Three New Flavonoid Glycosides, Byzantionoside B 6′-*O*-Sulfate and Xyloglucoside of (*Z*)-Hex-3-en-1-ol from *Ruellia patula*

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Three new flavonoid glycosides, demethoxycentaureidin 7-*O*-β-D-galacturonopyranoside, pectolinarigenin 7-*O*-α-L-rhamnopyranosyl-(1‴→4″)-β-D-glucopyranoside and 7-*O*-α-L-rhamnopyranosyl-(1‴→4″)-β-D-glucuronopyranoside, a new megastigmane glucoside, byzantionoside B 6′-*O*-sulfate, and a new (*Z*)-hex-3-en-1-ol *O*-β-D-xylopyranosyl-(1″→2′)-β-D-glucopyranoside, were isolated from leaves of *Ruellia patula* Jacq., together with 12 known compounds, β-sitosterol glucoside, vanilloside, bioside (decaffeoyl verbascoside), acteoside (verbascoside), syringin, benzyl alcohol *O*-β-D-xylopyranosyl-(1″→2′)-β-D-glucopyranoside, cistanoside E, roseoside, phenethyl alcohol *O*-β-D-xylopyranosyl-(1″→2′)-β-D-glucopyranoside, (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside, isoacteoside and 3,4,5-trimethoxyphenol *O*-α-L-rhamnopyranosyl-(1″→6′)-β-D-glucopyranoside. Their structures were elucidated by means of spectroscopic analyses.

Key words *Ruellia patula*; Acanthaceae; flavonoid glycoside; demethoxycentaureidin; pectolinarigenin; byzantionoside B 6′-*O*-sulfate

Ruellia patula Jacquin [syn. *Dipteracanthus patulus* (Jacq.) Nees.] (Acanthaceae) is a hairy small undershrub, found in Arabia, Africa, Sri Lanka, southwest India and Myanmar. *R. patula* is used in the treatment of gonorrhea, syphilis, eye sores, renal infection, cough, wounds, scalds, toothache, stomachache and kidney stones.[1)] An extract of it exhibited a hypertensive effect and has a cardiotonic property.[2)] The Kani tribe of Kilamalai, India, uses this plant as a remedy for the bite of a certain species of spider, the tiger spider.[3)] The present study deals with the isolation and structural elucidation of three new flavonoid glycosides (**1**—**3**), one new sulfated megastigmane glucoside (**4**), and one new aliphatic glycoside (**5**) (Fig. 1), and 12 known compounds from the leaves of this plant.

Results and Discussion

From a methanolic extract of the leaves of *R. patula*, 17 compounds (**1**—**17**) were isolated. The structures of the known compounds were determined to be β-sitosterol glucoside (**6**),[4)] vanilloside (**7**),[5)] bioside (decaffeoyl verbascoside) (**8**),[6)] acteoside (verbascoside) (**9**),[7)] syringin (**10**),[8)] benzyl alcohol *O*-β-D-xylopyranosyl-(1″→2′)-β-D-glucopyranoside (**11**),[9)] cistanoside E (**12**),[10)] roseoside (**13**),[11)] phenethyl alcohol *O*-β-D-xylopyranosyl(1″→2′)-β-D-glucopyranoside (**14**),[12)] (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**15**),[13)] isoacteoside (**16**),[14)] and 3,4,5-trimethoxyphenol *O*-α-L-rhamnopyranosyl-(1″→6′)-β-D-glucopyranoside (**17**)[15)] by comparing their spectroscopic data with those reported in the literature.

Compound **1** was obtained as a pale yellow amorphous powder and had the molecular formula of $C_{23}H_{22}O_{13}$ which was established from the positive-ion mode high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS). The IR spectrum contained bands at 3330 cm^{-1} for a hydroxy group, 1700 and 1652 cm^{-1} for carbonyl groups, and 1614 cm^{-1} for an aromatic ring(s). The UV spectrum exhibited the characteristic absorptions at 348 (band I) and 235 (band II) nm for a flavonoid skeleton. The ^{1}H-NMR spectrum of **1** displayed two singlet proton signals at δ_H 6.97 and 6.79, and those of three aromatic protons coupled in an ABX system at δ_H 7.09 (1H, d, J=8.6 Hz), 7.47 (1H, d, J=2.2 Hz), and 7.53 (1H, dd, J=8.6, 2.2 Hz), together with two methoxy signals at δ_H 3.78 and 3.85, and one anomeric proton signal at δ_H 5.09 (1H, d, J=7.2 Hz) (Table 1). The ^{13}C-NMR spectrum exhibited six signals assignable to a β-galacturonopyranosyl moiety,[16)] two signals for methoxy groups, 14 sp^2 signals and one carbonyl carbon signal (δ_C 182.1). Of the 14 sp^2 carbons, seven were expected to bear an oxygen atom from their chemical shifts. The presence of a galacturonopyranosyl moiety was confirmed by HPLC analysis of a hydrolyzate of

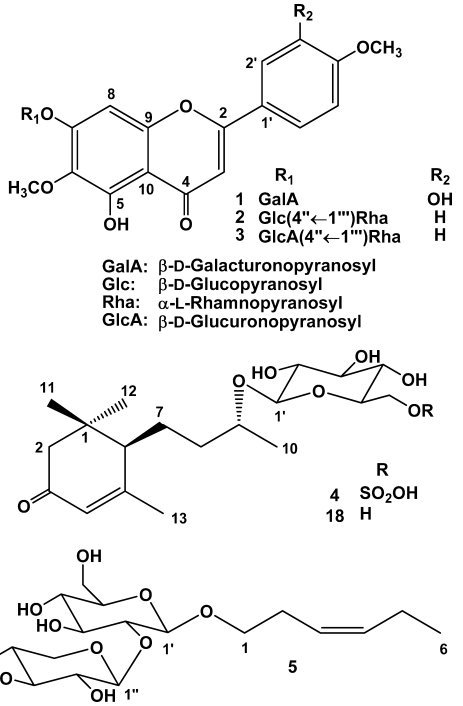

Fig. 1. Structures of New Compounds

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Table 1. ^{13}C- and ^{1}H-NMR Spectral Data for Compounds **1**—**3** (DMSO-d_6, 100, 400 MHz, Respectively)

Position	**1**		**2**		**3**	
	C	H	C	H	C	H
2	164.0	—	163.8	—	163.7	—
3	103.2	6.79 s	103.2	6.88 s	103.2	6.90 s
4	182.1	—	182.3	—	182.2	—
5	152.3	—	152.4	—	152.3	—
6	132.5	—	132.8	—	132.7	—
7	156.6	—	156.0	—	156.0	—
8	94.2	6.97 s	94.4	7.01 s	94.3	7.04 s
9	152.0	—	152.0	—	151.9	—
10	105.6	—	105.7	—	105.6	—
1′	122.8	—	122.7	—	122.7	—
2′	112.9	7.47 d 2.2	128.3	8.01 d 8.9	128.2	8.03 d 8.8
3′	146.8	—	114.6	7.09 d 8.9	114.5	7.11 d 8.8
4′	151.2	—	162.4	—	162.3	—
5′	112.1	7.09 d 8.6	114.6	7.09 d 8.9	114.5	7.11 d 8.8
6′	118.6	7.53 dd, 8.6, 2.2	128.3	8.01 d 8.9	128.2	8.03 d 8.8
1″	100.0	5.09 d 7.2	97.8	5.28 d 7.5	97.7	5.28 d 7.5
2″	73.1	3.17 m	73.5	3.14 m	73.4	3.20 m
3″	73.8	3.60 m	79.0	3.50 m	77.8	3.52 m
4″	71.9	3.60 m	76.0	3.59 m	75.9	3.55 m
5″	76.8	3.70 m	77.7	3.48 m	76.3	3.71 m
6″	171.5	—	63.9	3.79 m 3.86 m	173.4	—
1‴			100.0	5.23 br s	99.9	5.26 br s
2‴			70.3	3.91 m	70.3	3.90 m
3‴			68.4	3.71 m	68.4	3.72 m
4‴			72.0	3.45 m	71.9	3.44 m
5‴			70.4	3.45 m	70.4	3.69 m
6‴			18.0	1.08 d 6.4	17.9	1.09 d 6.0
6-OCH_3	60.1	3.78 s	60.2	3.78 s	60.1	3.79 s
4′-OCH_3	55.6	3.85 s	55.5	3.84 s	55.5	3.86 s
5-OH		12.90 s		12.88 s		12.87 s

H: chemical shifts, multiplicity and *J* in Hz. m: multiplet or overlapped signal.

1 and the absolute configuration of the sugar was simultaneously determined to be of the D-series using a chiral detector. From the above evidence and the eleven degrees of unsaturation of the aglycone, **1** was expected to be a flavonoid β-D-galacturonopyranoside with two methoxy groups. These physico-chemical data were in good agreement with those for structure of demethoxycentaureidin *O*-β-D-galacturonopyranoside (**1**).[17,18] The bathochromic shifts of 30 nm for band I on the additions of $AlCl_3$ and $AlCl_3$/HCl relative to the MeOH solution indicated the presence of a 5-hydroxy group (δ_H 12.90). While, on the addition of NaOMe, the insignificant bathochromic shift (27 nm) of band I, which was far less than 60 nm, as well as the absence of a band II shift on the addition of NaOAc indicated that the 4′- and 7-*O*-hydroxy groups must carry some substituents.[19] Further NMR analyses were performed, including ^{1}H–^{1}H correlation spectroscopy (COSY), and heteronuclear single quantum coherence (HSQC) and heteronuclear multiple-bound correlation (HMBC) spectroscopies. In the HMBC spectrum of **1**, H-3 (δ_H 6.79) showed cross peaks with C-10, C-1′, C-2 and C-4 (δ_C 105.6, 122.8, 164.0 and 182.1, respectively), and the proton signal of H-8 (δ_H 6.97) showed cross peaks with C-10, C-6, C-9 and C-7 (δ_C 105.6, 132.5, 152.0 and 156.6, respectively) (Fig. 2). Rare four bond couplings were observed between H-3 and C-5 (δ_C 152.3), and H-8 and C-4 due to the W-letter conformation. The proton signals of the methoxy groups at δ_H 3.78 and 3.85 were correlated with C-6 (δ_C 132.8) and C-4′ (δ_C 151.2), respectively. The signal of the anomeric proton of the galacturonopyranosyl moiety (δ_H 5.09) was correlated with C-7 (δ_C 156.6), confirming the sugar linkage to the hydroxy group at the C-7 position of the flavonoid skeleton. Therefore, the structure of compound **1** was elucidated to be 5,7,3′-trihydroxy-6,4′-dimethoxyflavone 7-*O*-β-D-galacturonopyranoside, namely demethoxycentaureidin 7-*O*-β-D-galacturonopyranoside, as shown in Fig. 1.

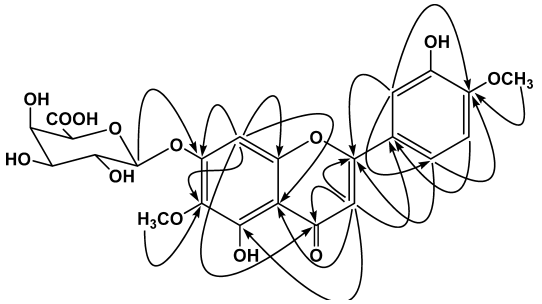

Fig. 2. Diagnostic HMBC Correlations for **1** Arrowheads Denote C and Arrow Ends H

Compound **2** was obtained as a pale yellow amorphous powder and it had the molecular formula of $C_{29}H_{34}O_{15}$ as determined by negative-ion mode HR-ESI-MS. The IR and UV spectra in methanol were similar to those of compound **1**. The ^{13}C-NMR spectroscopic data for the A and C rings were essentially the same as those of **1**, and in the ^{1}H-NMR spec-

trum, a pair of doublets at δ_H 7.09 (2H, d, J=8.9 Hz) and 8.01 (2H, d, J=8.9 Hz), which are characteristic of a *p*-disubstituted benzene ring, were observed, instead of three aromatic protons coupled in the ABX system, which were assigned to those on the B-ring of **1**. The NMR chemical shifts for aglycone were in complete accordance with those reported for pectolinarigenin.[20] In addition, two anomeric protons at δ_H 5.23 (1H, br s, H-1‴) and 5.28 (1H, d, J=7.5 Hz, H-1″) were observed. Acid hydrolysis of **2** liberated D-glucose and L-rhamnose, and the ^{13}C-NMR spectral data indicated that the terminal sugar was α-L-rhamnopyranoside. On the other hand, C-4″ of the β-glucopyranosyl unit was shifted downfield, δ_C 76.0, suggesting that the interglycosidic linkage was (1→4), which was confirmed by the HMBC correlation between the anomeric proton (H-1‴) of the rhamnopyranosyl moiety at δ_H 5.23 and C-4″ of the glucopyranosyl moiety. The HMBC spectroscopic data also displayed the correlation between the anomeric proton of the glucopyranosyl moiety (δ_H 5.28) and C-7 (δ_C 156.0) of the aglycone, being an evidence that the glycosylation had occurred at the hydroxyl group at C-7. On the basis of these spectral data, the structure of compound **2** was elucidated to be 5,7-dihydroxy-6,4′-dimethoxyflavone 7-*O*-α-L-rhamnopyranosyl-(1‴→4″)-β-D-glucopyranoside, namely pectolinarigenin 7-*O*-α-L-rhamnopyranosyl-(1‴→4″)-β-D-glucopyranoside, as shown in Fig. 1.

Compound **3** was obtained as a pale yellow amorphous powder and exhibited the molecular formula of $C_{29}H_{32}O_{16}$, which was determined by positive-ion mode HR-ESI-MS. Compound **3** was an analogous compound to **2**, except for the presence of a substituted β-glucuronopyranosyl moiety, instead of the 4″-substituted glucopyranose that was seen in **2**. The absolute configurations of these sugars were determined to be of the D-series. The HMBC spectrum also confirmed the glycosidic linkage to the hydroxy group at the C-7 position, since H-1‴ signal of the rhamnose at δ_H 5.26 was coupled with C-4″ of the glucuronic acid at δ_C 75.9. Therefore, the structure of compound **3** was elucidated to be 5,7-dihydroxy-6,4′-dimethoxyflavone 7-*O*-α-L-rhamnopyranosyl-(1‴→4″)-β-D-glucuronopyranoside, namely pectolinarigenin 7-*O*-α-L-rhamnopyranosyl-(1‴→4″)-β-D-glucuronopyranoside, as shown in Fig. 1.

Compound **4** was obtained as an amorphous powder and its elemental composition was determined to be $C_{19}H_{32}O_{10}S$ by HR-ESI-MS. Comparison of the ^{1}H- and ^{13}C-NMR spectral data of compound **4** showed a remarkable similarity to those of the known megastigmane glucoside byzantionoside B (**18**), except for the carbon signal at the 6′-position of the β-glucopyranosyl moiety (Table 2).[21,22] The downfield shift exhibited by the 6′-position of the β-glucopyranosyl moiety suggested the attachment of some substituent at this position, which was confirmed by measurement of mass spectra. The ESI-MS spectra showed a single peak at *m*/*z* 497.1424 in the positive-mode and *m*/*z* 451.1985 in the negative-mode corresponding to $C_{19}H_{31}O_{10}Na_2S$ $[M-H+2Na]^+$ and $C_{19}H_{31}O_{10}S$ $[M-H]^-$, respectively, showing the attachment of a sulfuryl moiety at the 6′-position. Consequently, the structure of compound **4** was established to be (6*R*,9*R*)-9-hydroxy-megastigman-4-en-3-one 9-*O*-β-D-glucopyranoside, 6′-*O*-sulfate, namely byzantionoside B 6′-*O*-sulfate, as shown in Fig. 1.

Table 2. ^{13}C- and ^{1}H-NMR Spectral Data for Compounds **4** and **5** (100, 400 MHz, Respectively)

Position	**4**[a]		**5**[b]	
	C	H	C	H
1	37.3	—	69.2	3.72 2H m
2	48.2	1.95 d 17.4	28.3	2.23 2H br q 6.7
		2.43 d 17.4		
3	202.3	—	126.1	5.33 m
4	125.3	5.80 br s	133.6	5.33 m
5	170.5	—	21.0	1.92 2H br tq 6.6, 6.6
6	52.3	1.99 m	15.0	0.89 t 6.6
7	26.5	1.46 m		
		1.96 m		
8	37.7	1.60 2H m		
9	75.7	4.08 qt 5.8, 5.8		
10	19.9	1.17 d 6.2		
11	27.5	1.08 s		
12	29.0	1.07 s		
13	25.0	2.05 d 1.2		
1′	102.3	4.32 d 7.8	102.1	4.24 d 7.7
2′	75.1	3.13 dd 8.8, 7.8	83.2	3.21 m
3′	77.8	3.32 dd 8.8, 8.8	77.6	3.18 m
4′	71.6	3.28 dd 8.9, 8.9	70.6	3.10 m
5′	75.9	3.25 m	77.2	3.16 m
6′	68.3	3.64 dd 11.5, 5.0	61.8	3.63 m
		3.85 dd 11.5, 6.0		3.70 m
1″			105.7	4.35 d 7.3
2″			75.2	2.99 m
3″			76.7	3.11 m
4″			70.4	3.41 m
5″			66.7	3.27 m
				3.66 m

a) Recorded for CD_3OD. *b*) Recorded for DMSO-d_6. m: multiplet or overlapped signal.

Compound **5** was obtained as a white amorphous powder and it had the molecular formula of $C_{17}H_{30}O_{10}$, which was deduced from positive-ion mode HR-ESI-MS. The aglycone portion consisted of six carbons; one terminal methyl, two olefinic, two methylene and one oxymethylene carbon. Considering the coupling patterns observed on ^{1}H-NMR spectroscopy, the structure of the aglycone portion was determined to be hex-3-en-l-o1. The geometry of the double bond was determined to be *cis* by comparison of the ^{13}C-NMR chemical shifts with those of the reported (*Z*)-hex-3-en-1-o1.[23] Sugar analysis revealed the presence of D-xylose and D-glucose, and the ^{13}C-NMR spectrum exhibited five signals assignable to the terminal xylopyranose and six signals to an inner glucopyranose (Table 2). Coupling constants of two anomeric proton signals at δ_H 4.24 (1H, d, J=7.7 Hz) on δ_C 102.1 and 4.35 (1H, d, J=7.3 Hz) on δ_C 105.7 indicated the mode of linkage was β (Table 2).[16] The linkage of these sugars was determined to be xylopyranosyl-(1→2)-glucopyranoside, because a ^{13}C-NMR signal that was assignable to the 2-position of glucose was significantly shifted downfield (δ_C 83.2) compared with that of an unsubstituted β-glucopyranoside[24] and the HMBC spectrum also supported the position of linkage. Accordingly, the structure of compound **5** was determined to be (*Z*)-hex-3-en-1-ol *O*-β-D-xylopyranosyl-(1″→2′)-β-D-glucopyranoside, as shown in Fig. 1.

Flavonoids and phenylethanoids were isolated from *R. patula*. Structurally related aglycone of compound **1**, centaureidin, was reported to possess antioxidant activity[25] and com-

mon aglycone of compounds **2** and **3**, pectolinarigenin, is also known to have a preventing activity of hepatic injury caused by D-galactosamine *via* antioxidant mechanism.[26] Phenylethanoids were also reported to have radical scavenging acitivity.[27]

Experimental

General Experimental Procedures Optical rotation data were measured on a JASCO P-1030 polarimeter. IR and UV spectra were obtained on a Horiba FT-710 Fourier transform infrared spectrophotometer and a JASCO V-520 UV/Vis spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM α-400 spectrometer with tetramethylsilane as an internal standard. A circular dichroism (CD) spectrum was measured on a JASCO J-720 spectropolarimeter. HR-ESI mass spectra were taken on LTQ Orbitrap XL and QSTAR XL mass spectrometers. A highly porous synthetic resin, Diaion HP-20, was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 [(E. Merck, Darmstardt, Germany) 70—230 mesh]. Reversed-phase [octadecylsilanized silica gel (ODS)] open CC (RPCC) was performed on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) (Φ=2 cm, L=40 cm, 10 g fractions being collected). The droplet countercurrent chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–1-PrOH (9 : 12 : 8 : 2) were used as the mobile and stationary phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column [Inertsil ODS-3; GL Science, Tokyo, Japan; (Φ=6 mm, L=25 cm, flow rate: 1.0 ml/min), using a refractive index and/or UV detector. Precoated silica gel 60 F_{254} plates (E. Merck; 0.25 mm in thickness) were used for TLC analyses, with visualization by spraying with a 10% H_2SO_4 solution in ethanol and then heating to around 150 °C on a hotplate.

Plant Material The leaves of *R. patula* were collected in March 2007 from the Orman Botanical Garden in Giza, Egypt. A voucher specimen of the plant has been deposited in the Herbarium of Faculty of Pharmacy, Minia University, Egypt (Minia-07-Mar-RP).

Extraction and Isolation Powdered air-dried leaves (2.80 kg) of *R. patula* was exhaustively extracted with 70% methanol (5 l×5) and then concentrated under reduced pressure to yield a viscous gummy material. This residue (330 g) was dissolved in 250 ml of water and defatted with *n*-hexane (1 l×5). The aqueous layer was evaporated to remove a trace amount of organic solvent, and then extracted with EtOAc and 1-BuOH, successively (1 l×5 each). The EtOAc and 1-BuOH fractions were concentrated under reduced pressure to give 14 g and 137 g of residues, respectively. The remaining aqueous layer was concentrated to furnish a water-soluble fraction (60 g).

The EtOAc fraction (14 g) was subjected to silica gel CC (520 g) (Φ= 55 mm, L=60 cm). The column was eluted initially with $CHCl_3$ (5 l), and then with a $CHCl_3$–MeOH gradient mixture, 500 ml fractions being collected. Similar fractions were combined, affording 16 fractions. The second fraction was precipitated with MeOH to provide 6 (35 mg).

The 1-BuOH fraction (137 g) was fractionated by CC on a highly porous synthetic resin, Diaion HP-20 (Φ=40 mm, L=55 cm). The column was eluted initially with H_2O (3 l) and then with a MeOH–H_2O stepwise gradient of increasing MeOH content, *i.e.*, 20% (2 l), 50% (2 l), 80% (2 l), and 100% MeOH (3 l). The effluent was collected in fractions (500 ml each). Similar fractions were combined to provide three fractions. The first fraction (I) eluted with 20% MeOH (4.33 g) was subjected to silica gel CC (160 g) (Φ=30 mm, L=50 cm), using $CHCl_3$–MeOH gradients, yielding six fractions. The fourth fraction, I-4 (316 mg), was purified by DCCC to give three fractions. The second fraction, I-4-2 (256 mg), was purified by HPLC to produce compound **7** (6.56 mg) and compound **8** (2.48 mg). The fifth fraction, I-5 (1.13 g), was purified by RPCC, affording four fractions. The fourth fraction, I-5-4 (219.9 mg), was subjected to DCCC to give three fractions. The second fraction, I-5-4-2 (108.2 mg), was purified by HPLC to furnish compound **4** (34.6 mg).

The second fraction (II) eluted with 50% MeOH (6.36 g) on Diaion HP-20 CC was chromatographed on silica gel (350 g), (Φ=40 mm, L=55 cm), using a $CHCl_3$–MeOH gradient system, 200 ml fractions being collected. Similar fractions were combined to yield seven fractions. The seventh fraction, II-7 (4.65 g), was purified by RPCC, producing five fractions. The third fraction, II-7-3 (1.58 g), was subjected to silica gel (75 g) CC with isocratic elution with $CHCl_3$–MeOH–H_2O (17 : 6 : 1), providing seven fractions. The fourth fraction, II-7-3-4 (124 mg), was purified by HPLC to furnish compound **1** (10 mg) and compound **2** (21.1 mg). The fourth fraction, II-7-4 (1.63 g), was subjected to silica gel (75 g) CC using an isocratic system of $CHCl_3$–MeOH–H_2O (17 : 6 : 1), giving five fractions. The third fraction II-7-4-3 (123.4 mg) was purified by HPLC to afford compound **9** (7 mg).

The last fraction (III) eluted with 80% MeOH (5.0 g) was subjected to silica gel CC (200 g) (Φ=35 mm, L=60 cm), using $CHCl_3$–MeOH mixtures, yielding seven fractions. The third fraction, III-3 (500 mg), was purified by RPCC, affording nine fractions. The third fraction, III-3-3 (13.4 mg), was purified by HPLC to yield compound **10** (4.1 mg) and compound **11** (5.2 mg). The fourth fraction, III-3-4 (15.3 mg), was purified by HPLC to give compound **12** (3.2 mg) and compound **13** (10.4 mg). The fifth fraction, III-3-5 (78.4 mg), was purified by HPLC to furnish compound **14** (5.7 mg) and compound **5** (4 mg). The sixth fraction, III-3-6 (31.5 mg), was purified by HPLC to yield compound **15** (8 mg). The ninth fraction, III-3-9 (98 mg), was subjected to DCCC, giving three fractions. The second fraction, III-3-9-2 (16 mg), was purified by HPLC to afford compound **16** (2.3 mg). The fourth fraction, III-4 (953 mg), was purified by RPCC, producing three fractions. The third fraction, III-4-3 (101 mg), was purified by HPLC to give compound **17** (5.5 mg). The fifth fraction, III-5 (82.7 mg), was subjected to silica gel (50 g) CC using an isocratic system of $CHCl_3$–MeOH–H_2O (17 : 6 : 1), affording five fractions. The fifth fraction, III-5-5 (40 mg), was purified by HPLC to furnish compound **3** (4.8 mg).

Demethoxycentaureidin 7-*O*-β-D-Galacturonopyranoside (**1**): Pale yellow amorphous powder. $[\alpha]_D^{25}$ −32.9° (c=1.00, DMSO); IR ν_{max} (film) cm^{-1}: 3330, 2930, 1700, 1652, 1614, 1512, 1458, 1360, 1269, 1140, 1020, 953; UV λ_{max} (MeOH) nm (log ε): 235 (3.88), 348 (3.86); +NaOMe 238 (3.90), 375 (3.80); +$AlCl_3$ 230 (3.85), 378 (3.94); +$AlCl_3$/HCl 230 (3.89), 378 (3.85); +NaOAc 238 (3.88), 375 (3.90); ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6): Table 1; HR-ESI-MS (positive-ion mode) m/z: 529.0944 $[M+Na]^+$ (Calcd for $C_{23}H_{22}O_{13}Na$: 529.0952).

Pectolinarigenin 7-*O*-α-L-Rhamnopyranosyl-(1‴→4″)-β-D-glucopyranoside (**2**): Pale yellow amorphous powder. $[\alpha]_D^{25}$ −59.5° (c=1.40, DMSO); IR ν_{max} (film) cm^{-1}: 3350, 2921, 1652, 1608, 1571, 1509, 1460, 1419, 1357, 1294, 1262, 1181, 1052, 834; UV λ_{max} (MeOH) nm (log ε): 240 (3.83), 355 (3.86); +NaOMe 242 (3.82), 380 (3.89); +$AlCl_3$ 243 (3.89), 390 (3.93); +$AlCl_3$/HCl 245 (3.82), 390 (3.97); +NaOAc 240 (3.85), 355 (3.87); ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6): Table 1; HR-TOF-ESI-MS (negative-ion mode) m/z: 621.1836 $[M-H]^-$ (Calcd for $C_{29}H_{33}O_{15}$: 621.1825).

Pectolinarigenin 7-*O*-α-L-rhamnopyranosyl-(1‴→4″)-β-D-glucuronopyranoside (**3**): Pale yellow amorphous powder. $[\alpha]_D^{25}$ −42.3° (c=0.48, DMSO); IR ν_{max} (film) cm^{-1}: 3390, 2950, 1712, 1655, 1607, 1510, 1462, 1357, 1258, 1183, 1106, 1073, 1034, 831; UV λ_{max} (MeOH) nm (log ε): 225 (4.18), 345 (4.21); +NaOMe 225 (4.23), 375 (4.21); +$AlCl_3$ 225 (4.29), 370 (4.25); +$AlCl_3$/HCl 227 (4.20), 370 (4.26); +NaOAc 238 (4.21), 375 (4.14); ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6): Table 1; HR-ESI-MS (positive-ion mode) m/z: 659.1577 $[M+Na]^+$ (Calcd for $C_{29}H_{32}O_{16}Na$: 659.1583).

Byzantionoside B 6′-*O*-Sulfate (**4**): Off-white amorphous powder. $[\alpha]_D^{25}$ +10.5° (c=1.13, MeOH); IR ν_{max} (film) cm^{-1}: 3420, 2963, 1650, 1377, 1257, 1220, 1069, 1012; UV λ_{max} (MeOH) nm (log ε): 236 (3.31), 280 (3.02); ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): Table 2; CD $\Delta\varepsilon$ (nm): +0.43 (320), +1.21 (240) (c=2.4×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 497.1424 $[M-H+2Na]^+$ (Calcd for $C_{19}H_{31}O_{10}Na_2S$: 497.1428).

(*Z*)-Hex-3-en-1-ol *O*-β-D-Xylopyranosyl-(1″→2′)-β-D-glucopyranoside (**5**): White amorphous powder. $[\alpha]_D^{25}$ +34.2° (c=0.26, MeOH); IR ν_{max} (film) cm^{-1}: 3390, 2927, 1075, 1040; ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6): Table 2. HR-ESI-MS (positive-ion mode) m/z: 417.1730 $[M+Na]^+$ (Calcd for $C_{17}H_{30}O_{10}Na$: 417.1731).

Analysis of the Sugar Moiety About 1 mg each of compounds **1**—**5** was hydrolyzed with 1 M HCl (1.0 ml) at 80 °C for 2 h. The reaction mixtures were neutralized with Amberlite MB-3 and then partitioned with an equal amount of EtOAc (1.0 ml), the resulting water layers being analyzed for their sugar components. The sugars were identified by HPLC on an amino column [Shodex Asahipak NH_2P-50 4E (4.6 mm×250 mm), CH_3CN–H_2O (4 : 1), 1 ml/min], using chiral detector (JASCO OR-2090*plus*), in comparison with authentic sugars (D-galacturonolactone, D-glucuronolactone, D-xylose, D-glucose and L-rhamnose). Compound **1** gave a peak for D-galacturonolactone at the retention time of 11.5 min. Compound **2** gave peaks for L-rhamnose and D-glucose at the retention times of 6.2 min and 11.4 min, respectively. Compound **3** gave peaks for L-rhamnose and D-glucuronolactone at the retention times of 6.2 min and 12.2 min, respectively. Compound **4**

gave a peak for D-glucose at the retention time of 11.4 min. Compound **5** gave peaks for D-xylose and D-glucose at the retention times of 7.4 min and 11.4 min, respectively.

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