

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26) 4-Hydroxybenzyl β-D-glucopyranoside (**14**). ^{1}H-NMR (CD_3OD, 500 MHz) δ [3.68 (1H, dd, J=5.3, 11.9 Hz), 3.89 (1H, dd, J=2.0, 11.9 Hz), 6′-H_2], 4.31 (1H, d, J=7.6 Hz, 1′-H), 4.55, 4.81 (1H each, both d, J=11.2 Hz, 7-H_2), 6.74, 7.23 (2H each, both d, J=8.6 Hz, 3,5, 2,6-H); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C 129.7 (1-C), 131.2 (2,6-C), 116.0 (3,5-C), 158.3 (4-C), 71.7 (7-C), 102.9 (1′-C), 75.1 (2′-C), 78.0 (3′-C), 71.8 (4′-C), 78.1 (5′-C), 62.7 (6′-C).
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