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# THE FLAVONOIDS OF *ALLIUM URSINUM**

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**Key Word Index**—*Allium ursinum*; Liliaceae; flavonoid glycosides; antiplatelet aggregation activity.

**Abstract**—From wild garlic *Allium ursinum* three new flavonoid glycosides were identified as kaempferol 3-*O*-*β*-neohesperidoside-7-*O*-[2-*O*-(*trans*-*p*-coumaroyl)]-*β*-D-glucopyranoside, kaempferol 3-*O*-*β*-neohesperidoside-7-*O*-[2-*O*-(*trans*-feruloyl)]-*β*-D-glucopyranoside, kaempferol 3-*O*-*β*-neohesperidoside-7-*O*-[2-*O*-(*trans*-*p*-coumaroyl)-3-*O*-*β*-D-glucopyranosyl]-*β*-D-glucopyranoside and characterized as the peracetates. Additionally, two known flavonoid glycosides kaempferol 3-*O*-*β*-glucopyranoside and kaempferol 3-*O*-*β*-neohesperidoside were isolated. The isolated compounds showed an inhibition of human platelet aggregation.

## INTRODUCTION

During a systematic study on the chemistry of *Allium* species from southern Italy we analysed the wild garlic *Allium ursinum* whose thiosulphinate profile of the room temperature extract was found to be in good agreement with literature data [1]. We report here that this organism also elaborates five flavonoid glycosides, three of which are new products, with anti-aggregation platelet activity.

## RESULTS AND DISCUSSION

Samples of the wild garlic *Allium ursinum* were collected in the Laceno Lake area (Campania, Italy), during June 1993. The plant materials were extracted at room temperature with ethanol; the *n*-butanol soluble fraction, on repeated chromatographic separations, yielded the flavonol glycosides **1**–**5**. The structures of **1** and **2** were assigned on the basis of their mass, UV and $^1$H NMR spectral data and comparison with the literature data [2].

The new compound **3** showed a pseudomolecular ion peak at *m/z* 901 in the negative-ion FAB mass spectrum. The UV spectrum of **3** showed the characteristic bands of a kaempferol, substituted at position 3 and 7, as indicated by the bathochromic shift on addition of diagnostic reagents [3] (see Table 1). A band at 318 nm suggested the presence of a *p*-coumaroyl chromophore. The $^1$H and $^{13}$C NMR spectra confirmed this hypothesis (Table 2). In particular the $^1$H NMR spectrum showed the typical pairs of doublets, one ($\delta$7.71 and 6.40) with a coupling of 16 Hz, identifying the *trans* olefinic double bond protons, and the second pair caused by the four ring protons.

The glycosidic nature of the compound was clearly indicated by a number of oxymethylene and oxymethine signals in proton and $^{13}$C NMR spectra. The structure of the sugar moieties could be entirely established on the basis of NMR data. Particular attention was paid to the very crowded mid-field region of the $^1$H NMR spectrum, characterized by the strong overlapping of many signals. In order to overcome this difficulty, we acetylated **3** to improve the signal resolution, thus obtaining the dodecaacetate **3a** (see Experimental). The $^1$H NMR spectrum and most two-dimensional NMR experiments, used for structural determination, were performed on compound **3a**. Three anomeric proton signals at $\delta$4.90, 5.25 and 5.61 were identifiable in the $^1$H NMR spectrum (see Table 3). When the anomeric proton $\delta$4.90 (*d*, $J$ = 1.3 Hz) was used as a starting point, analysis of COSY and HOHAHA experiments allowed the identification, in sequence, of four oxymethine and one methyl groups (see Table 3). All these oxymethine protons, except H-5, turned out to be linked to an acetylated carbon atom, as indicated by their low-field resonances, thus suggesting a terminal 6-deoxyhexose unit in the pyranose form. The sugar moiety was identified as rhamnose due to the small couplings of H-2 with H-1 and H-3, implying its equatorial position, and to the large couplings observed for the remaining methine protons. An intense nOe between H-3 and H-5 further indicated their axial position.

* Part 1 in the series 'Chemistry of the genus *Allium*'.
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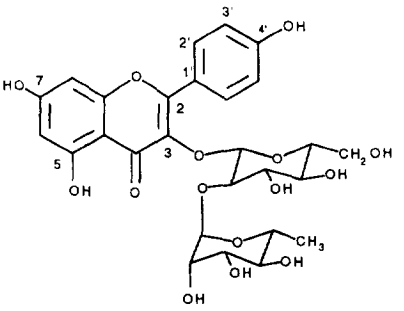

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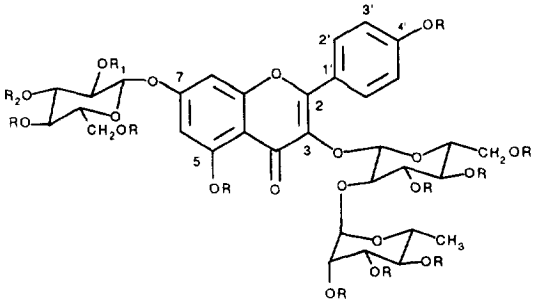

| | R | $R_1$ | $R_2$ |
|---|---|---|---|
| **2** | H | H | H |
| **3** | H | *trans-p*-coumaroyl | H |
| **3a** | Ac | *trans-p*-acetoxycoumaroyl | Ac |
| **4** | H | *trans*-feruloyl | H |
| **4a** | Ac | *trans*-acetoxyferuloyl | Ac |
| **5** | H | *trans*-p-coumaroyl | Glc |
| **5a** | Ac | *trans-p*-acetoxycoumaroyl | Glc $Ac_4$ |

Table 1. UV spectral absorptions of **3** and **5**

| | $\lambda_{max}$ in | | | | |
|---|---|---|---|---|---|
| | MeOH | NaOMe | $AlCl_3$ | $AlCl_3$/HCl | NaOAc |
| **3** | 355 | 395 | 391 | 390 | 360 |
| | 318 *sh* | 370 *sh* | 354 | 354 | 315 |
| | 300 | 296 | 322 | 316 | 299 |
| | 266 *sh* | 267 | 300 | 299 | 267 *sh* |
| | 226 | 242 | 271 | 272 | |
| | | | 264 | 267 | |
| **5** | 355 | 400 | 390 | 394 | 355 |
| | 317 | 370 | 350 | 350 | 313 |
| | 301 | 299 | 320 | 320 | 292 |
| | 267 | 275 | 299 | 300 | 265 *sh* |
| | 226 | 243 *sh* | 273 | 275 | |
| | | | 262 | 267 *sh* | |

The small coupling observed for the anomeric proton, together with the lack of nOe of H-1 with H-3 and H-5, pointed to an $\alpha$ configuration of the rhamnose unit [4].

The second anomeric proton at $\delta$5.61 (*d*, $J$ = 7.8 Hz) was proven to belong to a $\beta$-glucopyranose due to the large couplings of all the oxymethine protons identified by COSY and HOHAHA experiments, which evidenced the $^1H$–$^1H$ connectivities. In addition the high-field chemical shift of H-2 ($\delta$3.73) clearly indicated that the hydroxy group at C-2 was glycosylated rather than acetylated. This hypothesis was corroborated by analysis of a ROESY NMR spectrum showing a dipolar interaction of the last proton with the anomeric one of rhamnose, thus securing a $\beta$-neohesperidoside structure.

The last anomeric proton at $\delta$5.25 (*d*, $J$ = 7.5 Hz) was attributed to a $\beta$-glucopyranose, on the basis of the same arguments as those for the characterization of previous sugar units. Dipolar interactions in the ROESY spectrum of this anomeric proton with H-6 and H-8 kaempferol protons positioned this glucose residue at C-7 of the aglycone. Consequently, the neohesperidoside moiety was linked at C-3, since UV and $^{13}C$ NMR spectra of **3** clearly indicated that kaempferol was substituted at position 3 and 7.

Once the structure of compound **3a** was secured, except for the location of the *trans-p*-coumaroyl residue, we could achieve full assignment of the $^1H$ and $^{13}C$ NMR spectra of the parent compound **3** through analysis of a COSY and a $^1H$–$^{13}C$ one-bond correlation experiment. The downfield shift of the H-2 signal of the $\beta$-glucose attached at C-7 in the $^1H$ NMR spectrum of **3** (see Table 2) is consistent with an acylation in this position, thus defining the *trans-p*-coumaroyl linkage site.

In addition to **3**, the ethanolic extract contains very small quantities of the related compound **4** which differs in having an extra methoxy group. The presence of **4** which shows chromatographic properties very similar to **3** can be pointed out in the NMR spectra of **3** in which little amounts ( $<10\%$) of **4** are visible, differing from **3** only in the signals relative to the *trans-p*-coumaroyl residue.

We succeeded in isolating **4** as the dodeca-acetate **4a**, after treatment with $Ac_2O$/Py during the purification of **3a**. The compound **4a** in the FABMS showed a molecular ion at *m/z* 1437 $(M + H)^+$. $^1H$ NMR spectrum of **4a** was almost identical to that of **3a** (see Table 3) in the kaempferol and sugar moieties. A singlet at $\delta$3.85 pointed to a methoxy group located on the ring of the coumaric residue, accordingly with the change in its aromatic proton resonances. A doublet at $\delta$7.05 (1H, $J$ = 8 Hz) was coupled with a signal at $\delta$7.11 (*dd*, 1H, $J$ = 8.1 and 2.4 Hz) in turn coupled with a broad singlet at $\delta$7.09 (1H, *d*, $J$ = 2.4 Hz) characterizing a 3-hydroxy-4-methoxy or a 3-methoxy-4-hydroxy disubstituted coumaric acid. A dipolar interaction in the ROESY spectrum of **4a** between the methoxy protons and the H-2 of the acyl residue (doublet at $\delta$7.09), located the methoxy group at position 3 and consequently the OH group at C-4, thus defining the structure.

As far as the new compound **5** is concerned, much of the same can be said as for **3**. The negative-ion FABMS of **5** showed a quasi-molecular ion peak at *m/z* 1063 $(M\text{-}H)^-$. A comparison of the various spectral features of

Table 2. $^{13}C$ and $^{1}H$ assignments* for compounds **3** and **5**

| | **3** | | **5** | |
|---|---|---|---|---|
| Pos. | $\delta_C$ (mult.) | $\delta_H$ (mult., J Hz) | $\delta_C$ (mult.) | $\delta_H$ (mult., J Hz) |
| 2 | 159.20 (C) | | 159.32 (C) | |
| 3 | 134.74 (C) | | 134.66 (C) | |
| 4 | 179.52 (C) | | 179.46 (C) | |
| 5 | 162.97 (C) | | 162.99 (C) | |
| 6 | 100.57 (CH) | 6.41 (*d*, 2.1) | 100.53 (CH) | 6.41 (*d*, 2.1) |
| 7 | 164.05 (C) | | 163.92 (C) | |
| 8 | 95.75 (CH) | 6.73 (*d*, 21) | 95.75 (CH) | |
| 9 | 157.93 (C) | | 157.90 (C) | |
| 10 | 107.92 (C) | | 107.95 (C) | |
| 1′ | 122.86 (C) | | 122.44 (C) | |
| 2′–6′ | 132.28 (CH) | 8.09 (*d*, 8.6) | 132.30 (CH) | 8.09 (*d*, 8.9) |
| 3′–5′ | 116.15 (CH) | 6.91 (*d*, 8.6) | 116.39 (CH) | 6.90 (*d*, 8.9) |
| 4′ | 161.58 (C) | | 162.32 (C) | |
| 3-O-Glc | | | | |
| 1 | 100.24 (CH) | 5.76 (*d*, 7.5) | 100.24 (CH) | 5.75 (*d*, 7.2) |
| 2 | 80.05 (CH) | 3.64 (*dd*, 7.5, 9.2) | 80.03 (CH) | 3.64 (*dd*, 7.2, 8.9) |
| 3 | 78.94 (CH) | 3.57 (*t*, 9.2) | 78.96 (CH) | 3.57 (*t*, 8.9) |
| 4 | 71.85 (CH) | 3.29 (*t*, 9.2) | 71.84[b] (CH) | 3.29[a] |
| 5 | 78.41 (CH) | 3.23 (*ddd*, 2.1, 5.8, 9.2) | 78.40 (CH) | 3.24[a] |
| 6 | 62.63 ($CH_2$) | 3.74 (*dd*, 2.1, 12.0) | 62.64 ($CH_2$) | 3.73 (*dd*, 2.1, 12.0) |
| | | 3.50 (*dd*, 5.8, 12.0) | | 3.50 (*dd*, 5.8, 12.0) |
| Rha | | | | |
| 1 | 102.63 (CH) | 5.25 (*bs*, 1.3) | 102.62 (CH) | 5.26 (*bs*, 1.3) |
| 2 | 72.39 (CH) | 4.02[a] | 72.40 (CH) | 4.02[a] |
| 3 | 72.28 (CH) | 3.79[a] | 72.29 (CH) | 3.79[a] |
| 4 | 74.02 (CH) | 3.36[a] | 74.04 (CH) | 3.34[a] |
| 5 | 69.91 (CH) | 4.05[a] | 69.92 (CH) | 4.05[a] |
| 6 | 17.55 ($CH_3$) | 0.98 (*d*, 6.2) | 17.56 ($CH_3$) | 0.98 (*d*, 6.5) |
| 7-O-Glc | | | | |
| 1 | 99.83 (CH) | 5.38 (*d*, 7.9) | 99.75 (CH) | 5.42 (*d*, 7.9) |
| 2 | 74.82 (CH) | 5.14 (*dd*, 7.9, 9.3) | 73.64 (CH) | 5.31 (*dd*, 7.9, 9.6) |
| 3 | 76.02 (CH) | 3.78 (*t*, 9.3) | 84.67 (CH) | 4.02[a] |
| 4 | 71.36 (CH) | 3.55 (*t*, 9.3) | 69.85 (CH) | 3.67[a] |
| 5 | 78.59 (CH) | 3.66 (*ddd*, 2.0, 9.3, 12.0) | 78.21 (CH) | 3.70[a] |
| 6 | 62.38 ($CH_2$) | 3.99 (*dd*, 12.0, 2.0) | 62.30 ($CH_2$) | 3.99[a] |
| | | 3.79[a] | | 3.80[a] |
| Glc | | | | |
| 1 | | | 104.98 (CH) | 4.47[a] |
| 2 | | | 74.77 (CH) | 3.23[a] |
| 3 | | | 77.71[c] (CH) | 3.34[a] |
| 4 | | | 71.37[b] (CH) | 3.34[a] |
| 5 | | | 78.21[c] (CH) | 3.35[a] |
| 6 | | | 62.54 ($CH_2$) | 3.91[a] |
| | | | | 3.68[a] |
| TPC | | | | |
| 1 | 127.12 (C) | | 126.88 (C) | |
| 2–6 | 131.25 (CH) | 7.48 (*d*, 8.6) | 131.36 (CH) | 7.47 (*d*, 8.6) |
| 3–5 | 116.84 (CH) | 6.81 (*d*, 8.6) | 117.00 (CH) | 6.80 (*d*, 8.6) |
| 4 | 161.41 (C) | | 161.98 (C) | |
| 7 | 147.27 (CH) | 7.71 (*d*, 16.0) | 147.54 (CH) | 7.70 (*d*, 15.8) |
| 8 | 114.83 (CH) | 6.40 (*d*, 16.0) | 114.59 (CH) | 6.38 (*d*, 15.8) |
| 9 | 168.27 (C) | | 168.33 (C) | |

*The spectra are in $CD_3OD$ (Bruker AMX-500).

[a]Superimposed by other signals.

[b,c]Signals with the same superscript are interchangeable.

Table 3. $^1$H assignments* for compounds **3a–5a**

| | **3a** | **4a** | **5a** |
|---|---|---|---|
| Pos. | $\delta_H$ (mult., $J$ Hz) | $\delta_H$ (mult., $J$ Hz) | $\delta_H$ (mult., $J$ Hz) |
| 6 | 6.68 (*d*, 2.2) | 6.68 (*d*, 2.4) | 6.64 (*d*, 2.0) |
| 8 | 6.98 (*d*, 2.2) | 6.98 (*d*, 2.4) | 6.94 (*d*, 2.0) |
| 2′–6′ | 7.99 (*d*, 8.5) | 7.99 (*d*, 8.8) | 7.98 (*d*, 8.8) |
| 3′–5′ | 7.19 (*d*, 8.5) | 7.19 (*d*, 8.8) | 7.18 (*d*, 8.8) |
| 3-O-Glc | | | |
| 1 | 5.61 (*d*, 7.8) | 5.61 (*d*, 7.8) | 5.59 (*d*, 7.5) |
| 2 | 3.73 (*dd*, 7.8, 9.5) | 3.74 (*dd*, 7.8, 9.5) | 3.73 (*dd*, 7.5, 9.5) |
| 3 | 5.29 (*t*, 9.5) | 5.29 (*t*, 9.5) | 5.28 (*t*, 9.5) |
| 4 | 4.94 (*dd*, 9.5, 10.2) | 4.94 (*dd*, 9.5, 10.2) | 4.93 (*t*, 9.5) |
| 5 | 3.57 (*ddd*, 2.4, 4.1, 10.2) | 3.57 (*ddd*, 2.4, 4.1, 10.2) | 3.56 (*ddd*, 2.3, 4.0, 9.5) |
| 6a | 3.96 (*dd*, 4.1, 12.2) | 3.96 (*dd*, 4.1, 12.2) | 3.95 (*dd*, 4.0, 12.2) |
| 6b | 3.90 (*dd*, 2.4, 12.2) | 3.90 (*dd*, 2.4, 12.2) | 3.88 (*dd*, 2.3, 12.2) |
| Rha | | | |
| 1 | 4.90 (*d*, 1.3) | 4.90 (*d*, 1.3) | 4.89 (*d*, 1.3) |
| 2 | 5.04 (*d*, 3.4) | 5.04 (*d*, 3.4) | 5.04 (*d*, 3.4) |
| 3 | 5.41 (*dd*, 3.4, 10.2) | 5.41 (*dd*, 3.4, 10.2) | 5.40 (*dd*, 3.4, 9.8) |
| 4 | 5.02 (*t*, 10.2) | 5.02 (*t*, 10.2) | 5.02 (*t*, 9.8) |
| 5 | 4.38 (*dq*, 6.4, 10.2) | 4.38 (*dq*, 6.4, 10.2) | 4.38 (*m*) |
| 6 | 0.88 (*d*, 6.4) | 0.88 (*d*, 6.4) | 0.87 (*d*, 6.44) |
| 7-O-Glc | | | |
| 1 | 5.25 (*d*, 7.5) | 5.24 (*d*, 7.5) | 5.11 (*d*, 7.5) |
| 2 | 5.46 (*dd*, 7.5, 9.5) | 5.46 (*dd*, 7.5, 9.5) | 5.44 (*dd*, 7.5, 9.5) |
| 3 | 5.41 (*t*, 9.5) | 5.41 (*t*, 9.5) | 4.10 (*t*, 9.5) |
| 4 | 5.20 (*t*, 9.5) | 5.20 (*t*, 9.5) | 5.05 (*t*, 9.5) |
| 5 | 3.99 (*ddd*, 2.4, 4.1, 9.5) | 3.99 (*ddd*, 2.4, 4.1, 9.5) | 3.98 (*ddd*, 2.7, 5.7, 9.5) |
| 6a | 4.28 (*dd*, 4.1, 12.2) | 4.28 (*dd*, 4.1, 12.2) | 4.26 (*dd*, 2.7, 12.2) |
| 6b | 4.23 (*dd*, 2.4, 12.2) | 4.23 (*dd*, 2.4, 12.2) | 4.23 (*dd*, 5.7, 12.2) |
| Glc | | | |
| 1 | | | 4.62 (*d*, 8.1) |
| 2 | | | 4.93 (*t*, 8.1) |
| 3 | | | 5.08[a] |
| 4 | | | 5.08[a] |
| 5 | | | 3.70 (*ddd*, 2.0, 4.0, 9.5) |
| 6a | | | 4.39 (*dd*, 4.0, 12.2) |
| 6b | | | 4.06 (*dd*, 2.0, 12.2) |
| TPC | | | |
| 2 | 7.53 (*d*, 8.5) | 7.09 (*d*, 2.4) | 7.57 (*d*, 8.5) |
| 3 | 7.12 (*d*, 8.5) | | 7.15 (*d*, 8.5) |
| 5 | 7.12 (*d*, 8.5) | 7.05 (*d*, 8.1) | 7.15 (*d*, 8.5) |
| 6 | 7.53 (*d*, 8.5) | 7.11 (*dd*, 2.4, 8.1) | 7.57 (*d*, 8.5) |
| 7 | 7.70 (*d*, 15.9) | 7.68 (*d*, 15.9) | 7.73 (*d*, 15.9) |
| 8 | 6.33 (*d*, 15.9) | 6.32 (*d*, 15.9) | 6.37 (*d*, 15.9) |
| $OCH_3$ | | 3.85 (*s*) | |

*The spectra are in $CDCl_3$ (Bruker AMX-500). Additional $^1$H signals for the acetyl groups; compound **3a**: signals at $\delta$2.45, 2.33, 2.30, 2.12, 2.10, 2.07, 2.06, 2.02, 2.01, 1.99, 1.98, 1.87 (each 3H, *s*); compound **4a**: signals at $\delta$2.45, 2.33, 2.28, 2.12, 2.10, 2.07, 2.06, 2.04, 2.02, 1.99, 1.98, 1.87 (each 3H, *s*); compound **5a**: signals at $\delta$2.44, 2.33, 2.31, 2.12, 2.10, 2.08, 2.07, 2.05, 2.04, 2.02, 1.99 (each 3H, *s*), 1.98 (6H, *s*), 1.94, 1.86 (each 3H, *s*).

[a]Superimposed by other signals.

**5** and **3** showed a close structural relationship, and defined the differences between them. Compounds **5** and **3** showed virtually identical $^1$H and $^{13}$CNMR resonances (Table 2) for the aglycone moiety, and differ in some additional NMR signals of the sugar component of **5**. The NMR spectra of **5** showed signals related to a fourth sugar residue in agreement with the molecular weight. Thus, **5** appeared to be a glycosyl derivative of **3**.

Acetylation of **5** afforded the pentadeca-acetate **5a**, whose $^1$H NMR spectrum showed well-resolved multiplets in the mid-field region. A COSY together with

a HOHAHA experiment allowed us to identify the spin systems and to follow the proton sequences. In particular the additional anomeric proton ($\delta$4.62, *d*, $J$ = 8.1 Hz) was shown to belong to a $\beta$-glucopyranose on the basis of the large coupling constants observed among all the oxymethine protons. Thus, **3** and **5** were shown to be related molecules, differing by the additional $\beta$-glucopyranose residue in **5**. Once the structure of **5a** was secured, except in the location of this $\beta$-glucoside residue, we could achieve full assignment of the $^1$H and $^{13}$C spectra of **5** through analysis of COSY, HOHAHA and HETCOR spectra (see Table 2). In order to determine the site of glucosylation we compared the $^{13}$C NMR spectra of the glycosides **3** and **5**. The intense downfield shift in **5** of C-3 of the acylated $\beta$-glucose residue ($\delta$84.67, $\Delta$ = 8.65 ppm, $\beta$-effect) together with the upfield shifts of C-4 ($\delta$69.85, $\Delta\delta$ = 1.51) and C-2 (73.64, $\Delta\delta$ = 1.18 ppm) suggested that the glycosidic linkage was positioned at C-3. Therefore the structure of **5** could be entirely determined. If the hexopyranoses are assumed to belong to the most commonly found D and L series for glucose and rhamnose respectively, the absolute stereochemistry of compounds **3**–**5** could be assigned.

The isolated compounds **1**–**5** were evaluated for their inhibitory activity on platelet aggregation induced by collagen. This action has been already shown for kaempferol [5]. Compounds **1** and **2** were active inhibitors of platelet response to collagen. The effects of these compounds are reported in Fig. 1 as percentage of aggregation at a concentration of $10^{-3}$ M. Compounds **3**–**5** in the same conditions showed no significant activity. The data obtained suggested that both the increase of sugar residues and the presence of a *trans-p*-coumaroyl group lower the inhibitory activity.

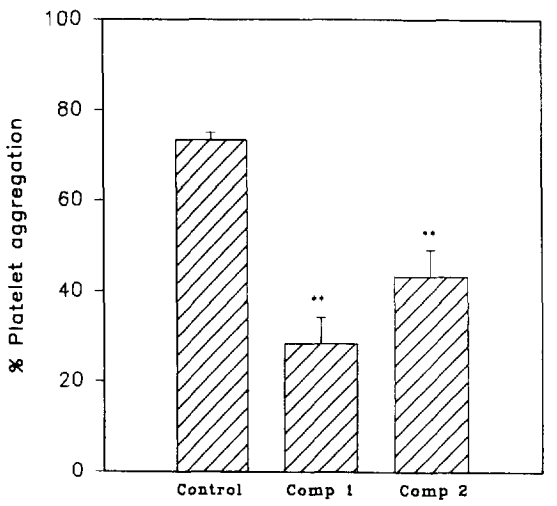


Fig. 1. Effect of compounds **1** and **2** on collagen-induced platelet aggregation at a concentration of $10^{-3}$ M (Control, 74.33 ± 1.76%; compound **1**, 28.33 ± 5.93%; Compound **2**, 43.33 ± 5.93%). $^{**}P < 0.01$, Dunnett test versus control group.

## EXPERIMENTAL

*General methods.* FABMS (recorded in glycerol matrix) were measured on a VG ZAB mass spectrometer (Xe atoms of energy of 2–6 kV). NMR Chemicals shifts were referenced 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 $^1$H–$^{13}$C connectivities were determined with a HETCOR experiment, optimized for an average of coupling of 125 Hz. $^1$H 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 [6]. Nuclear Overhauser effect (nOe) measurements were performed by 2D ROESY experiments. Medium-pressure liquid chromatography (MPLC) was performed on a Buchi 861 apparatus using a $SiO_2$ (230–400 mesh) and RP-18 columns. HPLC in isocratic mode was performed on a Varian apparatus equipped with a RI-4 refractive index detector. HPLC in gradient mode was performed on a Varian 5000 apparatus equipped with a UV detector.

*Plant material. Allium ursinum* was collected in June 1993 near Laceno Lake (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 (University of Naples, Italy).

*Isolation procedure.* The collected plant samples (60 g, dry weight after extraction) were homogenized and extracted with EtOH (5 × 800 ml) at room temperature. The combined extracts were filtered, the solvent was evaporated *in vacuo* to give an aqueous suspension which was extracted with EtOAc and subsequently with *n*-BuOH. Evaporation of the *n*-BuOH extract afforded 2.0 g of a dark brown residue, which was chromatographed by MPLC on a $SiO_2$ (230–400 mesh) column using a solvent gradient system from EtOAc to MeOH. Fractions containing flavonoid glycosides (EtOAc–MeOH, 1:1) were purified by MPLC on a RP-18 (40–63 $\mu$m) column with a gradient system from $H_2O$ to MeOH. The known compounds **1** (10 mg) and **2** (27 mg) were obtained by elution with $H_2O$–MeOH (4:6) and $H_2O$–MeOH (7:3), respectively. The new compounds **3**, **4** and **5** were obtained by elution with $H_2O$–MeOH (6:4). Further purification was achieved by reversed-phase HPLC on a Hibar LiChrospher RP-18 5 $\mu$m (4 × 250 mm) column with a mobile phase consisting of a gradient starting from MeOH 35% in $H_2O$ (5 min) and then increasing linearly to MeOH 45% in $H_2O$ (40 min). Detection was effected at 317 nm, thus obtaining a mixture of **3** and **4** (28 mg, relative ratio 9:1 by $^1$H NMR integration, $R_t$ = 18 min) and pure **5** (40 mg, $R_t$ = 25 min). Mixture of **3** and **4** (10 mg) was acetylated with $Ac_2O$ and pyridine (1:1) overnight at room temperature, concentrated under reduced pressure, and subjected to HPLC (column: Hibar LiChrospher Si-60 5 mm; eluant: *n*-hexane–EtOAc, 7:3) which afforded compounds **3a**

(9 mg) and **4a** (1 mg). Compound **5** (10 mg) subjected to the same treatment gave **5a**.

Kaempferol 3-*O*-β-neohesperidoside-7-*O*-(2-*O*-(*trans*-*p*-coumaroyl))-β-D-glucopyranoside (**3**): UV spectra see Table 1; $^1$H and $^{13}$C NMR spectra see Table 2; FABMS (negative ion) *m/z* 901 $(M - H)^-$.

Kaempferol 3-*O*-β-neohesperidoside-7-*O*-(2-*O*-(*trans*-*p*-coumaroyl))-β-D-glucopyranoside dodeca-acetate (**3a**): $[\alpha]_D^{25} = -40$ ($CHCl_3$); $^1$H NMR see Table 3; FABMS (positive ion) *m/z* 1407 $(M + H)^+$.

Kaempferol 3-*O*-β-neohesperidoside-7-*O*-(2-*O*-feruloyl)-β-D-glucopyranoside dodeca-acetate (**4a**): $[\alpha]_D^{25} = -36°$ ($CHCl_3$); $^1$H NMR see Table 3; FABMS (positive ion) *m/z* 1437 $(M + H)^+$.

Kaempferol 3-*O*-β-neohesperidoside-7-*O*-(2-*O*-(*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosil)-β-D-glucopyranoside (**5**): $[\alpha]_D^{25} = -45$ (MeOH); UV spectra see Table 1; $^1$H and $^{13}$C NMR spectra see Table 2; FABMS (negative ion) *m/z* 1063 $(M - H)^-$.

Kaempferol 3-*O*-β-neohesperidoside-7-*O*-(2-*O*-(*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside pentadeca-acetate (**5a**): $^1$H NMR see Table 3; FABMS (positive ion) 1695 $(M + H)^+$.

*Preparation of human platelets.* Venous blood was withdrawn from healthy donors into plastic syringes containing 1/10 vol. of 3.8% (w/v) sodium citrate and mixed immediately. The donors had not taken any medication for at least 10 days before blood collection. Platelet-rich plasma (PRP) was prepared by centrifugation of the citrated blood at $120 \times g$ for 10 min. Platelet-poor plasma (PPP) was obtained from centrifugation of the residue at $1100 \times g$ for 15 min. All procedures were performed at room temp.

*Measurements of platelet aggregation.* Platelet aggregation was measured using an ELVI 840 light transmission aggregometer. PPP was used to adjust to 100% transmittance. For all experiments 250 μl of PRP and 5 μl of the methanolic test samples soln, or vehicle, were incubated at 37°C for 1 min with stirring. Aggregation was induced by adding collagen (5 $\mu g\,ml^{-1}$).

*Statistical analysis.* Results obtained were analysed by ANOVA one-way followed by Dunnett's test for multiple comparison; a value of $P < 0.05$ was taken as significant.

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