New Pentacyclic Cucurbitane Glucosides from the Fruits of *Citrullus colocynthis* SCHRAD.

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Colocynthins A – C (**1** – **3**, resp.), new pentacyclic cucurbitane type triterpene glucosides, have been isolated from the AcOEt-soluble fraction of the fruits of *Citrullus colocynthis*, along with three known compounds, β-sitosterol 3-*O*-β-D-glucopyranoside, elaterinide, and bryoamaride. Their structures were determined on the basis of ^{1}H- and ^{13}C-NMR spectra, DEPT, and COSY, NOESY, HMQC, and HMBC experiments.

Introduction. – *Citrullus colocynthis* SCHRAD. (Cucurbitaceae) is reputed because of its purgative effects and has been suggested to possess antitumor activity [1 – 3]. This plant has been used to treat constipation, oedema, bacterial infections, cancer, and diabetes, and as an abortifacient in Iran [4]. The ethnobotanical uses of this plant include its use as an abortifacient, cathartic, cytotoxic, purgative, and vermifuge and for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, and tumours [5 – 8]. The previous phytochemical studies on the *Citrullus colocynthis* have resulted in the isolation of various steroids, flavonoids, and triterpenes, especially the bitter principles cucurbitacins [9 – 11]. The ethnopharmacological and chemotaxonomic importance of the genus *Citrullus* prompted us to carry out further studies on *C. colocynthis*. As a result, we have isolated three new pentacyclic cucurbitane type triterpene glucosides, **1** – **3**, along with β-sitosterol 3-*O*-β-D-glucopyranoside [12], elaterinide [13], and bryoamaride [14].

Result and Discussion. – 1. *Chemistry.* The MeOH extract of the whole plant was divided into fractions soluble in hexane, $CHCl_3$, AcOEt, BuOH, and H_2O. Column chromatography of the AcOEt soluble fraction provided three new bitter principles which are named as colocynthins A – C (**1** – **3**, resp.; *Fig. 1*), along with β-sitosterol 3-*O*-β-D-glucopyranoside, elaterinide, and bryoamaride, respectively. The compounds **1** – **3** were glycosides which gave a copious foam on shaking with H_2O, a positive *Molisch*'s test result, as well as *Salkowski* and *Liebermann – Burchard* color reactions for triterpenes. Acid hydrolysis provided D-glucose which could be identified through the measurement of its optical rotations, as well as by co-TLC with an authentic sample.

Colocynthin A (**1**) was isolated as an off-white amorphous solid. The IR spectrum showed bands for OH (3400 cm^{-1}), C=O (1715 cm^{-1}), conjugated C=O (1680 cm^{-1}), and olefinic (1610 – 1650 cm^{-1}) functionalities. The HR-FAB-MS (positive-ion mode) provided an $[M+H]^+$ peak at m/z 659.3422, indicating the molecular formula

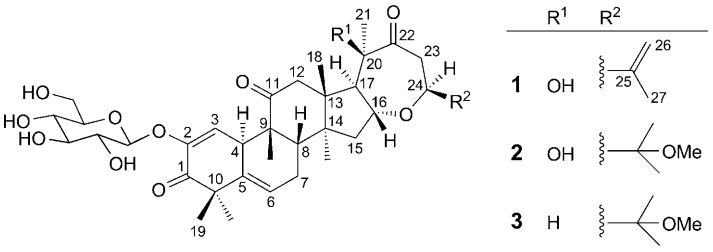

Fig. 1. *Structures of coloynthins A – C* (**1** – **3**, resp.)

$C_{36}H_{50}O_{11}$, as well as a fragment ion $[M-162+H]^+$ at *m/z* 497.2832 due to the loss of the glucose moiety. The ratio of C- to H-atoms in the molecule indicated twelve degrees of unsaturation. The EI-MS spectrum showed a peak at *m/z* 496 ($[M-162]^+$) and further significant peaks of cucurbitacins at *m/z* 402 ($[M$ – side chain$]^+$) and *m/z* 164 ($C_{10}H_{12}O_2$); the latter arising from the retro-*Diels – Alder* fragmentation of the ring *B* characteristic of cucurbitacins with a ring *A* diosphenol system [15]. The NMR data described in detail below indicated three CO groups, three olefinic bonds, and a glucose moiety. Because no other unsaturated function was indicated by the spectral data, the five remaining unsaturations were accounted for by five rings, suggesting a pentacyclic triterpene structure. The broad-band-decoupled and DEPT ^{13}C-NMR spectra (*Table 1*) showed 36 signals: seven Me groups, six CH_2 groups, including an O-bearing C-atom, twelve CH groups, including seven O-bearing C-atoms, eleven quaternary C-atoms, including one O-bearing C-atom, six olefinic C-atoms at δ(C) 147.1, 123.5, 136.9, 122.8, 146.4, and 113.6, and three CO groups at δ(C) 199.7, 214.4, and 215.5. An O-bearing CH_2 group at δ(C) 62.8 and five O-bearing CH groups at δ(C) 101.7, 72.2, 77.9, 70.3, and 78.4 were due to the glucose residue.

The ^{1}H-NMR spectrum (*Table 2*) displayed signals for seven tertiary Me groups at δ(H) 1.02, 1.07, 1.26, 1.31, 1.40, 1.44, and 1.73 (7*s*). The terminal CH_2 H-atoms were observed at δ(H) 4.67 (br. *s*, 1 H) and 4.80 (br. *s*, 1 H), respectively. The signals of two trisubstituted C=C moieties were observed at δ(H) 6.10 (*d*, $J=2.4$, 1 H) and 5.85 (br. *s*, 1 H), respectively [16]. The O-bearing CH group H-atoms of the aglycone were observed at δ(H) 4.48 (*dd*, $J=9.5, 7.5$, 1 H) and 4.24 (*dd*, $J=8.6, 1.6$, 1 H). The signal of the anomeric H-atom of the glucose moiety was identified at δ(H) 4.69 (*d*, $J=7.8$, 1 H) in addition to further O-bearing CH group H-atoms at δ(H) 3.41 – 3.58 and H-atoms of an O-bearing CH_2 group at δ(H) 3.98 (*dd*, $J=10.2, 4.5$, 1 H) and 3.70 (*dd*, $J=10.2, 2.6$, 1 H), respectively. The large coupling constant of the anomeric H-atom allowed us to assign the β-configuration to the glucose moiety. The presence of an olefinic C-atom signal at δ(C) 123.5 and an olefinic quaternary C-atom signal at δ(C) 147.1 suggested that the 2,3 position was unsaturated. This assignment was supported by the strong UV absorption at λ_{max} 270 nm and also by the high-field position of the C(1) signal in the ^{13}C-NMR spectrum. A comparison of the NMR spectra of **1** with those of bryoamaride [13] revealed that the glucose moiety and the *A*, *B*, and *C* ring part of the aglycone of both compounds share the same structure, the difference being in the side chain.

The signal for an H-atom of an O-bearing CH group at δ(H) 4.48 showed a correlation to the signal at δ(C) 73.1 in the HMQC spectrum, ^2J couplings to the signals

Table 1. *^{13}C-NMR Data of Compounds* **1**–**3**. Measured in CD_3OD; δ(C) in ppm.

	1	**2**	**3**
C(1)	199.7	199.4	199.6
C(2)	147.1	147.5	147.2
H–C(3)	123.5	123.7	123.3
H–C(4)	40.2	40.0	40.5
C(5)	136.9	137.1	136.8
H–C(6)	122.8	123.0	122.6
CH_2(7)	24.4	24.6	24.4
H–C(8)	42.5	42.3	42.6
C(9)	50.4	50.5	50.7
Me_β–C(9)	18.7	18.4	18.2
C(10)	50.8	49.9	51.0
Me_α–C(10)	28.5	28.7	28.8
C(11)	214.4	214.6	214.2
CH_2(12)	48.4	48.7	48.4
C(13)	48.9	48.9	49.1
C(14)	49.3	50.0	49.5
Me_α–C(14)	21.0	22.8	23.0
CH_2(15)	46.5	46.8	45.3
H–C(16)	73.1	73.4	80.5
H–C(17)	55.4	56.0	50.1
Me(18)	20.1	20.3	11.8
Me(19)	20.6	20.4	20.6
C(20) or H–C(20)	80.8	80.6	32.7
Me(21)	24.8	24.7	21.4
C(22)	215.5	215.2	213.5
CH_2(23)	38.7	38.4	46.9
H–C(24)	82.0	82.3	84.5
C(25)	146.4	83.7	84.0
CH_2(26) or Me(26)	113.6	23.4	23.1
Me(27)	21.5	23.2	22.9
H–C(1_{Glc})	101.7	101.5	101.5
H–C(2_{Glc})	72.2	72.4	72.0
H–C(3_{Glc})	77.9	78.2	77.8
H–C(4_{Glc})	70.3	70.1	70.5
H–C(5_{Glc})	78.4	78.0	78.3
CH_2(6_{Glc})	62.8	62.2	62.5
MeO	–	61.0	60.8

of C-atoms C(15) (δ(C) 46.5) and C(17) (δ(C) 55.4), and ^3J couplings to the signals of C-atoms C(14) (δ(C) 49.3), C(20) (δ(C) 80.8), and C(24) (δ(C) 82.0) in the HMBC, confirming that C(16) and C(24) were linked *via* an ether bond to form a seven membered ring. The position of the sugar moiety at C(2) was confirmed a HMBC of the signal for the anomeric H-atom at δ(H) 4.69 with the signal for C(2) at δ(C) 147.1. The presence of a terminal CH_2 group was confirmed by the low-field shift of Me(27) to δ(H) 1.73, as well as a HMBC showing ^3J correlations of the signals of both the olefinic H-atoms at δ(H) 4.67 and 4.80 with the signal for Me(27) at δ(C) 21.5. The assignments of H_α–C(23), H_β–C(23), and H–C(24) were confirmed by selective decoupling

Table 2. *^{1}H-NMR Data of Compounds* **1**–**3**. Measured in CD_3OD; δ(H) in ppm, J in Hz.

	1	**2**	**3**
H−C(3)	6.10 (*d*, *J* = 2.4)	6.13 (*d*, *J* = 2.4)	6.08 (*d*, *J* = 2.5)
H−C(4)	3.53 (br. *s*)	3.51 (br. *s*)	3.50 (br. *s*)
H−C(6)	5.85 (br. *s*)	5.88 (br. *s*)	5.87 (br. *s*)
H_α−C(7)	2.09–2.19 (*m*)	2.13–2.15 (*m*)	2.11–2.14 (*m*)
H_β−C(7)	2.39–2.41 (*m*)	2.43–2.45 (*m*)	2.41–2.43 (*m*)
H−C(8)	2.41–2.44 (*m*)	2.45–2.48 (*m*)	2.43–2.47 (*m*)
Me_β−C(9)	1.40 (*s*)	1.43 (*s*)	1.35 (*s*)
Me_α−C(10)	1.31 (*s*)	1.38 (*s*)	1.40 (*s*)
H_α−C(12)	2.57 (*d*, *J* = 14.5)	2.58 (*d*, *J* = 14.3)	2.49 (*d*, *J* = 12.2)
H_β−C(12)	3.15 (*d*, *J* = 14.5)	3.19 (*d*, *J* = 14.3)	3.01 (*d*, *J* = 12.2)
Me_α−C(14)	1.07 (*s*)	1.12 (*s*)	1.09 (*s*)
H_α−C(15)	1.48 (*dd*, *J* = 11.5, 3.4)	1.50 (*dd*, *J* = 11.2, 3.7)	1.48 (br. *d*, *J* = 11.2)
H_β−C(15)	1.90–1.93 (*m*)	1.92 (*dd*, *J* = 11.2, 9.1)	1.90 (br. *d*, *J* = 11.2)
H−C(16)	4.48 (*dd*, *J* = 9.5, 7.5)	4.45 (*dd*, *J* = 9.1, 7.2)	3.91–3.94 (*m*)
H−C(17)	2.47 (*d*, *J* = 9.5)	2.48 (*d*, *J* = 7.2)	2.24 (*dd*, *J* = 12.4, 9.2)
Me(18)	1.02 (*s*)	1.04 (*s*)	0.87 (*s*)
Me(19)	1.26 (*s*)	1.29 (*s*)	1.25 (*s*)
C(20) or H−C(20)	–	–	1.89–1.91 (*m*)
Me(21)	1.44 (*s*)	1.47 (*s*)	1.09 (*d*, *J* = 6.7)
H_α−C(23)	2.74 (*dd*, *J* = 17.5, 8.6)	2.77 (*dd*, *J* = 17.2, 8.4)	1.92 (br. *d*, *J* = 12.3)
H_β−C(23)	2.91 (*dd*, *J* = 17.5, 1.6)	2.90 (*dd*, *J* = 17.2, 1.8)	2.35 (br. *d*, *J* = 12.3)
H−C(24)	4.24 (*dd*, *J* = 8.6, 1.6)	4.22 (*dd*, *J* = 8.4, 1.8)	4.19 (*d*, *J* = 8.2)
CH_2(26) or Me(26)	4.67 (br. *s*), 4.80 (br. *s*)	1.45 (*s*)	1.39 (*s*)
Me(27)	1.73 (*s*)	1.55 (*s*)	1.45 (*s*)
H−C(1_{Glc})	4.69 (*d*, *J* = 7.8)	4.71(*d*, *J* = 7.6)	4.74(*d*, *J* = 7.8)
H−C(2_{Glc})	3.44 (*t*, *J* = 8.2)	3.46–3.49 (*m*)	3.48 (*t*, *J* = 8.5)
H−C(3_{Glc})	3.49–3.52 (*m*)	3.49–3.51 (*m*)	3.53–3.57 (*m*)
H−C(4_{Glc})	3.53–3.58 (*m*)	3.50–3.53 (*m*)	3.48–3.52 (*m*)
H−C(5_{Glc})	3.41–3.44 (*m*)	3.40–3.44 (*m*)	3.40–3.45 (*m*)
H_a−C(6_{Glc})	3.98 (*dd*, *J* = 10.2, 4.5)	3.97 −3.99 (*m*)	4.01 (*dd*, *J* = 10.5, 4.8)
H_b−C(6_{Glc})	3.70 (*dd*, *J* = 10.2, 2.6)	3.72 (*dd*, *J* = 10.4, 2.4)	3.74 (*dd*, *J* = 10.5, 2.4)
MeO	–	3.52 (*s*)	3.49 (*s*)

experiments. Irradiation of the double *doublet* at δ(H) 4.24 (*dd*, *J* = 8.6, 1.6) allowed the location of H_α−C(23) and H_β−C(23) at 2.74 and 2.91, respectively. Irradiation at δ(H) 2.91 resulted in the double *doublet* of H−C(24) collapsing to a *doublet* (*J* = 8.6 Hz). Similarly, irradiation at δ(H) 2.74 collapsed the signal of H−C(24) into a *doublet* (*J* = 1.6 Hz). The HMBCs allowed us to assign one CO group to C(22), as its signal showed ^2J correlations with both the signals for the H-atoms at C(23) and a ^3J correlation with the signal for H−C(24). A OH group at C(20) was inferred by the low-field shifts of C(20) δ(C) 80.8 and Me(21) δ(C) 24.8 in the ^{13}C-NMR spectrum and confirmed by ^2J and ^3J correlations of H−C(17) with C(20) and C(21), respectively.

The relative configuration of **1** was derived by comparison of the ^{13}C-NMR data with those of bryoamaride and by the NOE experiments, which showed NOE enhancement of β-oriented Me(18) (4.5%) on irradiation of H−C(16). On the other hand, enhancements of α-oriented Me(21) (4.3%) and H−C(24) (10.4%) were

observed when H_α–C(23) was irradiated. The large coupling constant (9.5 Hz) between H_β–C(16) and H_α–C(17) supported the *D*/*E* ring junction. The relative (*R**) and (*R**) configurations were assigned to C(16) and C(20), respectively, on the basis of biogenetic considerations, as well as due to the similarity of the NMR chemical shifts of C(16) and C(20) with related cucurbitacins [17]. The NOESY interactions of **1** illustrated in *Fig. 2* were in complete agreement to the assigned structure of colocynthin A (**1**) as 16*α*,24*α*-epoxy-2,20*β*-dihydroxy-3,11,22-trioxocucurbita-1,5,25-triene 2-*O*-*β*-D-glucopyranoside.

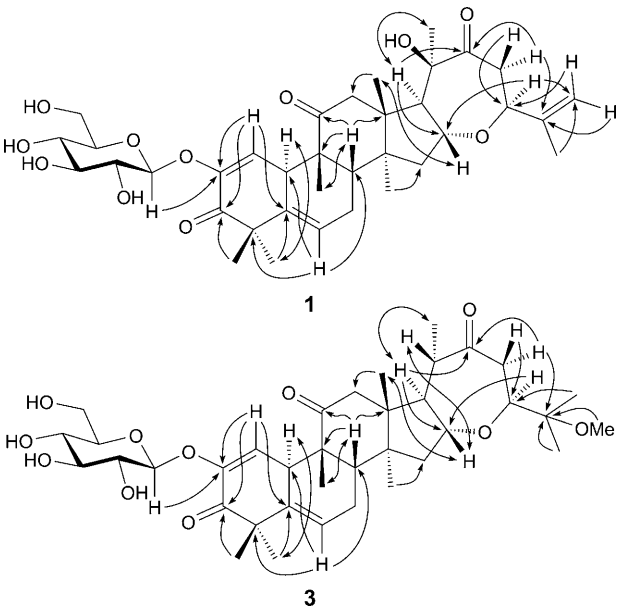

Fig. 2. *Important HMBC* (H→C) *and NOESY* (H↔H) *correlations of compounds* **1** *and* **3**

Colocynthin B (**2**) was isolated as an off-white amorphous solid. The HR-FAB-MS of **2** showed a signal at 691.3681 ($[M+H]^+$), and in conjunction with the ^{13}C-NMR data (*Table 1*), the molecular formula was determined to be $C_{37}H_{54}O_{12}$, indicating eleven degrees of unsaturation. The 36 signals in the ^{13}C-NMR spectrum were consistent with a triterpenoid backbone bearing a sugar moiety. The EI-MS showed an $[M-162]^+$ peak at *m*/*z* 528, as well as a peak at *m*/*z* 496 due to the loss of MeOH revealing the presence of a MeO group. The latter could further be confirmed by a Me group *singlet* at δ(H) 3.52 and a signal at δ(C) 61.0. The position at C(25) could be confirmed through low-field shift of C(25) as well as HMBCs; the signal at δ(H) 3.52 showing ^3J correlation with C(25). The rest of the ^{1}H- and ^{13}C-NMR data were similar to **1**. Acid hydrolysis of **2** gave, besides D-glucose, an aglycone which could be identified as cucurbitacin T by comparison of physical and spectral data in the literature [18]. The position of the glucose moiety was confirmed at C(2) as the signal for the anomeric C-atom showed a ^2J correlation with the signal for C(2). Moreover, the UV spectrum of the aglycone showed a sharp absorption at 300 nm in conformity to the presence of an

enol OH group. Thus, colocynthin B (**2**) was characterized as cucurbitacin T 2-*O*-*β*-D-glucopyranoside.

Colocynthin C (**3**) was obtained as an off-white amorphous solid. The molecular formula was established as $C_{37}H_{54}O_{11}$ by HR-FAB-MS in the positive-ion mode, which gave an $[M+H]^+$ peak at *m/z* 675.3732 ($C_{37}H_{55}O_{11}^+$; calc. 675.3744). The ^{1}H- and ^{13}C-NMR spectra of **3** suggested that it was closely related to **2**, except the high-field shift of C(20) (δ(C) 32.7) and occurrence of the signal of H–C(17) as a double *doublet* instead of a *doublet* and the signal of C(21) was also observed as a *doublet*. Hence, compound **3** is a 20-deoxy derivative of **2**. The COSY, HMBC, and NOESY correlations allowed us to assign the structure of colocynthin C (**3**) as 16*α*,24*α*-epoxy-2-hydroxy-25-methoxy-3,11,22-trioxocucurbita-1,5-diene 2-*O*-*β*-D-glucopyranoside.

The known compounds were identified by comparison of physical and spectroscopic data with literature values as *β*-sitosterol 3-*O*-*β*-D-glucopyranoside [12], elaterinide [13], and bryoamaride [14], respectively, of which bryoamaride has already been reported from this plant.

Experimental Part

General. Silica gel 230–400 mesh (SiO_2; *E. Merck*) was used for column chromatography (CC). Silica gel plates (*Si 60 F_{254}*, *E. Merck*) were used for thin layer chromatography (TLC). Optical rotations: *JASCO DIP-360* digital polarimeter. UV Spectra: *Hitachi UV-3200* spectrophotometer. IR Spectra: *JASCO 302-A* spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: in CD_3OD, on a *Bruker AMX-400* spectrometer, for ^{1}H at 400 MHz, and ^{13}C at 100 MHz; chemical shifts δ in ppm rel. to TMS as internal standard; coupling constants *J* in Hz. EI-MS: *JEOL JMS-HX-110* mass spectrometer. FAB-MS and HR-FAB-MS: *JEOL JMS-DA-500* instrument, with thioglycerol as matrix.

Plant Material. The fresh fruits of *Citrullus colocynthis* SCHRAD. (Cucurbitaceae) were collected from Karachi, Pakistan in September 2005 and identified by Prof. Dr. *Suraiya Khatoon*, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen (48/G.H.No. 67874/KUH) has been deposited.

Extraction and Isolation. The fresh fruits (10 kg) were chopped in small pieces and extracted thrice for 10 d each with MeOH (3 × 50 l) at r.t. The MeOH extract was evaporated under reduced pressure to afford a dark residue (500 g) which was suspended in H_2O (750 ml) and successively extracted with hexane (5 l), $CHCl_3$ (3 l), AcOEt (3 l), and BuOH (1 l), resp. The AcOEt-soluble fraction (30 g) was subjected to CC over SiO_2, successively eluting with hexane/$CHCl_3$, $CHCl_3$, and $CHCl_3$/MeOH in increasing order of polarity to obtain five major fractions, *Frs. A–E*, resp. *Fr. B* (3.8 g) obtained from $CHCl_3$/MeOH 9.8:0.2 was further purified by CC eluting with $CHCl_3$/MeOH 9.5:0.5 to afford colocynthin C (**3**; 14 mg) and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (25 mg). *Fr. C* (1.1 g), eluted with $CHCl_3$/MeOH 9.5:0.5, showed two major spots on TLC. Further purification by CC using $CHCl_3$/MeOH 9.2:0.8 as eluent afforded colocynthin A (**1**; 12 mg) and colocynthin B (**2**; 10 mg) from the top and tail fractions, resp. *Fr. D* (3.6 g), obtained from $CHCl_3$/MeOH 9.0:1.0, was chromatographed over SiO_2 with $CHCl_3$/MeOH 8.6:1.4 as eluent, and the resulting binary mixture finally resolved by prep. TLC, solvent system $CHCl_3$/MeOH 9.0:1.0 to yield elaterinide (R_f 0.6; 13 mg) and bryoamaride (R_f 0.3; 16 mg), resp.

Colocynthin A (*=16α,24α-Epoxy-2,20β-dihydroxy-3,11,22-trioxocucurbita-1,5,25-triene 2-*O*-β-D-Glucopyranoside = (4*R**,9β,16α,24*S**)-20-Hydroxy-9,10,14-trimethyl-1,11,22-trioxo-16,24-epoxy-4,9-cyclo-9,10-secocholesta-2,5,25-trien-2-yl β-D-Glucopyranoside*; **1**). Amorphous powder. M.p. 182–183°. $[\alpha]_D^{25} = +5.0$ ($c = 0.01$, MeOH). UV (MeOH): 270 (3.60), 258 (3.50). IR (KBr): 3400, 2965, 1715, 1680, 1610–1650, 1465, 1215. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 496 (12, $[M-162]^+$), 478 (18), 402 (15), 384 (20), 366 (9), 340 (25), 164 (100). HR-FAB-MS (pos.): 659.3422 ($[M+H]^+$, $C_{36}H_{51}O_{11}^+$; calc. 659.3431).

Colocynthin B (*=16α,24α-Epoxy-2,20β-dihydroxy-25-methoxy-3,11,22-trioxo-cucurbita-1,5-diene 2-*O*-β-D-Glucopyranoside = (4*R**,9β,16α,24*S**)-20-Hydroxy-25-methoxy-9,10,14-trimethyl-1,11,22-trioxo-

*16,24-epoxy-4,9-cyclo-9,10-secocholesta-2,5-dien-2-yl β-*D*-Glucopyranoside*; **2**). Amorphous powder. M.p. 185–186°. $[\alpha]_D^{25} = +6.5$ ($c = 0.03$, MeOH). UV (MeOH): 275 (4.34). IR (KBr): 3425, 1715, 1685, 1660, 1600, 1460, 1220. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 528 (9, $[M-162]^+$), 510 (11), 496 (20), 478 (18), 402 (15), 384 (22), 164 (100). HR-FAB-MS (pos.): 691.3681 ($[M+H]^+$, $C_{37}H_{55}O_{12}^+$; calc. 691.3693).

Colocynthin C (*=16α,24α-Epoxy-2-hydroxy-25-methoxy-3,11,22-trioxo-cucurbita-1,5-diene 2-*O*-β-*D*-Glucopyranoside = (4*R**,9β,16α,24*S**)-25-Methoxy-9,10,14-trimethyl-1,11,22-trioxo-16,24-epoxy-4,9-cyclo-9,10-secocholesta-2,5-dien-2-yl β-*D*-Glucopyranoside*; **3**). Amorphous powder. M.p. 178–180°. $[\alpha]_D^{25} = +4.0$ ($c = 0.02$, MeOH). UV (MeOH): 270 (4.20). IR (KBr): 3430, 1715, 1685, 1650, 1600, 1445, 1115. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 512 (10), 480 (12), 386 (18), 368 (21), 164 (100). HR-FAB-MS (pos.): 675.3732 ($[M+H]^+$, $C_{37}H_{55}O_{11}^+$; calc. 675.3744).

Acid Hydrolysis of Compounds ***1****,* ***2****, and* ***3***. Each compound (5 mg) in MeOH (5 ml) containing 1N HCl (5 ml) was refluxed for 4 h, concentrated under reduced pressure, diluted with H_2O (10 ml), and extracted with AcOEt (2 × 50 ml). The org. phase provided a complex mixture of aglycon degradation products from **1** and **3** which could not be separated due to paucity of material. The pure aglycon from **2** was obtained through prep. TLC (solvent system $CHCl_3$/MeOH 9.9:0.1), showed $[\alpha]_D^{28} = -53.2$ ($c = 1.1$, $CHCl_3$), and was identified as cucurbitacin T through comparison of data reported in the literature [18]. The aq. phase was concentrated, and D-glucose was identified by the sign of its optical rotation ($[\alpha]_D^{25} = +52.3$ from **1**, $+51.1$ from **2**, and $+52.0$ from **3**, resp.) and co-TLC with an authentic sample.

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