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THE FLAVONOIDS OF *ALLIUM NEAPOLITANUM**

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Key Word Index—*Allium neapolitanum*; Liliaceae; flavonol glycosides; branched trisaccharides; anti-platelet aggregation activity.

Abstract—An investigation of the extracts from *Allium neapolitanum* has led to the isolation of 13 flavonoid glycosides, based on kaempferol, quercetin and isorhamnetin. Four of them are new compounds and have been identified as: kaempferol 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-4-*O*-β-D-glucopyranosyl]-β-D-glucopyranoside}, isorhamnetin 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-6-*O*-β-D-glucopyranosyl]-β-D-glucopyranoside}, isorhamnetin 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-6-*O*-β-D-glucopyranosyl]-β-D-glucopyranoside}-7-*O*-β-D-glucopyranoside and isorhamnetin 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-6-*O*-β-D-gentiobiosyl]-β-D-glucopyranoside}. The isolated compounds were evaluated for their anti-aggregation human platelet activity.

INTRODUCTION

In the Italian flora, the genus *Allium* is represented by about 50 bulbous species, many of them widely distributed in the Mediterranean area [1]. *Allium neapolitanum* Cyr. is a perennial bulbous herb with a characteristic smell of garlic, which grows in pastures, cultivated grounds and dry, open habitats of the Mediterranean region [2].

A chemical study of *A. neapolitanum* was undertaken during a systematic survey on *Allium* species from southern Italy. It is similar to *A. ursinum* a plant which is known to produce flavonoid glycosides with anti-aggregation platelet activity [3]. We now report the isolation of 13 flavonoid glycosides, four of which are new compounds from *A. neapolitanum*.

RESULTS AND DISCUSSION

Whole plants of *A. neapolitanum* were extracted with water at room temperature and the aqueous extracts subjected to a Amberlite XAD-2 chromatography. The methanol soluble fraction, on repeated chromatographic separations (see Experimental), yielded the flavonol glycosides **1**–**13** (Fig. 1). The structures of the known compounds (**1**–**9**) were assigned by comparison of their mass, UV and ^{1}H NMR spectral data with those reported in the literature [4, 5].

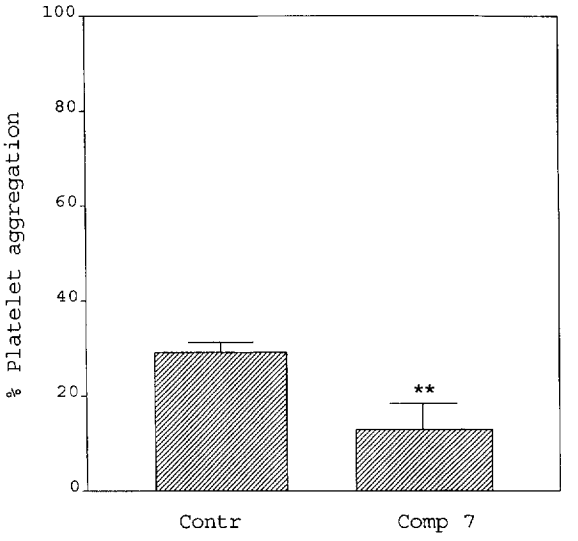

Fig. 1. Inhibitory effect of compound **7** (10^{-5} M) on collagen-induced platelet aggregation. (Control 29.18±2.05%; compound **7**, 13.00±5.40%.) $^{**}P < 0.01$, two tailed Student's test versus control group.

The new compound **10** showed a pseudomolecular

* Part 2 in the series 'Chemistry of the genus *Allium*'. For Part 1, see ref. [3]

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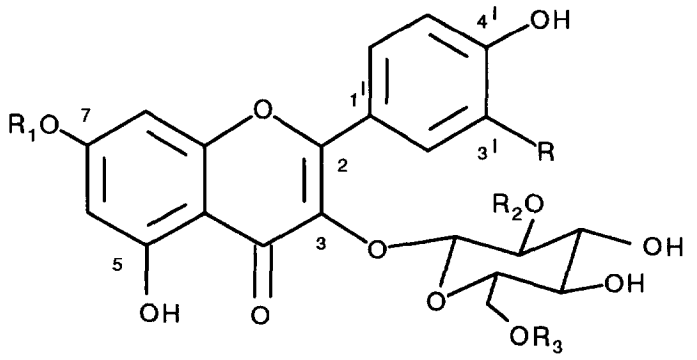
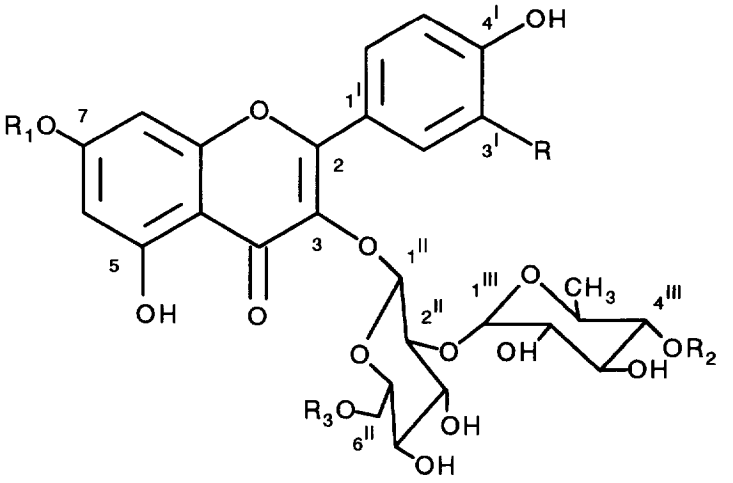


	R	R_1	R_2	R_3
1	H	H	H	H
2	OH	H	H	H
3	OMe	H	H	H
4	H	H	α-Rha	H
5	OMe	H	H	β-Glc
6	H	β-Glc	α-Rha	H
7	H	β-Glc (1 → 2) β-Glc	H	H
8	OH	β-Glc (1 → 2) β-Glc	H	H
9	OMe	β-Glc	H	β-Glc

ion peak at m/z 755 $[M-H]^-$ in the negative-ion FAB-mass spectrum. Its UV spectrum contained the characteristic bands of a kaempferol, substituted at position 3, as indicated by the bathochromic shift on addition of diagnostic reagents [6] (Table 1). The ^1H and ^{13}C NMR spectra (Table 2) confirmed this structural hypothesis and the presence of a number of oxymethylene and oxymethine signals indicated the glycosidic nature of the compound. Signals attributable to three anomeric protons and to the relevant carbon atoms in the ^1H and ^{13}C NMR spectra, respectively (Table 2), were easily identified so determining the number of sugar residues.

To define the nature of the sugars, **10** was acetylated to give the nonacetate **10a** (see Experimental), whose ^1H NMR spectrum showed a better resolution of the oxymethine and oxymethylene proton resonances. Three anomeric proton signals at δ4.67, 4.88 and 5.68 were identifiable in the ^1H NMR spectrum (see Table 2).

Using the anomeric proton at δ4.67 (d, J = 8.1 Hz) as a starting point, analysis of COSY and HOHAHA experiments allowed the identification, in sequence, of four oxymethine and one oxymethylene groups (Table 2). All these protons, except H-5^{IV}, were linked to an acetoxylated carbon atom, as indicated by their low-field resonances, thus suggesting a terminal hexose unit in the pyranose form. The sugar moiety was identified as β-glucose on the basis of the large couplings observed for all the oxymethine protons, implying their axial position. The second anomeric proton at δ4.88 (*bs*) was shown to belong to an α-rhamnose



	R	R_1	R_2	R_3
10	H	H	β-Glc ^{IV}	H
11	OMe	H	H	β-Glc ^{IV}
12	OMe	β-Glc ^{V}	H	β-Glc ^{IV}
13	OMe	H	H	β-Glc ^{V} (1→6) β-Glc ^{IV}

Table 1. UV spectral absorptions of **10–13**

	λ_{max} in					
	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
10	355	400	400	400	387	344
	300 sh	324	353	353	313	285
	266	270	301	306	277	256
			273	272		
11	356	418	404	403	407	360
	267	331	365	361	324	271
	256	284	302 sh	306 sh	274	256
			277 sh	278 sh		
			267	266		
12	354	426	403	405	422	363
	269 sh	280	360	366	369	272
	256	250 sh	305 sh	305	271 sh	257
			267	268	256	
13	355	420	406	402	410	358
	268	332	344	340	320	271 sh
	257	282	305	305 sh	278	258
			280	277		
			268 sh	265 sh		

Table 2. ^{13}C and ^{1}H assignments* for compounds **10** and **10a**

C/H	**10**		**10a**
	δ_C (mult.)	δ_H (mult., *J* Hz)	δ_H (mult., *J* Hz)
2	158.4 (C)		
3	134.3 (C)		
4	179.2 (C)		
5	161.3 (C)		
6	101.9 (CH)	6.20 (*d*, 2.1)	6.84 (*d*, 2.2)
7	163.1 (C)		
8	94.8 (CH)	6.39 (*d*, 2.1)	7.28 (*d*, 2.2)
9	158.4 (C)		
10	105.8 (C)		
1^{I}	123.1 (C)		
2^{I}–6^{I}	132.1 (CH)	8.07 (*d*, 8.7)	8.05 (*d*, 8.7)
3^{I}–5^{I}	116.1 (CH)	6.92 (*d*, 8.7)	7.21 (*d*, 8.7)
4^{I}	161.3 (C)		
3-*O*-Glc^{II}			
1	100.2 (CH)	5.73 (*d*, 7.6)	5.68 (*d*, 7.8)
2	80.0 (CH)	3.63 (*dd*, 7.6, 9.2)	3.73 (*dd*, 7.8, 9.3)
3	78.9 (CH)	3.58 (*t*, 9.2)	5.30 (*t*, 9.3)
4	71.3 (CH)	3.31 (*t*, 9.2)	4.95 (*t*, 9.3)
5	78.1 (CH)	3.25 (*ddd*, 1.7, 5.5, 9.2)	3.29 (*ddd*, 2.2, 4.0, 9.3)
6	62.4 (CH_2)	3.76 (*dd*, 1.7, 11.8)	4.00 (*dd*, 4.0, 12.5)
		3.50 (*dd*, 5.5, 11.8)	3.50 (*dd*, 2.2, 12.5)
2-*O*-Rha^{III}			
1	102.4 (CH)	5.25 (*bs*)	4.88 (*bs*)
2	72.2 (CH)	4.06[a]	5.01 (*bd*, 3.7)
3	72.2 (CH)	4.05[a]	5.32 (*dd*, 3.7, 10.0)
4	84.1 (CH)	3.59[a]	3.58 (*t*, 10.0)
5	68.5 (CH)	4.15[a]	4.21 (*dq*, 10.0, 6.2)
6	17.7 (CH_3)	1.08 (*d*, 6.2)	1.00 (*d*, 6.2)
4-*O*-Glc^{IV}			
1	105.8 (CH)	4.54 (*d*, 8.0)	4.67 (*d*, 8.1)
2	75.9 (CH)	3.19 (*dd*, 8.0, 9.0)	4.89 (*dd*, 8.1, 9.3)
3	78.4 (CH)	3.32[a]	5.07 (*t*, 9.3)[a]
4	71.8 (CH)	3.29[a]	5.06 (*t*, 9.34)
5	77.9 (CH)	3.25[a]	3.72 (*ddd*, 2.8, 4.7, 9.3)
6	62.4 (CH_2)	3.79 (*dd*, 11.8, 2.1)	4.19 (*dd*, 4.7, 12.1)
		3.66 (*dd*, 5.2, 11.8)	4.15 (*dd*, 2.8, 12.1)

*The spectra are recorded in CD_3OD for **10** and in $CDCl_3$ for **10a**.
[a]Superimposed by other signals; [b,c] signals with the same superscript are interchangeable.

which must be in the pyranose form as suggested by the coupling constants typical of a six-membered ring (Table 2) [7]. The high-field chemical shift of H-4^{III} (δ3.58) clearly indicated that the hydroxyl group at C-4 was glycosylated rather than acetylated. The last anomeric proton at δ5.68 (*d*, J = 7.8 Hz) by analysis of NMR data (see Table 2) was attributed to a 2-*O*-glycosylated-β-glucopyranose. The low-field chemical shift of its anomeric proton (δ5.68) indicated the linkage of this residue at C-3 of the aglycone [3].

The build up of the whole sugar moiety from the above substructures was inferred from some key peaks appearing in the ROESY-NMR spectrum: dipolar interaction of H-1^{IV} with H-4^{III} as well as that of rhamnose anomeric proton with H-2^{II}. A retrospective analysis of the NMR data of **10** allowed us to achieve full assignments of the ^{1}H and ^{13}C NMR resonances (Table 2). From these data **10** is identified as kaempferol 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-4-*O*-β-D-glucopyranosyl]-β-D-glucopyranoside}.

Compound **11** gave a FAB (negative-ion mode) mass spectrum with a quasi molecular ion peak at *m/z* 785 $[M-H]^-$. Examination of the ^{1}H and ^{13}C NMR spectra (Tables 3 and 4) indicated the presence of isorhamnetin, substituted in position 3, as confirmed by the UV spectral data (Table 1). In addition to the isorhamnetin moiety, NMR spectra showed signals corresponding to three saccharide residues. The sugar moieties were determined using the peracetylated compound **11a**, using as starting points the anomeric protons in the ^{1}H NMR spectrum for the analysis of COSY and HOHAHA experiments. The anomeric protons resonating at δ4.16 (*d*, J = 8.0 Hz) and δ4.94 (*bs*) were attributed to terminal residues of β-glucopyranose and α-rhamnopyranose, respectively (see Table 5 for assignments). The last anomeric proton

Table 3. ^{13}C assignments* for compounds **11**, **12** and **13**

C	**11**, δ_C (mult.)	**12**, δ_C (mult.)	**13**, δ_C (mult.)
2	158.1 (C)	158.3 (C)	158.2 (C)
3	134.4 (C)	135.4 (C)	134.4 (C)
4	179.1 (C)	179.6 (C)	179.1 (C)
5	163.0 (C)	162.5 (C)	163.1 (C)
6	99.7 (CH)	100.2 (CH)	99.7 (CH)
7	165.7 (C)	164.2 (C)	165.6 (C)
8	94.7 (CH)	95.61 (CH)	94.8 (CH)
9	158.3 (C)	159.5 (C)	158.3 (C)
10	106.0 (C)	107.6 (C)	106.0 (C)
1^I	123.5 (C)	123.1 (C)	123.3 (C)
2^I	114.6 (CH)	114.8 (CH)	114.6 (CH)
3^I	150.6 (C)		150.6 (C)
4^I	148.3 (C)		148.4 (C)
5^I	116.0 (CH)	116.0 (CH)	116.0 (CH)
6^I	123.2 (CH)	124.0 (CH)	123.3 (CH)
OCH_3	57.1 (CH_3)	57.1 (CH_3)	57.1 (CH_3)
3-*O*-Glc^{II}			
1	100.4 (CH)	100.2 (CH)	100.6 (CH)
2	80.1 (CH)	80.2 (CH)	80.1 (CH)
3	78.5 (CH)	78.5 (CH)	78.4 (CH)
4	71.7 (CH)	71.6 (CH)	71.5 (CH)
5	77.7 (CH)	77.7 (CH)	77.7 (CH)
6	69.3 (CH_2)	69.3 (CH_2)	69.4 (CH_2)
2-*O*-Rha^{III}			
1	102.8 (CH)	102.7 (CH)	102.7 (CH)
2	72.4 (CH)	72.3 (CH)	72.4 (CH)
3	72.3 (CH)	72.3 (CH)	72.3 (CH)
4	73.8 (CH)	73.8 (CH)	73.9 (CH)
5	69.9 (CH)	69.9 (CH)	69.9 (CH)
6	17.4 (CH_3)	17.4 (CH_3)	17.4 (CH_3)
6-*O*-Glc^{IV}			
1	104.4 (CH)	104.2 (CH)	104.5 (CH)
2	75.0 (CH)	74.8 (CH)	75.1 (CH)
3	78.0 (CH)	77.9 (CH)	78.0 (CH)
4	71.3 (CH)	71.3 (CH)	71.4 (CH)
5	77.5 (CH)	77.5 (CH)	76.3 (CH)
6	62.5 (CH_2)	62.5 (CH_2)	69.6 (CH_2)
6-*O*-Glc^{V}			
1			104.6 (CH)
2			75.1 (CH)
3			77.9 (CH)
4			71.7 (CH)
5			77.8 (CH)
6			62.4 (CH_2)
7-*O*-Glc^{V}			
1		101.5 (CH)	
2		74.4 (CH)	
3		77.9 (CH)	
4		71.5 (CH)	
5		77.6 (CH)	
6		62.5 (CH_2)	

*The spectra are recorded in CD_3OD.

signal at δ5.48 (d, J = 7.7 Hz) proved to belong to a β-glucopyranose unit. The high-field chemical shift values observed for H-2^{II} (δ3.84) and H-6^{II} (δ3.18 and 3.59) indicated the glycosylation sites. Diagnostic peaks in the ROESY NMR spectrum between H-1^{III} (δ4.94) and H-2^{II} (δ3.84) and between H-1^{IV} (δ4.16) and H-6^{II} (δ3.18 and 3.59) unambiguously defined the sugar moiety. Thus **11** is characterized as isorhamnetin 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-6-*O*-β-D-glucopyranosyl]-β-D-glucopyranoside}.

Compound **13** gave in the FAB (negative-ion mode) mass spectrum a quasi molecular ion peak at m/z 947 $[M-H]^-$, 162 mass units shifted relative to **11**, thus suggesting the presence of a further hexose sugar unit. This hypothesis was corroborated by comparison of the ^1H and ^{13}C NMR spectra of **11** and **13**, which showed almost identical resonances (Tables 3 and 4) for the aglycone moiety, differing in some additional signals in **13** related to the fourth sugar residue. Acetylation of **13** and successive COSY and HOHAHA analysis of the acetylated derivative (**13a**) revealed a β-glucopyranosyl structure for the fourth sugar residue. Comparison of the H-6^{IV} chemical shifts of **13a** with the corresponding shifts for **11a** (δ4.09, 3.91 for **11a** and 3.75, 3.38 for **13a**, Table 5), indicated that in **13a** the additional β-glucose was linked at C-6^{IV}. A ROESY NMR spectrum of **13a** confirmed this structure showing an interprotonic contact between H-1^V and the above-mentioned H-6^{IV}. The above data defined the structure of **13a** and consequently that of the parent compound **13** as isorhamnetin 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-6-*O*-β-D-gentiobiosyl]-β-D-glucopyranoside}.

Compound **12** differs from **11** only in the presence of an additional sugar residue at 7-*O* position of the isorhamnetin. Its structure was derived by UV, FAB MS, ^1H and ^{13}C NMR spectra and by comparison with **11**. In particular the ^1H and ^{13}C NMR spectra of **11** and **12** showed almost identical resonances in the mid-field region (Tables 3 and 4), indicating that the sugar moiety linked at C-3 of the aglycone was unchanged. Some additional resonances in the mid-field region of ^1H and ^{13}C NMR spectra of **12**, when compared to **11**, indicated the presence of a fourth sugar in **12**. This was confirmed by analysis of FAB (negative-ion-mode) mass spectrum which showed that **12** had the same mass as **13**, thus indicating its tetraglycosidic nature. The UV spectrum of **12** showed characteristic absorption bands for a isorhamnetin, substituted at positions 3 and 7 (Table 1), suggesting that the fourth sugar residue was linked at C-7. Dipolar interactions of H-1^V with H-6 and H-8 of the isorhamnetin in the ROESY NMR spectrum of **12**, together with the down field chemical shifts of H-6 (δ6.50) and H-8 (δ6.82) in the ^1H NMR spectrum of **12** (Table 4) confirmed this hypothesis. Analysis of NMR experiments on the pereacetylated compound **12a** established that the additional residue was an hexose in the pyranose form, as indicated by the high-field chemical shift of H-5^V (δ3.97, Table 5). Unfortunately, it was impossible to determine all the coupling constants between the protons belonging to this sugar residue because of their overlapping NMR resonances. However, the structure of the sugar moiety was clarified by chemical analysis. Thus **12** was sub-

Table 4. ^{1}H assignments* for compounds **11**–**13**

H	**11**, δ_H (mult., *J* Hz)	**12**, δ_H (mult., *J* Hz)	**13**, δ_H (mult., *J* Hz)
6	6.19 (*d*, 2.1)	6.50 (*d*, 2.1)	6.21 (*d*, 2.1)
8	6.42 (*d*, 2.1)	6.82 (*d*, 2.1)	6.45 (*d*, 2.1)
2^I	8.08 (*d*, 2.1)	8.08 (*d*, 2.1)	8.08 (*d*, 2.1)
5^I	6.94 (*d*, 8.3)	6.96 (*d*, 8.4)	6.96 (*d*, 8.4)
6^I	7.61 (*dd*, 2.1, 8.3)	7.70 (*dd*, 2.1, 8.4)	7.64 (*dd*, 2.1, 8.4)
MeO-	4.02 (*s*)	4.02 (*s*)	4.01 (*s*)
3-*O*-Glc^{II}			
1	5.85 (*d*, 7.3)	5.80 (*d*, 7.7)	5.82 (*d*, 7.3)
2	3.69 (*dd*, 7.3, 9.4)	3.69 (*dd*, 7.7, 9.4)	3.67[a]
3	3.66 (*t*, 9.4)	3.64 (*t*, 9.4)	3.64[a]
4	3.42 (*t*, 9.4)	3.32[a]	3.36[a]
5	3.56[a]	3.58[a]	3.52[a]
6a	4.08[a]	4.03[a]	4.03[a]
6b	3.70[a]	3.70[a]	3.70[a]
2-*O*-Rha^{III}			
1	5.22 (*bs*)	5.22 (*bs*)	5.22 (*bs*)
2	4.06 (*bd*, 3.5)	4.03 (*bd*, 3.2)	4.03 (*bd*, 3.2)
3	3.80 (*dd*, 3.5, 9.7)	3.80 (*dd*, 3.2, 9.5)	3.80 (*dd*, 3.2, 9.5)
4	3.36 (*t*, 9.7)	3.36 (*t*, 9.5)	3.35[a]
5	4.07 (*dq*, 9.7, 6.2)	4.10 (*dq*, 9.5, 6.3)	4.07[a]
6	0.93 (*d*, 6.2)	0.97 (*d*, 6.3)	0.93 (*d*, 6.2)
6-*O*-Glc^{IV}			
1	4.21 (*d*, 7.6)	4.18 (*d*, 7.7)	4.28 (*d*, 7.7)
2	3.08 (*dd*, 7.6, 9.0)	3.00 (*dd*, 7.7, 9.1)	3.19[a]
3	3.18 (*t*, 9.0)	3.09 (*t*, 9.1)	3.30[a]
4	3.20 (*t*, 9.0)	3.18 (*t*, 9.1)	3.26[a]
5	2.98 (*m*)	2.88 (*m*)	3.09[a]
6a	3.72[a]	3.75[a]	4.02[a]
6b	3.52 (*dd*, 5.5, 11.8)	3.51[a]	3.60[a]
6-*O*-Glc^{V}			
1			4.21 (*d*, 7.7)
2			3.05 (*dd*, 7.7, 9.1)
3			3.16 (*t*, 9.1)
4			3.18[a]
5			2.97[a]
6a			3.87 (*dd*, 2.1, 11.9)
6b			3.68[a]
7-*O*-Glc^{V}			
1		5.11 (*d*, 7.0)	
2		3.52[a]	
3		[b]	
4		[b]	
5		[b]	
6a		3.98[a]	
6b		3.79[a]	

*The spectra are recorded in CD_3OD.
[a]Superimposed by other signals; [b]unassignable.

mitted to acid methanolysis followed by silylation with trisil-Z. GC analysis of the released saccharides showed a sugar composition of glucose and rhamnose in a relative ratio 3:1, thus pointing to a glucose residue linked at C-7. A β-configuration at C-1^V was assigned on the basis of the vicinal coupling constant of 7.0 Hz observed in the ^{1}H NMR spectrum of **12** for the anomeric proton (δ5.11, Table 5). Compound **12** is identified from the above data as isorhamnetin 3-*O*-{[2-*O*-α-L-rhamnopyranosyl-6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside}-7-*O*-β-D-glucopyranoside.

By assuming glucose and rhamnose to belong to the most commonly found D- and L-forms, respectively, the absolute stereochemistry of **10**–**13** could be defined.

The isolated compounds **7**–**13** were evaluated for their inhibitory activity on platelet aggregation

Table 5. ^{1}H assignments* for compounds **11a**–**13a**

H	**11a**, δ_H (mult., *J* Hz)	**12a**, δ_H (mult., *J* Hz)	**13a**, δ_H (mult., *J* Hz)
6	6.79 (*d*, 2.2)	6.67 (*d*, 2.2)	6.80 (*d*, 2.2)
8	7.43 (*d*, 2.2)	7.14 (*d*, 2.2)	7.40 (*d*, 2.2)
2^I	8.18 (*d*, 1.8)	8.05 (*d*, 1.8)	8.09 (*d*, 1.8)
5^I	7.19 (*d*, 8.3)	7.18 (*d*, 8.4)	7.21 (*d*, 8.7)
6^I	8.16 (*dd*, 1.8, 8.3)	7.93 (*dd*, 1.8, 8.4)	8.03 (*dd*, 1.8, 8.7)
MeO-	3.97 (*s*)	3.95 (*s*)	3.96 (*s*)
3-*O*-Glc^{II}			
1	5.48 (*d*, 7.7)	5.57 (*d*, 7.8)	5.54 (*d*, 7.8)
2	3.84 (*dd*, 8.0, 9.9)	3.78 (*dd*, 7.8, 9.3)	3.82 (*dd*, 7.8, 9.6)
3	5.36 (*t*, 9.9)	5.34 (*t*, 9.3)	5.35 (*t*, 9.6)
4	4.76 (*t*, 9.9)	4.78 (*t*, 9.3)	4.82 (*t*, 9.6)
5	3.57 (*ddd*, 1.5, 8.0, 9.9)	3.58 (*ddd*, 1.8, 6.5, 9.3)	3.59 (*ddd*, 2.5, 6.5, 9.6)
6a	3.18 (*dd*, 1.5, 13.6)	3.65 (*dd*, 1.8, 12.1)	3.64 (*dd*, 2.5, 12.1)
6b	3.59 (*dd*, 8.0, 13.6)	3.90 (*dd*, 6.5, 12.1)	3.44 (*dd*, 6.5, 12.1)
2-*O*-Rha^{III}			
1	4.94 (*bs*)	4.91 (*bs*)	4.93 (*bs*)
2	5.06 (*bd*, 3.4)	5.06 (*bd*, 3.4)	5.07 (*bd*, 3.4)
3	5.49 (*dd*, 3.4, 10.2)	5.44 (*dd*, 3.4, 10.2)	5.49 (*dd*, 3.4, 10.0)
4	5.04 (*t*, 10.2)	5.03 (*t*, 10.2)	5.03 (*t*, 10.0)
5	4.50 (*dq*, 6.2, 10.2)	4.43 (*dq*, 6.2, 10.2)	4.45 (*dq*, 6.2, 10.0)
6	0.92 (*d*, 6.2)	0.90 (*d*, 6.2)	0.90 (*d*, 6.2)
6-*O*-Glc^{IV}			
1	4.16 (*d*, 8.0)	4.17 (*d*, 8.1)	4.18 (*d*, 8.1)
2	4.75 (*dd*, 8.0, 9.6)	4.76 (*dd*, 8.1, 9.3)	4.74 (*dd*, 8.1, 10.0)
3	4.94 (*t*, 9.6)	4.95 (*t*, 9.3)	4.91 (*t*, 10.0)
4	4.88 (*t*, 9.6)	4.93 (*t*, 9.3)	4.81 (*t*, 10.0)
5	2.64 (*ddd*, 2.2, 4.3, 9.6)	2.98 (*ddd*, 2.2, 4.3, 9.3)	2.84 (*ddd*, 2.5, 5.9, 10.0)
6a	4.09 (*dd*, 4.3, 12.6)	4.09 (*dd*, 4.3, 12.1)	3.75 (*dd*, 2.5, 11.2)
6b	3.91 (*dd*, 2.2, 12.6)	3.91 (*dd*, 2.2, 12.1)	3.38 (*dd*, 5.9, 11.2)
6-*O*-Glc^{V}			
1			4.42 (*d*, 8.1)
2			4.91 (*dd*, 8.1, 9.6)
3			5.14 (*t*, 9.6)
4			5.05 (*t*, 9.6)
5			3.66 (*ddd*, 2.5, 4.7, 9.6)
6a			4.26 (*dd*, 4.7, 12.1)
6b			4.09 (*dd*, 2.5, 12.1)
7-*O*-Glc^{V}			
1		5.21[a]	
2		5.30[a]	
3		5.30[a]	
4		5.20[a]	
5		3.97[a]	
6a		4.34 (*dd*, 5.0, 12.5)	
6b		4.23 (*dd*, 2.2, 12.5)	

*The spectra are in $CDCl_3$. Additional ^{1}H signals for the acetyl groups; **11a**: signals at δ2.49, 2.35, 2.33, 2.14, 2.12, 2.05, 2.03, 2.02, 2.01, 1.98, 1.97, 1.40 (each 3H, *s*); **12a**: signals at δ2.48, 2.36, 2.13, 2.11 (each 3H, *s*), 2.05, 2.04, 2.02, 2.00 (each 6H, *s*), 1.98, 1.96, 1.61 (each 3H, *s*); **13a**: signals at δ2.49, 2.37, 2.33, 2.13, 2.12 (each 3H, *s*), 2.04, 2.02 (each 6H, *s*), 2.01, 2.00, 1.99, 1.98, 1.96, 1.94 (each 3H, *s*).

[a]Superimposed by other signals.

induced by collagen. This action has been already shown for kaempferol [8]. Compound **7** (10^{-5} M) was the only active inhibitor of platelet response to collagen. Its inhibitory effect was not increased by increasing concentration. The effect of this compound is reported in Fig. 1 as percentage of aggregation at a concentration of 10^{-5} M.

EXPERIMENTAL

General methods. FAB-MS (recorded in glycerol matrix) were measured on a VG ZAB mass spectrometer (XE atoms of energy of 2–6 kV). ^{1}H and ^{13}C NMR measurements were performed on a Bruker AMX-500 spectrometer. Chemical shifts were ref-

erenced to the residual solvent signal ($CDCl_3$: $\delta_H = 7.26$, $\delta_C = 77.0$; CD_3OD: $\delta_H = 3.34$, $\delta_C = 49.0$). Signals of methyl, methylene and methine carbon atoms were distinguished by DEPT experiments. One-bond heteronuclear ^1H–^{13}C connectivities were determined by means of HETCOR and HMQC [9] experiments ($^1J_{CH}$ of 125 Hz). During the HMQC acquisition time ^{13}C broad-band decoupling was performed by using the GARP sequence [10]. ^1H connectivities were determined by using COSY and HOHAHA experiments; the 2D HOHAHA experiments were performed in the phase-sensitive mode (TPPI) using the MLEV-17 (mixing time 125 ms) sequence for mixing [11]. NOE measurements were performed by 2D ROESY experiments. MPLC were performed on a Buchi 861 apparatus using a SiO_2 (230-400 mesh) and RP-18 columns. HPLC were performed on a Varian apparatus equipped with UV and refractive index detectors. Hibar LiChrospher columns were used.

Plant material. Allium neapolitanum was collected in April 1995 near Salerno (Campania, Italy). The plants were frozen immediately after collection and kept frozen until extraction. A reference specimen has been deposited at the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, Italy.

Isolation procedure. The collected plant samples (117 g, dry wt after extraction) were homogenized and extracted with H_2O ($\times 2$, 2 l for 8 hr each): the aqueous extracts were decanted and passed through a column of Amberlite XAD-2 (1 kg), which was washed with distilled H_2O (1 l) and eluted with MeOH (2 l) to give, after removal of the solvent, a dark brown material (4.5 g) which was chromatographed on a Sephadex LH-20 column (4 × 100 cm) with MeOH–H_2O (2:1) as eluent. Frs (5 ml) were collected and analysed by TLC on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5) and $CHCl_3$–MeOH–H_2O (40:9:1). Frs 97–107 contained less polar compounds (30 mg). Further fractionation of the material by reversed-phase HPLC using H_2O–MeOH (2:3) afforded **1** (7 mg) and **2** (2 mg). Frs 86–96 (85 mg) purified by reversed-phase HPLC using H_2O–MeOH 4.2:5.8 give **3** (33 mg). Frs 75–85 (104 mg) were submitted to reversed-phase MPLC using gradient solvent system from H_2O to MeOH. Elution with H_2O–MeOH (3:2) gave **4** (29 mg). Elution with H_2O–MeOH (1:1) gave **5** (8 mg). Frs 65–74 (182 mg) were purified by reversed-phase MPLC using H_2O–MeOH (1:1) to give a further 27 mg of pure **5**, and then H_2O–MeOH (3:2) to give **10** which was purified by reversed-phase HPLC with a 15 min linear gradient starting from a mixture of H_2O, acidified with HOAc at pH 3.6 (soln. A), and MeOH (soln. B) A–B = 6.5:3.5 to a mixture of A–B = 5.5:4.5. Detection was effected at 280 nm. Compound **9** (6 mg) was collected. Frs 58–64 (274.8 mg) were further purified by reversed-phase MPLC with H_2O–MeOH (3:2) to give **11** (54 mg). Frs 40–57 (1.2 g) contained a complex mixture of flavonoid glycosides. Fractionation was pursued by DCCC using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the descending mode, the lower phase was the mobile phase, flow rate 10 ml hr^{-1}; 6 ml frs were collected and monitored by TLC on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5) and $CHCl_3$–MeOH–H_2O (40:9:1). Frs 38–48 (160 mg) were further purified by reversed-phase MPLC (same conditions as before). Elution with H_2O–MeOH (7:3) gave **6** (5 mg) and **7** (20 mg). Elution with H_2O–MeOH (3:2) afforded a fr. (30 mg) containing **9** and **13**, which were purified by reversed-phase HPLC using the linear gradient system above described. Pure **9** (4 mg) and **13** (1 mg) were collected. Frs 27–34 (115 mg) were submitted to reversed-phase MPLC. Elution with H_2O–MeOH (7.5:2.5) gave **8** (4 mg). Elution with H_2O–MeOH (7:3) gave **12** (2 mg).

Acetylation of flavonoid glycosides. The acetylations were performed with Ac_2O and pyridine (1:1) overnight at room temp. The concd reaction mixts were subjected to HPLC (column: Hibar LiChrospher Si-60 5 mm; eluent: *n*-hexane–EtOAc, 3:7).

Methanolysis of **12**. Sugar analysis. A solution of **12** (1 mg) in anhydrous 2M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 hr. After cooling, neutralization with Ag_2CO_3, and centrifugation, the supernatant was evapd to dryness under N_2. The residue was trimethylsilylated with TRISIL Z (Pierce Chemical Co.) for 15 min at room temp. GLC analysis gave peaks which co-eluted with those of methylrhamnoside and methylglucoside.

Kaempferol 3-O-{[2-O-α-L-*rhamnopyranosyl*-4-O-β-D-*glucopyranosyl*]-β-D-*glucopyranoside*} (**10**). $[\alpha]_D^{25} = -57°$ (MeOH); FAB-MS (negative ion) *m/z* 755 $[M-H]^-$; UV spectra see Table 1; ^1H and ^{13}C NMR spectra see Table 2.

Isorhamnetin 3-O-{[2-O-α-L-*rhamnopyranosyl*-6-O-β-D-*glucopyranosyl*]-β-D-*glucopyranoside*} (**11**). $[\alpha]_D^{25} = -56°$ (MeOH); FAB-MS (negative ion) *m/z* 785 $[M-H]^-$; UV spectra see Table 1; ^{13}C NMR and ^1H NMR spectra see Tables 3 and 4, respectively.

Isorhamnetin 3-O-{[2-O-α-L-*rhamnopyranosyl*-6-O-β-D-*glucopyranosyl*]-β-D-*glucopyranoside*}-7-O-β-D-glucopyranoside (**12**). $[\alpha]_D^{25} = -43°$ (MeOH); FAB-MS (negative ion) *m/z* 947 $[M-H]^-$; UV spectra see Table 1; ^{13}C and ^1H NMR spectra see Tables 3 and 4, respectively.

Isorhamnetin 3-O-{[2-O-α-L-*rhamnopyranosyl*-6-O-β-D-*gentiobiosyl*]-β-D-*glucopyranoside*} (**13**). $[\alpha]_D^{25} = -42°$ (MeOH); FAB-MS (negative ion) *m/z* 947 $[M-H]^-$; UV spectra see Table 1; ^{13}C and ^1H NMR spectra see Tables 3 and 4, respectively.

Platelet preparation. Blood (20 ml) was withdrawn by cardiac puncture from rabbits into a plastic syringe containing 1/10 vol. of 3.8% sodium citrate and mixed immediately. Platelet-rich plasma (PRP) was prepared by centrifugation of the anticoagulated blood at 250 *g* for 10 min. Platelet-poor plasma (PPP) was obtained from centrifugation of the residue at 650 *g* for 10 min. All procedures were performed at room temp.

Assessment of platelet aggregation. Platelet aggregation was monitored in an Elvi Pyton (Born) light

transmission aggregometer. PPP was used to adjust to 100% transmittance. For all experiments 250 μl of PRP and 5 μl of the aqueous test sample soln, or vehicle, were left in contact at room temp. for 10 min, then were incubated at 37° for 1 min with stirring and collagen (5 μg ml^{-1}) was added as aggregating agent.

Statistical analysis. Results obtained are expressed as mean ± S.E.M. and analysed by two tailed unpaired Student's test.

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