Phenolic Derivatives from *Ruprechtia polystachya* and Their Inhibitory Activities on the Glucose-6-phosphatase System

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Two new compounds, 5-methyl-2-(2-methylbutanoyl)phloroglucinol 1-*O*-(6-*O*-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside (**1**) and *trans*-2,3-dihydrokaempferol 3-*O*-(4-*O*-sulfo)-*α*-L-arabinopyranoside (**2**), together with 14 known flavonoids, *trans*-dihydrokaempferol 3-*O*-*α*-L-arabinopyranoside (**3**), *trans*-taxifolin 3-*O*-*α*-L-arabinofuranoside (**4**), quercetin 3-*O*-*α*-L-rhamnopyranoside (**5**), quercetin 3′-*O*-*α*-L-arabinofuranoside (**6**), catechin 3-*O*-*α*-L-rhamnopyranoside (**7**), *trans*-taxifolin 3-*O*-*α*-L-arabinopyranoside (**8**), *cis*-dihydrokaempferol 3-*O*-*α*-L-arabinopyranoside (**9**), catechin (**10**), myricetin 3-*O*-*α*-L-rhamnopyranoside (**11**), quercetin 3-*O*-*α*-L-arabinopyranoside (**12**), quercetin 3-*O*-*α*-L-arabinofuranoside (**13**), quercetin 3-*O*-(3″-galloyl)-*α*-L-rhamnopyranoside (**14**), quercetin 3-*O*-(2″-galloyl)-*α*-L-rhamnopyranoside (**15**), and epicatechin 3-*O*-gallate (**16**), were isolated from the leaves of *Ruprechtia polystachya* GRISEB. (Polygonaceae). Their structures were established on the basis of extensive 1D- and 2D-NMR experiments as well as MS analyses. All compounds, except **1**, showed inhibition of the enzyme glucose-6-phosphatase in intact microsomes.

Introduction. – *Ruprechtia polystachya* GRISEB. (Polygonaceae) is a tree distributed from Mexico to northern Argentina and Uruguay where it is known as '*vivaro bianco*', '*vivaro crespo*', or '*marmelero*'; it is also widespread in the Mediterranean area, especially in Spain and Egypt, as ornamental tree [1]. The genus *Ruprechtia* is one of the smallest and least explored Polygonaceae genera and comprises *ca.* 17 species. Few phytochemical and biological studies were reported for plants of this genus: a new isocarbostyril derivative, secalonic acid A, lichexanthone, and other phenolic compounds were identified in *R. tangarana* [2], some sterols isolated from *R. triflora* showed antitubercular activity [3], while a study of the EtOH extract of *R. apetala* reported the complete inhibition of the enzyme acetylcholinesterase, which is implicated in some neurodegenerative disorders [4]. However, no phytochemical studies on *R. polystachya* have been yet reported in literature.

As part of an ongoing research program on plants acclimatized at the El Zoharia Research Garden of Cairo, we performed a phytochemical study of *R. polystachya* leaves and describe here the isolation and structure elucidation of two new, **1** and **2**, together with fourteen, *i.e.*, **3**–**16**, known phenolic derivatives (*Fig.*).

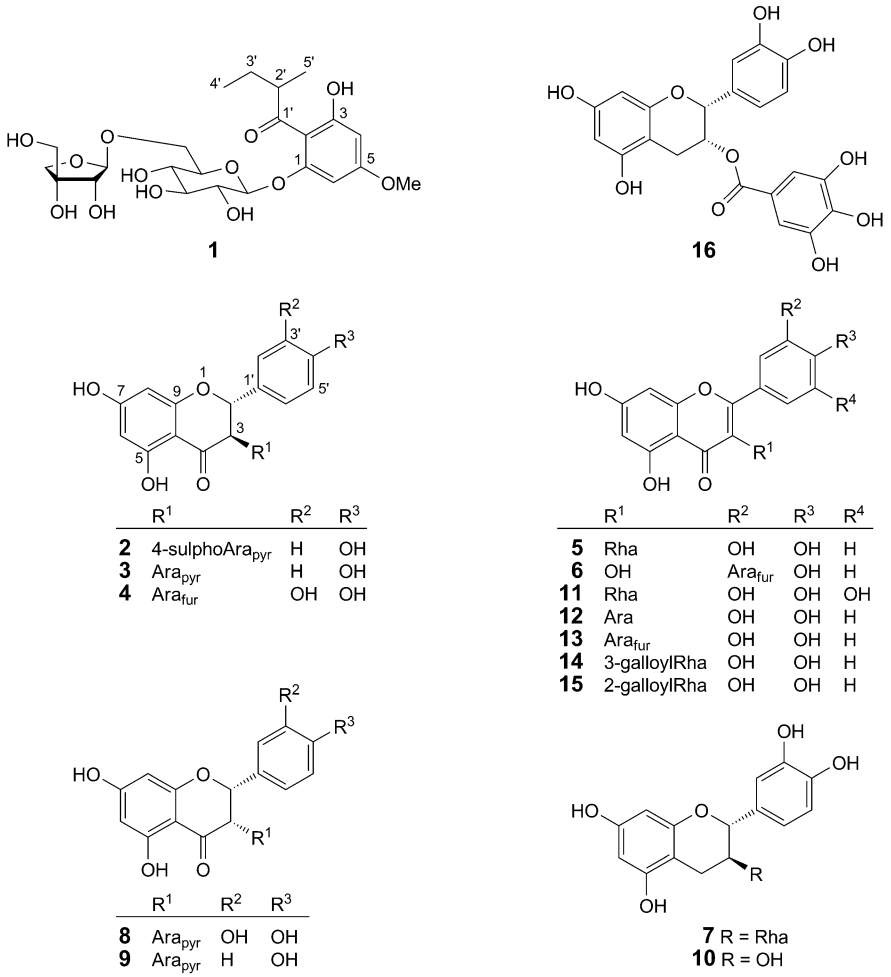

Figure. *Chemical structures of isolated compounds from* Ruprechtia polystachya (Ara$_{pyr}$ = α-L-arabinopyranose; Ara$_{fur}$ = α-L-arabinofuranose; Rha = α-L-rhamnopyranose)

Since some flavonoids were reported to have antihyperglycaemic activity [5], we assayed, in a preliminary biological study, all compounds isolated from *R. polystachya* as inhibitors of enzyme glucose-6-phosphatase. The hyperglycemia in diabetic patients is mainly due to an enhanced endogenous glucose production by liver and kidney through an increase in glycogenolysis and gluconeogenesis; the enzyme glucose-6-phosphatase (G-6-Pase EC 3.1.3.9) catalyzes the last step of both metabolic ways; accordingly, inhibition of G-6-Pase could be useful in the control of hyperglycemia present in diabetic patients [6]. According to the model of a catalytic subunit and transporters [7][8], the G-6-Pase is constituted by a relatively unspecific catalytic subunit, the active center of which is located in the lumen of the endoplasmic reticulum. In consequence, the substrate, glucose-6-phosphate, produced in the cytoplasm, has to

be transported by the T1 transporter into the endoplasmic reticulum cistern. The products of the enzyme reaction, phosphate and glucose, are exported by the T2 and T3 (also called GLUT 7) transporters, respectively. When the G-6-Pase activity is measured using intact microsomes, all the components of the system are required for the enzyme function; on the other hand, when the microsomal membrane is disrupted by the use of histones [9], only the catalytic subunit participates in the reaction.

Results and Discussion. – 1. *Structure Elucidation.* Extensive chromatographic purification of the $CHCl_3$/MeOH and MeOH extracts of the leaves of *R. polystachya* afforded compounds **1**–**16**. Compound **1** showed a *quasi*-molecular-ion peak at *m*/*z* 517.2008 ($[M-H]^-$) in the HR-ESI-MS spectrum (negative-ion mode) and a fragment ion at *m*/*z* 223.2015 ($[M-H-132-162]^-$). The ^{1}H-NMR spectrum (*Table 1*) showed in the aliphatic region signals for a 2-methylbutanoyl moiety at δ(H) 3.92–3.98 (*m*, H–C(2′)), 1.81–1.86 (*m*, H_a–C(3′)), 1.40–1.45 (*m*, H_b–C(3′)), 1.15 (*d*, *J* = 6.5, Me(5′)), 0.92 (*t*, *J* = 7.0, Me(4′)), and one MeO signal at δ(H) 3.85 (*s*, MeO), while, in the aromatic region, two *meta*-coupled *doublet*s appeared at δ(H) 6.16 (*d*, *J* = 1.8, H–C(4)) and 6.33 (*d*, *J* = 1.8, H–C(6)), indicating an asymmetrically substituted phloroglucinol [10]. The ^{1}H-NMR spectrum displayed also typical signals for sugar moieties, showing those of two anomeric H-atoms at δ(H) 5.04 (*d*, *J* = 7.5, H–C(1_{Glc})) and 4.96 (*d*, *J* = 1.8, H–C(1_{Api})), respectively. The chemical shifts of all the individual H-atoms of the sugar moieties were ascertained by a combination of 1D-TOCSY and DQF-COSY spectroscopic analyses, and the ^{13}C-NMR chemical shifts (*Table 1*) of the corresponding C-atoms were assigned unambiguously from the HSQC spectrum. Chemical shifts, multiplicities of the signals, and the absolute values of the coupling constants in the NMR spectra indicated the presence of one β-glucopyranose and one β-apiofuranose moiety [11]. Apiofuranosyl configuration was also confirmed by comparing ^{1}H,^{1}H scalar coupling constants with those reported for methyl apiofuranosides and DL-apiose, as well as NOE correlations. The 2D-NOESY spectrum of **1** exhibited cross-peaks between H–C(2_{Api}) and the H-atoms of the $HOCH_2$ group, H–C(2_{Api}) and H_b–C(4_{Api}), indicating that H–C(2_{Api}), the $HOCH_2$ group, and H_b–C(4_{Api}) were on the same face of the sugar ring [12]. Direct evidence of the glycosylation site and interglycosydic linkage was obtained from the HMBC correlations of H–C(1_{Api}) (δ(H) 4.96) with C(6_{Glc}), and of H–C(1_{Glc}) (δ(H) 5.04) with C(1). Other key correlations were observed between H–C(2′) (δ(H) 3.92–3.98) and C(1′) (δ(C) 211.0), H–C(2′) (δ(H) 3.92–3.98) and C(3′) (δ(C) 28.8), H–C(2′) (δ(H) 3.92–3.98) and C(4′) (δ(C) 11.7); MeO (δ(H) 3.85) and C(5) (δ(C) 167.3); H–C(4) (δ(H) 6.16) and C(2) (δ(C) 106.9), H–C(4) (δ(H) 6.16) and C(5) (δ(C) 167.3); H–C(4) (δ(H) 6.16) and C(6) (δ(C) 95.0); H–C(6) (δ(H) 6.33) and C(2) (δ(C) 106.9), H–C(6) (δ(H) 6.33) and C(3) (δ(C) 164.4). The positions of MeO and OH groups in the phloroglucinol ring were deduced from 2D-NOESY experiment. NOE Correlations were observed between MeO and H–C(4) (δ(H) 6.16); MeO and H–C(6) (δ(H) 6.33); H–C(6) (δ(H) 6.33) and H–C(1_{Glc}) (δ(H) 5.04), substantiating the presence of OH at C(3) and of MeO at C(5), respectively. The absolute configuration of the sugar units was assigned after hydrolysis of **1** with 1N HCl. The hydrolysate was treated with 1-(trimethylsilyl)-1*H*-imidazole and subsequently analyzed by GC on a chiral column. The sugars were identified as D-glucopyranose and D-apiofuranose by comparison of their retention

Table 1. *^{1}H- and ^{13}C-NMR Data* (600 MHz, CD_3OD) *of* **1** *and* **2**. δ in ppm, J in Hz. Atom numbering as indicated in the *Figure*.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	160.8	–	–
H–C(2)	–	106.9	5.20 (*d*, *J* = 10.0)	83.4
H–C(3)	–	164.4	4.81 (*d*, *J* = 10.0)	76.3
H–C(4)	6.16 (*d*, *J* = 1.8)	96.5	–	179.2
C(5)	–	167.3	–	161.6
H–C(6)	6.33 (*d*, *J* = 1.8)	95.0	5.93 (*d*, *J* = 2.0)	97.3
C(7)	–	–	–	165.1
H–C(8)	–	–	5.90 (*d*, *J* = 2.0)	96.0
C(9)	–	–	–	158.0
C(10)	–	–	–	104.9
C(1′)	–	211.0	–	122.7
H–C(2′)	3.92–3.98 (*m*)	47.0	7.38 (*d*, *J* = 8.0)	130.1
CH_2(3′)	1.81–1.86 (*m*), 1.40–1.45 (*m*)	28.8	6.84 (*d*, *J* = 8.0)	116.2
H–C(4′)	0.92 (*t*, *J* = 7.0)	11.7	–	160.5
H–C(5′)	1.15 (*d*, *J* = 6.5)	16.0	6.84 (*d*, *J* = 8.0)	116.2
H–C(6′)	–	–	7.38 (*d*, *J* = 8.0)	130.1
*Me*O–C(5)	3.85 (*s*)	56.0	–	–
H–C(1_{Glc})	5.04 (*d*, *J* = 7.8)	101.5	–	–
H–C(2_{Glc})	3.48 (*dd*, *J* = 9.0, 7.8)	74.6	–	–
H–C(3_{Glc})	3.48 (*t*, *J* = 9.0)	78.3	–	–
H–C(4_{Glc})	3.41 (*t*, *J* = 9.0)	71.4	–	–
H–C(5_{Glc})	3.60–3.65 (*m*)	77.0	–	–
CH_2(6_{Glc})	4.03 (*dd*, *J* = 12.0, 3.0), 3.58 (*dd*, *J* = 12.0, 4.5)	68.4	–	–
H–C(1_{Api})	4.96 (*d*, *J* = 1.8)	110.8	–	–
H–C(2_{Api})	3.89 (*d*, *J* = 1.8)	77.9	–	–
H–C(3_{Api})	–	80.5	–	–
CH_2(4_{Api})	3.98 (*d*, *J* = 10.0), 3.76 (*d*, *J* = 10.0)	74.8	–	–
H–C(5_{Api})	3.57 (br. *s*)	65.5	–	–
H–C(1_{Ara})	–	–	3.86 (*d*, *J* = 7.2)	101.2
H–C(2_{Ara})	–	–	3.57 (*dd*, *J* = 8.5, 7.2)	71.0
H–C(3_{Ara})	–	–	3.82 (*dd*, *J* = 8.5, 3.0)	71.5
H–C(4_{Ara})	–	–	4.58–4.61 (*m*)	73.1
CH_2(5_{Ara})	–	–	3.57 (*dd*, *J* = 11.4, 3.5), 4.23 (*dd*, *J* = 11.4, 2.0)	60.5

times with those of authentic samples derivatized under the same conditions. On the basis of these data, the structure of compound **1** was established as 5-methyl-2-(2-methylbutanoyl)phloroglucinol 1-*O*-(6-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside.

Compound **2** showed a *quasi*-molecular-ion peak at *m*/*z* 499.1940 ($[M-H]^-$) in the negative-ion-mode HR-ESI-MS. Other product ion peaks were observed at *m*/*z* 419.1951 ($[M-H-80]^-$) and 287.1213 ($[M-H-80-132]^-$), corresponding to the loss of 80 amu followed by one pentose moiety. The ^{1}H-NMR spectrum (*Table 1*) showed aromatic signals at δ(H) 7.38 (*d*, *J* = 8.0, H–C(2′), H–C(6′)), 6.84 (*d*, *J* = 8.0,

H–C(3′), H–C(5′)), 5.93 (*d*, *J* = 2.0, H–C(6)), and 5.90 (*d*, *J* = 2.0, H–C(8)), and two signals at δ(H) 5.20 (*d*, *J* = 10.0, H–C(2)) and 4.81 (*d*, *J* = 10.0, H–C(3)) attributed to a dihydrokaempferol aglycone [13]. The *trans* configuration was determined from the chemical shifts and coupling constants of H–C(2) and H–C(3). The ^{1}H-NMR spectrum displayed also signals between δ(H) 3.55 and 4.61 typical of a sugar moiety (*Table 1*), with one anomeric H-atom signal at δ(H) 3.86 (*d*, *J* = 7.2, H–C(1_{Ara})). Chemical shifts, multiplicities of the signals, and the values of the coupling constants in the ^{1}H-NMR spectrum established the presence of one α-arabinopyranose moiety. Arabinose in the pyranose form was also evident from the ^{13}C-NMR data (*Table 1*) [14]. The structure of the aglycone and sugar unit were deduced using 1D-TOCSY and 2D-NMR experiments. These data revealed that the arabinopyranosyl unit was substituted at C(4) by a sulfo or phospho group, as indicated by downfield shifts of the H–C(4_{Ara}) (δ(H) 4.58–4.61) and C(4_{Ara}) (δ(C) 73.1) signals. An unambiguous determination of the glycosylation site was obtained from the HMBC spectrum which showed key correlation peaks between the H-atom signal at δ(H) 3.86 (H–C(1_{Ara})) and C(3) of the aglycone (δ(C) 76.3). The configuration of the sugar unit was assigned after hydrolysis of **2** with 1N HCl and GC analysis of trimethylsilylated sugars through a chiral column. The sugar unit was determined as L-arabinopyranose. To determine the presence of a phosphate or a sulfate group, compound **2** was subjected to complete exchange of H to D for spectrometric analysis [15]. The HR-ESI-MS spectrum in the negative-ion mode showed a *quasi*-molecular-ion peak at *m*/*z* 505.1821 ($[M-D]^-$) corresponding to weight of **2** when all acidic and OH H-atoms exchanged for D-atoms. The fragment-ion peak observed at *m*/*z* 425.2063 corresponded to the loss of a group of weight 79.9758. The theorical value for the sulfate group is 79.957, whereas that for the phosphate group is 80.973, establishing that the group esterifying the arabinopyranose moiety was sulfate [15]. Consequently, the structure of **2** was established as *trans*-2,3-dihydrokaempferol 3-*O*-(4-*O*-sulfo-α-L-arabinopyranoside), a new sulfated flavonoid.

Compounds **3**–**16** were identified as *trans*-dihydrokaempferol 3-*O*-α-L-arabinopyranoside (**3**) [13], *trans*-taxifolin 3-*O*-α-L-arabinofuranoside (**4**) [16], quercetin 3-*O*-α-L-rhamnopyranoside (**5**) [17], quercetin 3′-*O*-α-L-arabinofuranoside (**6**) [18], catechin 3-*O*-α-L-rhamnopyranoside (**7**) [19], *trans*-taxifolin 3-*O*-α-L-arabinopyranoside (**8**) [13], *cis*-dihydrokaempferol 3-*O*-α-L-arabinopyranoside (**9**) [13], catechin (**10**) [20], myricetin 3-*O*-α-L-rhamnopyranoside (**11**) [21], quercetin 3-*O*-α-L-arabinopyranoside (**12**) [17], quercetin 3-*O*-α-L-arabinofuranoside (**13**) [22], quercetin 3-*O*-(3″-galloyl)-α-L-rhamnopyranoside (**14**) [23], quercetin 3-*O*-(2″-galloyl)-α-L-rhamnopyranoside (**15**) [24], and epicatechin 3-*O*-gallate (**16**) [20] by comparison of their spectral ^{1}H- and ^{13}C-NMR, and MS data with the literature values. To the best of our knowledge, this is the first report describing the structure of flavonoids in *Ruprechtia* genus.

2. *Biological Studies.* The inhibitory activities of the compounds isolated from *R. polystachya* leaves were evaluated towards the G-6-Pase system *in vitro* (*Table 2*). G-6-Pase Assay was carried out using intact and disrupted microsomes [25–27] in the absence (control) or presence of 50 μM of each one of the isolated compounds **1**–**16**; phlorizin, a known inhibitor of T1 transporter of the G-6-Pase system [28], was used as positive control at the same concentration as the tested compounds. With the exception of the pholoroglucinol glycoside **1**, all compounds inhibited the enzyme towards intact microsomes, **11**, **12**, **15**, and **16** being the most active ones with 69, 57, 71, and 64% of

inhibition in comparison with control, respectively. On the other hand, compounds **4**, **8**, and **9** showed the lowest inhibitory effects on intact microsomal G-6-Pase. Compounds **5**, **6**, and **7** exerted inhibitory activities (29, 21, and 20%, resp.) of the enzyme in intact microsomes similar to that shown by phlorizin. Compounds **2**, **3**, **10**, **13**, and **14** showed inhibition of *ca.* 35–50% towards the intact microsomal G-6-Pase. Compounds **3**, **5**, **11**, **14**, and **16** were the only ones that moderately inhibit the G-6-Pase of disrupted microsomes.

Table 2. *Effects of Flavonoids Isolated from* R. polystachya *on Hepatic Microsomal Glucose-6-phosphatase System*

Compound	Intact microsomes				Disrupted micosomes	
	Activity[a])	$p <$ [b])	Inhibition [%][c])	IC_{50}[d])	Activity	Inhibition [%][c])
Control	3.13 ± 0.35				6.18 ± 1.02	
Phlorizin	2.25 ± 0.13		28 ± 3	466 ± 19[e])	6.23 ± 0.44	–
1	3.10 ± 0.15		1		6.39 ± 1.37	–
2	1.84 ± 0.24	0.05	41 ± 6		6.41 ± 1.34	–
3	1.97 ± 0.25	NS	37 ± 7		5.86 ± 0.79	5
4	2.53 ± 0.10		19 ± 2		8.03 ± 0.32	–
5	2.23 ± 0.27		29 ± 7		5.72 ± 0.80	7
6	2.47 ± 0.37		21 ± 9		6.40 ± 1.72	–
7	2.50 ± 0.46		20 ± 12		7.83 ± 0.17	–
8	2.66 ± 0.22		15 ± 6		6.34 ± 0.07	–
9	2.73 ± 0.24		13 ± 6		8.61 ± 0.65	–
10	2.03 ± 0.47	0.5	35 ± 12		6.36 ± 0.05	–
11	0.98 ± 0.33	0.005	69 ± 6	63 ± 19[f])	5.98 ± 0.64	3
12	1.35 ± 0.52	0.05	57 ± 13	34 ± 13[f])	6.95 ± 0.49	–
13	1.96 ± 0.20	NS	37 ± 5		5.05 ± 0.24	18 ± 4
14	1.57 ± 0.34	0.05	50 ± 9	83 ± 25[f])	5.37 ± 0.65	13 ± 11
15	0.92 ± 0.09	0.0001	71 ± 2	28 ± 1[f])	6.45 ± 1.30	–
16	1.14 ± 0.15	0.0005	64 ± 4	48 ± 6[f])	5.24 ± 0.34	15 ± 6

[a]) Activity is expressed as nmol of phosphate release/h/mg of protein. Values are means ($n = 3$) ± standard deviation. [b]) Statistical significance was estimated by the *t-Student* test. [c]) Results are expressed as the percentage of inhibition in comparison with the control. Values are means ($n = 3$) ± standard deviation. When not reported, the standard deviation was higher than the mean. [d]) IC_{50} Values were determined in intact microsomes using 1 mM glucose-6-phosphate as substrate and increasing concentrations of the compounds. Values are expressed in μM and are means ($n = 4$) ± standard deviation. [e]) Reference value [5]. [f]) Statistically significant at $p < 0.00005$ in relation to phlorizin.

The inhibition of the G-6-Pase in intact microsomes, together with the lack of activity in disrupted microsomes, by flavonoids isolated from *R. polystachya* indicate that they inhibit one of the transporters of the G-6-Pase system, probably the glucose-6-phosphate transporter (T1). This hypothesis was also supported by the literature data [29]. The slight inhibition of the enzyme in disrupted microsomes by compounds **3**, **5**, **11**, **14**, and **16** should be due to the effects on the catalytic subunit, since, under these conditions, there is no need of the transporter activity. It is interesting that the majority of the isolated compounds displayed a behavior similar to that of phlorizin that mainly inhibits the T1 transporter with little effect on the catalytic subunit of the G-6-Pase

system. IC_{50} Values were recorded for compounds showing more than 50% inhibition. All the values obtained for compounds **11**, **12**, **14**, **15**, and **16** were lower than those reported for phlorizin, with the IC_{50} value of compound **15** being very close to that reported earlier [5].

Compound **3** exhibited approximately three times higher inhibitory capacity on the G-6-Pase of intact microsomes than **9**, suggesting that the *trans*-configuration of the *C*-ring substituents is important for the biological activity. Compound **8** showed approximately half of the biological activity (15% inhibition of intact microsome G-6-Pase) of compound **3** (37%), with the structural differences between them being the presence of a OH group at C(3′) and the *cis*-configuration. Among quercetin derivatives, compound **6**, which showed the lowest biological activity, was the only one possessing the sugar moiety at C(3′). On the contrary, the inhibitory capacity on G-6-Pase of intact microsomes increased if the sugar was at C(3) (compounds **11**, **12**, and **13**). As it was reported earlier [5], the presence of a galloyl group in the rhamnopyranosyl moiety (compounds **14** and **15**) considerably increased the biological activity. The galloyl moiety is likely to be also responsible for the strong activity of compound **16**.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*, Sweden). TLC: Precoated *Kieselgel 60* F_{254} plates (0.25 mm, *Merck*, D-Darmstadt); detection with $Ce(SO_4)_2/H_2SO_4$ and NTS/PEG. Prep. HPLC: *Shimadzu LC-8A* series pumping system (*Shimadzu*, Kyoto, Japan), equipped with a *Waters R401* refractive index detector (*Waters Co.*, Milford, MA, USA) and *Shimadzu* injector using a *μ-Bondapack* C_{18} semi-prep. column (300 × 7.8 mm; 5 μm). GC: *Dani GC 1000* instrument with a L-*CP-Chirasil-Val* column (0.32 mm × 25 m); temp. of both the injector and detector, 200°; a temp. gradient system was used for the oven, starting at 100° for 1 min and increasing up to 180° at a rate of 5°/min. Optical rotations: *Perkin–Elmer 241* polarimeter equipped with a Na lamp (589 nm) and a 1-dm microcell. NMR Spectra: *Bruker DRX-600* spectrometer using the UXNMR software package; δ in ppm rel. to CD_3OD (δ(H) 3.31, δ(C) 49.0 ppm), *J* in Hz. HR-MS: *Q-TOF Premier Instrument* (*Waters Co.*, Milford, MA, USA), equipped with a nanoelectrospray ion source. ESI-MS: *LCQ Advantage ThermoFinnigan* spectrometer (*ThermoFinnigan*, San Jose, CA, USA), equipped with an Xcalibur software.

Plant Material. The leaves of *R. polystachya* were collected in El Zoharia Research Garden of Cairo, Egypt, in May 2007, and identified by Dr. *Mamdouh Shokry* (El Zoharia Research Garden, Cairo, Egypt). A voucher specimen (No. 2211 *Ruprechtia polystachya*/1) was deposited with the Herbarium Hortii Botanici Pisani, Pisa, Italy.

Extraction and Isolation. Dried leaves of *R. polystachya* (900 g) were macerated with hexane, $CHCl_3$, $CHCl_3$/MeOH 9:1, and MeOH by exhaustive maceration (3 × 2 l) at r.t., to give 7.9, 17.6, 14.8, and 74.1 g of the respective residues. A portion (5.0 g) of the $CHCl_3$/MeOH extract was chromatographed on *Sephadex LH-20* (5 × 100 cm) with MeOH as eluent at flow rate 0.8 ml/min. Fractions of 15 ml were collected and combined into nine fractions, *Frs. A – I*, according to TLC analyses. *Frs. B* (136 mg), *E* (118 mg), *F* (84 mg), and *I* (20 mg) were separately purified by RP-HPLC with MeOH/H_2O 2:3 to give compound **1** (t_R 33 min; 2.1 mg) from *Fr. B*, with MeOH/H_2O 45:55 to give compound **3** (t_R 18 min; 8.6 mg) from *Fr. E*, with MeOH/H_2O 55:45 to give compounds **4** (t_R 7 min; 3.5 mg) and **5** (t_R 13 min; 5.3 mg), from *Fr. F*, and with MeOH/H_2O 1:1 to give compound **6** (t_R 25 min; 2.5 mg) from *Fr. I*. The MeOH extract was partitioned between BuOH and H_2O. A portion (5.0 g) of the BuOH-soluble fraction was separated by CC (*Sephadex LH-20* (5 × 100 cm); MeOH; flow rate 0.8 ml/min) to give twelve pooled fractions, *Frs. A–L*, according to TLC analyses. *Fr. C* (112 mg) was purified by RP-HPLC with MeOH/H_2O 25:75 to give compound **2** (t_R 15 min, 4.8 mg). *Frs. D* (171 mg), *F* (175 mg), and *G* (149 mg) were

separately purified by RP-HPLC with MeOH/H_2O 2:3 to give compounds **7** (t_R 8 min; 5.3 mg) from *Fr. D*, compounds **8** (t_R 13 min; 3.2 mg,) and **9** (t_R 23 min; 2.7 mg) from *Fr. F*, compounds **10** (t_R 6 min; 8.1 mg), **11** (t_R 19 min; 3.7 mg), **12** (t_R 28 min; 8.3 mg), and **13** (t_R 35 min; 4.5 mg) from *Fr. G. Frs. I* (121 mg) and *K* (160 mg) were separately submitted to RP-HPLC with MeOH/H_2O 1:1 to give compounds **14** (t_R 22 min; 4.6 mg) and **15** (t_R 27 min; 2.9 mg) from *Fr. I*, and compound **16** (t_R 6 min; 8.3 mg) from *Fr. K*.

*5-Methyl-2-(2-methylbutyanoyl)phloroglucinol 1-*O*-(6-*O*-β-*D*-Apiofuranosyl)-β-*D*-glucopyranoside* (= *3-Hydroxy-5-methoxy-2-(2-methylbutanoyl)phenyl 6-*O*-β-*D*-Apiofuranosyl-β-*D*-glucopyranoside*; **1**). Yellowish amorphous powder. $[\alpha]_D^{25} = -83$ ($c = 0.1$, MeOH). UV (MeOH): 286 (3.93). ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 1*. HR-ESI-MS: 517.2008 ($[M-H]^-$, $C_{23}H_{33}O_{13}^-$; calc. 517.1921), 223.2015 ($[M-H-132-162]^-$).

trans-*2,3-Dihydrokaempferol 3-*O*-(4-*O*-Sulfo-α-L-arabinopyranoside)* (= rel-(*2*R,*3*R)*-2,3-Dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-3-[(4-*O*-sulfo-α-L-arabinopyranosyl)oxy]-4*H*-1-benzopyran-4-one*; **2**). Yellowish amorphous powder. $[\alpha]_D^{25} = -18$ ($c = 0.1$, MeOH). UV (MeOH): 293 (4.02), 334 (sh, 3.45). ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 1*. HR-ESI-MS: 499.1940 ($[M-H]^-$, $C_{20}H_{19}O_{13}S^-$; calc. 499.0547), 419.1951 ($[M-H-80]^-$), 287.1213 ($[M-H-80-132]^-$).

Acid Hydrolysis of Compounds **1** *and* **2**. A soln. of each compound (2.0 mg) in 1N HCl (1 ml) was stirred at 80° in a stoppered reaction vial for 4 h. After cooling, the soln. was evaporated under N_2. Each residue was dissolved in 1-(trimethylsilyl)-1*H*-imidazole and pyridine (0.2 ml), and the soln. was stirred at 60° for 5 min. After drying, the residue was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC. The sugars were identified by comparison of their retention times (t_R) with those of authentic samples of D-glucose, D-apiose, and L-arabinose (*Sigma–Aldrich*), treated with 1-(trimethylsilyl)-1*H*-imidazole in pyridine.

Glucose-6-phosphatase Assay. Glass-*Teflon* homogenizer, centrifuge *RC5C Sorvall Instrument* (*Du Pont Delawer*, USA), ultracentrifuge *Beckman* model *L5–75* (*Spinco Beckman Instrument*, CA, USA), *Shaker Bath* model *2564* (*Forma Scientific*, USA), *Novaspect II* spectrophotometer (*Pharmacy LKB*, Sweden) were used. All chemicals used were of anal. grade and obtained from *Sigma–Aldrich* (USA). Liver microsomes were purified, as described in [25], from rats fasted overnight; in brief: livers were homogenized in 3 volumes of 0.32M sucrose 3 mM $MgCl_2$, centrifuged at 20000*g* for 20 min at 4°, the pellet was discarded, and the supernatant was centrifuged at 105000*g* for 1 h at 4°, and the pellet constituted the microsomal fraction. The microsomal fraction was resuspended in 0.25 mM sucrose, 1 mM $MgCl_2$, and 5 mM *HEPES* pH 6.5 to give a final protein concentration of 1 mg/ml and frozen at −80° until use. Proteins were estimated using a modification of the *Lowry* method [26]. Enzymatic assays were performed by the method of *Burchell et al.* [27] with intact and disrupted (histone treated) microsomes [9]. To study the effect of the isolated flavonoid, the glucose-6-phosphatase (G-6-Pase) assay was carried out using intact and disrupted microsomes in the absence (control) or in the presence of 50 μM of each one of the isolated compounds **1**–**16**. The compounds were added to the G-6-Pase assay at a final concentration of 50 μM. The final concentration of DMSO in the control and exper. assays was 0.5%. All the microsomes used were at least 95% intact, as determined by the hydrolysis of mannose-6-phosphate [28]. IC_{50} Determination was carried out for those compounds that showed an inhibition higher than 50% of the G-6-Pase activity in intact microsomes *i.e.*, **11**, **12**, **14**, **15**, and **16**, as described by *Arion et al.* [30] using 1 mM G-6-Pase as mentioned above in the absence (0% inhibition) or in the presence of increasing concentrations of the tested compounds.

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Received November 22, 2010